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QUANTITATIVE NEAR-INFRARED SPECTROSCOPY IN HUMAN SKELETAL MUSCLE

METHODOLOGICAL ISSUES AND CLINICAL APPLICATION

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Quantitative near-infrared spectroscopy in human skeletal muscle

Methodological issues and clinical application

Een wetenschappelijke proeve op het gebied
van de Medische Wetenschappen

Proefschrift

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aan de Katholieke Universiteit Nijmegen,
volgens besluit van het College van Decanen
in het openbaar te verdedigen
op woensdag 24 april 2002
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door

Mireille Christine Petrine van Beekvelt
geboren op 13 juni 1969
te Geldrop

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In memory of my father

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List of abbreviations

ADP	adenosine diphosphate
ANOVA	analysis of variance
ANT	adenine nucleotide translocator
AO	arterial occlusion
ATP	adenosine triphosphate
ATT	adipose tissue thickness
BF	blood flow
BR	brachioradialis muscle
CK	creatine kinase
CK_m	mitochondrial creatine kinase
COX	cytochrome oxidase
CPEO	chronic progressive external ophthalmoplegia
Cr	creatine
CV	coefficient of variation
DPF	differential path-length factor
e^-	electron
EMG	electromyography
FBF	forearm blood flow (in $ml \cdot min^{-1} \cdot 100ml^{-1}$)
FBF_{NIRS}	FBF derived from NIRS
FBF_{Pleth}	FBF derived from strain-gauge plethysmography
FDS	flexor digitorum superficialis muscle
FID	free induction decay
FRDA	Friedreich ataxia
GASTR	gastrocnemius muscle
Hb	haemoglobin
Hb_{diff}	difference between O_2Hb and HHb ($Hb_{diff} = O_2Hb - HHb$)
HHb	deoxyhaemoglobin
HMb	deoxymyoglobin
IO	inter-optode distance
IO_{35}	inter-optode distance of 35 mm
IO_{50}	inter-optode distance of 50 mm
Mb	myoglobin
MELAS	mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes
MI	mitochondrial inner membrane
MO	mitochondrial outer membrane

MRC	medical research council
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
^{31}P-MRS	phosphorus magnetic resonance spectroscopy
MtDNA	mitochondrial DNA
MVC	maximal voluntary contraction
$m\dot{V}O_2$	muscle oxygen consumption (in $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$)
$m\dot{V}O_{2(Fick)}$	$m\dot{V}O_2$ derived from the Fick-method
$m\dot{V}O_{2(NIRS_{AO})}$	$m\dot{V}O_2$ derived from NIRS using venous occlusion
$m\dot{V}O_{2(NIRS_{VO})}$	$m\dot{V}O_2$ derived from NIRS using arterial occlusion
NIRS	near-infrared spectroscopy
O_2	oxygen
O_2Hb	oxyhaemoglobin
O_2Mb	oxymyoglobin
OD	optical density
OI	oxygenation index
PCr	phosphocreatine
PF	plantar flexion
pH_i	intracellular pH
P_i	inorganic phosphate
PME	phophomonoesters
POX	pulse oximeter
PVD	peripheral vascular disease
QUADRI	quadriceps muscle
r	correlation coefficient
RRF	ragged red fibres
SaO_2	arterial O_2 saturation
SD	standard deviation
SE	standard error
SOL	soleus muscle
STPD	standard temperature pressure dry
SvO_2	venous O_2 saturation
t_{50}	half-recovery time
Tc	time constant
tHb	total haemoglobin
VO	venous occlusion
ΔO_2Hb	reoxygenation rate

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Introduction

OUTLINE

Background
Near-infrared spectroscopy
- *Quantitative NIRS measurements*
  - *Venous occlusion method*
  - *Arterial occlusion method*
  - *Oxygen consumption*
  - *Blood flow*
  - *Venous oxygen saturation*
  - *Half-recovery time*
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- *NIRS measurements during exercise*
  - *Sustained vs. rhythmic isometric exercise*
  - *Arm vs. leg measurements*

Aim of the thesis
Thesis outline
- *Methodological issues*
- *Clinical application*

BACKGROUND

Light is a form of electromagnetic waves that transport energy through a medium. There are many applications of electromagnetic waves that have become established in normal daily life, varying from radio and TV to microwave and solarium. In the medical field, electromagnetic waves are also frequently used, for instance, with X-rays and magnetic resonance imaging (MRI). The various techniques are determined by the frequency of the wave and by the accompanying wavelength (Fig. 1).

The part of the electromagnetic spectrum between 10 - 10^6 nanometer we call light. The light spectrum contains, besides the visible light, also infrared and ultraviolet light. Whereas ultraviolet and visible light are almost completely absorbed in the skin, near-infrared light penetrates much deeper into the tissue. Propagation of light through biological tissue depends on reflection, scattering, and absorption [Jöbsis 1977]. While reflection is mainly determined by the angle of the light beam in relation to the surface of the tissue, scattering and absorption of the light within the tissue are dependent on the wavelength. While scattering of the photons simply decreases with increasing wavelength, the absorption pattern is more complicated. Above 1300 nm, light is completely absorbed

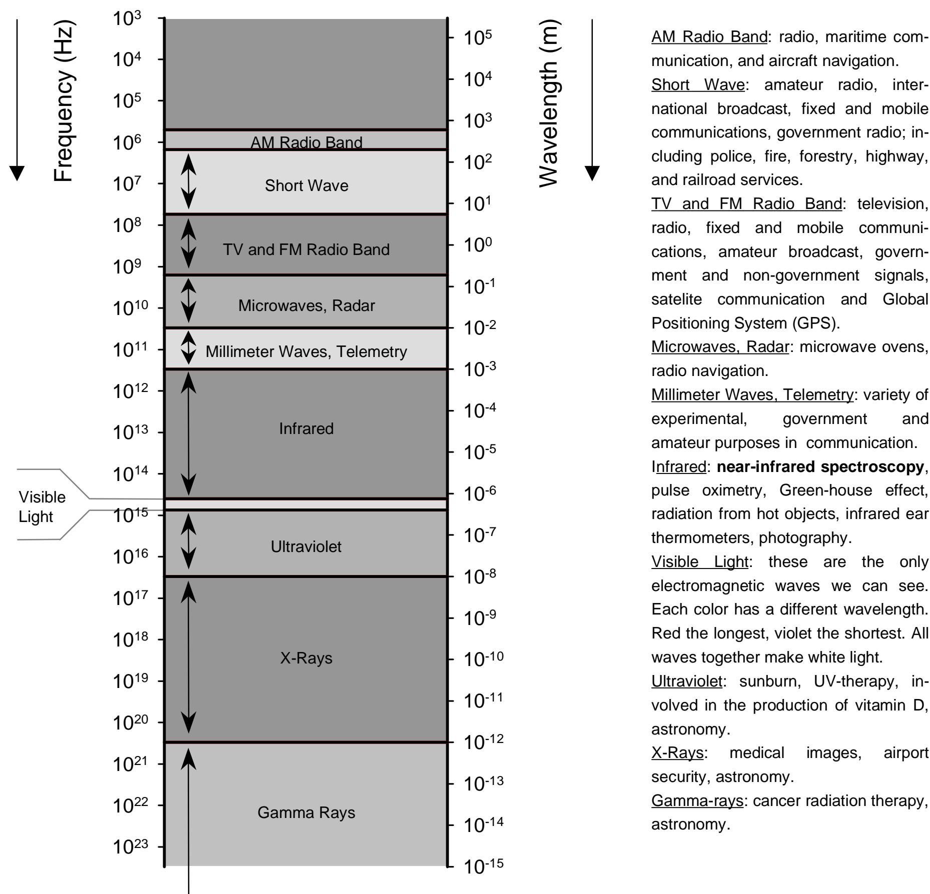

Fig. 1 The electromagnetic spectrum. Both frequency and wavelength are presented together with the most common applications.

by water in the most superficial layers of the skin. In the visible range of the light, below 700 nm, light is completely absorbed by haemoglobin and myoglobin, being the oxygen transporters of the blood and within the muscle cell, respectively. In the near-infrared region between 700-1300 nm, the light absorption of haemoglobin and myoglobin is much less and a significant amount of near-infrared light can, therefore, effectively travel through the tissue. This characteristic of relative transparency of the tissue for light in the near-infrared region is used by near-infrared spectroscopy (NIRS). By choosing distinct wavelengths of near-infrared light and knowledge of the chromophores that absorb that specific light, it becomes possible to follow concentration changes of that chromophore.

The near-infrared spectrophotometer, as used in this thesis, generates light at three wavelengths, which is transported to the tissue by means of an optical fibre bundle called optode. A second optode transports the light to the detector and is placed paralld to the light source, directly on the skin over the muscle or other tissue of interest (Fig. 2). The light penetrates into skin, subcutaneous fat layer and muscle, and is either scattered or absorbed within the tissue. The light scattering originating from the source occurs in any direction, but the light detected by the second optode is thought to describe a banana shape [Cui et al. 1991].

As mentioned above, the chromophores absorbing the light of these specific wavelengths are mainly haemoglobin and myoglobin. By using more than one wavelength it becomes possible to differentiate between the oxygenated and the deoxygenated form of haemoglobin/myoglobin and this again enables to obtain information about tissue oxygenation. The measurement of changes in tissue oxygenation that can now be followed noninvasively and directly in the muscle makes near-infrared spectroscopy an

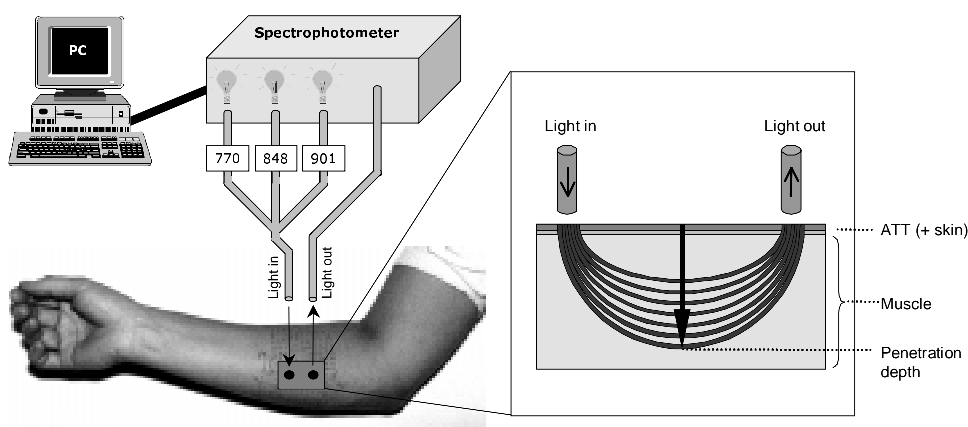

Fig. 2 Schematic presentation of the set-up with the spectrophotometer emitting light of 3 wavelengths (770, 848, and 901 nm) The light is transported into the tissue by an optical fibre (optode). The light that is detected by the receiver is thought to describe a banana-shape travelling through the tissue [Cui et al. 1991]. Typically, the interoptode distance used in this thesis was 35 and 50 mm.

interesting tool for clinical purposes. For instance, during surgery to check the blood (=oxygen) supply, at the intensive care to monitor brain oxygenation, or as a diagnostic tool in disorders that affect energy production.

Impaired energy production can be found in some metabolic myopathies and in mitochondrial myopathies. A screeningstest in this perspective is the ischemic forearm test where patients have to perform high intensity work under ischemic conditions [Sinkeler et al. 1986, Wevers et al. 1986]. By taking frequent blood samples during exercise and recovery, lactate and ammonia concentrations are measured. With this test, several metabolic myopathies can be distinguished because patients with inherited defects in glyco(geno)lysis are unable to produce lactate while patients with myoadenylate deficiency are unable to produce ammonia (Fig. 3). However, mitochondrial myopathies can not be distinguished from the controls with this test.

Mitochondrial myopathies share the clinical characteristics of exercise intolerance, undue fatigue, and lactic acidosis during low- to moderate-intensity work. The diagnostic confirmation can be hard due to aspecific clinical symptoms and is often based on a muscle biopsy, an unpleasant, invasive, and expensive procedure. A simple functional test that can discriminate between presumable mitochondrial patients and non-mitochondrial patients would, therefore, be of utmost importance to decrease the number of muscle biopsy samples and to increase the number of definite diagnoses.

This, and the fact that mitochondrial myopathies have a major impact on the body has been the scope from which this thesis originates. By applying NIRS to mitochondrial myopathies it might be possible to develop a functional test that, in addition, can distinguish patients suspected to have a mitochondrial myopathy from healthy controls.

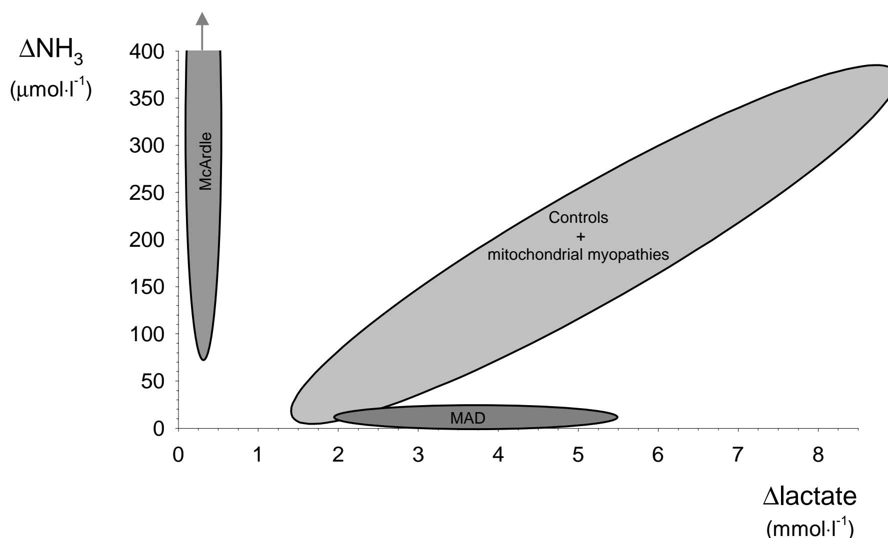

Fig. 3 Changes in lactate and ammonia during the ischemic forearm test. Patients with myoadenylate deficiency (MAD) lacked ammonia production while patients with myophosphorylase deficiency (McArdle's disease) lacked lactate production. mitochondrial myopathies could not be discriminated from the controls.

The application of NIRS in metabolic and mitochondrial myopathies was pioneered by Bank & Chance in 1994 [Bank et al. 1994] who found an abnormal oxygenation during treadmill exercise in 6 patients with various metabolic myopathies and 1 patient with mitochondrial myopathy. This was followed by two other studies using NIRS in mitochondrial myopathies [Abe et al. 1997, Gellerich et al. 1998]. Although all studies showed abnormalities in oxygenation pattern, these abnormalities were not uniform and present in all individual patients. Therefore, we have chosen for a quantitative instead of qualitative NIRS approach and measured oxygen consumption and blood flow in patients with mitochondrial myopathies. However, near-infrared spectroscopy is still a relatively young technique, especially when it concerns quantitative measurements. Because the methodological constraints of the method have not yet been fully exploited, we have, in parallel to the clinical application of NIRS in neuromuscular disorders, studied some important methodological aspects of *in vivo* near-infrared spectroscopy in human skeletal muscle.

NEAR-INFRARED SPECTROSCOPY

NIRS is, as mentioned above, an optical method that can be used for the noninvasive measurement of tissue oxygenation and haemodynamics. It is based on the relative tissue transparency for light in the near-infrared region and on the oxygen-dependent absorption changes of haemoglobin and myoglobin. Haemoglobin, myoglobin, and to a lesser extent cytochrome oxidase are the most important chromophores absorbing near-infrared light in muscle tissue. Haemoglobin is the main component of the erythrocytes and the oxygen carrier of the blood. Myoglobin is present within the muscle cell and facilitates intracellular oxygen transport. Due to identical spectral characteristics, it is not possible with NIRS to distinguish between haemoglobin and myoglobin. Cytochrome oxidase is the terminal enzyme of the mitochondrial respiratory chain reaction transferring the electrons to molecular oxygen. Because the amount of cytochrome oxidase in muscle is relatively low as compared with haemoglobin and myoglobin, changes in cytochrome oxidase are lost within the noise of the signal. Therefore, we neglected the contribution of cytochrome oxidase in the *in vivo* muscle studies of this thesis.

Both haemoglobin and myoglobin can be divided into two major forms; oxyhaemoglobin/myoglobin (O_2Hb/O_2Mb) and deoxyhaemoglobin/myoglobin (HHb/HMb). Because oxy- and deoxy- forms have different absorption spectra (Fig. 4), it becomes possible to obtain local information about tissue oxygenation by choosing the right wavelengths for application of the Lambert-Beer law. This law enables calculation of the optical density in a homogeneous, non-scattering medium. Because biological tissue is not

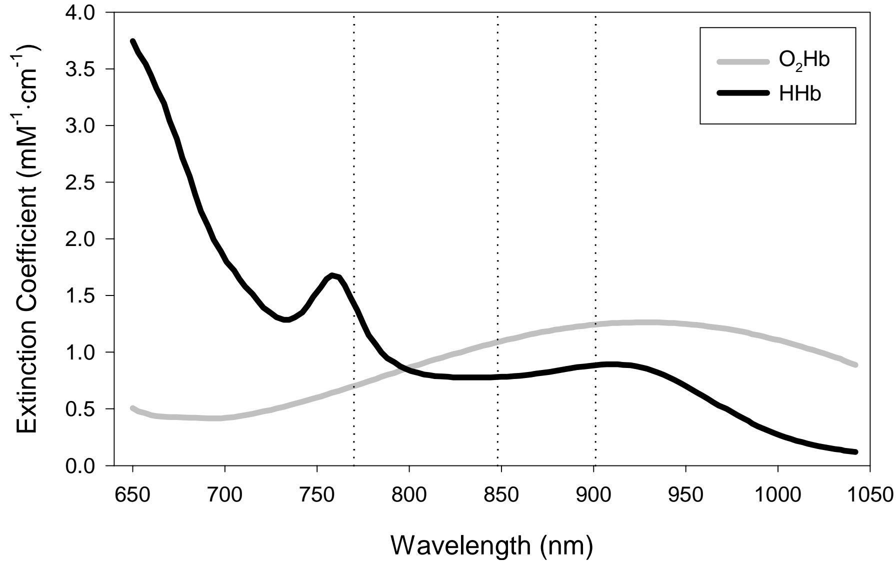

Fig. 4 Extinction coefficients of adult oxyhaemoglobin (O_2Hb) and deoxyhaemoglobin (HHb) as determined by Wray et al. [Wray et al. 1988]. Dotted lines represent the 3 wavelengths used (770, 848, and 901 nm).

homogeneous and considered as a scattering medium, a dimensionless path-length correction factor, also known as differential path-length factor (DPF), must be incorporated. The modified Lambert-Beer law [Delpy et al. 1988] is then given by:

$$OD(\lambda) = \varepsilon(\lambda) \cdot c \cdot L \cdot DPF + OD(\lambda)_R$$

where $OD(\lambda)$ is a dimensionless factor known as the optical density of the medium, $\varepsilon(\lambda)$ is the extinction coefficient of the chromophore ($mM^{-1} \cdot cm^{-1}$) (Fig. 4), c is the concentration (mM) of the chromophore, L is the distance (cm) between light entry and light exit point, λ is the wavelength (nm) and $OD(\lambda)_R$ represents the light losses due to scattering and absorption by other, oxygen-independent, chromophores in the tissue. Assuming that $OD(\lambda)_R$ is constant during the measurement, the change in optical density can be converted into a change in chromophore concentration by the following equation:

$$\Delta c = \left(\frac{\Delta OD(\lambda)}{\varepsilon(\lambda) \cdot L \cdot DPF} \right)$$

This equation is valid for a medium with one chromophore. At least as many wavelengths are needed as there are chromophores present, resulting in a set of linear equations. With the spectral extinction coefficients of the various chromophores, the solution of a multiple set of equations leads to an algorithm as is used in most NIRS systems [Matcher

et al. 1995]. With this algorithm it becomes possible to convert changes in optical absorbance to changes in concentration of the chromophores studied.

In this thesis we used a continuous-wave near-infrared spectrophotometer (Oxymon, Biomedical Engineering Department, University of Nijmegen, NL) that generates light at 901, 848 and 770 nm [Van der Sluijs et al. 1998] (Fig. 5). Near-infrared light is transmitted from the source to the tissue and back to the detector by flexible fibre optic bundles called optodes. The absorption changes at the discrete wavelengths are converted into concentration changes of O_2Hb and HHb with the spectral extinction coefficients [Wray et al. 1988] (Fig. 4) of the chromophores incorporated into a three-wavelength algorithm [Livera et al. 1991, Matcher et al. 1995]. The matrix notation of this algorithm is described as:

$$\Delta O_2Hb = 1.776 \cdot \Delta OD_{901} + 0.081 \cdot \Delta OD_{848} + -1.157 \cdot \Delta OD_{770}$$

$$\Delta HHb = -0.221 \cdot \Delta OD_{901} + -1.020 \cdot \Delta OD_{848} + 1.642 \cdot \Delta OD_{770}$$

$$\Delta Cyt = -0.611 \cdot \Delta OD_{901} + 0.635 \cdot \Delta OD_{848} + -0.014 \cdot \Delta OD_{770}$$

This way, absolute concentration changes of O_2Hb and HHb can be monitored in the tissue. As for reasons explained above, we neglected the contribution of cytochrome oxidase in our muscle studies. Apart from O_2Hb and HHb, a third variable is derived from the sum of O_2Hb and HHb and reflects the total amount of haemoglobin in the tissue (tHb). Changes in tHb reflect, therefore, changes in blood volume within the NIRS volume of interest. Another calculated variable, called Hb_{diff}, is derived from the difference between O_2Hb and HHb ($Hb_{diff} = O_2Hb - HHb$) and is used to obtain a better signal-to-noise ratio. In some studies, Hb_{diff} is confusingly called the oxygenation index (OI) as this variable is not an index, but a subtraction.

Fig. 5 Near-infrared spectrophotometer (Oxymon, Biomedical Engineering Department, University of Nijmegen, NL) as has been used in this study for the measurement of tissue oxygenation and haemodynamics in human skeletal muscle.

To correct for scattering of photons in the tissue, a differential path-length factor (DPF) of 4.0 was used for the calculation of absolute concentration changes [Duncan et al. 1996, Ferrari et al. 1992]. Concentration changes of O_2Hb, HHb and tHb can be measured with a sample frequency of up to 50 Hz while sampled data are displayed real-time and stored on disk for off-line analysis.

Quantitative NIRS measurements

Fig. 6 shows the experimental set-up and the NIRS signals that are presented real-time on screen during the measurement. Using a simple physiological intervention (occlusion) in order to control the circulation to a certain amount, it is possible to calculate various quantitative variables in arm or leg, both at rest as well as during exercise, for instance, by using an arm ergometer.

Venous occlusion method

Venous occlusion can be applied by inflating a cuff to a pressure of approximately 50 mmHg. Such an occlusion blocks venous outflow, but does not impede arterial inflow. As a result, venous blood volume as well as venous pressure increases. The increase in blood volume is monitored by NIRS as an increase in O_2Hb, HHb, and tHb signals (Fig. 6A: 465-485 s). When occlusion is maintained long enough, venous pressure will eventually

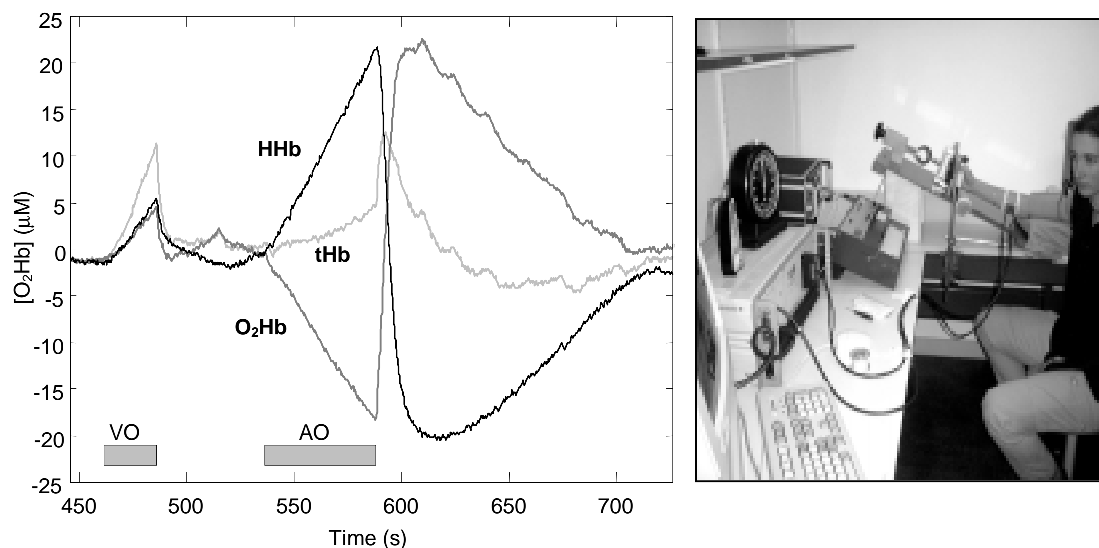

Fig. 6 A) Real-time NIRS tracings of oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb), and total haemoglobin (tHb) in response to 20 s of venous occlusion (VO) and 45 s of arterial occlusion (AO). Note that recovery after VO is very rapid while recovery from AO takes more than two minutes. B) Experimental set-up as used in the majority of our studies with the NIRS optodes placed on the forearm and a pneumatic cuff around the upper arm to apply occlusion. NIRS data were sampled at 10 or 20 Hz, displayed real-time on screen and stored on disk for off-line analysis.

equal the pressure in the cuff, arterial inflow and venous outflow will become the same and blood volume will stabilise. After release of the venous occlusion, all signals rapidly return to pre-exercise levels. By using the venous occlusion method it is possible to calculate blood flow, O_2 consumption and venous saturation.

Arterial occlusion method

Arterial occlusion is applied by inflating the cuff to a pressure of at least 60 to 80 mmHg above systolic pressure. This way, both venous outflow and arterial inflow are blocked and systemic circulatory changes are sufficiently eliminated in the limb. Lacking the supply of well oxygenated blood, muscle metabolism fully depends on the available O_2 in local capillaries and muscle cells. Depletion of local available O_2 stores during arterial occlusion is monitored by NIRS as a decrease in O_2Hb and a concurrent increase in HHb while tHb remains constant (Fig. 6A: 530-590 s). A hyperaemic response can be observed after release of the arterial occlusion. Blood volume increases rapidly, resulting in a fresh pool of O_2Hb and a quick wash-out of HHb. Using the arterial occlusion method including the recovery phase, it is possible to calculate O_2 consumption, reoxygenation rate and the half-recovery times of the signals.

Oxygen consumption

Measurement of muscle O_2 consumption is of great importance in the investigation of *in vivo* muscle metabolism in health and disease. Whereas the more conventional techniques like strain-gauge plethysmography combined with blood gas analysis are invasive and provide regional values of the total limb, therefore, including other than muscle tissue, NIRS is noninvasive and measures local oxygenation directly in the muscle.

As described above, NIRS O_2 consumption ($m\dot{V}O_2$) can be calculated both by means of venous and arterial occlusion. Using venous occlusion, $m\dot{V}O_2$ is calculated from the rate of increase in HHb [De Blasi et al. 1997] (Fig. 7A) since venous outflow is blocked and the increase in HHb is thought to be solely due to the O_2 consumed. Calculation of $m\dot{V}O_2$ from arterial occlusion assumes that tHb remains constant [De Blasi et al. 1997] and can then be derived from the rate of decrease in O_2Hb (Fig. 7B) or from the rate of decrease in Hb_{diff} divided by 2. Obstruction of inflow and outflow results in a static compartment of blood where the decrease of O_2 from O_2Hb is directly related to consumption.

Concentration changes of HHb, O_2Hb, and Hb_{diff} are expressed in $\mu M \cdot s^{-1}$ and converted to millilitres O_2 per minute per 100 gram tissue ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$) taking into account that each Hb molecule binds four O_2 molecules and that the molar volume of gas

is 22.4 L assuming STPD conditions. A value of 1.04 kg·L^{-1} was used for muscle density [Vierordt 1906]. This results in the following equation:

$$m\dot{V}O_2 = Abs(((\Delta O_2Hb \times 60)/(10 \times 1.04)) \times 4) \times 22.4/1000 \quad \text{in } mlO_2 \cdot min^{-1} \cdot 100g^{-1}$$

Note that ΔO_2Hb can be replaced by ΔHHb or by $\Delta Hb_{diff}/2$.

Blood flow

NIRS blood flow measurements are similar to the well-established method of strain-gauge plethysmography. Venous occlusion is used to provoke a blood volume increase in the part of the limb distal from the pneumatic cuff. Within the initial period of the occlusion, the increase in blood volume per time is a measure for the blood flow. Strain-gauge plethysmography measures blood volume changes by changes in limb circumference and can not distinguish between the various tissues of the limb. NIRS measures blood volume changes directly in the muscle of interest by monitoring changes in the haemoglobin/myoglobin content.

Blood flow (BF) in arm or leg can be measured during venous occlusion by evaluating the linear increase in tHb within the first seconds of the venous occlusion [De Blasi et al. 1997, Van Beekvelt et al. 1998] (Fig. 7A). Venous outflow is blocked and the increase in

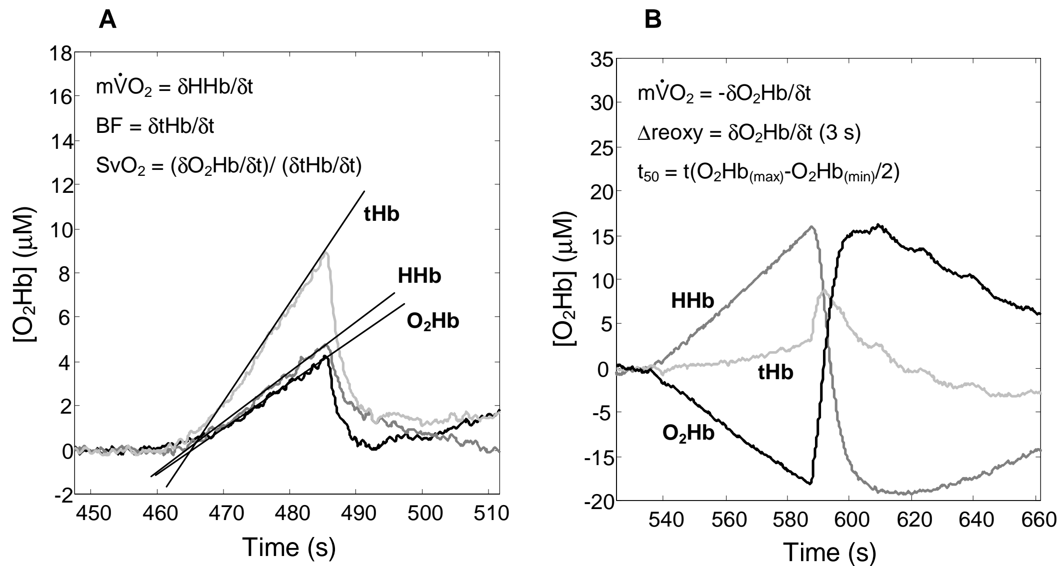

Fig. 7 Quantitative NIRS measurements during A) venous occlusion and B) arterial occlusion. During venous occlusion, blood flow (BF), muscle oxygen consumption ($m\dot{V}O_2$), and venous saturation (SvO_2) can be calculated. Using arterial occlusion and its recovery phase, it is possible to calculate $m\dot{V}O_2$, reoxygenation rate (ΔO_2Hb), and half-recovery times (t_{50}) of the signals.

tHb (= oxy- plus deoxyhaemoglobin/myoglobin) is directly related to arterial inflow. Concentration changes of tHb are expressed in $\mu M \cdot s^{-1}$ and converted to millilitres blood per minute per 100 millilitres tissue ($ml \cdot min^{-1} \cdot 100ml^{-1}$) using either the individual Hb-concentration ([Hb] in $mmol \cdot L^{-1}$) obtained from blood samples or male and female values derived from literature. The molecular weight of Hb (64.458 $gmol^{-1}$) and the molecular ratio between Hb and O_2 (1:4) have to be taken into account. BF can than be calculated by the following equation:

$$BF = (((\Delta tHb \times 60)/(([Hb] \times 1000)/4)) \times 1000)/10 \quad \text{in } ml \cdot min^{-1} \cdot 100ml^{-1}$$

Venous oxygen saturation

Another variable that can be derived from venous occlusion is peripheral venous oxygen saturation (SvO_2) and has been described by Yoxall & Weindling [Yoxall et al. 1996]. With the measurement of SvO_2 direct information about O_2 extraction can be derived. SvO_2 can be of special interest in the neonatology where knowledge of cerebral haemodynamics and oxygenation are of primary importance for survival of the premature neonate since peripheral SvO_2 is thought to provide an index of the adequacy of global oxygenation [Yoxall et al. 1996].

SvO_2 can be calculated from the ratio of increase in ΔO_2Hb to increase in ΔtHb during venous occlusion (Fig. 7A):

$$SvO_2 = \frac{\Delta O_2Hb}{\Delta O_2Hb + \Delta HHb}$$

This is independent of the optical path-length since absolute quantification of the chromophore concentration changes is not required. As described by Yoxall and Weindling [Yoxall et al. 1997], SvO_2 is best determined from the first 5 s of the venous occlusion while an occlusion is considered to be satisfactory for analysis if there is a steady baseline before the occlusion, a rise in both O_2Hb and HHb during the occlusion, and a return to baseline after release of the occlusion.

Half-recovery time

The recovery of O_2Hb after exercise or ischemia represents the time needed for resaturation of deoxygenated haemoglobin and myoglobin and is thought to reflect both the influx of oxygenated arterial blood and the continued O_2 consumption during recovery [Chance et al. 1992, McCully et al. 1994a, McCully et al. 1994b].

Two different approaches are known to calculate half-recovery times from NIRS signals. The first approach was described by Chance et al. [Chance et al. 1992] as the time needed for half recovery of O_2Hb from maximum deoxygenation at the end of occlusion to maximum re-oxygenation during hyperaemia (t_{50}) (Fig. 8A). Another more elegant method to calculate half-recovery times or time constants (Tc) is by fitting the recovery signal to a mono-exponential curve from which a time constant or tau value can be derived [McCully et al. 1994b, McCully et al. 1994a](Fig. 8B).

The latter method is identical to the determination of phosphocreatine (PCr) recovery that is commonly used in phosphorus magnetic resonance spectroscopy (^{31}P-MRS) (Chapter 5). However, as can be seen in Fig. 8 this method is only possible when the hyperaemic response is small or absent because in case of a hyperaemic response, the signal is no longer mono-exponential.

Reoxygenation rate

Another variable that can be calculated in relation to recovery from arterial occlusion or exercise is the rate of O_2Hb reoxygenation. Whereas the half-recovery is a function of time, the reoxygenation rate reflects the velocity at which the recovery starts off after release of exercise or ischemia.

The reoxygenation rate (ΔO_2Hb in $\mu M \cdot s^{-1}$) was calculated as the rate of increase in O_2Hb during the initial 3 s after cessation of occlusion and/or exercise (Fig. 7B). This variable reflects the initial inflow of O_2Hb over a fixed time period and is, therefore, not influenced by the presence or absence of a hyperaemic response. Whereas the recovery

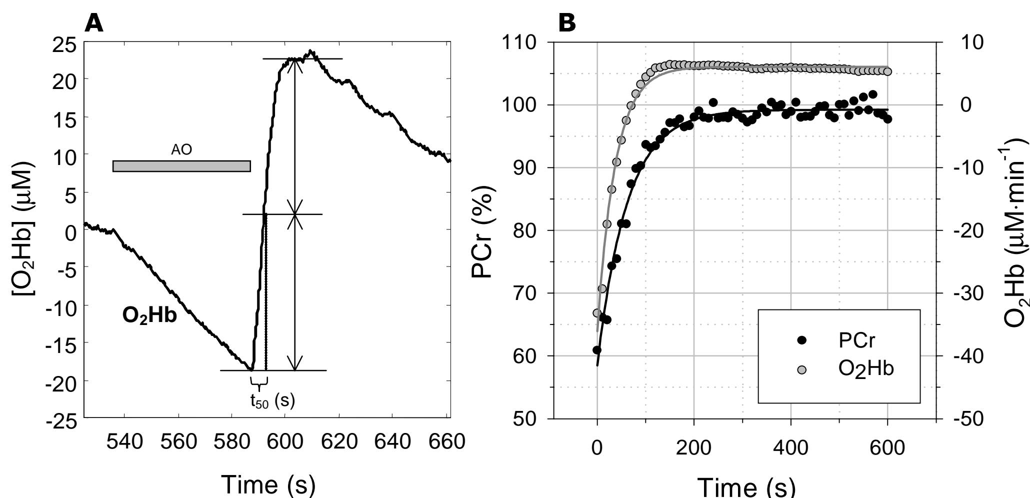

Fig. 8 Half-time recovery, calculated as A) the time needed for half recovery of O_2Hb from maximum deoxygenation at the end of arterial occlusion or exercise to maximum re-oxygenation during hyperaemia, and B) the tau value derived from a mono-exponential curve-fit of O_2Hb recovery as is standard for calculation of PCr resynthesis with ^{31}P-MRS.

time (or half-recovery time) includes all processes for total recovery of vascular O_2Hb, muscular O_2Mb as well as the continued oxygen consumption during recovery, the reoxygenation rate is thought to reflect the fast initial recovery rate at which primarily vascular components are restored. It is directly related to microvascular function and can, therefore, be a functional new variable in the investigation of disorders concerning O_2 delivery or in the discrimination between disorders of O_2 delivery and O_2 consumption.

NIRS measurements during exercise

Another major advantage of NIRS is the fact that oxygenation and haemodynamics can also easily been monitored during exercise. This is of great importance since local measurement of $m\dot{V}O_2$ and blood flow during exercise has, so far, been impossible using the more conventional techniques like strain-gauge plethysmography and blood gas analysis. Moreover, it enables the complementary use of NIRS with ^{31}P-MRS and the measurement of muscle oxygenation in exercise-related pathology like e.g. vascular disease or mitochondrial impairment. ^{31}P-MRS has developed over the last three decades as a "gold standard" for noninvasive measurement of skeletal muscle metabolism during exercise and recovery, monitoring the kinetics of intramuscular high-energy phosphates and pH. However, it is unable to monitor high-energy phosphates and pH simultaneously with tissue oxygenation and the direct measurement of muscle oxygenation by NIRS might, therefore, well fill this gap.

Besides the qualitative monitoring of tissue oxygenation and haemodynamics during and after exercise, NIRS has also been used for the quantitative calculation of $m\dot{V}O_2$ and BF during various exercise protocols [Colier et al. 1995, De Blasi et al. 1992, De Blasi et al. 1993, De Blasi et al. 1994, De Blasi et al. 1997, Hamaoka et al. 1996, Sako et al. 2001, Van Beekvelt et al. 1999, Van Beekvelt et al. 2001b, Van Beekvelt et al. 2001a, Van Beekvelt et al. 2002a, Van Beekvelt et al. 2002b]. Quantitative NIRS measurements of $m\dot{V}O_2$ from O_2Hb during exercise can be calculated under ischemic conditions, either by application of arterial occlusion during or after the exercise task, or during exercise at a workload that is high enough for a complete obstruction of muscle perfusion. In all cases, tHb is assumed to stay constant. Whereas at rest this assumption is mostly met, during exercise tHb often shows large fluctuations caused by redistribution of blood due to compression of the blood vessels by the contracting muscle. Quantitative NIRS BF measurements during exercise can only be calculated using venous occlusion.

Because there is a large diversity in the protocols described in the literature, varying in exercise intensity, type of exercise (sustained vs. rhythmic), and time of $m\dot{V}O_2$ determination, most studies can not be compared with each other.

Sustained vs. rhythmic isometric exercise

Basically, two types of muscle isometric exercise can be discriminated. Sustained or static isometric exercise where muscle contraction is exerted continuously, and rhythmic or dynamic isometric exercise in which short periods of contraction alternate with periods of relaxation [Lind et al. 1967a]. The advantage of sustained exercise for NIRS $m\dot{V}O_2$ and BF measurements is that the occlusion can be truly applied during exercise. This in contrast to rhythmic exercise where the alternating contraction and relaxation of the muscle results in corresponding changes in the NIRS signals (Fig. 9). When occlusion is applied during exercise, the movement artefacts interfere with the linear response to occlusion, needed to calculate $m\dot{V}O_2$ and BF. On the other hand, it is known that blood flow will be impaired when exercise exceeds 25-30% of the maximum voluntary contraction force (MVC) [Barcroft et al. 1939, Kahn et al. 1998, Lind et al. 1967b] due to compression of the small blood vessels within the muscle. This means that sustained exercise above 30% MVC is per definition ischemic and probably does not lead to an increased oxidative metabolism. NIRS measurements of $m\dot{V}O_2$ and BF during sustained exercise are, therefore, limited to low-intensity work. Using rhythmic exercise with immediate post-exercise application of occlusion, $m\dot{V}O_2$ can be measured over a broad range of exercise intensities when oxidative metabolism can increase to a higher extent due to the temporary obstruction of the blood flow.

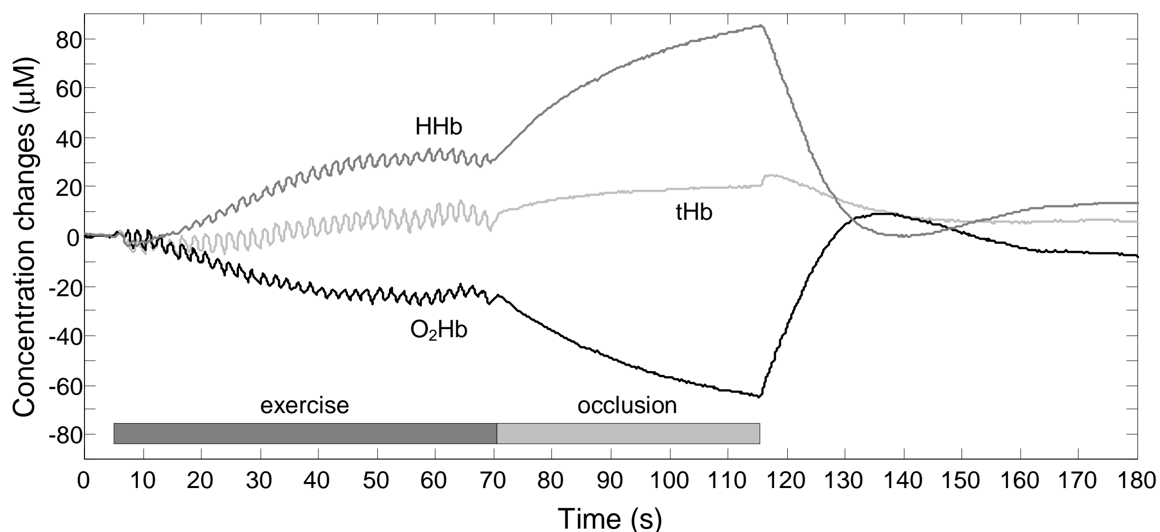

Fig. 9 Individual NIRS O_2Hb, HHb, and tHb signals during exercise, arterial occlusion, and recovery. Rhythmic isometric handgrip exercise was performed at 70% of the subject's maximum voluntary contraction force (MVC). Rhythmic changes, corresponding to muscle contraction and relaxation are clearly visible in the NIRS signals during exercise.

In this thesis, NIRS muscle O_2 consumption in relation to exercise was measured in several ways. $m\dot{V}O_2$ during sustained low-intensity exercise was measured using venous occlusion (Chapter 2) and using arterial occlusion (Chapter 2, 4 and 7). In Chapter 3 and 8 (postscriptum), $m\dot{V}O_2$ was measured from arterial occlusion immediately after cessation of rhythmic isometric exercise at various exercise intensities.

Arm vs. leg measurements

Apart from the study described in Chapter 5, we have focussed on arm measurements. The advantage of arm measurements concerns the small muscle mass that enables exercise tests at a broad range of work-intensities without substantially increasing the cardiovascular load. This is of special importance in the investigation of patients where exercise tests can be potentially hazardous e.g. in patients with heart failure or cardiac conduction abnormalities known to be associated with some mitochondrial myopathies. Another advantage of arm measurements is that the skinfold thickness of the arm is normally substantially lower than that of the leg. This because mathematical simulations have shown that skinfold thickness confounds NIRS measurements [Matsushita et al. 1998, Niwayama et al. 1999, Yamamoto et al. 1998]. Moreover, arm measurements have limited blood pooling as compared with the leg, the muscles are easy accessible, and exercise tasks can be perfectly standardised.

AIM OF THE THESIS

Near-infrared spectroscopy enables the noninvasive investigation of local oxygenation and haemodynamics in skeletal muscle. Because muscle oxidative capacity is impaired in mitochondrial disorders, NIRS can be very useful in the diagnostic work-up of patients suspected to suffer from such a disease. However, NIRS is still a relatively new technique and the methodological constraints have not yet been fully exploited. The aim of this thesis is, therefore, twofold. The *first objective* is to investigate some of the major methodological aspects necessary for the legitimate use of NIRS in the investigation of normal and pathological states of *in vivo* muscle metabolism. The *second objective* is to investigate the possibilities of NIRS for the investigation of neuromuscular disorders in order to develop a noninvasive screeningstest that is functional in a wide range of patients suspected to have a metabolic/mitochondrial myopathy.

THESIS OUTLINE

According to the two objectives described above, this thesis is divided into two main parts. The first part of the thesis (Chapter 2-6) is focussed on the methodological aspects of NIRS. The second part (Chapter 7-9) is focussed on the clinical application of NIRS

on neuromuscular disorders. Finally, in Chapter 10, a summary of the main results is given and these results are discussed with respect to their implications for future studies and their clinical relevance.

Methodological issues

We have studied the performance of near-infrared spectroscopy as an optical method that enables calculation of quantitative oxygen consumption and blood flow. In Chapter 2, NIRS was compared with the more conventional, but invasive Fick-method. It describes whether quantitative NIRS measurements at rest and during exercise correlates with the Fick-method, and whether NIRS reveals local differences that are not detectable by the more established though more regional Fick method. In Chapter 3 the reproducibility of *in vivo* quantitative NIRS $m\dot{V}O_2$ in human skeletal muscle at various work intensities of isometric exercise is described.

Propagation of the near-infrared light within the tissue is complex as it travels through multiple layers of the inhomogeneous medium. The subcutaneous layer can vary considerably due to individual differences in adipose tissue thickness (ATT) and may confound NIRS measurements. Computer simulations showed that ATT had a confounding effect on NIRS, but only a small number of *in vivo* NIRS measurements were done to verify this effect of ATT. In Chapter 4, we investigated the influence of ATT on *in vivo* NIRS measurements of $m\dot{V}O_2$ and forearm BF in a large group of healthy subjects.

In Chapter 5, NIRS was used in combination with the more established method of phosphorus magnetic resonance spectroscopy (^{31}P-MRS), currently considered as a "gold standard" for the noninvasive measurement of muscle metabolism. NIRS and ^{31}P-MRS have been used as complementary methods for the investigation of *in vivo* skeletal muscle metabolism, measured simultaneously and within the same volume of interest. This way, both oxidative and glycolytic processes of muscle metabolism could be studied at the same time. This approach was used in healthy subjects as well as in Friedreich ataxia (FRDA) patients, in which mitochondrial function is thought to be impaired, in order to stress the differences and similarities between both techniques.

Chapter 6 describes the normal range and biological variability of the various quantitative NIRS variables measured in the healthy resting muscle.

Clinical application

A brief introduction on the clinical applications of NIRS in neuromuscular disorders and an overview of the current literature are given in Chapter 7. In Chapter 8, $m\dot{V}O_2$ and BF at rest and during low-intensity exercise was examined in patients with a specific mitochondrial myopathy and compared with healthy controls. In the postscriptum of

Chapter 8 the additional analysis of this patient group after incorporation of the confounding effect of adipose tissue thickness on *in vivo* NIRS measurements (Chapter 4) is described.

Although NIRS has recently been used for diagnosis of metabolic/ mitochondrial myopathies, it had never been used in other neuromuscular disorders, such as inflammatory myopathies. In Chapter 9, we used NIRS to study the effect of corticosteroid treatment in dermatomyositis, a muscle disorder characterised by complement mediated capillary necrosis, resulting in ischemia and hypoperfusion.

PART 1

METHODOLOGICAL ISSUES

2

Performance of near-infrared spectroscopy in measuring local oxygen consumption and blood flow in skeletal muscle

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SUMMARY

The aim of this study was to investigate local oxygen consumption ($m\dot{V}O_2$) and forearm blood flow (FBF) in resting and exercising muscle by use of near-infrared spectroscopy (NIRS) and to compare the results with the global $m\dot{V}O_2$ and FBF derived from the well-established Fick-method and plethysmography. $m\dot{V}O_2$ was derived from 1) NIRS using venous occlusion, 2) NIRS using arterial occlusion, and 3) the Fick-method ($m\dot{V}O_{2(Fick)}$). FBF was derived from 1) NIRS and 2) strain-gauge plethysmography. Twenty-six healthy subjects were tested at rest and during sustained isometric handgrip exercise. Local variations were investigated with two independent and simultaneously operating NIRS systems at two different muscles and two measurement depths. $m\dot{V}O_2$ increased more than fivefold in the active flexor digitorum superficialis muscle, and it increased 1.6 times in the brachioradialis muscle. The average increase in $m\dot{V}O_{2(Fick)}$ was twofold. FBF increased 1.4 times independent of the muscle or the method. It is concluded that NIRS is an appropriate tool to provide information about local $m\dot{V}O_2$ and local FBF because both place and depth of the NIRS measurements reveal local differences that are not detectable by the more established, but also more global, Fick method.

INTRODUCTION

Near-infrared spectroscopy (NIRS) is a noninvasive, continuous, and direct method to determine oxygenation and haemodynamics in tissue. It enables the study of local differences in muscle oxygen consumption and delivery. NIRS has also shown to be a sensitive tool in the discrimination between normal and pathological states. Abnormal oxygenation due to insufficient delivery has been found with NIRS in patients with heart failure [Belardinelli et al. 1995, Mancini et al. 1994a, Matsui et al. 1995, Wilson et al. 1989] and peripheral vascular disease [Cheatle et al. 1991, Komiyama et al. 1994, Kooijman et al. 1997, McCully et al. 1994b]. NIRS was also used to characterise patients with metabolic myopathies, in which abnormalities in oxygenation pattern are related to oxygen extraction instead of oxygen delivery [Abe et al. 1997, Bank et al. 1994, Gellerich et al. 1998]. Our recent study showed that NIRS makes it possible to quantify differences in oxygen consumption and forearm blood flow at rest as well as during exercise and discriminates between patients with mitochondrial myopathies and healthy persons [Van Beekvelt et al. 1999].

Quantification of muscle oxygen consumption ($m\dot{V}O_2$) and blood flow using NIRS has become possible by incorporating a differential path-length factor in the Lambert-Beer law [Delpy et al. 1988] and applying an occlusion to control circulation in the limb. $m\dot{V}O_2$ has been measured with NIRS during arterial occlusion [Cheatle et al. 1991, Colier et al. 1995, De Blasi et al. 1993, De Blasi et al. 1997, Van Beekvelt et al. 1998] as well as during venous occlusion [De Blasi et al. 1994, De Blasi et al. 1997, Homma et al. 1996a, Van Beekvelt et al. 1998]. Muscle blood flow has been measured with NIRS during venous occlusion [De Blasi et al. 1994, Van Beekvelt et al. 1998] and by use of an intravascular tracer [Edwards et al. 1993].

Comparison of the NIRS method to quantify blood flow with more established methods has been reported in only a few studies. NIRS blood flow measurement at rest, obtained by an intravascular tracer, was compared with venous occlusion plethysmography by Edwards et al. [Edwards et al. 1993], and De Blasi et al. [De Blasi et al. 1994] compared NIRS flow measurement with plethysmographic flow measurement, both simultaneously measured during venous occlusion. Quantitative NIRS $m\dot{V}O_2$ measurement, however, has never been compared with a more established method. Although Homma et al. [Homma et al. 1996a] showed that there was a relationship between the deoxygenation pattern estimated by NIRS during venous occlusion and the O_2 consumption obtained by a more established method, they did not calculate quantitative values for $m\dot{V}O_2$.

The present study was undertaken to determine whether quantitative measurement of NIRS $m\dot{V}O_2$ and FBF of human skeletal muscle at rest as well as during exercise correlates with the more established methods of combining blood gas analysis, pulse

oximetry, and plethysmography and whether the depth and the place of the NIRS measurements reveal local differences that are not detectable by the more established, though global, Fick method.

MATERIALS AND METHODS

Subjects

Twenty-six healthy volunteers (16 male, 10 female) participated in this study. The study was approved by the Faculty Ethics Committee and all subjects gave their written informed consent. The subject characteristics were 28.8 ± 7.7 yr in age, 178.9 ± 10.9 cm in height and 70.0 ± 11.1 kg in weight (means ± SD). One subject used medication (Pulmicort puffs) that, to our knowledge, does not affect muscle peripheral circulation. Skinfold thickness was measured between the NIRS optodes using a skinfold caliper (Holtain Ltd., Crymmych, UK) and divided by two to determine the adipose tissue thickness (fat + skin layer; ATT) covering the muscle. All, but two of the subjects were right-handed.

Near-infrared Spectroscopy (NIRS)

NIRS is based on the relative tissue transparency for light in the near-infrared region and on the oxygen-dependent absorption changes of haemoglobin and myoglobin. By using a continuous-wave near-infrared spectrophotometer (Oxymon, Biomedical Engineering Department, University of Nijmegen, NL) that generates light at 905, 850 and 770 nm [Van der Sluijs et al. 1998] it is possible to differentiate between oxy- and deoxyhaemoglobin/myoglobin (O_2Hb/O_2Mb and HHb/HMb, respectively). Because of identical spectral characteristics, it is not possible to distinguish between Hb and Mb. The absorption changes at the discrete wavelengths are converted into concentration changes of O_2Hb and HHb by using the algorithm described by Livera et al. [Livera et al. 1991]. To correct for scattering of photons in the tissue, a differential path-length factor (DPF) of 4.0 was used for the calculation of absolute concentration changes [Duncan et al. 1996, Ferrari et al. 1992]. Data were sampled at 10 Hz, displayed real-time and stored on disk for off-line analysis.

The sum of O_2Hb and HHb concentrations ([O_2Hb] and [HHb], respectively) reflects the total amount of haemoglobin ([tHb]) and changes in [tHb] can be interpreted as changes in blood volume in the tissue. The difference between [O_2Hb] and [HHb] (= [Hb_{diff}]) is used for the calculation of oxygen consumption during arterial occlusion.

Simultaneous NIRS measurements were done on top of the flexor digitorum superficialis (FDS) muscle and on top of the brachioradialis (BR) muscle with two independent-operating NIRS systems. This was done to obtain unique information about

local differences in muscle $m\dot{V}O_2$ and blood flow between the agonistic flexor muscles initiating handgrip exercise and the synergistic brachioradialis muscle. In addition, two inter-optode distances (IO) of 35 mm and 50 mm were used to measure simultaneously at different depths, further referred to as IO_{35} and IO_{50}, respectively.

Strain-gauge plethysmography

Forearm blood flow was also measured by the more established method of strain-gauge plethysmography (Loosco, Amsterdam, NL) by using mercury-filled silicon gauges [Witney 1953]. A pneumatic arm cuff around the upper arm just above the elbow was inflated to 50 mmHg to apply venous occlusion. A wrist cuff inflated to 260 mmHg was used to exclude blood flow from the hand. The strain-gauge was stretched halfway around the forearm on top of the FDS and between the NIRS optodes to measure plethysmographic flow in the same region as the NIRS measurement. The strain-gauge was electronically calibrated. Strain-gauge plethysmography and NIRS data were recorded simultaneously.

Blood sampling

A Venflon catheter (BOC Ohmeda AB, Helsingborg, SE) was inserted into the antecubital vein. To sample blood from deep within the active muscle, thus avoiding mixture with skin circulation, the catheter was inserted in retrograde direction [Hartling et al. 1989, Mottram 1955]. A three-way stopcock was attached to allow for drawing blood into heparinized 1-ml syringes for measurement of blood gasses and Hb content (Synthesis 25, Instrumentation Laboratory, IT). Blood gas analysis took place directly after withdrawal of the blood. The catheter was washed out with sodium chloride (0.9%) to prevent it from clotting. Arterial saturation (SaO_2) was measured using a pulse oximeter (POX; N200, Nellcor Puritan-Bennet INC., USA) with the probe placed on the left index finger.

Protocol

The subject lay in a comfortable supine position, 15-20 min prior to the test. The right hand rested on a handgrip dynamometer with the upper arm at heart level and the forearm in an upward angle of 30° to avoid venous pooling of the blood. The arm was supported at the wrist and above the elbow so that there was no contact between forearm and dynamometer, and circulation in the forearm was completely unrestricted. The subject's maximum voluntary contraction (MVC) force was determined before the test. Pneumatic cuffs were placed around the upper arm and the wrist.

The experiment started with a 5-min rest period after placement of the instruments and insertion of the catheter (Fig. 1). At 4 min rest, the wrist cuff (260 mmHg) was inflated. One minute later, three consecutive venous occlusions (50 mmHg) were applied, followed by an arterial occlusion (260 mmHg). All venous occlusions lasted 20 s, and the arterial occlusion was maintained for 30-45 s. One minute of recovery separated the interventions. A blood sample was taken just before the first venous occlusion and again before arterial occlusion (Fig. 1).

After 5 min of recovery, the subject was asked to perform sustained isometric handgrip exercise at 10% MVC. The 10% level was marked on a display visible for the subject. The wrist cuff was inflated at the start of exercise. After 50 s of exercise, when NIRS signals had reached steady state, a blood sample was taken, immediately followed by rapid inflation of the arm cuff to apply venous occlusion while exercise was maintained (Fig. 1). Cuff inflation was kept at 50 mmHg for 20 s and than released. At the same time, the exercise was ended and the wrist cuff released.

When NIRS and plethysmographic signals had returned to preexercise levels after 5 min, a second exercise session was performed under identical conditions to determine $m\dot{V}O_2$ during arterial occlusion. A blood sample was taken at 50 s after the start of exercise, this time immediately followed by an arterial occlusion, which was released after 30-45 s while the exercise was ended and the wrist cuff released.

Forearm measurements

Forearm blood flow

Forearm blood flow was calculated from NIRS data (FBF_{NIRS}) by evaluating the linear increase in [tHb] within the first seconds of the venous occlusion [De Blasi et al. 1997, Van Beekvelt et al. 1998]. Concentration changes of tHb were expressed in micromolars per second ($\mu M \cdot s^{-1}$) and were converted to millilitres blood per minute per 100 millilitres tissue ($ml \cdot min^{-1} \cdot 100ml^{-1}$) by using the individual Hb concentration that was obtained from the blood samples. The molecular weight of Hb (64.458 $g \cdot mol^{-1}$) and the molecular ratio between Hb and O_2 (1:4) were taken into account.

To compare FBF_{NIRS} with a more established method, forearm blood flow was also measured by venous occlusion plethysmography (FBF_{Pleth}) [Witney 1953] (Fig. 1). The linear increase within the first seconds of the 20 s occlusion was considered for FBF_{Pleth} calculation. Volume changes were expressed in percentages and converted to millilitres blood per minute per 100 millilitres tissue ($ml \cdot min^{-1} \cdot 100ml^{-1}$) for the comparison with FBF_{NIRS}. FBF_{NIRS} and FBF_{Pleth} were both calculated from the same time period during venous occlusion and reflect, therefore, the local (FBF_{NIRS}) and the total (FBF_{Pleth}) flow in the forearm for that time period.

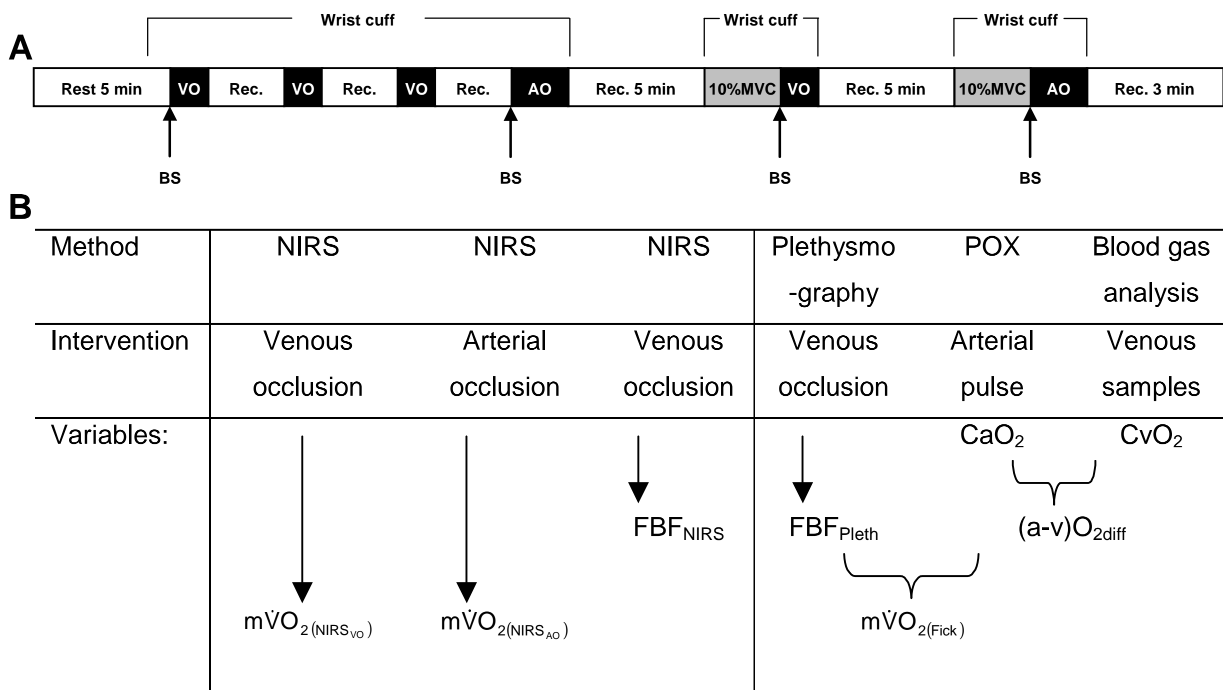

Fig. 1. Protocol (A) and measurements (B) as performed by each subject. Venous and arterial occlusion (VO and AO, respectively) are applied at rest and during sustained isometric handgrip exercise at 10% of the subject's maximal voluntary contraction force (10%MVC). BS = blood sample. See METHODS for a detailed description of the protocol. From this protocol, oxygen consumption was calculated with NIRS during VO ($m\dot{V}O_{2(NIRS_{VO})}$) and during AO ($m\dot{V}O_{2(NIRS_{AO})}$). Forearm blood flow was calculated during VO with NIRS (FBF_{NIRS}) and with plethysmography (FBF_{Pleth}). Oxygen consumption derived from Fick ($m\dot{V}O_{2(Fick)}$) was obtained by the combination of plethysmography, pulse oximetry (POX) and blood sampling. CaO_2 = arterial O_2 content, CvO_2 = venous O_2 content, $(a\text{-}v)O_{2diff}$ = arterio-venous O_2 difference.

Oxygen consumption

Muscle oxygen consumption ($m\dot{V}O_2$) was measured by three methods (Fig. 1). First, $m\dot{V}O_2$ was derived from NIRS using venous occlusion ($m\dot{V}O_{2(NIRS_{VO})}$) as the rate of increase in [HHb] [De Blasi et al. 1997]. Second, $m\dot{V}O_2$ was derived from NIRS using arterial occlusion ($m\dot{V}O_{2(NIRS_{AO})}$) by evaluating the rate of decrease in [Hb_{diff}] ([Hb_{diff}]=[O_2Hb]-[HHb]) with the assumption that [tHb] is constant [De Blasi et al. 1997]. Concentration changes of HHb and Hb_{diff} were expressed in micromolars per second ($\mu M \cdot s^{-1}$) and converted to millilitres O_2 per minute per 100 grams tissue ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$). A value of 1.04 $kg \cdot l^{-1}$ was used for muscle density [Vierordt 1906].

Third, $m\dot{V}O_2$ was derived from the combination of Blood samples, POX and plethysmography using Fick's law for the equation of $m\dot{V}O_2$ ($m\dot{V}O_{2(Fick)}$) assuming standard temperature pressure dry (STPD) conditions (Eq. 1).

$$m\dot{V}O_2 = FBF \times (a - v)O_{2diff} \quad \text{(Eq. 1)}$$

Where $(a\text{-}v)O_{2diff}$ is arteriovenous O_2 difference.

Arterial saturation was derived from POX while venous saturation was derived from the blood samples. The oxygen binding capacity of human haemoglobin (Hüfners factor = 1.39 $mlO_2 \cdot g^{-1}$) was taken into account. The dissolved O_2 in the blood was assumed to be 0.3 $mlO_2 \cdot 100ml^{-1}$.

Statistics

In some cases, parts of the measurements were missing because of a very low signal-to-noise ratio. A Shapiro-Wilk test was used to test all variables for normality ($P < 0.01$). Log-transformation was applied on variables that failed the normality test. To test the reproducibility of the three consecutive venous occlusions for the measurement of $m\dot{V}O_2$ and FBF, a two-way ANOVA with a mixed model was used and followed by Scheffé's method if significant differences were found. Statistical differences between measurement depth, measurement place, both methods for $m\dot{V}O_2$, and both methods for FBF and between rest and exercise were tested by means of the Student paired *t*-test. To protect against a type I error, an alpha of 0.01 was chosen. A Spearman correlation test was used to test the correlation between NIRS $m\dot{V}O_2$ and adipose tissue thickness. All data are reported as means ± SD. The level of statistical significance was set at $P \leq 0.05$.

RESULTS

Adipose tissue thickness (ATT) was 2.2 ± 0.8 mm on top of the flexor digitorum superficialis muscle and 2.6 ± 0.5 mm on top of the brachioradialis muscle. MVC force was 566 ± 125 N. No correlation was found between $m\dot{V}O_2$ measurements and ATT (Table 1).

Reproducibility

The reproducibility of the NIRS measurements for $m\dot{V}O_2$ and FBF, as well as the plethysmographic flow measurement was investigated by means of the repetition of three

venous occlusions. All values for $m\dot{V}O_2$ and FBF during the repeated measurements as well as the coefficient of variation are shown in Table 2.

Oxygen consumption from NIRS

Oxygen consumption, measured by NIRS during venous occlusion ($m\dot{V}O_{2(NIRS_{VO})}$) was not reproducible for FDS at IO_{35} as this value decreased slightly, but significantly when venous occlusion was repeated. Because no differences were expected, on the basis of the physiological background and the sufficient time for recovery, we decided that this value for $m\dot{V}O_{2(NIRS_{VO})}$ was not reliable and, therefore, excluded it from further analysis. On the contrary, FDS at IO_{50} and BR at IO_{35} were reproducible ($P > 0.05$) and, therefore, were calculated as the average $m\dot{V}O_2$ from the three occlusions.

Forearm blood flow from NIRS

No significant differences between the three consecutive venous occlusions at rest were found in the calculated flow measured by NIRS (FBF_{NIRS}). Therefore, FBF_{NIRS} was calculated as the average flow of the three occlusions.

Forearm blood flow from plethysmography

No significant differences between the three consecutive venous occlusions at rest were found in the plethysmographic flow measurement (FBF_{Pleth}) either. Therefore, FBF_{Pleth} was calculated as the average flow value obtained from the three occlusions.

Table 1. Correlation between ATT and $m\dot{V}O_{2(NIRS_{AO})}$ in the FDS and BR

		FDS IO_{35}	FDS IO_{50}	BR IO_{35}
ATT (mm)		2.2 ± 0.8 (1.3-4.5)	2.2 ± 0.8 (1.3-4.5)	2.6 ± 0.5 (1.8-3.7)
Spearman	r	-0.13	0.11	-0.20
	P	0.52	0.61	0.31

Values are mean ± SD (range) for adipose tissue thickness (ATT). Spearman correlation coefficient (r) and P values are given for the three near-infrared spectroscopy (NIRS) local muscle O_2 consumption ($m\dot{V}O_2$) measurements. FDS, flexor digitorum superficialis muscle; BR, brachio-radialis muscle; IO_{35} and IO_{50}, interoptode distances of 35 and 50 mm, respectively.

Table 2. Reproducibility of $m\dot{V}O_2$ and FBF during three consecutive venous occlusions

		VO-1	VO-2	VO-3	Comparison		*P* value	CV (%)
$m\dot{V}O_{2(NIRS_{VO})}$	FDS IO_{35} n = 24	0.09 ± 0.04	0.08 ± 0.04	0.08 ± 0.04	Overall	*	P = 0.01	30.6
					VO-3 vs. VO-1	*	P < 0.05	
					VO-3 vs. VO-2	NS	P > 0.05	
					VO-2 vs. VO-1	**	P < 0.01	
	FDS IO_{50} n = 20	0.08 ± 0.04	0.08 ± 0.04	0.07 ± 0.04	Overall		NS	25.4
	BR IO_{35} n = 26	0.14 ± 0.09	0.14 ± 0.11	0.13 ± 0.10	Overall		NS	23.3
FBF_{NIRS}	FDS IO_{35} n = 25	0.76 ± 0.33	0.70 ± 0.39	0.71 ± 0.35	Overall		NS	28.6
	FDS IO_{50} n = 21	0.61 ± 0.28	0.59 ± 0.34	0.54 ± 0.28	Overall		NS	30.3
	BR IO_{35} n = 26	1.41 ± 1.01	1.40 ± 1.09	1.44 ± 1.11	Overall		NS	20.4
FBF_{Pleth}	n = 24	2.07 ± 0.86	2.01 ± 0.73	2.07 ± 0.71	Overall		NS	16.7

*Values are mean (±SD) for $m\dot{V}O_2$ ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$) and forearm blood flow (FBF; $ml \cdot min^{-1} \cdot 100ml^{-1}$). First, second, and third venous occlusions are represented by VO-1, VO-2, and VO-3, respectively. Number of subjects (n) varies due to missing values in either VO-1, VO-2 or VO-3. CV, coefficient of variation. *P ≤ 0.05; **P ≤ 0.01; NS, not significant.*

NIRS $m\dot{V}O_2$ measurements

No significant differences between $m\dot{V}O_{2(NIRS_{VO})}$ and $m\dot{V}O_{2(NIRS_{AO})}$ were found in the BR muscle or for FDS at IO_{50} (Table 3). During exercise, $m\dot{V}O_2$ increased significantly for all measurements ($P \leq 0.01$) compared with at rest. Although $m\dot{V}O_{2(NIRS_{AO})}$ showed marked differences between the different muscles, the increase in $m\dot{V}O_{2(NIRS_{VO})}$ was roughly the same, independent of the measured muscle or the depth of the measurement (Table 3). This resulted in a significantly lower $m\dot{V}O_{2(NIRS_{VO})}$ in FDS at IO_{35} and FDS at IO_{50} as compared with $m\dot{V}O_{2(NIRS_{AO})}$, whereas no difference was found in the BR.

We decided to focus on $m\dot{V}O_{2(NIRS_{AO})}$ in the rest of this paper on the basis of *1)* the non-reproducibility of FDS at IO_{35}, *2)* the absence of expected local differences between active and inactive muscles during exercise, and *3)* a substantially lower coefficient of variance for $m\dot{V}O_{2(NIRS_{AO})}$ (16.2%) compared with $m\dot{V}O_{2(NIRS_{VO})}$ (32.6%) that was found in another study (unpublished data) that we performed.

Table 3. $m\dot{V}O_2$ values measured by NIRS during arterial occlusion vs. venous occlusion

		$m\dot{V}O_{2(NIRS_{AO})}$, $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$	$m\dot{V}O_{2(NIRS_{VO})}$, $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$	*P* value	
Rest	FDS IO_{35}	0.11 ± 0.03			
	FDS IO_{50}	0.09 ± 0.03	0.08 ± 0.04	NS	$P = 0.04$
	BR IO_{35}	0.13 ± 0.05	0.14 ± 0.10	NS	$P = 0.51$
Exercise	FDS IO_{35}	0.58 ± 0.27	0.21 ± 0.12	**	$P < 0.01$
	FDS IO_{50}	0.55 ± 0.22	0.19 ± 0.10	**	$P < 0.01$
	BR IO_{35}	0.20 ± 0.09	0.24 ± 0.21	NS	$P = 0.30$

Values are means ± SD; n = 22 for FDS IO_{35}; n = 26 for FDS IO_{50} and BR IO_{35}. No P value was calculated for FDS IO_{35} at rest because $m\dot{V}O_{2(NIRS_{VO})}$ was not reproducible. $m\dot{V}O_{2(NIRS_{AO})}$, $m\dot{V}O_2$ measured by arterial occlusion. To protect against a type I error, an α of 0.01 was chosen, $^{**}P \leq 0.01$.

Influence of depth

The $m\dot{V}O_{2(NIRS_{AO})}$ at rest was significantly lower ($P \leq 0.01$) in the deeper region of the FDS (IO_{50}) compared with the superficial region (IO_{35}) (Table 4). From rest to low-intensity exercise at 10% MVC, $m\dot{V}O_{2(NIRS_{AO})}$ increased more than five times independent of the measurement depth (Fig. 2). This increase during exercise was highly significant for both depths ($P \leq 0.01$). No significant difference ($P = 0.15$) was found between IO_{35} and IO_{50} during exercise.

The higher $m\dot{V}O_{2(NIRS_{AO})}$ at IO_{35} compared with IO_{50} was accompanied by a significantly higher FBF_{NIRS} at IO_{35} as compared with IO_{50} (Table 4). From rest to exercise, FBF_{NIRS} increased significantly ($P \leq 0.01$) at both depths (1.4 times), but did not match the fivefold increase in $m\dot{V}O_2$. The difference in FBF_{NIRS} between IO_{35} and IO_{50} found at rest was still present during exercise ($P \leq 0.01$).

Influence of place

No significant difference in $m\dot{V}O_{2(NIRS_{AO})}$ was found between FDS and BR at rest (Table 4). During exercise at 10% MVC, $m\dot{V}O_{2(NIRS_{AO})}$ in the BR increased significantly ($P \leq 0.01$) with a factor of 1.6, but did not match the increase in the FDS (Fig. 2). This resulted

in a significantly higher ($P \leq 0.01$) consumption during exercise in the FDS compared with the consumption in the BR.

Although no difference in resting $m\dot{V}O_2$ was found between both muscles, FBF_{NIRS} was significantly higher in the BR ($P \leq 0.01$) compared with the FDS. At the transition from rest to exercise, FBF_{NIRS} increased significantly ($P \leq 0.01$) in both muscles, and the relative increase was the same for both muscles.

Comparison Fick and NIRS method

$m\dot{V}O_2$ at rest was significantly higher ($P \leq 0.01$) for the Fick-method compared with the NIRS measurement at FDS IO_{35} (Table 4). During exercise $m\dot{V}O_2$ of both methods increased, but the increase in $m\dot{V}O_{2(NIRS_{AO})}$ was much larger than the increase in $m\dot{V}O_{2(Fick)}$ and resulted in a significantly higher ($P \leq 0.01$) $m\dot{V}O_{2(NIRS_{AO})}$.

The blood flow at rest measured with plethysmography was more than twice ($P \leq 0.01$) the flow measured with NIRS (Table 4). From rest to exercise, both FBF_{Pleth} and FBF_{NIRS} increased significantly with a factor of 1.4 and the difference between FBF_{NIRS} and FBF_{Pleth} that was found at rest was, therefore, maintained during exercise ($P \leq 0.01$).

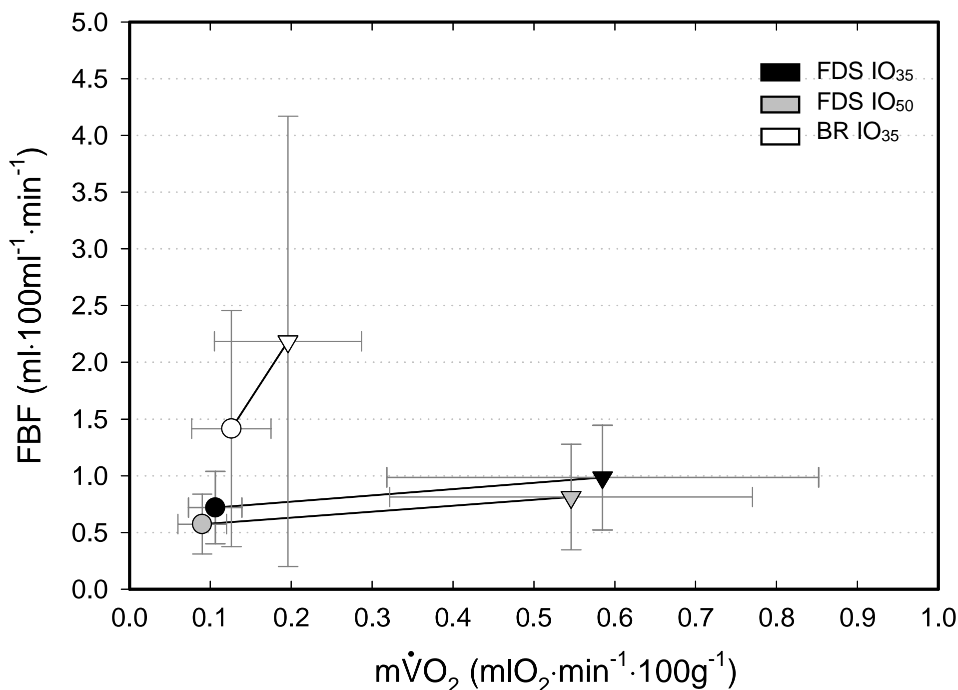

Fig. 2. Average and SD for NIRS $m\dot{V}O_2$ vs. NIRS FBF showing an increased $m\dot{V}O_2$ in the active flexor digitorum superficialis muscle (FDS) during isometric handgrip exercise with interoptode distances of 35 and 50 mm (IO_{35} and IO_{50}, respectively) compared with the relative inactive brachioradialis muscle (BR). Circles represents resting values, and triangles represents values during exercise. Significant differences are shown in Table 4.

Table 4. $m\dot{V}O_2$ and FBF values at rest and during sustained isometric handgrip exercise at 10% maximum voluntary contraction

	$m\dot{V}O_2$, $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$		FBF, $ml \cdot min^{-1} \cdot 100ml^{-1}$	
	Rest	Exercise	Rest	Exercise
NIRS FDS IO_{35}	0.11 ± 0.03	0.59 ± 0.27 †	0.72 ± 0.32	0.98 ± 0.46 †
NIRS FDS IO_{50}	0.09 ± 0.03 *	0.55 ± 0.22 †	0.57 ± 0.26 *	0.81 ± 0.47 *, †
NIRS BR IO_{35}	0.13 ± 0.05	0.20 ± 0.09 *, †	1.42 ± 1.04 *	2.18 ± 1.98 *, †
Fick/pleth.	0.15 ± 0.06 *	0.30 ± 0.12 *, †	2.06 ± 0.70 *	2.98 ± 1.26 *, †

*Values are means ± SD. *Significantly different ($P \leq 0.01$) from FDS IO_{35}; †significant difference ($P \leq 0.01$), rest vs. exercise.*

DISCUSSION

This study was performed to investigate the performance of NIRS for the quantitative measurement of local muscle oxygen consumption and blood flow in the human forearm. Two independently operating identical NIRS systems were used simultaneously to study local differences based on the activity level of the muscle as well as on the measurement depth. Furthermore, local differences were compared with the more established, though global, Fick method.

Methodological considerations

Because the penetration depth of the near-infrared light is limited to roughly half the distance between source and detector, ATT can be a substantial confounder in the measurement of muscle oxygenation [Binzoni et al. 1998, Homma et al. 1996b, Matsushita et al. 1998, Yamamoto et al. 1998]. However, in this study, there was no correlation between ATT and $m\dot{V}O_2$ and the results are, therefore, not biased by ATT. This is probably due to the relatively narrow range of low values that we found for ATT in our subject group (Table 1). Although we did not find a correlation between ATT and $m\dot{V}O_2$ measurements, the individual differences in ATT might have increased to some extent the variability within the group.

NIRS is unable to distinguish between changes in O_2Hb and O_2Mb or in HHb and HMb because of identical absorption spectra of Hb and Mb. Although there is no consensus yet about whether the NIRS signal originates from Hb [Seiyama et al. 1988, Wang et al. 1990] or Mb [Mole et al. 1999, Tran et al. 1999], this does not affect our results since we were interested in the amount of O_2 consumed independently whether it

came from Hb or Mb. Furthermore, we think that substantial desaturation of Mb is negligible in our study because the workload that we used was only 10% MVC.

The DPF for skeletal muscle has been measured by several investigators under different conditions and using different instrumentation [Delpy et al. 1988, Duncan et al. 1995, Essenpreis et al. 1993, Ferrari et al. 1992, van der Zee et al. 1992]. The average values found for DPF in the human forearm lie between 3.59 and 4.57. We have chosen a DPF of 4.0 because this reflects roughly the mean value. Because we chose one DPF value for the complete group, the inter-individual variation probably increased with 10-12%.

Reproducibility of NIRS measurements

Oxygen consumption

$m\dot{V}O_{2\,(NIRS_{VO})}$ was not uniformly reproducible over the three consecutive venous occlusions because $m\dot{V}O_2$ appeared to decrease over time when measured repeatedly at FDS IO_{35}. We did not expect to find differences between the three occlusions and have three possible explanations for this non reproducibility. The first one concerns technical conditions of the protocol, the second a change of physiological variables during the occlusion, and the third the NIRS method during venous occlusion being not stable enough to provide a reliable $m\dot{V}O_2$ value.

Concerning the chosen protocol variables, it is our opinion that there were no differences among the three occlusions given that we applied the occlusions at distinct time periods and all with the same duration for both occlusion and recovery. The non reproducibility was not caused by an insufficient recovery time between the determination of the MVC force and the first venous occlusion either because this effect was not present in the other measurements that were simultaneously performed.

Physiological changes, affecting optical properties of the tissue caused by the venous occlusion itself are not very likely since minor decrease (7%) of the optical path-length has been detected after 3 min of venous occlusion [Ferrari et al. 1992], whereas we applied only 20 s of occlusion.

Venous occlusion has recently been used to calculate $m\dot{V}O_2$ by use of NIRS [De Blasi et al. 1994, De Blasi et al. 1997, Homma et al. 1996a]. This method is thought to be preferable to arterial occlusion because the procedure is less inconvenient for the subject and can be repeated at short time intervals [De Blasi et al. 1997, Homma et al. 1996a]. However, venous occlusion is also more prone to ever occurring variations in flow within the arm due to changes in blood pressure and local vasoreactivity, whereas these influences are negligible during arterial occlusion because of the closed compartment, temporarily cut off from centrally mediated variations. The arterial occlusion method to

determine NIRS $m\dot{V}O_2$ measurements proved to be reproducible [Colier et al. 1995], but no data about the reproducibility of NIRS $m\dot{V}O_2$ measurement during venous occlusion are available.

The relative variability within our group, when looking at the SD in relation to the mean, was consistently higher for $m\dot{V}O_{2(NIRS_{VO})}$ compared with $m\dot{V}O_{2(NIRS_{AO})}$, both at rest and during exercise (Table 3). In an unpublished study that we performed in healthy subjects (n=78), it was found that the arterial occlusion method had a substantially lower coefficient of variance (16.2%) than the venous occlusion method (32.6)% while the absolute values were roughly the same compared with this study. Moreover, no differences were found between the active FDS and the inactive BR muscle during low-intensity work, whereas differentiation in oxygenation pattern was expected based on elementary physiological principles of agonistic and synergistic muscles. On the basis of the above-mentioned points and the lack of data from the literature, we have to conclude that the venous occlusion method does not provide a reliable quantitative value for $m\dot{V}O_2$.

Forearm blood flow

The reproducibility for the measurement of forearm blood flow obtained both by plethysmography (FBF_{Pleth}) and by NIRS during venous occlusion (FBF_{NIRS}) was good (Table 2). Although we found a higher coefficient of variation, our results are supported by De Blasi et al. [De Blasi et al. 1994] who studied the reproducibility of FBF_{NIRS}. They applied three to five repetitive venous occlusions with a 30-s interval between each measurement and found a coefficient of variance of 10.0 ± 5.5% for FBF_{NIRS} and 6.2 ± 4.1% for FBF_{Pleth}. Therefore, we conclude that FBF_{NIRS} is a valid method to measure local flow.

Influence of depth ($m\dot{V}O_{2(NIRS_{AO})}$ and FBF_{NIRS})

Table 4 shows a consistent difference between both depths, present in both $m\dot{V}O_2$ and FBF at rest and in FBF during exercise. During rest, $m\dot{V}O_2$ and FBF were slightly higher ($P \leq 0.01$) in the superficial region of the FDS compared with the deeper region. $m\dot{V}O_2$ during exercise increased more than fivefold for both measurements, and this eliminated the difference in $m\dot{V}O_2$ between superficial and deep. The difference between superficial flow and deep flow that was present at rest was maintained during exercise. The flow increase was the same for both depths, but flow did not increase with the same factor as the $m\dot{V}O_2$. The high demand for O_2 must, therefore, be partly met by an increase of O_2 extraction from the blood.

The reason for the difference in O_2 consumption at rest in relation to the depth of the measurement is unclear. It might be related to local and/or temporary differences in relation to the activity level of that specific part of the muscle.

Influence of place ($m\dot{V}O_{2(NIRS_{AO})}$ and FBF_{NIRS})

Concerning the measurement place, no significant difference was found in $m\dot{V}O_2$ between FDS and BR at rest. At the transition from rest to exercise, $m\dot{V}O_2$ in the BR did not increase as much as that in the FDS. Although this difference in $m\dot{V}O_2$ during exercise was expected because we localised the FDS as the most active muscle during handgrip exercise (unpublished 128-channel surface electromyogram data) and because the function of the BR is not directly related to handgrip exercise, it is the first time that these local differences in $m\dot{V}O_2$ are actually quantified. The flow increase was roughly the same for both muscles. In the case of the BR, the increase in O_2 consumption is equally matched by an increase in delivery. A possible explanation for the lag in delivery in relation to the consumption in the FDS might be an impaired flow within the muscle due to the increased intramuscular pressure enforced by the contracting muscle. It is known that the capillaries within the exercising muscle will be compressed when exercise exceeds 25-30% MVC, which will lead to obstruction of the blood flow [Barcroft et al. 1939, Kahn et al. 1998, Lind et al. 1967b]. These findings, however, give an estimation of the flow in the total limb whereas NIRS is focussed on the local flow within one muscle. If the flow in the total arm becomes obstructed at 25-30% MVC, it might be reasonable to assume that the local flow in the active muscle will be impeded at lower work intensities. This is supported by Barcroft & Millen [Barcroft et al. 1939] who hypothesised that ischemia and hyperaemia might both be present in the limb as a result of considerable differences in contraction strength from one muscle to another.

Comparison of Fick and NIRS arterial occlusion methods

According to Table 4, we found a consistent difference between both methods, present in both $m\dot{V}O_2$ and FBF. During rest, $m\dot{V}O_2$ and FBF were higher according to the Fick method compared with the NIRS measurements. FBF_{Pleth} during exercise was also higher than FBF_{NIRS}. The difference in $m\dot{V}O_2$ between both methods reversed during exercise, resulting in a high $m\dot{V}O_2$ measured by NIRS. As for the local $m\dot{V}O_2$ measured by NIRS (FDS) it might be expected to find a higher value during exercise as compared with the Fick method because the Fick method will reflect an average value of $m\dot{V}O_2$ in the forearm. The exercise performed was low-intensity work and was mainly generated by the FDS muscle, probably without much support from other forearm muscles. Therefore,

local $m\dot{V}O_2$ can increase more than fivefold, whereas the increase of $m\dot{V}O_2$ in the total forearm is only twofold.

The lower NIRS $m\dot{V}O_2$ at rest compared with Fick $m\dot{V}O_2$ is less clear. On the basis of the hypothesis of local vs. global measurement, no difference in resting $m\dot{V}O_2$ between both methods was expected. It implies a higher $m\dot{V}O_2$ elsewhere in the forearm, but we did not find this higher $m\dot{V}O_2$ in either the deeper region of the FDS or in the BR. This higher $m\dot{V}O_2$ will probably not been found in skin tissue or bone tissue either. Therefore, we conclude that the difference between NIRS and Fick is not physiological, but must have its origin somewhere else. A methodological explanation might be found in a systematic discrepancy between FBF_{pleth} and FBF_{NIRS}. FBF_{pleth} triples the flow that is measured with NIRS. This difference was both present at rest and during exercise. Our values for blood flow obtained by plethysmography were comparable with previous observations of blood flow in resting muscle [De Blasi et al. 1994, Edwards et al. 1993, Jorfeldt et al. 1990, Mottram 1955, Pallares et al. 1994, Witney 1953]. The discrepancy that we found between FBF_{pleth} and FBF_{NIRS} is in agreement with De Blasi et al. [De Blasi et al. 1994] who found that FBF_{pleth} was almost twice as high as FBF_{NIRS} measured on top of the brachioradialis muscle while correlation between both methods was good.

Variations in flow measurements might be due to a heterogeneous distribution of flow [Edwards et al. 1993] or fluctuations of blood flow over time, but there are also some methodological differences between plethysmography and NIRS. Plethysmographic flow reflects the total flow of the forearm. Apart from blood flowing through skeletal muscle, it contains blood coming from cutaneous tissues, bone, and tendons and might thus lead to a higher FBF_{Pleth}. NIRS flow reflects only the local flow in the NIRS region of interest. Furthermore, NIRS is limited to monitoring capillaries that have a diameter smaller than approximately 1 mm because of the absorption of light in vessels with larger diameters [Mancini et al. 1994b]. During rest, only part of the capillaries are perfused, and most of the blood flows through metarterioles or arteriovenous anastomoses. This blood will bypass the capillaries on its way from the arterial to the venous side of the circulation and will only partly contribute to the NIRS signal. Compared to FBF_{Pleth}, the FBF_{NIRS} will be underestimated and the $m\dot{V}O_2$ calculated from Fick will be overestimated. In addition, due to the lower haematocrit in capillaries, the FBF_{NIRS} will also be underestimated [De Blasi et al. 1994, Leenders et al. 1990].

Overall

Overall, we see that, at the transition from rest to sustained isometric handgrip exercise at a workload of 10% MVC, the blood flow increased homogeneously despite the difference in flow between different muscles and between different methods. This is not the case for oxygen consumption because the increase during exercise depends on the muscle that is

monitored as well as on the method used. Local oxygen consumption is high in the active muscle and much lower in the relative inactive muscle. This is in accordance with basic exercise physiology and is directly and noninvasively detectable by NIRS. The value for $m\dot{V}O_2$ as measured by the combination of blood samples, POX, and plethysmography lies in between the consumption value of the active and inactive muscle that we measured. This is in agreement with the assumption that the Fick method represents the average value for oxygen consumption in the total forearm as determined by blood sampling from mixed venous blood and the measurement of total blood flow.

The increase in FBF at the transition from rest to low-intensity work was roughly 1.4, whereas the average (Fick-method) increase in $m\dot{V}O_2$ was 2.0. Thus, the increase in oxygen consumption was higher than the increase in flow and, apparently, the increased demand for oxygen is met by an increase in extraction of O_2 from the blood. When we look at the NIRS data during exercise in the FDS as compared with the BR, we see that in the active FDS the increased demand for oxygen is mostly met by an increase in extraction, whereas in the relatively inactive BR it is almost completely met by the increase in flow. This is in agreement with De Blasi et al. [De Blasi et al. 1994] who also found an increase in $m\dot{V}O_2$ that was many times greater than the increase in flow after a period of ischemic exercise.

In conclusion, NIRS is a suitable tool to give new insight in the heterogeneity of local muscle metabolism. We have shown that NIRS is able to discriminate between the resting and exercising states of the muscle. With the use of two independent simultaneously operating NIRS systems, the technique also discriminates between physically active and less active muscle. O_2 consumption measured by the global Fick method during exercise lies in between our NIRS results measuring local O_2 consumption in the active FDS and the less active BR muscle. Furthermore, it is shown that the increase in blood flow during exercise is much more homogeneous compared with the local increase in $m\dot{V}O_2$.

3

In vivo quantitative near-infrared spectroscopy in skeletal muscle during incremental isometric handgrip exercise

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SUMMARY

The aim of this study was to investigate the performance of in vivo quantitative near-infrared spectroscopy (NIRS) in skeletal muscle at various workloads. NIRS was used for the quantitative measurement of O_2 *consumption (*$m\dot{V}O_2$*) in the human flexor digitorum superficialis muscle at rest and during rhythmic isometric handgrip exercise in a broad range of work intensities (10-90% MVC = maximum voluntary contraction force). Six subjects were tested on three separate days. No significant differences were found in* $m\dot{V}O_2$ *measured over different days with the exception of the highest workload. The within-subject variability for each workload over the three measurements days ranged from 15.7 - 25.6% and did not increase at the high workloads.* $m\dot{V}O_2$ *was* 0.14 ± 0.01 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ *at rest and increased roughly 19 times to* 2.68 ± 0.58 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ *at 72% MVC. These results show that local muscle oxygen consumption at rest as well as during exercise at a broad range of work intensities can be measured reliably by NIRS, applied to a uniform selected subject population. This is of great importance since direct local measurement of* $m\dot{V}O_2$ *during exercise is not possible with the conventional techniques. The method is robust enough to measure over separate days and at various workloads and can, therefore, contribute to a better understanding of human physiology in both the normal and pathological state of the muscle.*

INTRODUCTION

Understanding the mechanisms of local muscle metabolism at rest and during exercise depends on the ability to obtain reliable quantitative measurements of local muscle oxygen consumption and blood flow. The standard for measurement of oxygen consumption in arm or leg is the combination of strain-gauge plethysmography and blood gas analysis for determination of blood flow and arteriovenous O_2 difference, respectively. However, the volume of interest for both techniques is limited to the total limb and tissues other than muscle tissue influence muscle oxygen consumption and blood flow. Blood sampling is invasive and requires a high precision within a small time window while strain-gauge plethysmography is impossible during moderate to strenuous exercise.

Near-infrared spectroscopy (NIRS) is a noninvasive optical method to determine oxygenation and haemodynamics in the tissue. Although the measurement depth is restricted to the optode set-up, NIRS is able to obtain signals localised in muscle tissue. Using a modified Lambert-Beer law and a simple physiological intervention, it is possible to obtain a quantitative value for muscle oxygen consumption and blood flow. The reproducibility of forearm blood flow at rest using the venous occlusion method proved to be reliable within the test session, although the mean values of the within-subject variability varied between 10-29% [De Blasi et al. 1994, Van Beekvelt et al. 2001b, Van Beekvelt et al. 2001a]. NIRS blood flow showed good agreement with strain-gauge plethysmography [De Blasi et al. 1994, Homma et al. 1996a] although flow measured by plethysmography was generally two to three times higher than NIRS flow [De Blasi et al. 1994, Van Beekvelt et al. 2001b].

NIRS muscle oxygen consumption ($m\dot{V}O_2$) can be measured during both venous and arterial occlusion. It has been suggested that the venous occlusion method is to be preferred over the arterial occlusion method because venous occlusion is less inconvenient for the subject, the recovery is much faster, and $m\dot{V}O_2$ and flow can be measured simultaneously. However, we showed that $m\dot{V}O_2$ from venous occlusion appeared unreliable when repeated several times within one session [Van Beekvelt et al. 2001b]. In another study we found a coefficient of variation of 30.4% [Van Beekvelt et al. 2001a] for resting $m\dot{V}O_2$ during venous occlusion while the coefficient of variation was much lower (16.2%) for the arterial occlusion method. Therefore, it is our opinion that the arterial occlusion method is to be preferred over the venous occlusion method for the calculation of $m\dot{V}O_2$. Although $m\dot{V}O_2$ at rest proved to be reproducible within one test session [Van Beekvelt et al. 2001a] and between test sessions [Kragelj et al. 2000], no data are available concerning $m\dot{V}O_2$ during exercise. The accuracy of NIRS $m\dot{V}O_2$ measurements at rest has been addressed in an earlier study from our group by comparing NIRS $m\dot{V}O_2$ with $m\dot{V}O_2$ values obtained from the well-established Fick method [Van Beekvelt et al. 2001b].

Reliable measurements of local $m\dot{V}O_2$ during exercise are of great importance since this has not been possible with the standard methods of strain-gauge plethysmography and blood gas analysis. This also means that the accuracy of NIRS $m\dot{V}O_2$ cannot be studied during exercise. Apart from more insight in local regulation of tissue oxygenation at the change from rest to exercise or from low- to strenuous work intensities, reliable measurements during exercise are indispensable since some disorders are not recognisable at rest and become only apparent during exercise (e.g. metabolic/mitochondrial myopathies or peripheral vascular disease).

$m\dot{V}O_2$ using arterial occlusion can be calculated from the rate of decrease in oxyhaemoglobin [Cheatle et al. 1991, Colier et al. 1995, Kragelj et al. 2000, Van Beekvelt et al. 1999] or from the rate of decrease in Hb_{diff} reflecting the difference between oxy- and deoxyhaemoglobin ($[Hb_{diff}]=[O_2Hb]-[HHb]$) [Cheatle et al. 1991, De Blasi et al. 1992, De Blasi et al. 1993, De Blasi et al. 1997, Van Beekvelt et al. 2001a, Van Beekvelt et al. 2001b]. The calculation of $m\dot{V}O_2$ from the rate of decrease in Hb_{diff} is thought to be more accurate than the calculated $m\dot{V}O_2$ from oxyhaemoglobin, but to our knowledge no literature has yet addressed this issue.

The purpose of this study was, therefore, *1)* to test the the precision of the NIRS method by studying the day-to-day reproducibility of $m\dot{V}O_2$ at rest and at various workloads of isometric handgrip exercise on three separate measurement days and *2)* to compare $m\dot{V}O_2$ from arterial occlusion, calculated from the rate of decrease in O_2Hb and that from the rate of decrease in Hb_{diff}. With reproducible $m\dot{V}O_2$ values at rest and during exercise, NIRS can be used for direct investigation of muscle oxygen consumption and extend the current possibilities of regional measurements at rest to new possibilities of local measurements both at rest and during exercise, and in health and disease.

MATERIALS AND METHODS

Subjects

Seven healthy volunteers (1 female, 6 male) participated in this study. The Faculty Ethics Committee approved the study and all subjects gave their written informed consent. The average (± SE) characteristics of the subjects were 36 ± 4 yr in age, 175.3 ± 3.4 cm in height and 68.7 ± 2.7 kg in weight. Skinfold thickness was measured in between the NIRS optodes using a skinfold caliper (Holtain Ltd., Crymmych, UK) and divided by 2 to determine the adipose tissue thickness (ATT = fat + skin layer) covering the muscle. All subjects were right-handed.

Near-infrared Spectroscopy (NIRS)

NIRS is based on the relative tissue transparency for light in the near-infrared region and on the oxygen-dependent absorption changes of haemoglobin and myoglobin. Using a continuous-wave near-infrared spectrophotometer (Oxymon, Biomedical Engineering Department, University of Nijmegen, NL) that generates light at 905, 850 and 770 nm [Van der Sluijs et al. 1998] it is possible to differentiate between oxy- and deoxyhaemoglobin/myoglobin (O_2Hb/O_2Mb and HHb/HMb, respectively). Due to the overlap of the spectrum, it is not possible to distinguish between changes in haemoglobin and myoglobin. For convenience, both hemoglobin and myoglobin are referred to as hemoglobin in this paper. Although there is no consensus yet about whether the NIRS signal originates from Hb [Seiyama et al. 1988],[Chance et al. 1992, Wang et al. 1990] or from the combination of Hb and Mb [Hoofd 1999, Mole et al. 1999, Tran et al. 1999], this does not affect our results since we were interested in the amount of O_2 consumed independently whether it was supplied by Hb or Mb. The changes in absorption at the discrete wavelengths are converted into concentration changes of O_2Hb and HHb using a modified Lambert-Beer law in which a path-length factor is incorporated to correct for scattering of photons in the tissue [Livera et al. 1991]. The average values found for DPF in the human forearm lie between 3.59 and 4.57. In this paper, we have used a fixed value of 4.0 since direct measurement of the individual DPF is impossible using continuous-wave spectrophotometers. NIRS measurements were done on top of the flexor digitorum superficialis muscle (FDS) with an interoptode distance of 35 mm. In order to prevent variations in placement of the optodes and to avoid operator errors, the angle and place of the optodes were kept constant during the test using a special support that was attached to the skin with adhesive stickers. Waterproof markers on the arm avoided variation in placement over separate days. Data were sampled at 20 Hz, displayed real-time and stored on disk for off-line analysis.

Forearm measurements

Muscle oxygen consumption ($m\dot{V}O_2$) was measured by NIRS evaluating the rate of decrease in [Hb_{diff}] during arterial occlusion ((-d(Hb_{diff})/dt)/2). Hb_{diff}, also called the oxygenation index (OI), reflects the difference between O_2Hb and HHb ([Hb_{diff}]=[O_2Hb]-[HHb]). In addition, $m\dot{V}O_2$ was calculated by evaluating the rate of decrease in [O_2Hb] during arterial occlusion (-d(O_2Hb)/dt). The initial linear decrease after the start of occlusion was used to calculate the rate of decrease in Hb_{diff} and O_2Hb. The exact time window that was used for calculation of $m\dot{V}O_2$ varied over the different work intensities. Whereas the initial decrease in O_2Hb and Hb_{diff} was linear for the full 45 s during rest, it became shorter in time when exercise intensity increased. Concentration changes of Hb_{diff}

and O_2Hb were expressed in $\mu M \cdot s^{-1}$ and converted to $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$. A value of 1.04 $kg \cdot L^{-1}$ was used for muscle density [Vierordt 1906].

Protocol

All subjects came back three times on separate days to test the reproducibility of NIRS $m\dot{V}O_2$ at rest and during isometric handgrip exercise. All three tests of each subject were performed on the same time of the day. The subject sat in a comfortable chair and the right hand rested on a handgrip dynamometer with the upper arm at heart level and the forearm in an upward angle of 30° to avoid venous pooling of the blood. The arm was supported at the wrist and above the elbow to avoid contact between forearm and dynamometer assuming completely unrestricted circulation in the forearm. The subject's maximum voluntary contraction (MVC) force was determined prior to the test and followed by a 15-20 min recovery period before starting the test. A pneumatic cuff was placed around the upper arm and was used to apply arterial occlusion during the test.

After placement of the instruments, the experiment started with a 5 min rest period followed by an arterial occlusion to determine $m\dot{V}O_2$ at rest. Cuff inflation was kept at 260 mmHg for 45 s and than released. After 3 min of recovery, when NIRS signals had returned to baseline values, the subject performed seven sessions of rhythmic isometric handgrip exercise at incremental work intensities. Each session consisted of 1 min exercise at a contraction rate of $30 \cdot min^{-1}$ (duty cycle 50%). An arterial occlusion lasting 45 s was applied immediately at the end of exercise. All different exercise intensities were separated by 3 min of recovery. The work intensity was indicated visually and increased with each session in following way; 10-20-30-40-50-70-90% MVC. This way $m\dot{V}O_2$ could be determined at rest and immediately following exercise in a broad range of workloads.

Statistics

The day-to-day reproducibility for $m\dot{V}O_2$ calculated from Hb_{diff} and O_2Hb was determined by a one-way analysis of variance (ANOVA) for repeated measurements. In addition, the within-subject variability was calculated as the coefficient of variation (CV) for each subject ((SD/mean)·100) across the three days. The difference between $m\dot{V}O_2$ (Hb_{diff}) and $m\dot{V}O_2$ (O_2Hb) over the complete range from rest to 90% MVC was determined by a one-way ANOVA for repeated measurements. Differences of $P \leq 0.05$ were considered as statistically significant and any differences were further analysed with Student-Newman Keuls post-hoc test. All data are presented as mean ± SE.

RESULTS

Adipose tissue thickness (ATT) between the optodes ranged from 1.4 - 3.9 mm with mean and SE of 2.5 ± 0.2 mm. There was no significant difference in ATT between the three measurement days (P = 0.55) and the intra-observer variability was 3.8%. The maximum voluntary contraction force (MVC) ranged from 397 - 679 N with mean and SE of 542 ± 20 N. No significant difference in MVC was found between the three measurement days (P = 0.06). The within-subject variability in MVC measured over the different days was 3.3%. One subject was excluded from further analysis because of a poor signal-to-noise ratio.

Fig. 1 shows a typical example of the NIRS signals in an individual subject during a single exercise intensity at 20% MVC. During exercise, the compression of blood out of the muscles due to each contraction can be seen from the rhythmic rise and fall in all signals. Over the 60 s of exercise, O_2Hb decreased while HHb increased. This became more pronounced when work intensity increased. Over the whole range of exercise intensities (10 - 90% MVC), we saw a net increase in tHb during each 60 s work period. Only during the lowest workloads (10 - 20% MVC), we saw that tHb remained roughly constant in some subjects, but at no time a decrease in tHb was observed. During occlusion, a linear decrease in O_2Hb and a linear increase in HHb were seen after the start of the occlusion. In contrast to the situation at rest where the linear decrease/increase

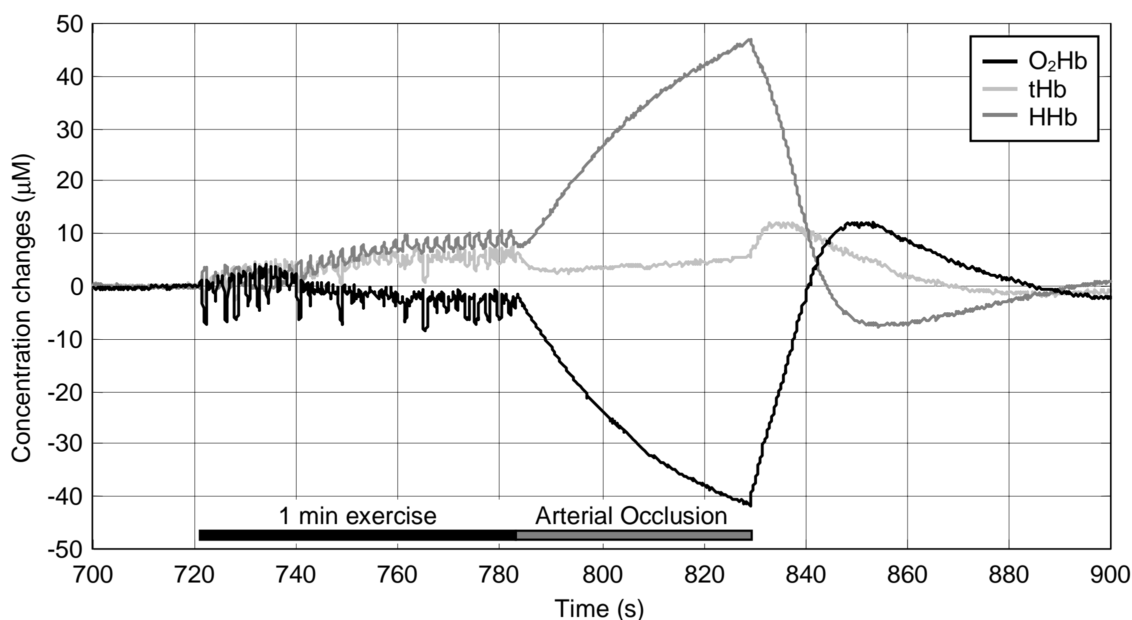

Fig. 1. Raw NIRS signal during 1 min of rhythmic isometric handgrip exercise at 20% of the maximum voluntary contraction force (MVC) followed by 45 s of arterial occlusion. O_2 consumption was calculated from the linear decrease in oxyhaemoglobin (O_2Hb) immediately after the start of the occlusion and from the linear decrease in Hb_{diff} ($[Hb_{diff}] = [O_2Hb] – [HHb]$) (not shown). The sum of $[O_2Hb]$ and [HHb] reflects the total amount of haemoglobin ([tHb]) and changes in tHb can be interpreted as changes in blood volume in the tissue.

was continued for the full 45 s of the occlusion, O_2Hb and HHb showed a tendency to plateau within the 45 s of occlusion after exercise. This plateau became more pronounced and faster in time when exercise intensity increased. In 15 out of 126 measurements there was a slight increase in tHb during the occlusion.

The intensity of each exercise session was based on the individual maximum force production as determined prior to the test. Because we were interested in the relationship between $m\dot{V}O_2$ and the amount of work performed we compared the intended force (based on MVC) with the average force that was actually produced during each 1 min contraction session. Fig. 2 shows that the actual performance was less than the intended workload and this became more pronounced at the high workloads of 70% and 90% MVC. Calculation of the true work intensities resulted in the corrected workloads of 9-19-28-36-44-61-72% MVC instead of 10-20-30-40-50-70-90% MVC. The within-subject variability of the work intensity that was performed over the different days ranged between 7.3-15.6% (10.5 ± 2.8%) for the various work intensities.

No significant differences were found in $m\dot{V}O_2$ measured over several days with the exception of the highest work level, which was significantly lower at the first day as compared with the second and third day both for $m\dot{V}O_2$ calculated from Hb_{diff} ($m\dot{V}O_2(Hb_{diff})$) (Table 1) and O_2Hb ($m\dot{V}O_2(O_2Hb)$) (P=0.01). The within-subject variability for $m\dot{V}O_2$, calculated as the coefficient of variation for each subject ((SD/mean)·100%) is shown in Table 2 for $m\dot{V}O_2(Hb_{diff})$ at rest and at 9% and 72% MVC. The within-subject variability for $m\dot{V}O_2(O_2Hb)$ was similar (data not shown). The mean within-subject variability for each workload is shown in Table 1 and ranged from

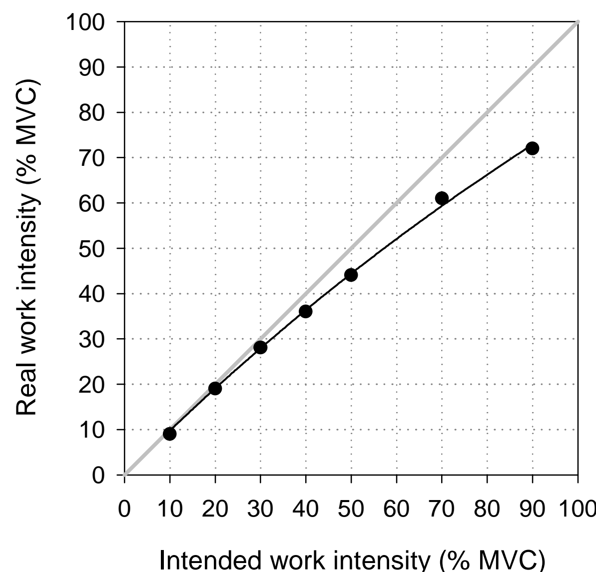

Fig. 2. Agreement in work intensity between the intended work, based on the maximum voluntary contraction force (MVC), and the real performed work. The straight grey line indicates the line of identity between both work intensities.

Table 1. Mean (± SE) values for O_2 consumption ($m\dot{V}O_2$) from Hb_{diff} at rest and during rhythmic isometric handgrip exercise.

	Rest	9% MVC	18% MVC	28% MVC	36% MVC	44% MVC	61% MVC	72% MVC
Day 1	0.14 ± 0.01	0.38 ± 0.07	0.84 ± 0.25	1.31 ± 0.44	1.52 ± 0.51	1.85 ± 0.63	2.45 ± 0.79	2.54 ± 0.73
Day 2	0.15 ± 0.02	0.43 ± 0.09	0.95 ± 0.23	1.48 ± 0.37	1.83 ± 0.43	2.16 ± 0.65	2.68 ± 0.71	3.13 ± 0.59
Day 3	0.15 ± 0.02	0.41 ± 0.07	0.88 ± 0.25	1.46 ± 0.45	1.97 ± 0.56	2.14 ± 0.68	2.36 ± 0.60	3.06 ± 0.59
Avg	0.15 ± 0.01	0.41 ± 0.07	0.89 ± 0.24	1.42 ± 0.41	1.77 ± 0.49	2.05 ± 0.64	2.50 ± 0.69	2.91 ± 0.63
P-value	0.99	0.32	0.34	0.49	0.10	0.24	0.49	0.02 * day 1 < day 2 day 1 < day 3
CV (%)	17.6	16.3	21.1	23.2	23.0	20.3	20.0	20.6

*$m\dot{V}O_2$ in $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$. MVC = maximal voluntary contraction force, CV = coefficient of variation, n = 6. *$P \leq 0.05$ for differences between the three days.*

Table 2. Within-subject variability for $m\dot{V}O_2$ from Hb_{diff} at rest and during isometric handgrip exercise at 9% and 72% of the maximal voluntary contraction force (MVC).

	Rest				9% MVC				72% MVC			
Subject	I	II	III	CV (%)	I	II	III	CV (%)	I	II	III	CV (%)
1	0.15	0.11	0.09	25.0	0.55	0.61	0.45	15.2	1.70	2.30	2.00	14.9
2	0.18	0.14	0.21	21.4	0.40	0.54	0.57	17.8	2.76	3.54	3.10	12.6
3	0.13	0.11	0.10	10.8	0.41	0.37	0.33	11.1	1.51	2.09	1.87	16.1
4	0.14	0.18	0.17	12.7	0.11	0.16	0.17	21.9	2.37	3.23	3.52	19.6
5	0.13	0.13	0.14	5.0	0.24	0.23	0.31	17.3	0.93	1.84	2.18	39.0
6	0.14	0.22	0.17	21.8	0.54	0.69	0.62	12.1	5.95	5.78	5.70	2.2
			Avg	17.6			*Avg*	16.3			*Avg*	20.6

$m\dot{V}O_2$ in $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$. CV = coefficient of variation ((SD/mean)·100%), I = day 1, II = day 2, III = day 3.

16.4 - 23.1% for $m\dot{V}O_2$ (Hb_{diff}) and 15.7 - 25.6% for $m\dot{V}O_2$ (O_2Hb). The variation over the different days was similar for all exercise levels tested. The 95% confidence interval for the within-subject variability of all subjects and all exercise intensities together was 15.6 - 20.9% for $m\dot{V}O_2$ (Hb_{diff}) and 15.6 - 21.9% for $m\dot{V}O_2$ (O_2Hb).

The average group value for resting $m\dot{V}O_2$ over the three measurement days was 0.15 ± 0.01 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ for $m\dot{V}O_2$ (Hb_{diff}) and 0.14 ± 0.01 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ for $m\dot{V}O_2$ (O_2Hb). Compared to the $m\dot{V}O_2$ at rest, $m\dot{V}O_2$ during exercise increased roughly 19 times up to 2.91 ± 0.63 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ for Hb_{diff} and 2.68 ± 0.58 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ for O_2Hb during 72% MVC (Fig. 3). The increase in $m\dot{V}O_2$ is closely related to the amount of work performed. The factor of increase in mean $m\dot{V}O_2$ between rest and exercise increased linearly up to 36% MVC, but became less at the higher workloads.

Using a repeated measurement ANOVA test over the whole range of work intensities, a significant difference ($P \leq 0.05$) was also found in $m\dot{V}O_2$ when $m\dot{V}O_2(Hb_{diff})$ was compared with $m\dot{V}O_2(O_2Hb)$. Apart from the 9% MVC workload, all mean $m\dot{V}O_2$ values calculated from O_2Hb were lower than those calculated from Hb_{diff}. The difference between both means was negligible up to a workload of 28% MVC, but became more pronounced from 36% MVC and up (Fig. 3). Calculation from Hb_{diff} and O_2Hb resulted in a reasonable identical $m\dot{V}O_2$ at low consumption rates (low intensity work), but $m\dot{V}O_2$ calculated from Hb_{diff} exceeded that of O_2Hb in 11 out of 12 measurements (61 and 72% MVC) at the two highest work intensities (high consumption rates).

DISCUSSION

The main finding of this study was that local $m\dot{V}O_2$ calculated by the noninvasive optical method of near-infrared spectroscopy (NIRS) was reproducible at rest and equally reproducible at a broad range of exercise intensities as no significant differences were found over the different days apart from the highest work level. This suggests that NIRS is capable of obtaining reliable quantitative measurements of O_2 consumption and is of great importance in the understanding of the mechanisms of local muscle metabolism at rest as well as during exercise.

By choosing an accurate distance between source and detector, NIRS is able to measure directly in the muscle of interest. Although NIRS has become more accepted nowadays and has been compared with other methods to study regional metabolism [Boushel et al. 1998, De Blasi et al. 1994, Edwards et al. 1993, Hamaoka et al. 1996, Homma et al. 1996a, Mancini et al. 1994b, Mancini 1997, McCully et al. 1994a, Sako et al. 2001, Van Beekvelt et al. 2001b], little is known about the reproducibility of the method. Furthermore, various methods are used in literature to calculate $m\dot{V}O_2$ and there is no consensus yet whether one is to be preferred over the other.

The average resting $m\dot{V}O_2$ of 0.15 ± 0.01 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ found with NIRS in this study was in agreement with the forearm $m\dot{V}O_2$ measured with the conventional invasive Fick method [Hartling et al. 1989, Holling 1939, Mottram 1955, Van Beekvelt et al. 2001b] (Table 3) though Mottram found a $m\dot{V}O_2$ value that was slightly higher than that of the others. Similar $m\dot{V}O_2$ values were also found by Wang et al. [Wang et al. 1990], Hamaoka et al. [Hamaoka et al. 1996] and Sako et al. [Sako et al. 2001] who used phosphorus magnetic resonance spectroscopy, which measures $m\dot{V}O_2$ indirectly from PCr kinetics. The present NIRS values for resting $m\dot{V}O_2$ were also comparable with our previous reports and with other NIRS studies as is shown in Table 3.

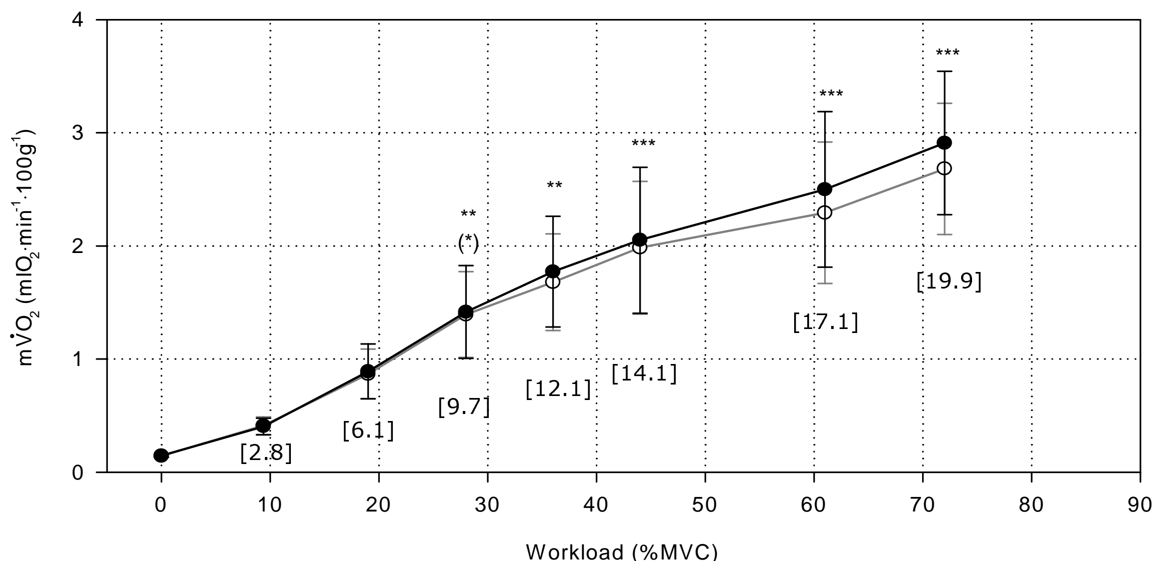

*Fig. 3. Group mean (± SE) over three measurement days for muscle oxygen consumption ($m\dot{V}O_2$) values during arterial occlusion at rest and after isometric handgrip exercise at various work intensities. Closed circles represent $m\dot{V}O_2$ calculated from Hb_{diff}, open circles represent $m\dot{V}O_2$ calculated from O_2Hb. *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.001 for differences between rest and the various work intensities for both O_2Hb and Hb_{diff} except (*) for difference between rest and 30% MVC in $m\dot{V}O_2$ calculated from Hb_{diff}. The factor of the increase in $m\dot{V}O_2$ (Hb_{diff}) between rest and exercise is given in brackets for each workload.*

The $m\dot{V}O_2$ that we found during exercise showed good agreement with the $m\dot{V}O_2$ that Hartling et al. [Hartling et al. 1989] and Hamaoka et al. [Hamaoka et al. 1996] found during rhythmic forearm contractions. In the present study, $m\dot{V}O_2$ increased to roughly 19 times the resting value at the highest work intensity (Fig. 2). The increase in $m\dot{V}O_2$ was linear up to 36% MVC, but became less at the higher intensities. This was also seen by Hamaoka et al [Hamaoka et al. 1996] where a tendency to plateau already appeared at 20% MVC. The deviation of linearity is in agreement with the common hypothesis that blood flow will be impaired when exercise exceeds 25-30% MVC [Barcroft et al. 1939, Kahn et al. 1998, Lind et al. 1967b] due to compression of the capillaries within the contracting muscle as a result of the increased intramuscular pressure. O_2 delivery at high intensities will probably lag behind and local O_2 availability will no longer be sufficient to meet the O_2 consumption rate. Another possible cause for the deviation of linearity between $m\dot{V}O_2$ and MVC might be related to the fact that more muscles will be involved when work intensity increases suggesting that the additional force production wil be more and more provided by the other muscles that are not directly measured by NIRS. Furthermore , $m\dot{V}O_2$ was measured immediately post-exercise and not during the actual exercise itself. Hereby underestimating the actual value.

Table 3. Mean (± SE) values for resting O_2 consumption ($m\dot{V}O_2$) from literature.

	Method	$m\dot{V}O_2$		Method	$m\dot{V}O_2$
Holling 1939	Fick	0.11	De Blasi et al. 1992	NIRS	0.11 ± 0.02
Mottram 1955	Fick	0.23 ± 0.02	De Blasi et al. 1993	NIRS	0.05 ± 0.01
Hartling et al. 1989	Fick	0.14 ± 0.04	De Blasi et al. 1994	NIRS	0.10 ± 0.01
Van Beekvelt et al. 2001b	Fick	0.15 ± 0.01	De Blasi et al. 1997	NIRS	0.06 ± 0.01
Wang et al. 1990	^{31}P-MRS	0.17	Niwayama et al. 1999	NIRS	0.21 ± 0.01
Hamaoka et al. 1996	^{31}P-MRS	0.16 ± 0.00	Van Beekvelt et al. 2001a	NIRS	0.11 ± 0.01
Sako et al. 2001	^{31}P-MRS	0.16 ± 0.00	Van Beekvelt et al. 2001b	NIRS	0.11 ± 0.00

$m\dot{V}O_2$ data from various studies were converted to $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ using 1.04 $kg \cdot L^{-1}$ for muscle density and 22.4 L as the molar volume of gas assuming STPD conditions. 1 mol of O_2 is assumed to synthesise 6 mol of ATP.

Quantitative measurement of NIRS $m\dot{V}O_2$ during exercise has been done by few other groups [Colier et al. 1995, De Blasi et al. 1993, De Blasi et al. 1994, Homma et al. 1996a]. However, no comparison can be made with most of these studies since the exercise task or muscle area were substantially different [Colier et al. 1995, De Blasi et al. 1992, De Blasi et al. 1993, De Blasi et al. 1994] or comparable quantitative values were not provided [Homma et al. 1996a]. Another difficulty in the comparison with other reported data is the actual amount of work that is performed. In this study, the work intensity of each exercise session was based on the individual maximum force production as is commonly used in dynamometry. Because we were interested in the relationship between $m\dot{V}O_2$ and the amount of work performed we compared the intended work intensity (based on % MVC) with the work intensity that was actually performed during each contraction session. The actual work intensity, determined as the average MVC produced over 1 min of rhythmic exercise, was less than the desired percentage of MVC and this became more pronounced at high intensity work (70-90% MVC). There are two logical explanations for this phenomenon. First, the exact force level over 1 s can only been achieved when the generated force curve describes a perfect square wave form. Since this is not the case and the previously determined MVC level is seen as maximum, the amount of performed work will mostly be less than the desired work. Second, performance decreased when exercise intensity increased because more time is needed to reach the desired MVC level and, consequently, less time is available to keep the force at that level. Moreover, the produced force at 70% and 90% MVC will further decrease due to fatigue. Therefore, we re-calculated the performed work intensities, resulting in the corrected workloads of 9-19-28-36-44-61-72% (± 1-2%) MVC instead of 10-20-30-40-50-70-90% MVC.

We investigated the reproducibility of $m\dot{V}O_2$ during arterial occlusion in this study because the day-to-day reproducibility of quantitative NIRS $m\dot{V}O_2$ during exercise has so far remained unclear. The day-to day reproducibility of $m\dot{V}O_2$ that we found was good. No significant differences between the three measurement days were found at rest or during exercise, apart from the highest work intensity (Table 1) where $m\dot{V}O_2$ of day 1 was significantly lower than that of day 2 and day 3. This is possibly due to familiarisation with the test. The within-subject variability for $m\dot{V}O_2$ at rest ranged from 5.1 - 25.0% with a mean of 17.6% for $m\dot{V}O_2$ (Hb_{diff}). The mean value for $m\dot{V}O_2$ (O_2Hb) was 22.2% and the within-subject variability ranged from 5.4 - 45.0%. This is in agreement with Kragelj et al. [Kragelj et al. 2000] who found a group CV of 21.8% for $m\dot{V}O_2$ calculated from Hb_{diff} and with our previous study [Van Beekvelt et al. 2001a] where we found a CV of 16.2% within one test session. During exercise, the group CV was roughly the same for both methods. The 95% confidence interval for the within-subject variability of all subjects and all exercise intensities was 15.6 - 20.9% for $m\dot{V}O_2$ (Hb_{diff}) and 15.6 - 21.9% for $m\dot{V}O_2$ (O_2Hb). There were few values that exceeded the 25% (Table 2), but these were randomly distributed over the subjects as well as over work intensities. Although this variability may seem high, it is most probably influenced by the differences in work that was actually performed. As is clearly shown in this study, the intended work intensity is not the same as the work intensity at which the work is performed. The within-subject variability for the work intensity that was performed over the different days was 10.5 ± 2.8% on average. This means that the actual work that was performed varied with 10% over the days and this, most probably, increased the within-subject variability of $m\dot{V}O_2$ with up to 10%.

Contrary to the within-subject variability, the variability in $m\dot{V}O_2$ between subjects was reasonably high at the higher work intensities. Similar variation in $m\dot{V}O_2$ between subjects was found with Fick at high intensity [Hartling et al. 1989]. The reason for this variability might probably be found in the variation of physiological and metabolic properties of the subjects. Moreover, in this study $m\dot{V}O_2$ was only measured in a single muscle and the amount of work done by other muscles was not known. This recruitment pattern is likely to vary between subjects and becomes especially important at high work intensities. To fully understand the influence of these different recruitment patterns on the variability of $m\dot{V}O_2$, multichannel NIRS can be of great help.

Surprisingly, the group CV did not increase at high intensities indicating that local $m\dot{V}O_2$, as measured by NIRS is not only reliable at rest, but also during exercise up to a work intensity of 61% MVC. This is of great importance since local measurement of $m\dot{V}O_2$ during exercise has so far been impossible using conventional techniques like strain-gauge plethysmography and blood gas analysis and since it enables complementary measurement with ^{31}P-MRS. However, all data presented in this paper were obtained from

healthy subjects. Whether the reproducibility in pathological situations is similar to that in normal muscle remains to be investigated.

Calculation of $m\dot{V}O_2$ using arterial occlusion can be derived from the rate of decrease in Hb_{diff} and O_2Hb. Both methods are used in the literature, but it was unclear whether they give similar results. We found a significant difference between $m\dot{V}O_2(Hb_{diff})$ and $m\dot{V}O_2(O_2Hb)$ which was negligible at low intensities, but became more pronounced during high intensity work (Fig. 3). When changes in O_2Hb and HHb are identical, but in opposite direction, then $m\dot{V}O_2$ calculated from O_2Hb and Hb_{diff} will also be identical. However, this can only be achieved when blood volume stays constant during the occlusion, which is not always the case. In our study, we found that there was a slight increase in tHb in 15 out of the 126 measurements (100% - abs(100% x ($dtHb/dO_2Hb$)) ≥ 70%). An increase in tHb has been reported before in at least 4 other studies [Cheatle et al. 1991, Colier et al. 1995, Kooijman et al. 1997, Kragelj et al. 2000] and is also shown in figures of 2 other papers [Ferrari et al. 1997, Tamaki et al. 1994]. This phenomenon is unanimously addressed to a redistribution of blood during the occlusion and not to an incomplete arterial occlusion [Cheatle et al. 1991, Colier et al. 1995, Kooijman et al. 1997, Kragelj et al. 2000]. Nevertheless, the increase in tHb results in a discrepancy between $m\dot{V}O_2$ calculated from O_2Hb and that calculated from Hb_{diff} simply because the increase in HHb is no longer equal to the decrease in O_2Hb. Since it is unknown whether the increase in blood volume originates from arterial or venous site and, therefore, whether the extra volume contains mostly O_2Hb or HHb, it remains unclear if the true consumption is reflected by the decrease in O_2Hb or by the increase in HHb. Hb_{diff} will give, when blood volume is not constant, either an underestimation (O_2Hb) or overestimation (HHb) of $m\dot{V}O_2$. However, it is our opinion that as long as the origin of the extra blood volume is not identified, Hb_{diff} is the best choice for calculation of $m\dot{V}O_2$ since choosing the wrong variable (O_2Hb or HHb) will increase the measurement error .

In conclusion, we have shown that local muscle oxygen consumption in normal muscle can be measured reliable by NIRS, both at rest as well as during exercise at a broad range of work intensities. This is of great importance since direct local measurement of $m\dot{V}O_2$ during exercise is not possible with the conventional techniques. NIRS can, therefore, contribute to a better understanding of muscle physiology as it can give more insight in the regulation of local tissue oxygenation in normal physiological situations, but enables also to detect abnormalities that become especially apparent during exercise, for instance in mitochondrial myopathies or peripheral vascular disease. To restrict measurement errors caused by blood volume changes during arterial occlusion it is preferable to calculate $m\dot{V}O_2$ from the rate of decrease in Hb_{diff} instead of O_2Hb until the origin of the blood volume increase is known.

4

Adipose tissue thickness affects *in vivo* quantitative near-infrared spectroscopy in human skeletal muscle

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SUMMARY

The influence of adipose tissue thickness (ATT) on in vivo near-infrared spectroscopy (NIRS) measurements was studied in the human flexor digitorum superficialis muscle at rest and during sustained isometric handgrip exercise. NIRS was used for the quantitative measurement of muscle O_2 consumption ($m\dot{V}O_2$) and forearm blood flow (FBF) in seventy-eight healthy subjects. Skinfold thickness ranged from 1.4 – 8.9 mm within the group. Resting $m\dot{V}O_2$ was 0.11 ± 0.04 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$, and FBF was 1.28 ± 0.82 $ml \cdot 100ml^{-1} \cdot min^{-1}$. There was a negative correlation ($r = -0.70$, $P \leq 0.01$) indicating a decrease in $m\dot{V}O_2$ with increasing ATT. $m\dot{V}O_2$ in the 10 leanest subjects appeared to be twice as high as that in the 10 subjects with the highest ATT. A poor correlation ($r = 0.29$, $P \leq 0.01$) was found between ATT and FBF. The gender difference that we found for $m\dot{V}O_2$ was due to the difference in ATT between female and male subjects. No correlation was found between maximum voluntary contraction (MVC) force and $m\dot{V}O_2$ nor between MVC and ATT, indicating that the contraction force did not confound our results. These results show that ATT has a substantial confounding effect on in vivo NIRS measurements, and that it is essential to incorporate this factor into future NIRS muscle studies in order to justify comparisons between various groups. To facilitate such comparisons, upper and lower boundaries for normal values of $m\dot{V}O_2$ and FBF in relation to ATT are presented.

INTRODUCTION

Near-infrared spectroscopy (NIRS) is an optical method for the measurement of tissue O_2 consumption and delivery. NIRS has been used mainly to monitor oxygenation changes in the brain and to a far lesser extent to study skeletal muscle tissue oxygenation. In the past couple of years, NIRS has become a more accepted technique for the noninvasive determination of local oxygen consumption and blood flow in human skeletal muscle. The advantage of measurement in skeletal musde is the ability to obtain local information about muscle oxygenation, with the possibility of calculating quantitative values for O_2 consumption and blood flow using simple physiological interventions such as arterial or venous occlusion [Cheatle et al. 1991, Colier et al. 1995, De Blasi et al. 1993, De Blasi et al. 1994, De Blasi et al. 1997, Homma et al. 1996a, Van Beekvelt et al. 1998, Van Beekvelt et al. 1999]. *In vivo* NIRS measurements in skeletal muscle, however, assess not only the muscle tissue, but also the overlying tissue. Propagation of the light within the tissue is complex, as the light travels through multiple layers of the inhomogeneous medium. The subcutaneous layer can vary considerably due to individual differences in adipose tissue thickness (ATT), which may confound the NIRS measurements in muscle. The maximum measurement depth of NIRS is thought to be roughly half the distance between source and detector [Cui et al. 1991, Homma et al. 1996b]. This means that a thick skin or fat layer prevents a substantial amount of light passing through the muscle tissue. Furthermore, the optical characteristics and, therefore, the path of the light, are different in adipose compared with muscle tissue [Matsushita et al. 1998]. Since adipose tissue metabolism is lower than muscle metabolism [Coppack et al. 1990a], muscle oxygen consumption will be underestimated. Although the problem of the effect of ATT on the interpretation of NIRS measurements is generally accepted, only few studies actually investigated this influence. *In vivo* measurements were performed by Homma et al. [Homma et al. 1996b], who investigated various source-detector distances in relation to ATT. They found that all subjects showed deoxygenation during exercise with a source-detector distance of 30 mm, whereas only the subjects with low ATT showed deoxygenation with a source-detector distance of 20 mm. This suggests that the light did not penetrate deeply enough to reach the active muscle tissue in the subjects with higher ATT. In other studies, mathematical models with two, three, or four different tissue layers were used to investigate the influence of ATT on the propagation of near-infrared light, thus simulating NIRS measurements [Matsushita et al. 1998, Niwayama et al. 1999, Yamamoto et al. 1998]. These simulations revealed a decrease in sensitivity with increasing ATT. Subsequently, a small number of *in vivo* NIRS measurements were carried out to verify the simulated effect of ATT, and these showed good agreement with the theoretical results. Both Yamamoto et al. [Yamamoto et al. 1998] and Matsushita et al. [Matsushita et al. 1998] suggested mathematical corrections, but these are not widely applicable because of the differences in available NIRS systems and techniques employed.

Based on the small number of *in vivo* measurements, the real *in vivo* effect of ATT on NIRS measurements remains uncertain. Furthermore, it is not known whether the effect of ATT on NIRS measurements is different for male and female subjects, which might be expected because of the different amounts of adipose tissue in the two sexes. Moreover, it is not known whether the effect of ATT on NIRS measurements changes during exercise. Thus far, attention has been focused on oxygen consumption, while nothing has been reported with regard to the influence of ATT on the measurement of blood flow by NIRS.

Therefore, the aim of the present study was to determine the influence of ATT on quantitative *in vivo* measurement of local $m\dot{V}O_2$ and forearm blood flow (FBF) in a large group of healthy subjects, and to investigate whether this influence is also present during exercise or is affected by gender differences.

METHODS

Subjects

A total of seventy-eight healthy volunteers (34 female, 44 male) participated in this study. The study was approved by the Faculty Ethics Committee, and all subjects gave their written informed consent. The mean (± SD) characteristics of the subjects were 28.2 ± 12.5 yr in age, 177.6 ± 9.0 cm in height and 71.6 ± 11.4 kg in weight. Four of the subjects were on medication that, to our knowledge, does not affect muscle peripheral circulation. Skinfold thickness was measured between the NIRS optodes using a skinfold caliper (Holtain Ltd., Crymmych, UK), and divided by 2 to determine ATT (= fat + skin layer) covering the muscle. All, but six of the subjects were right-handed. Although none of the subjects had consumed a large meal, the metabolic state of the subjects might have differed slightly within the group.

Near-infrared Spectroscopy (NIRS)

NIRS is based on the relative transparency of biological tissue to light in the near-infrared region, and on the oxygen-dependent absorption changes of haemoglobin (Hb) and myoglobin (Mb). Using a continuous-wave near-infrared spectrophotometer (Oxymon, Biomedical Engineering Department, University of Nijmegen, NL) that generates light at 905, 850 and 770 nm [Van der Sluijs et al. 1998] it is possible to differentiate between oxy- and deoxyhaemoglobin/myoglobin (O_2Hb/O_2Mb and HHb/HMb, respectively). Due to the overlap of the spectrum, it is not possible to distinguish between changes in Hb and Mb. Although there is no consensus about whether the NIRS signal originates mainly from Hb [Seiyama et al. 1988],[Chance et al. 1992, Wang et al. 1990] or from a combination of Hb and Mb [Hoofd 1999, Mole et al. 1999, Tran et al. 1999], this does

not affect our results, since we were interested in the amount of O_2 consumed irrespective of whether it was supplied by Hb or Mb. The absorption changes at the discrete wavelengths are converted into concentration changes of O_2Hb and HHb using a modified Lambert-Beer law in which a path-length factor is incorporated to correct for scattering of photons in the tissue. Although gender differences in differential path-length factor (DPF) have been reported [Duncan et al. 1996, Essenpreis et al. 1993, Ferrari et al. 1992, van der Zee et al. 1992], we used a fixed DPF of 4.0 for the calculation of absolute concentration changes. NIRS measurements were carried out on top of the flexor digitorum superficialis muscle (FDS), with an interoptode distance of 35 mm. Data were sampled at 10 Hz, displayed in real time, and stored on disk for off-line analysis.

Experimental protocol

All tests were performed at a room temperature of approximately 21 °C. The subject was seated for 15-20 min prior to the test. The right hand rested on a handgrip dynamometer with the upper arm at heart level and the forearm placed at an upward angle of 30° to avoid venous pooling of the blood. The arm was supported at the wrist and above the elbow to prevent contact between forearm and dynamometer, assuming completely unrestricted circulation in the forearm. The maximum voluntary contraction (MVC) force of the subject was determined before the test. A pneumatic cuff was placed around the upper arm in order to apply venous or arterial occlusion.

After placement of the instruments, the experiment started with a 5 min rest period followed by three venous occlusions (50 mmHg) and three arterial occlusions (260 mmHg). Venous occlusions lasted 30 s and were separated by 1 min recovery periods. Arterial occlusions lasted 45 s and were separated by 5 min recovery periods.

After 5 min of recovery, which allowed the NIRS signals to return to baseline values, the subject was asked to perform sustained isometric handgrip exercise at 10% of MVC. After 1 min of exercise, when NIRS signals had reached steady state, arterial occlusion was applied by rapid inflation of the arm cuff while exercise was maintained. Cuff inflation was kept at 260 mmHg for 45 s, and released simultaneously with the end of exercise. When all signals had returned to pre-exercise levels, a second and a third exercise session were performed at 20% and 30% of MVC, respectively.

Forearm measurements

Forearm blood flow

The sum of [O_2Hb] and [HHb] reflects the total amount of haemoglobin ([tHb]), and changes in [tHb] can be interpreted as changes in blood volume in the tissue. Forearm blood flow (FBF) was calculated by evaluating the rate of increase in [tHb] during venous

occlusion, as described previously [De Blasi et al. 1997, Van Beekvelt et al. 2001b]. The linear increase in the concentration changes within the first seconds of the 20 s occlusion was used for the calculation of FBF. Concentration changes of tHb were expressed in $\mu M \cdot s^{-1}$ and converted to millilitres blood per minute per 100 millilitres tissue ($ml \cdot min^{-1} \cdot 100ml^{-1}$) using an average Hb concentration of 7.5 $mmol \cdot L^{-1}$ for female subjects and 8.5 $mmol \cdot L^{-1}$ for male subjects. The molecular weight of Hb (64.458 $g \cdot mol^{-1}$) and the molecular ratio between Hb and O_2 (1:4) were taken into account. FBF at rest was calculated as the average of the three venous occlusions.

Oxygen consumption

Muscle oxygen consumption ($m\dot{V}O_2$) was measured by NIRS using arterial occlusion, by evaluating the rate of decrease in [Hb_{diff}] ([Hb_{diff}]=[O_2Hb]-[HHb]), assuming that [tHb] is constant [De Blasi et al. 1997]. Concentration changes of Hb_{diff} were expressed in $\mu M \cdot s^{-1}$, and converted to $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$. A value of 1.04 $kg \cdot L^{-1}$ was used for skeletal muscle density [Vierordt 1906]. $m\dot{V}O_2$ at rest was calculated as the average of the three arterial occlusions.

Statistics

A Shapiro-Wilk test was used to test all variables for normality ($P < 0.01$). Logarithmic transformation was applied on variables that failed the normality test. A coefficient of variation [(SD/mean)·100] was calculated to test the reproducibility of NIRS variables at rest. Statistical differences between rest and exercise and between gender were tested by means of the Student paired *t*-test. A Spearman correlation test was used to test the correlation between NIRS variables and log(ATT). All data are reported as means ± SD. The level of statistical significance was set at $P \leq 0.05$.

RESULTS

ATT had a mean (±SD) value of 3.7 ± 1.9 mm, with a range of 1.4 – 8.9 mm. As expected, ATT did not have a normal distribution within our subject group (Fig. 1). MVC was 465 ± 117 N.

Oxygen consumption and forearm blood flow

The coefficient of variation for forearm blood flow (FBF) during venous occlusion was 22.4%, and that for muscle O_2 consumption ($m\dot{V}O_2$) obtained during arterial occlusion

was 16.2% (Table 1). For further analysis, FBF and $m\dot{V}O_2$ were calculated as the means of the three consecutive measurements.

FBF was 1.28 ± 0.82 $ml \cdot min^{-1} \cdot 100ml^{-1}$ at rest while $m\dot{V}O_2$ at rest was 0.11 ± 0.04 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$. During exercise, $m\dot{V}O_2$ increased significantly to 0.43 ± 0.35 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ at 10% MVC, 0.70 ± 0.50 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ at 20% MVC, and 0.83 ± 0.68 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ at 30% MVC ($P \leq 0.01$).

Correlation between ATT and NIRS variables

Correlation coefficients for the relationships between NIRS variables and ATT are shown in Table 2. A clear correlation was found between ATT and $m\dot{V}O_2$ at rest (Fig. 2A). A poor correlation was found between ATT and $m\dot{V}O_2$ during exercise at the various workloads (Table 2). A weak correlation was found between ATT and FBF at rest (Fig. 2B).

Gender differences

NIRS data for female and male subjects are shown in Table 3. No difference were found in FBF values between female and male subjects. However, gender differences were present for $m\dot{V}O_2$ at rest and during exercise. Overall, female subjects had lower $m\dot{V}O_2$ values than male subjects. The difference between the two sexes became more pronounced during exercise when female subjects showed no further increase after 20% MVC while $m\dot{V}O_2$ in male subjects continued to increase at 30% MVC.

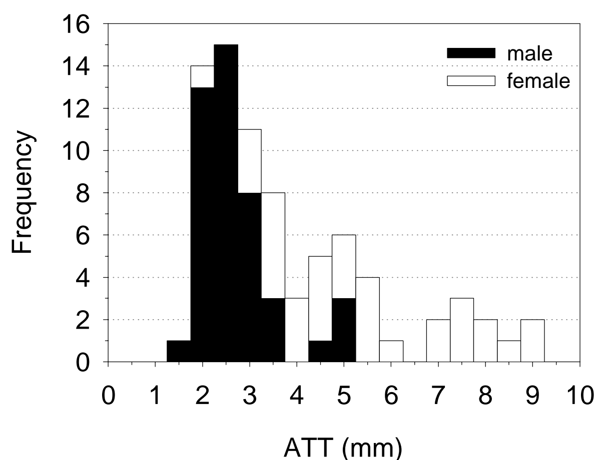

Fig. 1 Distribution of ATT for the 78 subjects. ATT is defined as skinfold thickness / 2. Data from male and female subjects are presented as stacked bars.

Table 1. Reproducibility of NIRS FBF and $m\dot{V}O_2$ measurements during three consecutive occlusions

	I	II	III	CV (%)
FBF ($ml \cdot min^{-1} \cdot 100ml^{-1}$)	1.26 ± 0.88	1.23 ± 0.78	1.34 ± 0.96	22.4
$m\dot{V}O_2$ ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$)	0.12 ± 0.05	0.11 ± 0.04	0.10 ± 0.04	16.2

FBF was measured during venous occlusion, and $m\dot{V}O_2$ during arterial occlusion. Values are means ± SD, and the coefficient of variance (CV) is given in each case. The first, second, and third occlusion are represented by I, II, and III, respectively.

Table 2. Correlation coefficients for the relationship between ATT and NIRS variables

	ATT	*a*	*b*	SE(*a*)	SE(*b*)	*r*	*P*-value
Rest	FBF	0.71	1.11	0.24	0.43	0.29	*
Rest	$m\dot{V}O_2$	0.18	-0.14	0.02	0.01	-0.70	**
10% MVC	$m\dot{V}O_2$	0.71	-0.54	0.10	0.18	-0.33	**
20% MVC	$m\dot{V}O_2$	1.20	-0.99	0.14	0.26	-0.41	**
30% MVC	$m\dot{V}O_2$	1.43	-1.18	0.19	0.36	-0.36	**

*The relationship is defined by the equation: FBF or $m\dot{V}O_2$ = a + b*log(ATT). Calculated values are given for all variables; a, b, standard error (SE) of a and b, and r. *$P \leq 0.05$; **$P \leq 0.01$.*

Table 3. Values for $m\dot{V}O_2$ and FBF at rest and during sustained isometric handgrip exercise for female and male subjects separately

		Female (33)	Male (44)	*P*-value
Rest	FBF	1.46 ± 0.74	1.14 ± 0.86	0.159
Rest	$m\dot{V}O_2$	0.08 ± 0.03	0.13 ± 0.03	**
10% MVC	$m\dot{V}O_2$	0.31 ± 0.18 ##	0.53 ± 0.41 ##	**
20% MVC	$m\dot{V}O_2$	0.48 ± 0.33 ##	0.87 ± 0.54 ##	**
30% MVC	$m\dot{V}O_2$	0.52 ± 0.40	1.07 ± 0.75 ##	**

*$m\dot{V}O_2$ values are given in $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$, and FBF values in $ml \cdot min^{-1} \cdot 100ml^{-1}$. Values are means ± SD. Significance of differences: **$P \leq 0.01$ for male compared with female subjects; ##$P \leq 0.01$ for differences in $m\dot{V}O_2$ between two consecutive workloads.*

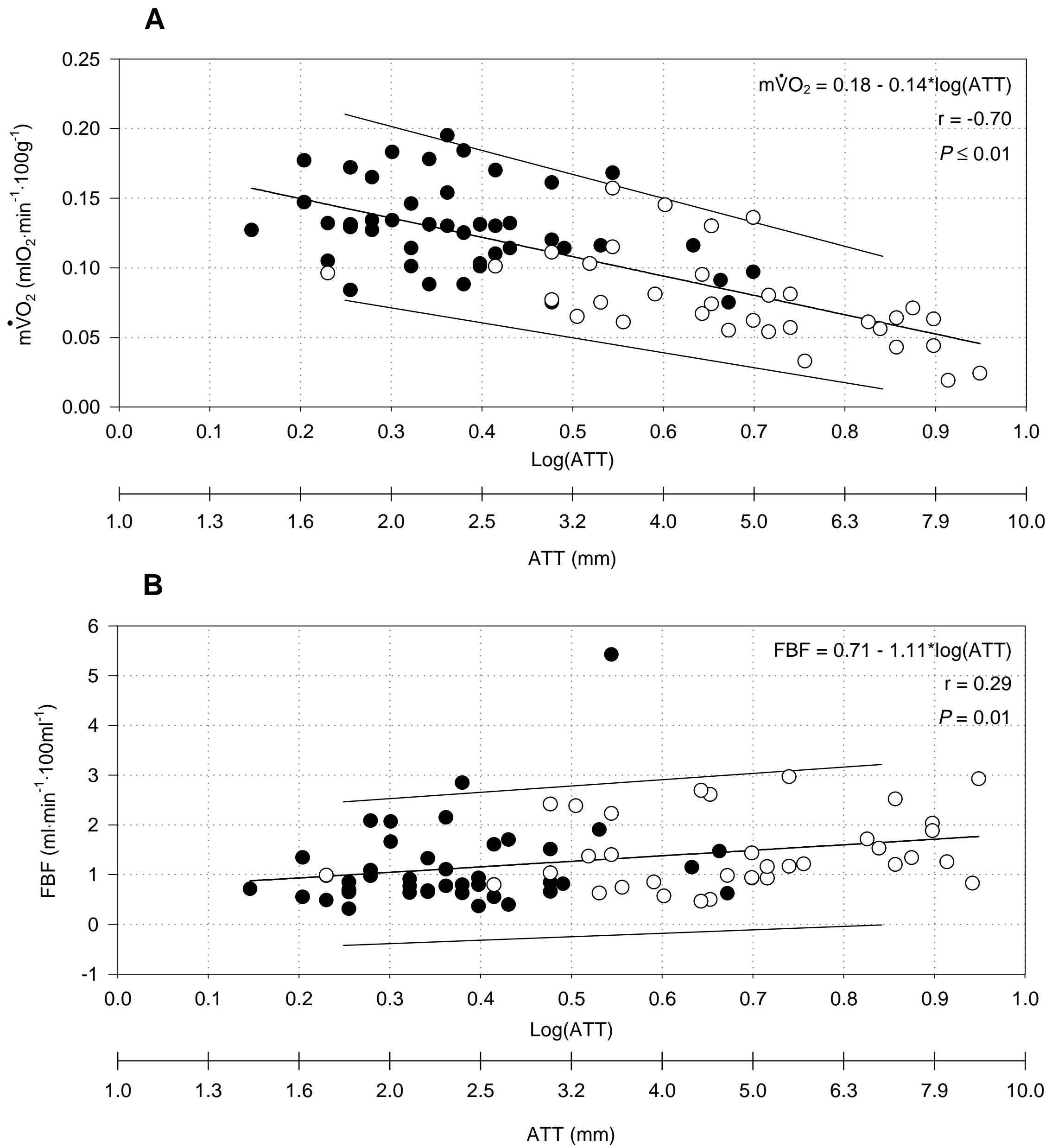

Fig. 2 Correlation between log(ATT) and NIRS variables at rest. A) m$\dot{V}O_2$ and B) FBF, measured in 78 healthy subjects. Data from male (△) and female (☗) subjects are shown separately.

DISCUSSION

The main finding of this study was a major decrease in $m\dot{V}O_2$ with increasing ATT (Fig. 2A). The 10 subjects with the highest values for ATT (ATT ≥ 6.7 mm) had a $m\dot{V}O_2$ that was 2.7 times lower ($P < 0.01$) than the $m\dot{V}O_2$ in the 10 leanest subjects (ATT ≤ 1.8 mm). Less clear was the relationship between FBF and ATT. Although correlation was weak, FBF was more than twice as high in the 10 subjects with the highest values for ATT as compared with the FBF in the 10 leanest subjects ($P < 0.01$). To our knowledge, this is the first time that the influence of ATT on NIRS has been quantified *in vivo* using a large subject population. Our findings show that it is of utmost importance that ATT is known and is incorporated into the analysis of NIRS measurements in muscle tissue and where comparison between subjects with varying ATT values is involved.

The maximum measurement depth of NIRS is thought to be roughly half the distance between source and detector [Cui et al. 1991, Homma et al. 1996b]. For an interoptode distance (IO) of 35 mm the light will, therefore, penetrate approximately 18 mm into the tissue. Hypothetically, when ATT is small, the contribution of light passing through adipose tissue will be minimal. When ATT is large, however, a substantial amount of light will pass through adipose tissue only, instead of through muscle tissue. A simple calculation using our data shows that 92% of the measurement depth in our leanest subject (1.4 mm ATT) compromises muscle tissue, while this value was only 49% for our subject with the largest ATT (8.9 mm) (Fig. 3). This means that the amount of light penetrating muscle tissue will be far less in the subject with high ATT than in the subject with the low ATT. Taking into account the complexity of the propagation of light within the tissue, the differences between subjects might become more pronounced [Matsushita et al. 1998]. $m\dot{V}O_2$ measured by NIRS will be underestimated as ATT increases, due to the mixture of consumption originating from muscle and adipose tissue, since adipose tissue is believed to have a low O_2 consumption [Arner et al. 1993, Coppack et al. 1990a, Frayn et al. 1993, Simonsen et al. 1994]. When compared with muscle tissue, both O_2 consumption [Coppack et al. 1990a] and O_2 extraction are much lower in adipose tissue [Coppack et al. 1990a, Coppack et al. 1990b, Frayn et al. 1993]. Coppack et al. [Coppack et al. 1990a] measured O_2 consumption values of 0.02 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ (range 0.01-0.03) for adipose tissue and of 0.17 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ (range 0.12-0.19) for muscle tissue (data converted from $\mu mol \cdot min^{-1} \cdot 100\ ml^{-1}$). This is in agreement with the results of Simonsen et al. [Simonsen et al. 1994] who found a similar low O_2 consumption (0.04 ± 0.01 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$) in adipose tissue. Although our $m\dot{V}O_2$ value was lower than that of Coppack et al. [Coppack et al. 1990a], it was still roughly three times higher than the O_2 consumption in adipose tissue. A possible explanation for the discrepancy in $m\dot{V}O_2$

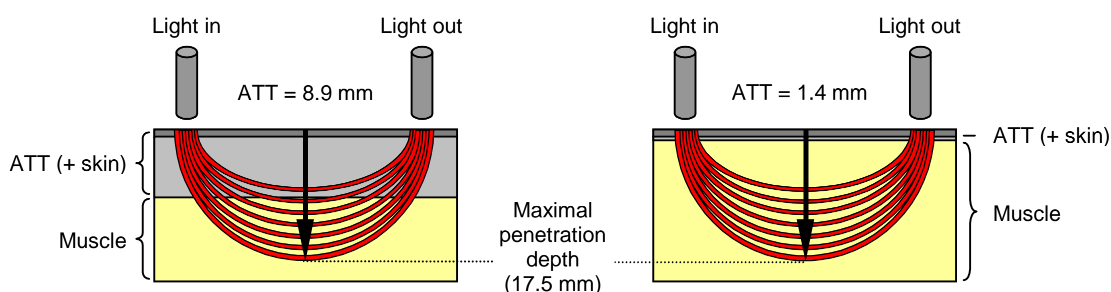

Fig. 3 Simplified schematic presentation of NIRS light penetration in relation to ATT (+skin) and muscle tissue in our subject with the greatest ATT (8.9 mm) and in our leanest subject (ATT = 1.4 mm). The model does not take into account the different light-scattering properties of fat and muscle. The light travels in a 'banana shape' form from source to detector [Cui et al. 1991], and the maximum penetration depth is roughly half the interoptode distance. Approximately 49% of the measurement depth (17.5 mm) in our subject with the largest ATT comprises muscle tissue, while this value was 92% for our leanest subject.

between the two studies might be an overestimation of muscle blood flow due to the plethysmographic flow method used by Coppack et al. [Coppack et al. 1990a]. Plethysmographic flow reflects the total forearm blood flow including flow originating from adipose tissue and skin. Since adipose tissue blood flow appears to be greater than muscle blood flow [Coppack et al. 1990a, Coppack et al. 1999], plethysmographic flow measurement will lead to an overestimation when used to represent muscle blood flow. As has been shown in other NIRS studies [De Blasi et al. 1994, Van Beekvelt et al. 2001b] flow measured by plethysmography was 2-3 times higher than the local muscle blood flow measured by NIRS.

Another contribution to the lower $m\dot{V}O_2$ that we found in our study might be a difference in body composition. As Coppack et al. [Coppack et al. 1990a] tested seven subjects with a maximum whole-body fat content of 28%, their population is most probably much leaner than ours, since we included a wide range of skinfold thicknesses. Therefore, our group mean for $m\dot{V}O_2$ will be underestimated.

Our *in vivo* test shows clearly that $m\dot{V}O_2$ measured by NIRS will be underestimated when ATT increases. These findings are supported by the decreased sensitivity found with increasing ATT in mathematical simulations [Matsushita et al. 1998, Niwayama et al. 1999, Yamamoto et al. 1998]. In an earlier study of Yamamoto et al. [Yamamoto et al. 1996], a decrease of 50% in absorbance was reported when the thickness of the fat layer increased from 5 to 10 mm, with a decrease of 25% when fat layer thickness increased from 2.5 to 5 mm. Niwayama found a similar decrease of 50%, but did not specify the range of ATT that caused this decrease. Based on Fig. 2A, we found similar results after

extrapolation of the regression line to an ATT of 10 mm. The $m\dot{V}O_2$ decreased by 50% as the ATT increased from 5 to 10 mm ATT (0.08 vs. 0.04 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$) and by 30% from 2.5 to 5 mm ATT (0.13 vs. 0.08 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$).

Only a small number of *in vivo* NIRS measurements have been carried out previously. The existence of an influence of ATT on NIRS measurements has been reported and verified previously, but no data have been available with regard to the normal range of local $m\dot{V}O_2$ in relation to ATT. With our data set, we were able to obtain reference values for the amounts by which $m\dot{V}O_2$ and FBF values are likely to fall/rise with increasing ATT. This is of great importance for the determination of abnormal $m\dot{V}O_2$ and FBF values, for example, in patients with disorders of O_2 delivery (e.g. heart failure or peripheral vascular disease) or O_2 consumption (e.g. metabolic and mitochondrial myopathies). Furthermore, with this data it is now possible to compare subjects with various ATT values, by normalising the data using the equation of the regression line. When we included other data from our laboratory and the converted data from Binzoni et al. [Binzoni et al. 1998] (skinfold thickness divided by 2), Niwayama et al. [Niwayama et al. 1999], and Yamamoto et al. [Yamamoto et al. 1998], practically all data points fell within the reference ranges from our data set (Fig. 4). Although the units used in the study of Yamamoto et al. [Yamamoto et al. 1998] were not specified, the rate of decrease in absorbance of [O_2Hb] as a function of ATT is comparable with our results. Half of the data points from the study of Niwayama et al. [Niwayama et al. 1999] fell just outside the upper reference line, but still confirmed the relationship between ATT and $m\dot{V}O_2$. The same trend of a decrease in $m\dot{V}O_2$ with increasing ATT was still present during exercise, but the correlation decreased due to an overall increase in variability.

In contrast to the clear correlation between ATT and $m\dot{V}O_2$, there was only a weak relationship between ATT and FBF. This weak correlation was inverse, such that FBF increased with increasing ATT. This is in agreement with Coppack et al. [Coppack et al. 1990a, Coppack et al. 1999] who found a higher blood flow in adipose tissue as compared with that in muscle in two separate studies. Skin vascularisation can be abundant and might, therefore, increase the confounding effect. However, interference of skin blood flow with NIRS measurements is thought to be negligible (< 5%) when source and detector are separated by more than 20 mm [Hampson et al. 1988].

Although none of the subjects had consumed a large meal before the test, we did not take the metabolic state of each individual subject into account because of practical limitations. It has been found that subcutaneous adipose tissue blood flow increased within 30 min after a mixed meal [Coppack et al. 1990a] or a glucose load [Simonsen et al. 1994], but a slight decline after a glucose infusion has also been reported [Coppack et al. 1999]. For forearm blood flow, there is little or no effect after a mixed meal [Coppack et al. 1990a, Coppack et al. 1999]. As for O_2 consumption, subcutaneous adipose tissue O_2

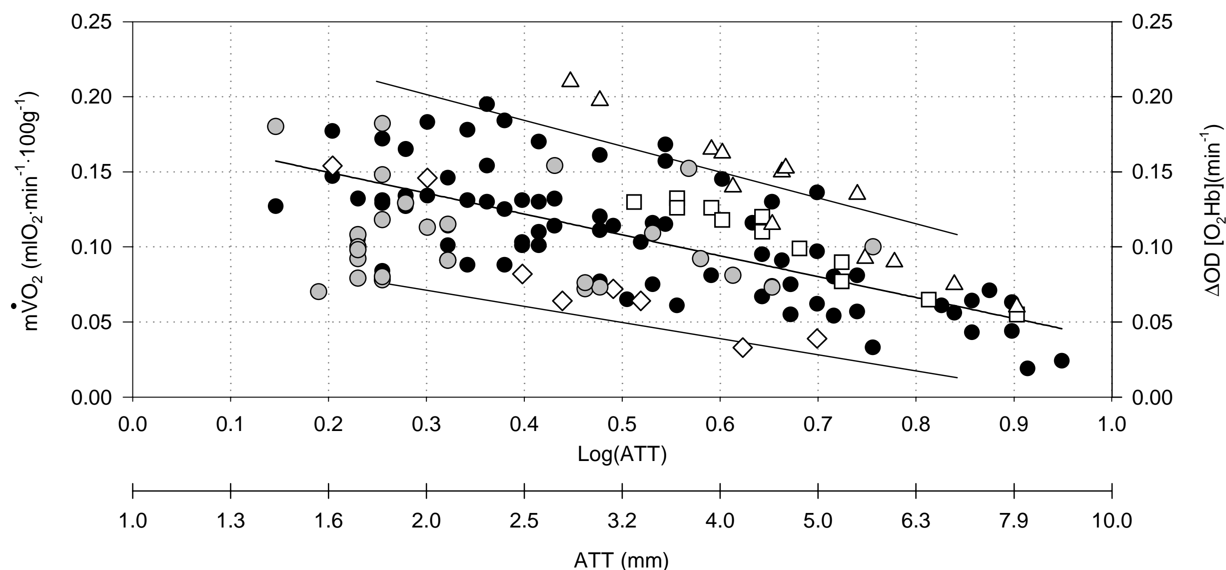

Fig. 4 Combined data supporting the confounding effect of ATT on NIRS mV̇O$_2$ measurements. Data from the present study (black circles) are shown, along with data from other studies in our laboratory (n=27; grey circles) and from Binzoni et al. 1998 (◇), Yamamoto et al. 1999 (□), and Niwayama et al. 1998 (△). All data are presented in units of mlO$_2$·min^{-1}·100g^{-1}, except the data from Yamamoto et al. 1999, which are presented in ΔOD[O$_2$Hb](min^{-1}), where OD represents absorbance.

consumption was reported to increase after a meal or a glucose load, doubling within 120 min [Coppack et al. 1990a, Simonsen et al. 1994]. Muscle O_2 consumption also increased after a meal, but to a smaller extent than in adipose tissue [Coppack et al. 1990a]. Although the postprandial effects described in these studies are based on responses after long non-physiological fasting times, the effect of food intake on adipose tissue, and to a lesser extent on muscle tissue, might still be substantially. In this light, our mV̇O$_2$ and FBF values might be slightly biased. However, since the aim of our study was to investigate the influence of ATT on NIRS measurements, postprandial effects would not change our findings, but are likely to result in chiefly higher variability within the group.

The present study also demonstrated differences between female and male subjects. Unlike FBF, all mV̇O$_2$ values were significantly lower in female than in male subjects (Table 3). Furthermore, apart from the expected gender differences such as height and weight, we also found a significant differences in ATT and MVC between the sexes. ATT in female subjects was 5.2 ± 1.9 mm, significantly greater than that in male subjects (2.5 ± 0.8 mm). As can be seen in Fig. 2A, there is a distinct separation of female and male subjects based on ATT, with very little overlap. Therefore, we conclude that the difference in mV̇O$_2$ found between the sexes can be explained by the difference in ATT. This is supported by the different values for DPF that have been found for men and

women in several studies [Duncan et al. 1996, Essenpreis et al. 1993, van der Zee et al. 1992].

During exercise, $m\dot{V}O_2$ might also be influenced by the contraction force. MVC force in female subjects was 374 ± 62 N, significantly lower than that in male subjects (537 ± 100 N). Since MVC and $m\dot{V}O_2$ were both lower in female subjects, MVC can theoretically be considered as another potential confounder for the measurement of $m\dot{V}O_2$ during exercise, as less absolute force produced might result in lower O_2 consumed. However, this is not likely, because no correlation was found between MVC and $m\dot{V}O_2$ in male subjects (r=-0.01, *P*=0.96) or in female subjects (r=-0.09, *P*=0.57). In addition, no correlation was found between MVC and ATT in female subjects (r=0.09, *P*=0.60) or in male subjects (r=0.16, *P*=0.31).

In conclusion, the present results suggest that ATT is a substantial confounder on *in vivo* NIRS measurements, and that it is essential to incorporate this factor in future NIRS muscle studies, in order to fully exploit the possibilities of NIRS and to justify comparisons between various groups.

5

Oxidative and anaerobic skeletal muscle metabolism simultaneously measured by NIRS and ^{31}P-MRS in healthy subjects and patients with Friedreich ataxia

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SUMMARY

Near-infrared spectroscopy (NIRS) and phosphorus magnetic resonance spectroscopy (^{31}P-MRS) have been used in this study as complementary methods for the investigation of in vivo *skeletal muscle metabolism, measured simultaneously and within the same volume of interest. In order to investigate the differences and similarities between both techniques, we studied muscle function in normal controls and a group of Friedreich ataxia (FRDA) patients in which mitochondrial function is thought to be impaired. Eleven healthy subjects and fifteen FRDA patients were tested during 2 phases of 3 min aerobic and ischemic isometric exercise and subsequent recovery. Immediately after the start of both exercise phases, a rapid deoxygenation and depletion of phosphocreatine (PCr) was found, accompanied by a moderate increase in the intracellular pH (pH_i). Subsequently, pH_i decreased indicating lactic acid formation during anaerobic glycolysis, while the rate of deoxygenation and PCr depletion slowed down. The results show that oxidative phosphorylation during exercise was present, even during ischemia when O_2 stores were thought to be depleted. Similar responses in NIRS and ^{31}P-MRS were found for FRDA patients and controls. Although PCr recovery after cessation of exercise was significantly delayed in FRDA, NIRS showed no indication for an impaired oxygen consumption. Our data are suggestive for either partial uncoupling of the electron-transport chain or reduced ATP synthesis at the level of complex V (ATPsynthase), the adenine nucleotide translocator (ANT), or mitochondrial creatine kinase. The results of this study demonstrated the additional value of NIRS in the complementary use with ^{31}P-MRS for the investigation of* in vivo *muscle metabolism enabling both oxidative and anaerobic processes to be measured noninvasively and simultaneously within the same muscle area.*

INTRODUCTION

Investigation of *in vivo* muscle metabolism has made a large progress over the last decades due to the development of various techniques enabling regional measurements in addition to whole body measurements. Whereas the first available local techniques, like blood and muscle biopsy samples, are invasive and provide a static view on muscle physiology at a given time point, it is now possible to get a noninvasive "on line" view in the intact and working human being. Phosphorus magnetic resonance spectroscopy (^{31}P-MRS) has developed over the last three decades as a "gold standard" for noninvasive measurement of skeletal muscle metabolism during exercise and recovery, monitoring the kinetics of intramuscular high-energy phosphates and pH. Near-infrared spectroscopy (NIRS), as another noninvasive technique, is a continuous and direct optical method enabling the measurement of oxygenation and haemodynamics in muscle tissue by monitoring changes in oxygenated and deoxygenated heme groups (haemoglobin/myoglobin). It has shown to be a sensitive tool that can detect local differences in muscle oxygen consumption ($m\dot{V}O_2$) and delivery [Van Beekvelt et al. 2001b] between muscles at different activity levels within the same region.

^{31}P-MRS monitors the intracellular high energy phosphates and pH while NIRS reflects the balance between oxygen delivery and consumption and gives direct insight in the oxygenation state of the local vascular system (haemoglobin) and the muscle cell (myoglobin). Therefore, simultaneous use of ^{31}P-MRS and NIRS as complementary methods has a high potential in establishing a reliable comprehensive picture of muscle metabolism *in vivo* [Cerretelli et al. 1997]. In this study, we have used the simultaneous measurement of ^{31}P-MRS and NIRS, applied approximately within the same volume of interest, during exercise and recovery to investigate the complementary benefits of both methods in monitoring anaerobic and oxidative pathways. We have investigated muscle metabolism in normal controls as well as in Friedreich patients. Friedreich Ataxia (FRDA) is an autosomal recessive neurodegenerative disorder characterised by an expanded $(GAA)_n$ trinucleotide repeat in intron 1 of the frataxin gene on chromosome 9q13 [Campuzano et al. 1996]. It is the most common hereditary ataxia occurring with a frequency of 1 in 50 000 in the Caucasian population. Frataxin is associated with the inner membrane of the mitochondria, and mutations in the frataxin gene are thought to lead to impaired mitochondrial function. Using ^{31}P-MRS, an impairment in the rate of mitochondrial ATP formation in FRDA has been reported, characterised by a delayed oxidative PCr recovery after exercise [Lodi et al. 1999, Vorgerd et al. 2000].

The aim of this study was to investigate the chronological features of oxidative and anaerobic processes during exercise and recovery by simultaneous use of ^{31}P-MRS and NIRS, measured within the same volume of interest. We compared healthy subjects with Friedreich ataxia patients suspected to have mitochondrial dysfunction, and hypothesised

that the complementary use of NIRS and ^{31}P-MRS would reveal the localisation of the functional limitation of muscle metabolism in FRDA.

MATERIALS AND METHODS

Subjects

Eleven healthy volunteers (5 female, 6 male) participated in this study. The study was approved by the Ethics Committee of the Ruhr University Bochum and all subjects gave their written informed consent. The average (± SD) characteristics of the subjects were 27 ± 10 yr in age, 171 ± 8 cm in height and 64 ± 12 kg in weight. Fifteen ambulatory FRDA patients (10 female, 5 male) from thirteen families, homozygous for the (GAA)n repeat expansion in the frataxin gene, participated in this study (age 30 ± 10 yr, height 174 ± 10 cm, and weight 64 ± 10 kg. Genetic testing for FRDA was performed from peripheral blood lymphocytes as previously described [Epplen et al. 1997]. Skinfold thickness was measured between the NIRS optodes using a skinfold caliper (Holtain Ltd., Crymmych, UK) and divided by 2 to determine the adipose tissue thickness (ATT = fat + skin layer) covering the muscle.

The standardised calf muscle metabolism test

The subjects lay in supine position with the upper body in an upward angle of 25°. The foot was fixed on a pedal ergometer designed for isometric plantarflexion exercise. The angle between the pedal and the horizontal plane was 70°. Force was recorded continuously and displayed visibly for the patient. The right calf was placed on a calf holder with an integrated radio-frequency surface coil. Maximum voluntary contraction force (MVC) was determined as the best of three contractions of 5 s duration with 1 min recovery in between. Energy metabolism was monitored during the following exercise protocol: 1 min rest, 3 min 30% MVC, 8 min rest, 3 min rest + arterial occlusion, 3 min 30% MVC + arterial occlusion, 10 min rest. Arterial occlusion was applied using an air cuff placed around the lower part of the right thigh. For the application of ischemia the cuff was rapidly inflated to 280-300 mmHg using compressed air. At the end of contraction or in case of emergency, the cuff could be deflated within seconds by the subject or the obligatorily accompanying person.

^{31}P magnetic resonance spectroscopy and energy metabolism

The MR spectra of the calf were obtained with a 4.7 Tesla 40-cm horizontal bore spectrometer (Bruker-Biospec 47/40, Bruker-Medizintechnik, Ettlingen, Germany) using a 5-cm diameter ^{1}H/^{31}P surface coil, placed under the right calf on top of the gastrocnemius muscle. The resonance frequencies were 200 MHz for ^{1}H and 81 MHz for

^{31}P. ^{1}H MR spectra were used to optimise magnetic field homogeneity (shimming). A line half-width lower than 48 Hz for the water signal was accepted. In the examinations of most controls, a value of 39 Hz was reached. An exitation radio frequency (RF) pulse length of 100 μs was used for ^{31}P MR spectroscopy. The flip angle was 60° at the centre of the coil. For each spectrum, 12 free induction decays (FIDs) were acquired in 10 s. The vector size was 2048 data points. In patients with FRDA half-widths of the water signal were between 53 and 70 Hz because of large magnetic field inhomogeneities. 24 FIDs were accumulated within 20 s for each spectrum, to obtain a good signal-to-noise ratio. Spectra were evaluated for PCr, P_i, the beta position of the phosphate in ATP, and for phosphomonoesters (PME). The area under each peak was corrected for partial spin saturation. Metabolite tissue levels were generally given in percent of [PCr] at initial rest ($\%[PCr]_i$). The intracellular pH was determined by the chemical shift of the P_i-peak (δ in ppm) relative to PCr: [$pH = 6.75 + \log((\delta - 3.27)/(5.69 - \delta))$] [Taylor et al. 1983]. The resonance frequency of the PCr signal was defined as 0 ppm.

Near-infrared Spectroscopy (NIRS)

NIRS is based on the relative tissue transparency for light in the near-infrared region and on the oxygen-dependent absorption changes of haemoglobin and myoglobin. Using a continuous-wave near-infrared spectrophotometer (Oxymon, Biomedical Engineering Department, University of Nijmegen, NL) that generates light at 905, 850 and 770 nm it is possible to differentiate between oxy- and deoxyhaemoglobin/myoglobin (O_2Hb/O_2Mb and HHb/HMb, respectively). Due to the overlap of the spectrum, it is not possible to distinguish between haemoglobin and myoglobin. The changes in absorption at the discrete wavelengths are converted into concentration changes of O_2Hb and HHb using a modified Lambert-Beer law in which a path-length factor is incorporated to correct for scattering of photons in the tissue. A fixed value of 4.0 for the differential path-length factor (DPF) was used to calculate absolute concentration changes. The NIRS optodes were placed in the calf holder with an interoptode distance of 40 mm and within the integrated radio-frequency surface coil for the ^{31}P-MRS measurements. This way, NIRS and ^{31}P-MRS could be measured simultaneously and at the same place on top of the gastrocnemius muscle.

O_2Hb and HHb were sampled at 20 Hz. Total haemoglobin (tHb) was calculated as the sum of O_2Hb and HHb and reflects changes in blood volume. All three signals were displayed real-time and stored on disk for off-line analysis. In order to compare NIRS signals with ^{31}P-MRS signals, raw NIRS signals were resampled to 0.1 Hz and averaged per group. NIRS muscle oxygen consumption ($m\dot{V}O_2$) was calculated from the signal sampled at 20 Hz. $m\dot{V}O_2$ at rest was calculated during arterial occlusion from the rate of linear decrease in O_2Hb. Likewise, $m\dot{V}O_2$ during exercise was calculated from the rate of

linear decrease in O_2Hb immediately after the start of exercise and on condition that tHb was constant. Concentration changes of O_2Hb were expressed in $\mu M \cdot s^{-1}$ and converted to $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ using a value of 1.04 $kg \cdot L^{-1}$ for muscle density. End-exercise O_2Hb deoxygenation was also calculated from the raw signal and determined by the amount of O_2Hb deoxygenation in μM from pre-exercise baseline to end-exercise (aerobic exercise) and from pre-occlusion baseline to end-exercise (ischemic exercise). The τ-value for O_2Hb recovery after exercise was calculated in similar way as the τ-value for PCr recovery using a mono-exponential curve fit.

Statistics

Data are presented as mean ± SD. Differences in ^{31}P-MRS and NIRS variables between FRDA and controls and between aerobic and ischemic exercise were analysed by the appropriate Student's t-test. A Welch test was used in case the standard deviation in both groups was not equal. Differences in $m\dot{V}O_2$ and intracellular pH at rest, aerobic exercise, and ischemic exercise were analysed by repeated measures analysis of variance (ANOVA) and any differences were further analysed with Student-Newman Keuls post-hoc test. Differences of $P < 0.05$ were considered as statistically significant.

RESULTS

No significant differences were found for age ($P = 0.34$), height ($P = 0.51$) or weight ($P = 0.95$) between control subjects and FRDA patients. Adipose tissue thickness (ATT) between the NIRS optodes ranged from 2.7 – 10.1 mm in the control group (mean ± SD: 6.6 ± 3.3 mm) and from 2.3 – 11.5 mm in FRDA (6.4 ± 2.5 mm). There was no significant difference in ATT between both groups ($P = 0.84$). The maximum voluntary contraction force (MVC) was 894 ± 212 N in the controls and 766 ± 227 N in FRDA ($P = 0.16$).

NIRS vs. ^{31}P-MRS in healthy subjects

Fig. 1A shows the NIRS O_2Hb, HHb, and tHb tracings measured continuously in an individual control subject during the complete test. Fig. 1B shows the accompanying PCr tracing, simultaneously measured by ^{31}P-MRS. Immediately after the start of exercise there was a rapid deoxygenation indicated by the decrease of O_2Hb and concurrent increase of HHb. At the same time, blood volume decreased abruptly within the first seconds, but stayed constant during the remainder of the contraction period. PCr depletion occurred also immediately from the start of exercise. A hyperaemic response in NIRS signals was seen after cessation of exercise, indicated by a rapid increase in blood volume above pre-exercise levels and resulting in reoxygenation with O_2Hb replenished

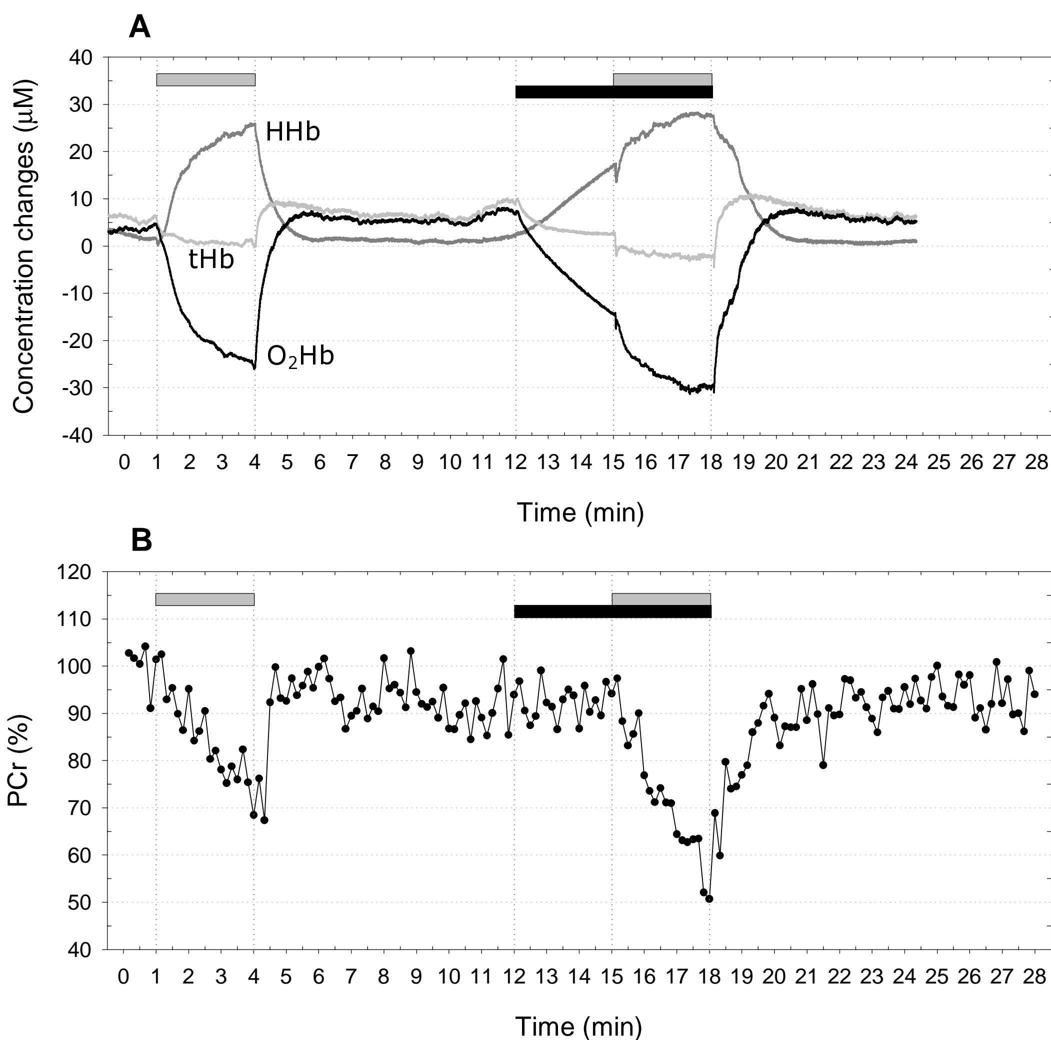

Fig. 1. Individual, simultaneously measured signals for near-infrared spectroscopy (NIRS) and phosphorus magnetic resonance spectroscopy (^{31}P-MRS) during aerobic and ischemic exercise and its recovery in a healthy volunteer. A) NIRS. Oxyhemoglobin (O_2Hb), deoxyhaemoglobin (HHb), and total haemoglobin (tHb) signals measured at 20 Hz and displayed real-time on screen. B) ^{31}P-MRS. Simultaneously measured phosphocreatine (PCr) tracing at 0.1 Hz. The grey bar represents the periods of sustained isometric exercise at 30% of the individual maximum voluntary contraction force (MVC). The black bar represents the period of arterial occlusion.

and HHb washed out. PCr also recovered quickly, but without an overshoot. During arterial occlusion at rest, O_2Hb decreased linearly with a concurrent linear increase in HHb after tHb had stabilised. No change in PCr was seen during the occlusion at rest. The start of the ischemic contraction period developed in a similar way as the aerobic contraction period, but the fast, initial deoxygenation was shorter in time and rapidly followed by a slower deoxygenation rate and, finally, a plateau in O_2Hb and HHb. PCr depletion during ischemic exercise was more pronounced and faster than that during aerobic exercise. A fast recovery of NIRS and PCr tracings was seen after cessation of exercise and occlusion.

Similar responses were found for the overall group (Fig. 2) although individual tracings varied. Fig. 2 shows the mean values (± SD) for NIRS O_2Hb, HHb and tHb in the controls after resampling the individual signals to 0.1 Hz in order to equal the time resolution of MRS. As described above, NIRS showed a rapid deoxygenation during exercise and a rapid reoxygenation after cessation of exercise. Blood volume was constant during occlusion and both exercise phases apart from a small initial drop at the start of exercise. At the end of the 3 min occlusion at rest, O_2 stores were not completely depleted since O_2Hb decreased further during the following ischemic exercise phase. Even during ischemic exercise, no plateau in O_2Hb was reached in 36% of the control subjects. Instead, these subjects showed a slow, but persistent deoxygenation until the end of exercise, indicating that the time of occlusion and ischemic exercise was insufficient for complete depletion of O_2Hb. In the other 64%, a plateau was only reached after an average time of 2½ min of ischemic exercise.

The mean PCr signal for the controls (Fig. 3B) was also comparable with the individual signal as described above, with a depletion during ischemic exercise that was more pronounced and faster than that during aerobic exercise. No change in PCr was seen during arterial occlusion at rest. End-exercise PCr depletion and end-exercise O_2Hb deoxygenation were more pronounced at the end of ischemic exercise as compared with aerobic exercise. While PCr returned to baseline levels after both exercise sessions, O_2Hb stayed slightly elevated after cessation of exercise.

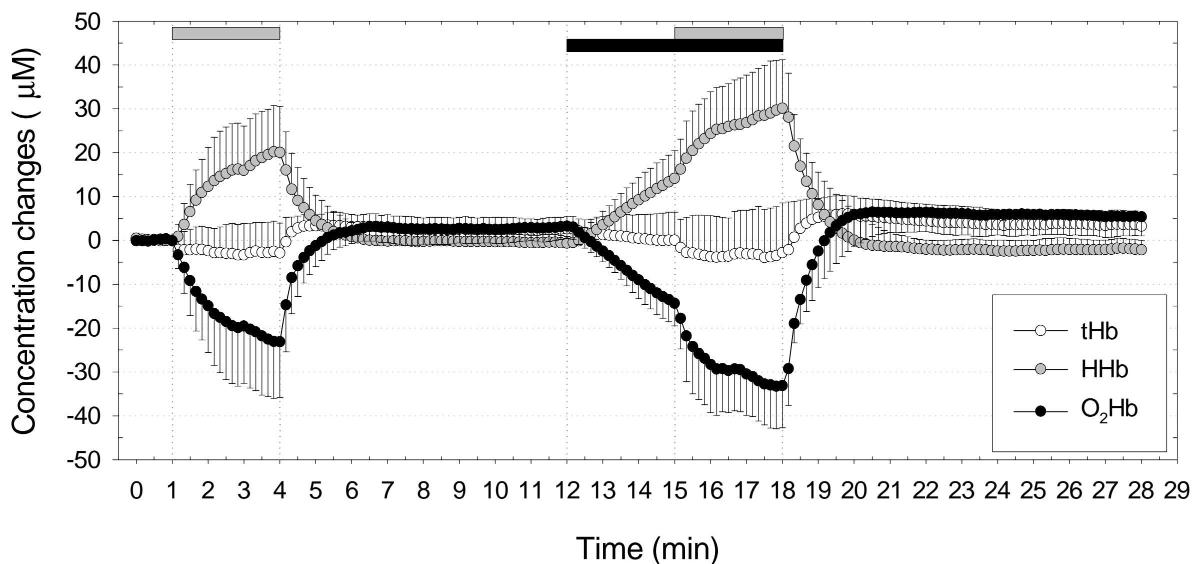

Fig. 2. Mean (±SD) NIRS signals in the healthy volunteers (n=11); O_2Hb, HHb, and tHb signals after resampling the individual tracings to 0.1 Hz to equal time resolution with MRS data. SD is presented one-sided for more clarity. The grey bar represents the periods of sustained isometric exercise at 30% MVC. The black bar represents the period of arterial occlusion.

Within the first minute of both exercise phases, intracellular pH (pH_i) showed a moderate initial alkalinisation that was subsequently followed by a decrease in pH_i at almost constant rate (Fig. 3C). During ischemic exercise, both the initial alkalinisation and the subsequent pH_i decrease were more pronounced as compared with the aerobic phase. The onset of the pH_i decrease during exercise seemed to be synchronised with the transitions in the rates of O_2Hb and PCr decrease.

FRDA versus healthy controls

Fig. 3 shows the combined data of FRDA and controls for O_2Hb, PCr, and pH_i during the complete test. The ATP levels in the calf remained constant throughout the whole protocol for both the patient group as well as the controls. NIRS and MRS in FRDA showed similar responses to occlusion and exercise as compared with the controls. Whereas a plateau in O_2Hb during ischemic exercise was seen in 64% of the control subjects, this was only seen in 31% in FRDA patients. The average time for these patients to reach a plateau was almost 2 min. In 69% of the patients, no depletion of O_2 stores was reached within the three min of exercise under arterial occlusion.

Quantitative MRS and NIRS variables for patients and controls are shown in Table 1. Depletion of PCr at the end of exercise was not significantly different between patients and controls for both exercise periods. Nor was there a significant difference in end-exercise intracellular pH.

However, the τ-value for PCr recovery was significantly slower in FRDA as compared with the controls, both after aerobic exercise and after ischemic exercise. Contrary to the difference in PCr recovery, no difference between FRDA and controls was found in the τ-value for O_2Hb recovery after exercise. The τ-value for O_2Hb recovery after aerobic exercise was significantly faster than PCr recovery in both FRDA and controls ($P < 0.01$ and $P = 0.04$, respectively). This difference was also present after ischemic exercise in FRDA ($P < 0.0001$), but was not significant in the controls ($P = 0.07$).

Oxygen consumption ($m\dot{V}O_2$) was calculated at rest during arterial occlusion and in the initial phase of aerobic and ischemic exercise with the assumption that tHb was constant. Especially during exercise this assumption could not always be met and resulted consequently in smaller groups for $m\dot{V}O_2$ analysis. No significant differences in $m\dot{V}O_2$ were found between FRDA patients and controls neither at rest nor during exercise (Table 1).

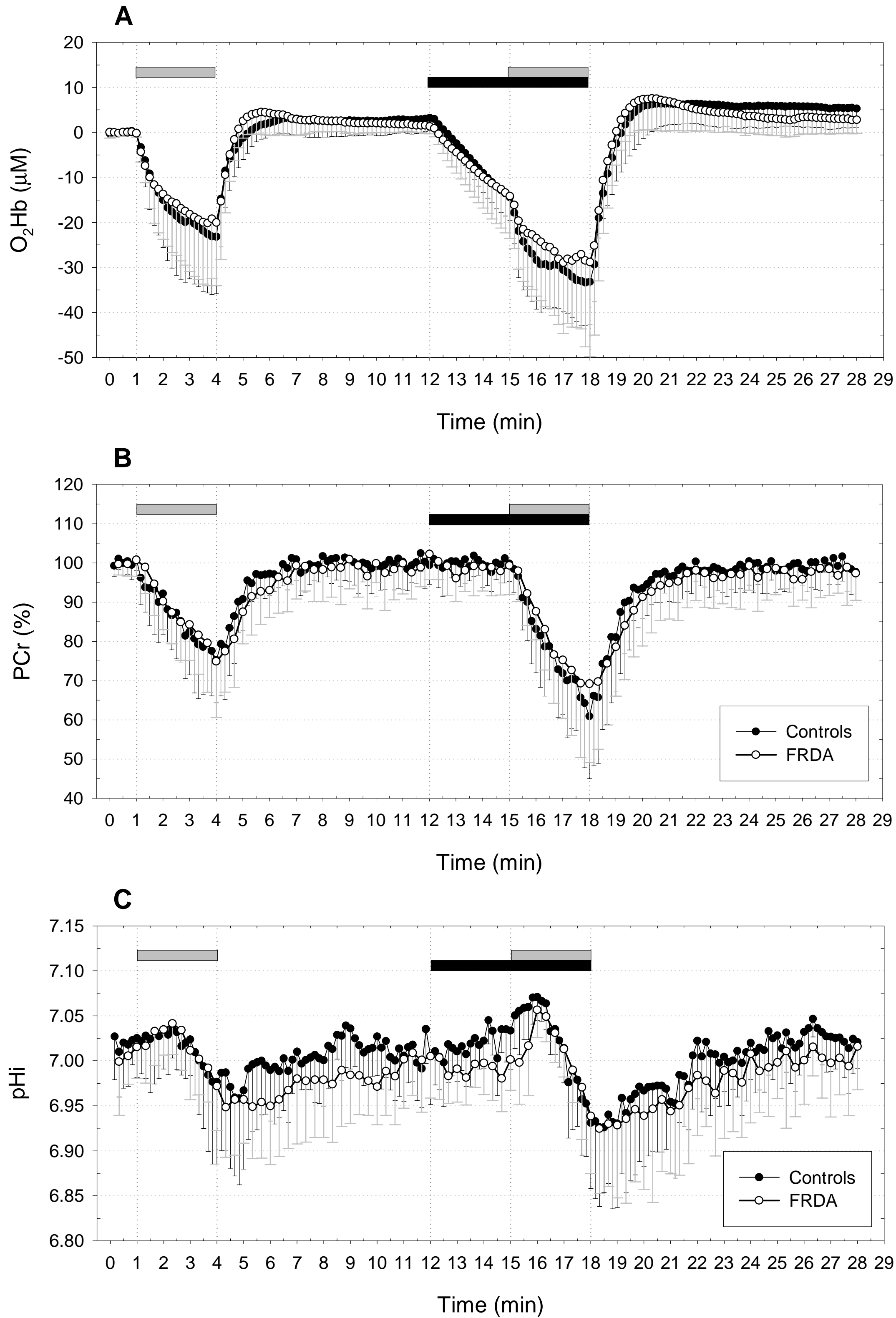

Fig. 3. Mean (±SD) values for NIRS and ^{31}P-MRS tracings in Friedreich ataxia (FRDA) patients and healthy controls. A) O_2Hb signals, B) PCr signals, and C) intracellular pH (pH_i). The grey bar represents the periods of sustained isometric exercise at 30% MVC. The black bar represents the period of arterial occlusion.

Table 1. Mean (± SD) values for phosphorus magnetic resonance spectroscopy (^{31}P-MRS) and near-infrared spectroscopy (NIRS) variables in Friedreich ataxia (FRDA) and healthy controls.

	Healthy subjects (11)		FRDA patients (15)		
	Mean ± SD	Range	Mean ± SD	Range	P-value
Initial values					
Pre-exercise intracellular pH	7.02 ± 0.03	6.96-7.11	7.01 ± 0.03	6.96-7.06	0.44
End-occlusion O_2Hb deoxygenation (µM)	-17.9 ± 4.9	-23.9 - -7.5	-15.7 ± 9.3	-38.0 - -5.9	0.45
$m\dot{V}O_2$ during AO ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$)	0.05 ± 0.02 (10)	0.02-0.07	0.05 ± 0.04 (14)	0.01-0.14	0.85
Aerobic exercise					
$m\dot{V}O_2$ ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$)	0.26 ± 0.18 (10)	0.10-0.57	0.27 ± 0.33 (8)	0.03-1.04	0.92
End-exercise O_2Hb deoxygenation (µM)	-25.1 ± 13.3	-46.8-3.3	-20.7 ± 14.1	-51.9-1.3	0.44
End-exercise [PCr] (%[PCr])	75.8 ± 10.7	59.6-90.4	74.4 ± 14.7	45.3-92.6	0.78
End-exercise pH	6.98 ± 0.09	6.77-7.08	6.97 ± 0.05	6.86-7.05	0.90
τ-value of O_2Hb recovery (s)	29.9 ± 23.2	6.1-73.5	22.7 ± 5.8	13.9-33.1	0.36
τ-value of PCr recovery (s)	52.6 + 22.5	19.5-94.3	82.6 + 40.8	39.8-178.6	0.04
Ischemic exercise					
$m\dot{V}O_2$ ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$)	0.16 ± 0.09 (7)	0.05-0.29	0.17 ± 0.14 (7)	0.04-0.44	0.96
End-exercise O_2Hb deoxygenation (µM)	-37.4 ± 9.6	-59.5-15.4	-32.5 ± 18.6	-64.6-8.5	0.44
End-exercise [PCr] (%[PCr])	61.0 ± 15.7	29.7-79.7	66.3 ± 18.7	20.3-98.0	0.45
End-exercise pH	6.94 ± 0.08	6.77-7.00	6.93 ± 0.06	6.79-7.01	0.90
τ-value of O_2Hb recovery (s)	41.7 ± 28.2	17.2-116.3	30.6 ± 13.3	10.1-52.1	0.25
τ-value of PCr recovery (s)	57.1 ± 25.2	26.0-105.3	86.3 ± 28.6	46.7-161.3	0.01

$m\dot{V}O_2$ = muscle oxygen consumption, AO = arterial occlusion, O_2Hb = oxyhaemoglobin, PCr = phosphocreatine, (n) = number of subjects included in calculation. $P \leq 0.05$ is considered statistically significant.

DISCUSSION

The main findings of this combined NIRS-MRS study were, *1)* that the O_2 stores trapped within the ischemic muscle are much slower consumed during contraction than assumed hitherto, and *2)* that although FRDA patients showed a slower PCr resynthesis, indicating a reduced rate in oxidative phosphorylation, NIRS showed no indication for an impaired oxygen delivery or consumption.

As can be seen in Fig. 1 and 3, the kinetics of deoxygenation and reoxygenation of O_2Hb paralleled those for depletion and regeneration of phosphocreatine apart from the tracings during arterial occlusion. Both O_2Hb deoxygenation and PCr depletion were more pronounced during ischemic exercise as compared with aerobic exercise. This indicates that muscle perfusion during aerobic exercise was not completely obstructed and O_2 stores were probably partly replenished during exercise. Moreover, at the start of ischemic exercise O_2 stores were already partly depleted due to the arterial occlusion that preceded exercise and that was continued during exercise. In this set-up, locally available O_2 stores were thought to be depleted by the end of the occlusion or early during ischemic exercise. The assumption of monitoring pure anaerobic PCr turn-over can only be met when O_2 stores are fully depleted. However, though the rate of O_2Hb deoxygenation slowed down within the first minute, NIRS showed a persistent decrease in O_2Hb almost during the full three minutes of occlusion (Fig. 3A). Although the majority of the controls reached a plateau in O_2Hb during ischemic exercise, depletion of O_2Hb was only reached after 2½ min. In 36% of the controls O_2 stores were not yet fully depleted at the end of exercise. Moreover, in FRDA, no plateau was reached before the end of exercise in 71% of the patients. This means that, not only at the start of ischemic exercise, but also during the remaining period, part of the energy production was still provided by oxidative phosphorylation indicating that the energy turn-over measured by ^{31}P-MRS reflects not solely anaerobic energy production, but a combination of anaerobic and oxidative pathways. These data are compatible with a recent study of Sako et al. [Sako et al. 2001] who showed that it took at least 5 min of arterial occlusion at rest before O_2Hb ceased to decline and reached full depletion. It was not until full depletion of O_2Hb was reached that PCr started to decrease [Sako et al. 2001]. Establishing pure anaerobic exercise can only be met when O_2 stores are completely depleted. Simultaneous use of NIRS and ^{31}P-MRS might, therefore, result in a reduction of the between-subject variability of PCr kinetics when ^{31}P-MRS is linked to depletion of O_2 stores measured directly by NIRS.

In this study, we investigated muscle metabolism in mitochondrial impairment by simultaneous use of ^{31}P-MRS and NIRS in order to obtain more insight in the localisation of the functional limitation of muscle metabolism in FRDA. It was shown that O_2Hb, PCr, and pH_i signals in FRDA paralleled those of the controls and apart from the τ-value

of PCr recovery, no significant differences in the quantitative variable were found between FRDA and healthy controls.

PCr recovery found in FRDA was comparable with the previous reported values [Vorgerd et al. 2000]. However, the τ-value of PCr recovery found in our controls was substantially higher than the τ-values reported in other studies [Hands et al. 1986, Keller et al. 1985, McCully et al. 1991, McCully et al. 1994a, Vorgerd et al. 2000] with the exception of the τ-value of 68 s after maximal exercise that was reported by McCully et al. [McCully et al. 1994a]. The higher tau values might be explained by the different exercise protocols used because McCully et al. [McCully et al. 1994a] showed that PCr recovery was related to work intensity. On the other hand, this can not explain the difference between our previous [Vorgerd et al. 2000] and our current study because both exercise protocols were identical.

The current differences between FRDA and controls were less pronounced than those found in the previous study [Vorgerd et al. 2000] because the slower PCr recovery in controls and the larger PCr depletion in FRDA patients in this study made the two groups come closer together. However, the differences remained statistically significant. Because both set-up and protocol were identical in the two studies, the differences are addressed to variation in subject population and not to methodological aspects.

O_2Hb recovery from aerobic exercise in the controls was in agreement with previously reported values [Chance et al. 1992, McCully et al. 1994a, McCully et al. 1994b]. PCr and O_2Hb recovery were slower after ischemic exercise as compared with aerobic exercise suggesting that both recovery parameters were related to the amount of ischemic load. This is in agreement with McCully et al. [McCully et al. 1994a] who found that PCr recovery time had almost doubled from submaximal to maximal exercise, and with Chance et al. [Chance et al. 1992] who found that O_2Hb recovery was related to workload. However, McCully et al. [McCully et al. 1994a] did not find an increase in O_2Hb recovery, but this might be due to the different sampling areas used for MRS and NIRS.

Since FRDA patients are thought to have impaired mitochondrial function we hypothesised that, compared with the controls, these patients would present a lower oxygen consumption ($m\dot{V}O_2$) measured by NIRS. This was not the case at rest nor during exercise. However, it has to be taken in mind that the percentage of subjects excluded from $m\dot{V}O_2$ analysis during exercise was much larger in the patient group (46%) as compared with the control group (9%). This was due to the fact that initial fluctuations in blood volume were much more pronounced in FRDA than in controls.

Resting $m\dot{V}O_2$ found in the controls was in agreement with calf $m\dot{V}O_2$ found by Colier et al. [Colier et al. 1995]. However, it was about 1/3 of the value that is commonly found by us and others for $m\dot{V}O_2$ in the forearm using the conventional invasive Fick

method [Hartling et al. 1989, Holling 1939, Mottram 1955, Van Beekvelt et al. 2001b] or derived indirectly by ^{31}P-MRS [Hamaoka et al. 1996, Sako et al. 2001, Wang et al. 1990]. With NIRS, $m\dot{V}O_2$ in the forearm is also mostly higher than our present $m\dot{V}O_2$ value measured in the calf [De Blasi et al. 1992, De Blasi et al. 1994, Niwayama et al. 1999, Van Beekvelt et al. 2001a, Van Beekvelt et al. 2001b, Van Beekvelt et al. 2002b]. The most probable reason for the low $m\dot{V}O_2$ found in the calf is the confounding effect of skinfold thickness on NIRS measurements [Matsushita et al. 1998, Niwayama et al. 1999, Van Beekvelt et al. 2001a, Yamamoto et al. 1998]. The skinfold thickness on top of the gastrocnemius muscle was 6.6 ± 3.3 mm and substantially higher (factor 3) than the skinfold thickness that we found on top of the flexor digitorum muscle (2.2-2.5 mm) [Van Beekvelt et al. 2001b, Van Beekvelt et al. 2002b]. After correction for skinfold thickness, as we described previously [Van Beekvelt et al. 2001a], the value of 0.05 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ was perfectly in agreement with the 0.07 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ found for a skinfold thickness of 6.6 mm [Van Beekvelt et al. 2001a] and with the 0.04 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ found in the gastrocnemius of a subject with high skinfold thickness described by Binzoni et al. [Binzoni et al. 1998]. Although skinfold thickness had a substantial confounding effect on $m\dot{V}O_2$ and should be taken into account in future research, it did not influence the comparison between FRDA and controls in this study since both groups were matched for skinfold thickness. On the other hand, adipose tissue is also known to decrease the signal-to-noise ratio in ^{31}P-MRS and the high skinfold thickness might, therefore, have increased the variability in both methods.

This study has shown that the simultaneous use of NIRS and ^{31}P-MRS, applied within the same volume of interest, supplies substantial additional information to enhance the investigation of *in vivo* muscle metabolism. Using NIRS to monitor oxidative processes during exercise it became clear that the commonly used assumption that local O_2 stores are depleted by arterial occlusion and ischemic exercise [Wackerhage et al. 1998] did not apply for a substantial number of subjects, hereby unnecessarily increasing the inter-subject variability of PCr kinetics. This was even more pronounced in FRDA where the majority of the patients showed no O_2 depletion at all before the end of exercise. Thus, ^{31}P-MRS measurements during ischemic exercise, as presented in the protocol that we used, does not necessarily mean the monitoring of pure anaerobic energy turn-over. Since the O_2 stores vary not only between subjects, but also between muscles it might be reasonable to wait for full depletion of O_2 stores before exercise is started. This way, the high variability known to exist in PCr kinetics during exercise might be decreased substantially.

Apart from a slower PCr recovery, we found no major abnormalities in oxidative processes and other MRS parameters in our FRDA patients. FRDA is caused by severely reduced levels of frataxin. Although the function of this mitochondrial protein is still

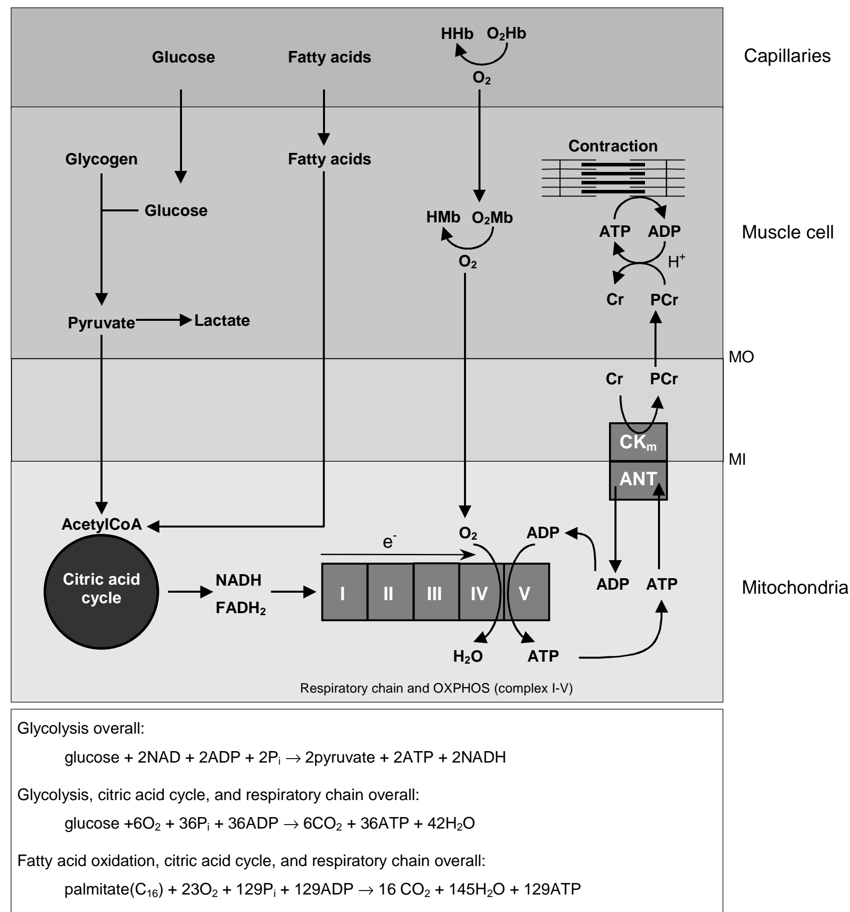

Fig. 4. Strongly simplified scheme of the pathway of oxidative phosphorylation (OXPHOS) from blood born substrates to adenosine triphosphate (ATP) with a final role for oxygen at complex IV. The respiratory chain is presented by the roman-numbered squares, existing of five complexes. Electrons (e^-) are transported from complex I to complex IV where they are adopted by O_2 to form H_2O. ATP then, is formed from adenosine diphosphate (ADP) by complex V, (ATPsynthase). Subsequently, phosphocreatine (PCr) can be resynthesised from creatine (Cr) by the adenine nucleotide translocator (ANT) and mitochondrial creatine kinase (CK_m). MO; mitochondrial outer membrane, MI; mitochondrial inner membrane.

unknown [Puccio et al. 2001] it is thought that defects in this protein lead to mitochondrial impairment. This is based on the findings that frataxin defects cause a specific Fe-S enzyme deficiency and intra-mitochondrial iron accumulation while complete absence of frataxin in the mouse leads to early embryonic lethality [Puccio et al. 2001]. PCr recovery in FRDA was delayed, suggesting impaired mitochondrial function. However, based on our NIRS measurements, muscle O_2 delivery and O_2 consumption, at rest and during exercise, seemed fully normal in FRDA. Combining NIRS and MRS data illustrates that in FRDA the mitochondrial electron transport chain can function undisturbed until the dissolved oxygen is reduced at the level of complex IV or cytochrome oxidase (Fig. 4). The delayed PCr recovery in the patients could be explained by partial uncoupling of mitochondria, meaning that oxygen is normally consumed but

ATP synthesis is decreased. This can be caused indirectly by an excess of free radicals, which can act as reactive oxygen species that may influence oxidative phosphorylation by damaging the mitochondrial inner membrane. However, the mitochondria in FRDA are probably not affected by free radicals as was shown in yeast models [Babcock et al. 1997]. Alternatively, the resynthesis of PCr could be hampered by an impaired function at the level of either complex V (ATPsynthase), the adenine nucleotide translocator (ANT), or mitochondrial creatine kinase (CK_m). Diminished activity of complex V would affect the mitochondrial ATP synthesis directly. Decreased activity of ANT would limit the transport of ATP over the inner mitochondrial membrane, and decreased mitochondrial creatine kinase activity would negatively influence the conversion of ATP into PCr. Defects in complex V, ANT, or CK_m may variously influence O_2 consumption during exercise. In the recovery phase, Friedreich ataxia patients showed a significantly slower PCr recovery rate than controls. However, in the recovery phase, NIRS monitors the sum of O_2 influx via the blood and the O_2 consumption in the muscle. Therefore, it is not surprising that the O_2Hb/O_2Mb signal in this phase is not significantly different between controls and Friedreich patients as the O_2 influx forms by far the major contribution to the NIRS signal in this phase. The NIRS data from this recovery phase do, therefore, not allow a reliable comparison of O_2 consumption between controls and patients. How a primary defect at the level of frataxin can influence PCr (re)synthesis basically remains to be established.

In conclusion, we showed the high potential of simultaneous use of NIRS and ^{31}P-MRS in the investigation of skeletal muscle metabolism. Used as complementary methods, both oxidative and anaerobic aspects of *in vivo* muscle metabolism can be monitored simultaneously and noninvasively. Furthermore, NIRS provides additional information in those situations where energy turn-over is still sufficient to replenish the intramuscular high-energy phosphates as, for instance, at rest or at the transition from rest to exercise. Future research in patients with proven mitochondrial impairment could enhance the understanding of muscle metabolism in the pathological muscle.

6

Quantitative *in vivo* near-infrared spectroscopy in resting human skeletal muscle; an overview of normal values, biological variability, and reproducibility in healthy muscle

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Submitted for publication

OUTLINE

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    Blood flow
    Venous oxygen saturation
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INTRODUCTION

Near-infrared spectroscopy (NIRS) is a noninvasive optical method for the direct measurement of local oxygenation and haemodynamics in muscle tissue. It is based on the relative transparency of the tissue for light in the near-infrared region and on the oxygen-dependent absorption changes of haemoglobin and myoglobin. The near-infrared spectrophotometer, as used in this thesis, generates light at three wavelengths. The light is transported to the tissue by means of an optical fibre bundle called optode and a second optode transports the light back to the detector. Both optodes are placed directly on the skin over the muscle of interest, and parallel to each other. The light penetrates into skin, subcutaneous fat layer and muscle, and is either scattered or absorbed within the tissue. The light scattering originating from the source occurs in any direction, but the light detected by the second optode is thought to describe a banana shape [Cui et al. 1991].

The chromophores absorbing the light of these specific wavelengths are mainly haemoglobin and myoglobin. By using more than one wavelength it becomes possible to differentiate between oxygenated and deoxygenated haemoglobin/myoglobin and enables thus information about tissue oxygenation. The measurement of changes in tissue oxygenation, noninvasive and directly measured in the muscle makes NIRS an interesting tool for clinical purposes. For instance, during surgery to check the blood (=oxygen) supply, at the intensive care to monitor brain oxygenation, or as a diagnostic tool in disorders that affect energy production.

Although NIRS has mainly been used to monitor oxygenation changes in the brain and to a far lesser extent to study skeletal muscle, it has now become a more accepted technique for the noninvasive determination of local oxygen consumption and blood flow in human skeletal muscle. The advantage of measurement in skeletal muscle is the ability to obtain local information about muscle oxygenation with the possibility of calculating quantitative values for O_2 consumption and blood flow using simple physiological interventions such as arterial or venous occlusion [Cheatle et al. 1991, Colier et al. 1995, De Blasi et al. 1993, De Blasi et al. 1994, De Blasi et al. 1997, Homma et al. 1996a, Van Beekvelt et al. 1998, Van Beekvelt et al. 1999].

In the past few years we have done numerous NIRS measurements spread over several studies and obtained thus quantitative NIRS data for a large group of healthy subjects. In this chapter we describe the various variables that can be obtained from NIRS and combined all resting data of our healthy population in order to provide information about normal range and biological variability of the various NIRS variables. Furthermore, an overview of the available data known from literature is given.

METHODS

A continuous-wave near-infrared spectrophotometer (Oxymon, Biomedical Engineering Department, University of Nijmegen, NL) that generates light at 905, 850 and 770 nm was used for all measurements. The changes in absorption at the discrete wavelengths are converted into concentration changes of O_2Hb and HHb using a modified Lambert-Beer law in which a path-length factor is incorporated to correct for scattering of photons in the tissue. A fixed value of 4.0 for the differential path-length factor (DPF) was used to calculate absolute concentration changes. This way, oxygenation and/or haemodynamic NIRS variables at rest were measured in the flexor digitorum superficialis muscle of 139 healthy volunteers (83 male, 56 female) with an interoptode distance of 35 mm. Data were sampled at 10 Hz or faster, displayed in real time, and stored on disk for off-line analysis.

SUBJECT POPULATION

The distribution and mean (±SD) values of age, height, weight, and arm circumference are shown in Fig. 1. Skinfold thickness was measured between the NIRS optodes using a skinfold caliper (Holtain Ltd., Crymmych, UK) and divided by 2 to determine the adipose tissue thickness (ATT = fat + skin layer) covering the muscle. The maximum voluntary contraction force (MVC) of each subject was determined at least 20 min before the test. Distribution and mean (±SD) values of ATT and MVC are also shown in Fig. 1. All, but ten of the subjects were right-handed. Although none of the subjects used a heavy meal, the metabolic state of the subjects might slightly differ within the group.

QUANTITATIVE NIRS MEASUREMENTS

NIRS enables calculation of various quantitative variables in arm or leg using a simple physiological intervention like venous or arterial occlusion. Venous occlusion can be applied by inflating a cuff to a pressure of approximately 50 mmHg. Such an occlusion blocks venous outflow, but does not impede arterial inflow. As a result, venous blood volume as well as venous pressure increase. The increase in blood volume is monitored by NIRS as an increase in oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb), and total haemoglobin (tHb) signals (Fig. 2). After release of the venous occlusion, all signals rapidly return to pre-exercise levels. Using the venous occlusion method it is possible to calculate blood flow, O_2 consumption and venous O_2 saturation.

Arterial occlusion is applied by inflating the cuff to a pressure of at least 60 to 80 mmHg above systolic pressure. This way, both venous outflow and arterial inflow are blocked and systemic circulatory changes are sufficiently eliminated in the limb. Lacking the supply of well-oxygenated blood, muscle metabolism fully depends on the available O_2 in local capillaries and muscle cells. Depletion of local available O_2 stores during

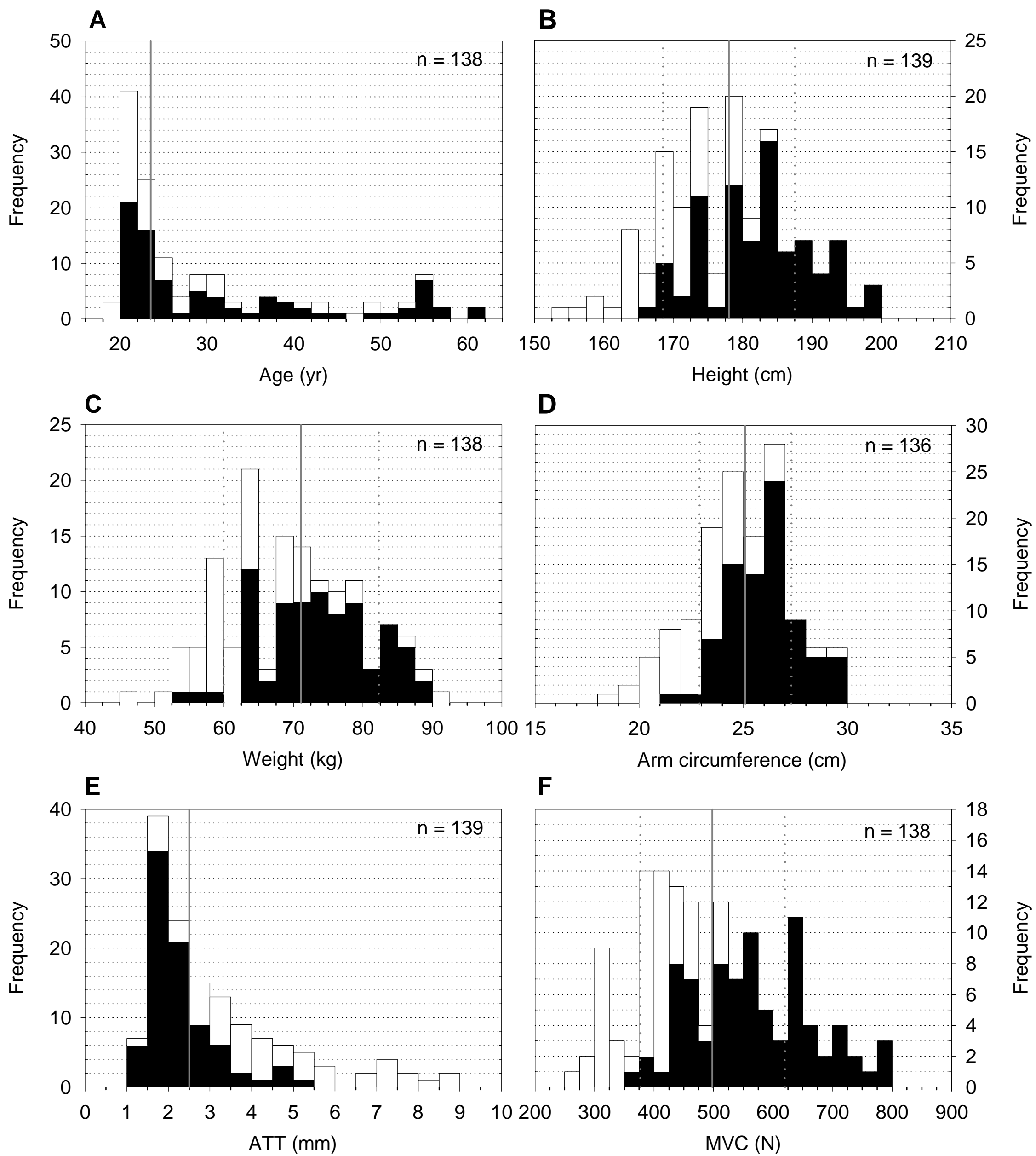

Fig. 1 Distribution and mean (±SD) values for the physical characteristics of the 139 healthy volunteers. Distribution is plotted as bars, mean as straight lines, and standard deviation as dotted lines. The median, instead of mean, is shown when distribution failed normality. Male (black bars) and female (white bars) subjects are presented separately. A) Distribution of age with median. B) Distribution of height with mean ± SD. C) Distribution of weight with mean ± SD. D) Distribution of arm circumference with mean ± SD. E) Distribution of adipose tissue thickness (ATT) with median. F) Distribution of maximal voluntary contraction force (MVC) with mean ± SD.

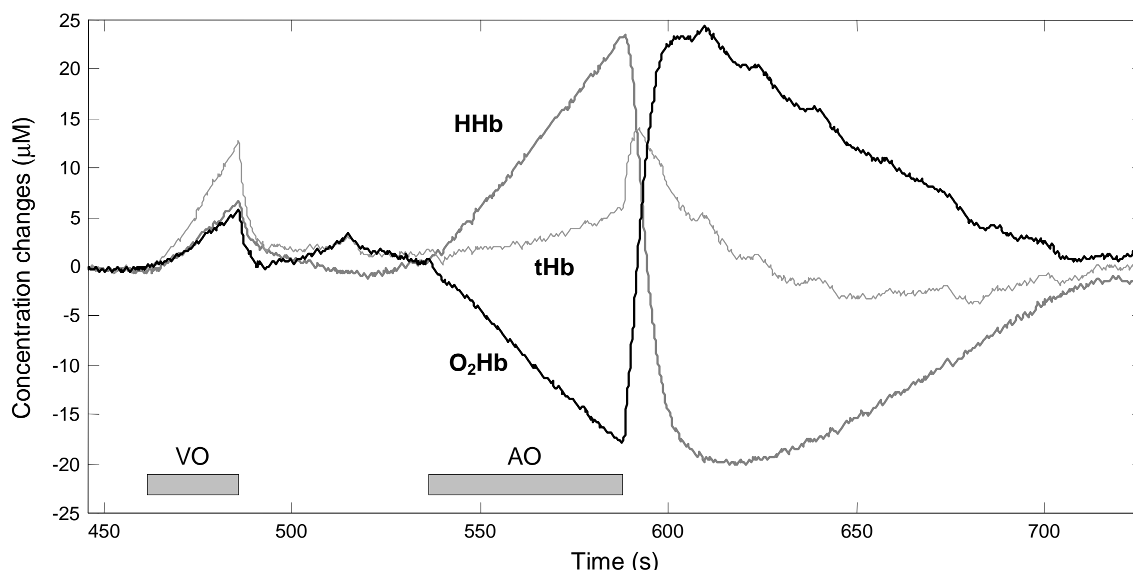

Fig. 2 Real-time NIRS tracings of oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb), and total haemoglobin (tHb) in response to 20 s of venous occlusion (VO) and 45 s of arterial occlusion (AO). Note that recovery after VO is very rapid while recovery from AO takes more than two minutes.

arterial occlusion is monitored by NIRS as a decrease in O_2Hb and a concurrent increase in HHb while tHb remains constant (Fig. 2). A hyperaemic response can be observed after release of the arterial occlusion. Blood volume increases rapidly, resulting in a fresh pool of O_2Hb and a quick washout of HHb. Using the arterial occlusion method and the recovery phase, it is possible to calculate O_2 consumption, reoxygenation rate, and the half-recovery times of the signals.

Mean (± SD) of all quantitative *in vivo* NIRS measurements are presented in Table 1. Forearm blood flow (FBF) and half-recovery times of O_2Hb and HHb did not have a normal distribution and are, therefore, presented as median in Table 1. When subjects were tested more than once, the additional results were not incorporated in the calculation of mean (± SD) values. However, these additional individual data were incorporated into the calculation of the reproducibility of the variable. The reproducibility of quantitative NIRS measurements was calculated as the within-subject variability and presented as coefficient of variation (CV) in Table 2. This within-subject variability represents a variable number of repeated measurements for the individual subjects consisting of at least three repeated measurements per individual subject. It does not discriminate between measurements within the same test session and measurements over several days. The separate data of variability within a test session and over several days are described in the previous chapters of this thesis and are in agreement with the overall CV presented here.

The various quantitative values that can be obtained from *in vivo* NIRS measurements are described in the following paragraphs, together with our own data and an overview of the available data from the literature.

Table 1. Quantitative NIRS variables in healthy subjects at rest

NIRS variable	Normality	Mean ± SD	Median	n	P_{25}	P_{75}
Venous occlusion						
Forearm blood flow ($ml \cdot min^{-1} \cdot 100ml^{-1}$)	No	-	0.88	104	0.63	1.35
Venous saturation (%)	Yes	59 ± 20	-	23	46	72
Oxygen consumption ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$)	Yes	0.12 ± 0.06	-	24	0.08	0.15
Arterial occlusion						
Oxygen consumption ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$)	Yes	0.11 ± 0.04	-	138	0.08	0.14
Reoxygenation rate ($\mu M \cdot s^{-1}$)	Yes	2.0 ± 1.0	-	33	1.3	2.8
Half-recovery time O_2Hb (s)	No	-	3.8	33	3.4	4.8
Half-recovery time HHb (s)	No	-	6.7	34	5.5	7.9
Half-recovery time tHb (s)	Yes	1.4 ± 0.8	-	29	1.0	1.8

Values are measured in the flexor digitorum superficialis muscle with an interoptode distance of 35 mm. Group values were tested for normality with the Kolmogorov-Smirnov test. SD: standard deviation, n: number of subjects, P_{25}: 25th percentile, P_{75}: 75th percentile.

Oxygen consumption

Method

Measurement of muscle O_2 consumption is of great importance in the investigation of *in vivo* muscle metabolism in health and disease. Whereas the more conventional techniques like strain-gauge plethysmography combined with blood gas analysis are invasive and provide regional values of the total limb, therefore, including tissues other than muscle tissue, NIRS is noninvasive and measures local oxygenation directly in the muscle.

As described above, NIRS O_2 consumption ($m\dot{V}O_2$) can be calculated both by means of venous and arterial occlusion. Using venous occlusion, $m\dot{V}O_2$ is calculated from the rate of increase in HHb [De Blasi et al. 1997] (Fig. 2) since venous outflow is blocked and the increase in HHb is thought to be solely due to the O_2 consumed under the assumption that arterial O_2 saturation is near 100%. However, this is not the case under hypoxic conditions e.g. at high altitude, and correction in the calculation of $m\dot{V}O_2$ from venous occlusion in these situations is required. Calculation of $m\dot{V}O_2$ from arterial occlusion assumes that tHb remains constant [De Blasi et al. 1997] and can then be derived from the rate of decrease in O_2Hb (Fig. 2) or from the rate of decrease in Hb_{diff} divided by 2. No inflow and outflow results in a static compartment of blood where the decrease of O_2 from O_2Hb is directly related to consumption.

Concentration changes of HHb, O_2Hb, and Hb_{diff} are expressed in $\mu M \cdot s^{-1}$ and converted to millilitres O_2 per minute per 100 gram tissue ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$) taking into account that each Hb molecule binds four O_2 molecules and that the molar volume of gas

Table 2. Within-subject variability of in vivo quantitative NIRS variables in healthy subjects at rest

NIRS variable	CV (%)	n_{CV}
Venous occlusion		
Forearm blood flow (ml·min^{-1}·100ml^{-1})	25.2	103
Venous saturation (%)	37.7	21
Oxygen consumption (mlO$_2$·min^{-1}·100g^{-1})	33.5	104
Arterial occlusion		
Oxygen consumption (mlO$_2$·min^{-1}·100g^{-1})	16.3	83
Reoxygenation rate (μM·s^{-1})	23.4	6
Half-recovery time O$_2$Hb (s)	16.9	6
Half-recovery time HHb (s)	16.1	6
Half-recovery time tHb (s)	53.9	6

CV: coefficient of variation, indicating the within-subject variability derived from repeated measurements. n_{CV}: number of subjects in calculation of CV.

is 22.4 L assuming STPD conditions. A value of 1.04 kg·L^{-1} was used for muscle density [Vierordt 1906]. This results in the following equation:

$$m\dot{V}O_2 = \text{Abs}\left(\left(\left(\Delta O_2Hb \times 60\right)/\left(10 \times 1.04\right)\right) \times 4\right) \times 22.4/1000 \quad \text{in mlO}_2\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}.$$

Note that ΔO_2Hb can be replaced by ΔHHb or by $\Delta Hb_{diff}/2$.

Literature

Quantitative values for resting $m\dot{V}O_2$ in arm or leg have been reported in several studies from 1991 on (Table 3). The mean value for $m\dot{V}O_2$ over all these studies was 0.11 ± 0.05 mlO$_2$·min^{-1}·100g^{-1}, but values ranged from 0.04 to 0.21 mlO$_2$·min^{-1}·100g^{-1} in the various studies. Slight differences can be expected based on the different muscle groups examined (arm vs. leg) and the chosen DPF. Moreover, as is concluded in the previous chapters of this thesis, the method used (venous vs. arterial occlusion) as well as the choice of analysis (O_2Hb vs. Hb_{diff} vs. HHb) can also result in different values for $m\dot{V}O_2$.

Data

Over the past few years and spread over several studies, we have measured resting $m\dot{V}O_2$ in the flexor digitorum superficialis muscle of 138 healthy subjects during arterial occlusion and in 104 healthy subjects during venous occlusion. The distribution of the

Table 3. Resting quantitative NIRS muscle oxygen consumption (in $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$) from literature.

Authors	DPF	IO	Method	Analysis	Muscle	n	Mean ± SD
Van Beekvelt et al. 2002b	4.0	35	AO	O_2Hb	FDS	6	0.14 ± 0.02
	4.0	35	AO	Hb_{diff}	FDS	6	0.15 ± 0.03
Van Beekvelt et al. 2001a	4.0	35	AO	Hb_{diff}	FDS	78	0.11 ± 0.04
Van Beekvelt et al. 2001b	4.0	35	AO	Hb_{diff}	FDS	26	0.11 ± 0.03
	4.0	50	AO	Hb_{diff}	FDS	26	0.09 ± 0.03
	4.0	35	AO	Hb_{diff}	BR	26	0.13 ± 0.03
	4.0	50	VO	HHb	FDS	26	0.08 ± 0.04
	4.0	35	VO	HHb	BR	26	0.14 ± 0.10
Kragelj et al. 2000*	?	40	AO	O_2Hb	Foot	6	0.08 ± 0.01
Van Beekvelt et al. 1999	4.0	35	AO	O_2Hb	FDS	27	0.11 ± 0.03
Paunescu et al. 1999	?	20-35	VO	HHb	GASTR	8	0.10
Niwayama et al. 1999	?	30	AO	O_2Hb	Forearm	15	0.12 ± 0.05
	?	30	AO	O_2Hb	Forearm	15	0.21 ± 0.03
Binzoni et al. 1998*	5.0	30	AO	O_2Hb	GASTR	8	0.08 ± 0.05
	5.0	30	AO	O_2Hb	GASTR	8	0.16
	5.0	30	AO	O_2Hb	GASTR	8	0.04
De Blasi et al. 1997*	4.16	35	VO	Hb_{diff}	BR	10	0.07 ± 0.02
	4.16	35	AO	Hb_{diff}	BR	10	0.06 ± 0.02
Colier et al. 1995*	4.3	45	AO	O_2Hb	SOL	11	0.06 ± 0.03
De Blasi et al. 1994*	4.3	30-40	VO	HHb	BR	11	0.10 ± 0.03
De Blasi et al. 1993*	3.1-5.1	28-32	AO	Hb_{diff}	BR day 1	7	0.03 ± 0.01
	3.1-5.1	28-32	AO	Hb_{diff}	BR day 2		0.05 ± 0.02
De Blasi et al. 1992*	3.59	30-35	AO	Hb_{diff}	BR	6	0.11 ± 0.06
Cheatle et al. 1991	5.4	30-50	AO	O_2Hb	Calf	21	0.20

*DPF: differential path-length factor, IO: interoptode distance in mm, AO: arterial occlusion, VO: venous occlusion, FDS: flexor digitorum superficialis muscle, BR: brachioradialis muscle, GASTR: gastrocnemius muscle, SOL: soleus muscle, n: number of subjects, ?: no data available. Values from studies marked with * are converted to $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$.*

$m\dot{V}O_2$ values for both methods is shown in Fig. 3. The mean values for $m\dot{V}O_2$ found in this large pool of healthy subjects (Table 1) were perfectly in agreement with the overall value found for all NIRS studies (0.11 ± 0.05 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$) (Table 3). Moreover, they are also in agreement with the values found in the more established, but invasive Fick method as well as with the values found with phosphorus magnetic resonance spectroscopy (^{31}P-MRS) which is thought to be the "gold standard" for noninvasive measurement of skeletal muscle metabolism during exercise (Table 4).

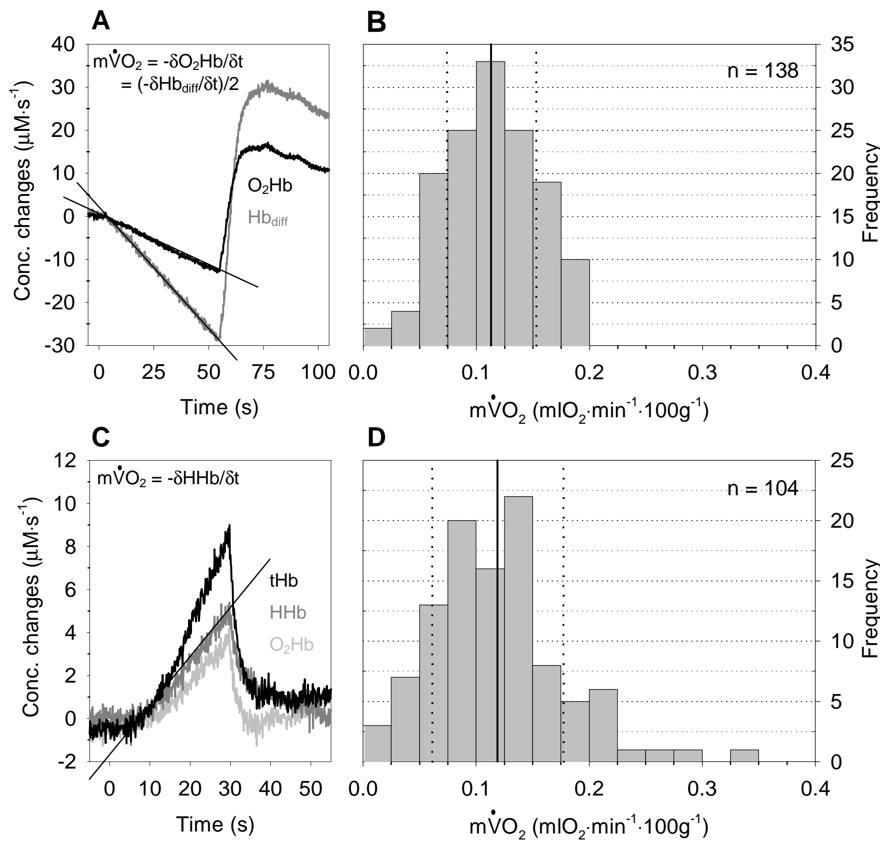

Fig. 3 Muscle oxygen consumption (m$\dot{V}O_2$ *) in the flexor digitorum superficialis muscle, A) calculated during arterial occlusion with B) distribution of individual values, and C) calculated during venous occlusion with* D) *distribution of individual values.*

The within-subject variability for m$\dot{V}O_2$ derived from repeated measurements was calculated for the majority of the subjects and presented in Table 2 as coefficient of variation (CV). Calculation of the coefficient of variation was done over a minimum of three repeated measurements either within one test session or over several days. Although the mean group values for resting m$\dot{V}O_2$ were similar for both venous and arterial occlusion methods, the within-subject variability was substantially different between both methods (Table 2). The CV for the venous occlusion method was more than twice as high than that for the arterial occlusion method (33.5% and 16.3%, respectively). This overall within-subject variability for m$\dot{V}O_2$ combined all available data and did not discriminate between repeated measurements within one test session and over several days. However, the values were roughly the same as those described in the previous chapters. Reproducibility of m$\dot{V}O_2$ derived from venous occlusion within one test session is described in Chapter 2. The reproducibility of m$\dot{V}O_2$ derived from arterial occlusion over several days is described in Chapter 3. In Chapter 4, reproducibility within one test session is evaluated for both m$\dot{V}O_2$ derived from venous and arterial occlusion.

Table 4. Forearm oxygen consumption measured by other methods.

Authors	Method	Flow measurement	Localisation	n	Mean ± SD
Van Beekvelt et al. 2001b	Fick	Plethysmography	Forearm	26	0.15 ± 0.06
Sako et al. 2001*	^{31}P-MRS	-	FDS	12	0.16 ± 0.02
Hamaoka et al. 1996*	^{31}P-MRS	-	FDS	5	0.16
Wang et al. 1990*	^{31}P-MRS	-	Forearm	1	0.17
Hartling et al. 1989*	Fick	Dye dilution	Forearm	5	0.14 ± 0.09
Wahren 1966	Fick	Plethysmography	Forearm	10	0.30 ± 0.09
Andres et al. 1956	Fick	Dye dilution	Forearm	8	0.26 ± 0.08
Mottram 1955	Fick	Plethysmography	Forearm	16	0.23 ± 0.07
Holling 1939*	Fick	Plethysmography	Forearm	34	0.11

*Mean (± SD) forearm oxygen consumption in $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$. Fick oxygen consumption is derived from the arteriovenous O_2 difference (derived by blood gas analysis) times blood flow. ^{31}P-MRS: phosphorus magnetic resonance spectroscopy, FDS: flexor digitorum superficialis muscle, n: number of subjects. Values from studies marked with * are converted to $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$.*

Blood flow

Methods

NIRS blood flow measurements are similar to the well-established method of strain-gauge plethysmography. Venous occlusion is used to provoke a blood volume increase in the part of the limb distal from the pneumatic cuff. Within the initial period of the occlusion, the increase in blood volume per time is a measure for the blood flow. Strain-gauge plethysmography measures blood volume changes by changes in limb circumference and can not distinguish between the various tissues of the limb. NIRS measures blood volume changes directly in the muscle of interest by monitoring changes in the haemoglobin/ myoglobin content.

Blood flow in arm or leg can be measured during venous occlusion by evaluating the linear increase in tHb within the first seconds of the venous occlusion [De Blasi et al. 1997, Van Beekvelt et al. 1998] (Fig. 2). Venous outflow is blocked and the increase in tHb (= oxy- plus deoxyhaemoglobin/myoglobin) is directly related to arterial inflow. Concentration changes of tHb are expressed in $\mu M \cdot s^{-1}$ and converted to millilitres blood per minute per 100 millilitres tissue ($ml \cdot min^{-1} \cdot 100ml^{-1}$) using either the individual Hb-concentration ([Hb] in $mmol \cdot L^{-1}$) obtained from blood samples or male and female values derived from literature. The molecular weight of Hb (64.458 $g mol^{-1}$) and the molecular ratio between Hb and O_2 (1:4) have to be taken into account. BF can than be calculated by the following equation:

$$BF = (((\Delta tHb \times 60)/(([Hb] \times 1000)/4)) \times 1000)/10 \quad \text{in } mlO_2 \cdot min^{-1} \cdot 100g^{-1}.$$

Literature

Quantitative values for resting BF using NIRS in arm or leg have been reported in only a few studies (Table 5). BF values ranged from 0.57 – 2.35 $ml \cdot min^{-1} \cdot 100ml^{-1}$ where the highest value was measured using an intravenous tracer instead of venous occlusion. In two studies, a comparison of quantitative forearm BF, derived from venous occlusion, was made between simultaneous measurement of NIRS and the well-established method of strain-gauge plethysmography [De Blasi et al. 1994, Van Beekvelt et al. 2001b]. Although good agreement was found between the two methods, and plethysmographic flow was in agreement with other studies (Table 6), plethysmographic flow appeared to be 2 to 3 times higher than NIRS flow. This is probably due to the different measurement volumes addressed by the two methods, since plethysmographic flow reflects the flow in the total forearm while NIRS flow reflects only the local flow within the muscle of interest.

Data

In our lab, we have measured resting forearm blood flow (FBF) in 104 healthy subjects spread over several studies (Table 1). Fig. 4 shows that FBF did not have a normal distribution, but was positively skewed. The within-subject variability for FBF derived from repeated measurements within one test session is presented in Table 2 as coefficient of variation (CV). Calculations of within-subject CV for FBF were done over a minimum of three reported measurements. The within-subject CV for FBF (n=104) was 25.2% and substantially higher than the within-subject CV for $m\dot{V}O_2$ calculation during arterial occlusion (16.3%).

The within-subject CV for plethysmographic flow (16.7%) as described in Chapter 2 was also lower than for the NIRS flow. A possible explanation for this discrepancy in inter-individual variability between NIRS and plethysmography might be the heterogeneous flow of muscle tissue which fully contributes to NIRS flow measurements and only partly to plethysmographic flow measurement since plethysmography also incorporates other tissues of the forearm with less heterogeneous flow patterns like bone and tendon. However, as described in Chapter 2, it might also be related to the venous occlusion method itself based on the fact that venous occlusion is more prone to ever-occurring variations in flow within the arm due to changes in blood pressure and local vasoreactivity, whereas these influences are negligible during arterial occlusion because of the closed compartment, temporarily cut off from centrally mediated variations.

Table 5. Resting quantitative NIRS blood flow values (in ml·min⁻¹·100ml⁻¹) from literature.

Authors	DPF	IO	Muscle	n	Mean ± SD
Van Beekvelt et al. 2001a	4.0	35	FDS	78	1.28 ± 0.82
Van Beekvelt et al. 2001b	4.0	35	FDS	26	0.72 ± 0.32
	4.0	50	FDS	26	0.57 ± 0.26
	4.0	35	BR	26	1.42 ± 1.04
Van Beekvelt et al. 1999	4.0	35	FDS	27	0.72 ± 0.33
Paunescu et al. 1999	?	20-35	GASTR	8	0.73
De Blasi et al. 1994	4.3	30-40	BR	11	1.9 ± 0.8
Edwards et al. 1993 # $	3.59	Through	Forearm	6	2.35 ± 1.11

DPF: differential path-length factor, IO: interoptode distance in mm, FDS: flexor digitorum superficialis muscle, BR: brachioradialis muscle, GASTR: gastrocnemius muscle, n: number of subjects, ?: no data available. # Blood flow measured by intravenous tracer instead of venous occlusion method. $ light detection through the arm instead of parallel optode placement.

Table 6. Forearm blood flow measured by other methods.

Authors	Method	Localisation	n	Mean ± SD
Van Beekvelt et al. 2001b	Plethysmography	Forearm	26	2.1 ± 0.7
De Blasi et al. 1994	Plethysmography	Forearm	11	3.4 ± 1.3
Edwards et al. 1993*	Plethysmography	Forearm	7	2.2 ± 1.1
Hartling et al. 1989	Dye dilution	Forearm	5	3.6 ± 2.2
Wahren 1966	Plethysmography	Forearm	10	3.8 ± 1.1
Andres et al. 1956	Dye dilution	Forearm	14	3.6
Mottram 1955	Plethysmography	Total forearm	16	3.2
	Estimation	Forearm muscle	16	2.9

*Mean (± SD) blood flow in ml·min⁻¹·100ml⁻¹. n: number of subjects. Values from studies marked with * are converted to $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$.*

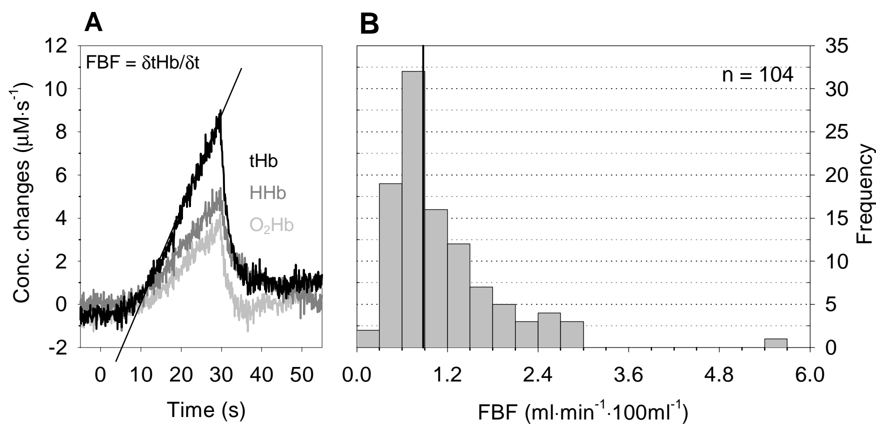

Fig. 4 Forearm blood flow (FBF) in the flexor digitorum superficialis muscle, A) calculated during venous occlusion. B) distribution of individual values. The straight line represents the median.

Venous oxygen saturation

Methods

Another variable that can be derived from venous occlusion is peripheral venous oxygen saturation (SvO_2) and has been described by Yoxall & Weindling [Yoxall et al. 1996]. SvO_2 can be of special interest in neonatology where knowledge of cerebral haemodynamics and oxygenation are of primary importance for survival of the premature neonate since peripheral SvO_2 is thought to provide an index of the adequacy of global oxygenation [Yoxall et al. 1996]. SvO_2 can be calculated from the ratio of increase in ΔO_2Hb to increase in ΔtHb during venous occlusion:

$$SvO_2 = \frac{\Delta O_2Hb}{\Delta O_2Hb + \Delta HHb}$$

This is independent of the optical path-length since absolute quantification of the chromophore concentration changes is not required. As described by Yoxall and Weindling [Yoxall et al. 1997], SvO_2 is best determined from the first 5 s of the venous occlusion while an occlusion is considered to be satisfactory for analysis if there is a steady baseline before the occlusion, a rise in both O_2Hb and HHb during the occlusion, and a return to baseline after release of the occlusion.

Literature

Apart from the two above mentioned studies (Table 7), no other publications on NIRS venous oxygen saturation of muscle tissue are known. In another study, cerebral oxygen saturation was determined in healthy newborn infants by occluding the jugular artery [Buchvald et al. 1999].

Data

We have calculated venous oxygen saturation in 24 subjects as is shown in Table 1. The distribution of the individual values passed the normality test and is shown in Fig. 5. Our mean value for SvO_2 (and median (56%) as well) was substantially lower than that found by Yoxall & Weindling [Yoxall et al. 1996, Yoxall et al. 1997] while the range was much wider. The reproducibility of SvO_2 (presented as CV in Table 2) was high and in many cases the assumptions as proposed by Yoxall & Weindling could not be fulfilled because of fluctuations in baseline levels that interfered with recovery tracings. When O_2Hb showed a decrease instead of increase, the data was excluded from analysis. However, in a number of subjects, O_2Hb shifted from increase to decrease within 10 s of occlusion and

Table 7. Resting quantitative NIRS venous oxygen saturation (in %) from literature.

			NIRS		Co-oximeter	
Authors	n	Localisation	Median	Range	Median	Range
Yoxall et al. 1997	19 adults	Forearm	71.3	50.1 – 80.0	71.9	50.4 – 92.0
Yoxall et al. 1996	16 infants	Forearm	71.6	50.7 – 79.7	75.9	60.8 – 90.7

n: number of subjects.

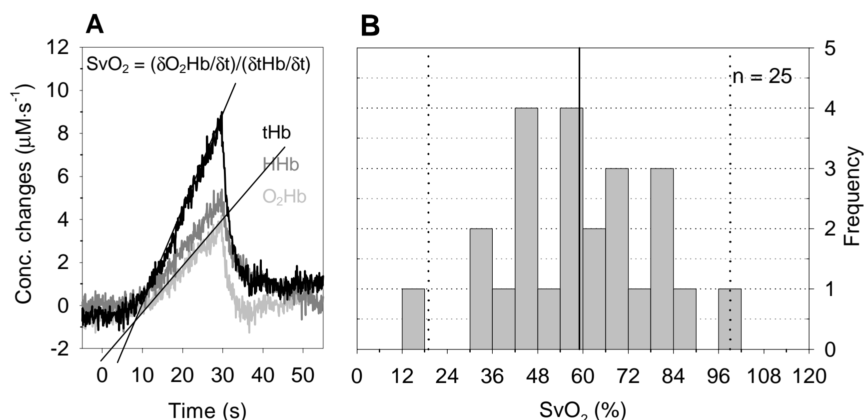

Fig. 5 Venous oxygen saturation (SvO_2) in the flexor digitorum superficialis muscle, A) calculated during venous occlusion. B) distribution of individual values. Mean is presented as a straight line, SD as dotted lines.

as long as the increase existed during the 5 s of analysis, data was included. We found that the range of SvO_2 was very wide ranging from 12 - 100% venous saturation. Considering that the measurements were all done at rest, it is our opinion that this variable is not accurate for *in vivo* measurements. Therefore, we have neglected the variable in the analysis of the studies described in this thesis.

Half-recovery time

Methods

The recovery of O_2Hb after exercise or ischemia represents the time needed for resaturation of deoxygenated haemoglobin and myoglobin and is thought to reflect both the influx of oxygenated arterial blood and the continued O_2 consumption during recovery [Chance et al. 1992, McCully et al. 1994a, McCully et al. 1994b].

Two different approaches are known to calculate half-recovery times from NIRS signals. The first approach was described by Chance et al. [Chance et al. 1992] as the time needed for half recovery of O_2Hb from maximum deoxygenation at the end of occlusion to maximum re-oxygenation during hyperaemia (t_{50}) (Fig. 6A). Another more elegant

method to calculate half-recovery times or time constants (Tc) is by fitting the recovery signal to a mono-exponential curve from which a time constant or tau value can be derived [McCully et al. 1994a] (Fig. 6B) [McCully et al. 1994b].

The latter method is identical to the determination of phosphocreatine (PCr) recovery that is commonly used in phosphorus magnetic resonance spectroscopy (^{31}P-MRS) (Chapter 5). However, as can be seen in Fig. 6 this method is only possible when the hyperaemic response is small or absent because in case of a distinct hyperaemic response, the signal is no longer mono-exponential.

Similar to the calculations of O_2Hb, but not described in literature, half-recovery time could also be calculated from HHb and tHb. Whereas recovery of O_2Hb reveals information about arterial inflow (and continued O_2 consumption), HHb reflects venous outflow (and continued O_2 consumption) while tHb reflects the balance between arterial inflow and venous outflow.

Literature

Half-recovery time measured by NIRS has so far been scarcely used (Table 8). However, this variable might be an interesting parameter in the investigation of trained versus untrained or health versus disease. Chance et al. [Chance et al. 1992] was the first to explore this variable in response to the intensive experiments on recovery times of PCr using ^{31}P-MRS. They measured the recovery time for haemoglobin/myoglobin desaturation in the quadriceps of elite male and female rowers following work near maximal voluntary contraction force. The recovery from desaturation after submaximal exercise ranged between 10 and 80 s while resaturation times were found to increase with intensity of work.

This was followed by McCully et al. [McCully et al. 1994b] who used recovery times to study exercise induced changes in elderly subjects with peripheral vascular disease (PVD) and found that the time constant of recovery (mono-exponential curve fit) measured in the calf was significantly delayed in PVD. In another study of this group [McCully et al. 1994a], NIRS and ^{31}P-MRS were simultaneously used and O_2Hb recovery was compared with PCr recovery after maximal and submaximal plantar flexion. It was found that the rate of recovery was much slower for PCr than for O_2Hb.

Contrary to Chance et al. [Chance et al. 1992] McCully et al. [McCully et al. 1994a] did not find a difference in O_2Hb recovery between maximal and submaximal exercise while PCr recovery was longer after maximal than after submaximal exercise. In a study that we performed (unpublished data), we found, in agreement with Chance et al. [Chance et al. 1992], that O_2Hb recovery increased with intensity of work.

Data

Because of the hyperaemic response that was seen in the arm in practically all subjects, we have calculated half-recovery time in the forearm as the time needed for half recovery of O_2Hb from maximum deoxygenation at the end of occlusion to maximum re-oxygenation during hyperaemia (t_{50}). Equally, the t_{50} of HHb was calculated as the time needed for half recovery of HHb from the maximum accumulation of HHb at the end of occlusion to the maximum wash-out during hyperaemia while t_{50} of tHb was calculated as the half-time from the end of occlusion to peak blood volume increase. All half-recovery times presented here are preceded by an arterial occlusion of 45 s. The half-recovery time is most probably influenced by the duration of the arterial occlusion. In what way the duration of occlusion affects the recovery times remains to be investigated.

While tHb passed the normality test, the distribution of O_2Hb and HHb was deviated from Gaussian distribution. Measured at rest in the forearm of 34 healthy subjects (Table

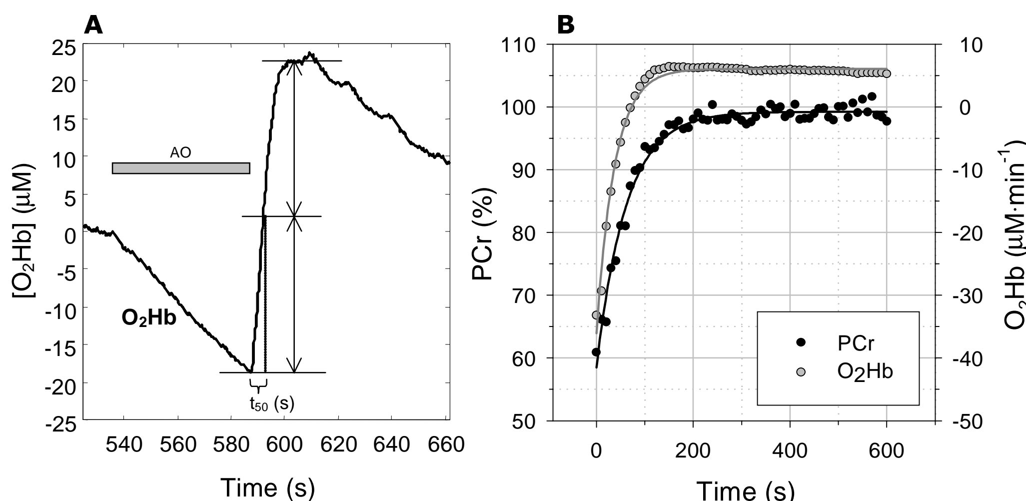

Fig. 6 Half-time recovery, calculated as A) the time needed for half recovery of O_2Hb from maximum deoxygenation at the end of arterial occlusion or exercise to maximum re-oxygenation during hyperaemia, and B) the tau value derived from a mono-exponential curve-fit of O_2Hb recovery after exercise as is standard for calculation of PCr resynthesis with ^{31}P-MRS.

Table 8. Half-recovery time (s) after exercise from literature.

Authors	Exercise protocol	Muscle	n	Mean ± SD
McCully et al. 1994a	Submax PF, incremental, 5 min	GASTR	5	28 ± 6
	Max PF, 64 s	GASTR	5	29 ± 6
McCully et al. 1994b	PF, 3.4-9.1 kg, 1 min	GASTR	6	22 ± 9
Chance et al. 1992	100% MVC	QUADRI	7	36 ± 4

n: number of subjects, PF: plantar flexion, GASTR: gastrocnemius muscle, QUADRI: quadriceps muscle.

1), we found that half-recovery time (t_{50}) after cessation of arterial occlusion at rest was fastest in tHb. Replenishment of O_2Hb in the capillary bed and muscle cells was significantly slower ($P<0.001$), but still faster than the washout of HHb ($P<0.01$) (Friedman with post-test). The distribution of the half-recovery times is shown in Fig. 7. Both outliers for $t_{50}(O_2Hb)$ and t_{50}(HHb) originated from the same subject, but this was not the case for t_{50}(tHb). In all subjects, recovery of tHb was fastest (n=29). In all, but two subjects (n=33), O_2Hb was faster than HHb recovery. The within-subject variability for the various half-recovery times derived from repeated measurements was calculated over several days in 6 subjects (Table 2). Reproducibility was good for $t_{50}(O_2Hb)$ and t_{50}(HHb), but poor for t_{50}(tHb).

The faster recovery of tHb as compared with O_2Hb was in agreement with Chance et al. who found that the increase in blood volume during recovery from exercise was also clearly faster than that of resaturation, as might be expected because the former represents a restoration of blood volume and the latter a repayment of oxygen deficit [Chance et al. 1992]. The slower recovery that was found for HHb, representing venous outflow is probably due to the delay in vasodilation indicated by the increased blood volume that reflects the mismatch between arterial inflow and venous outflow.

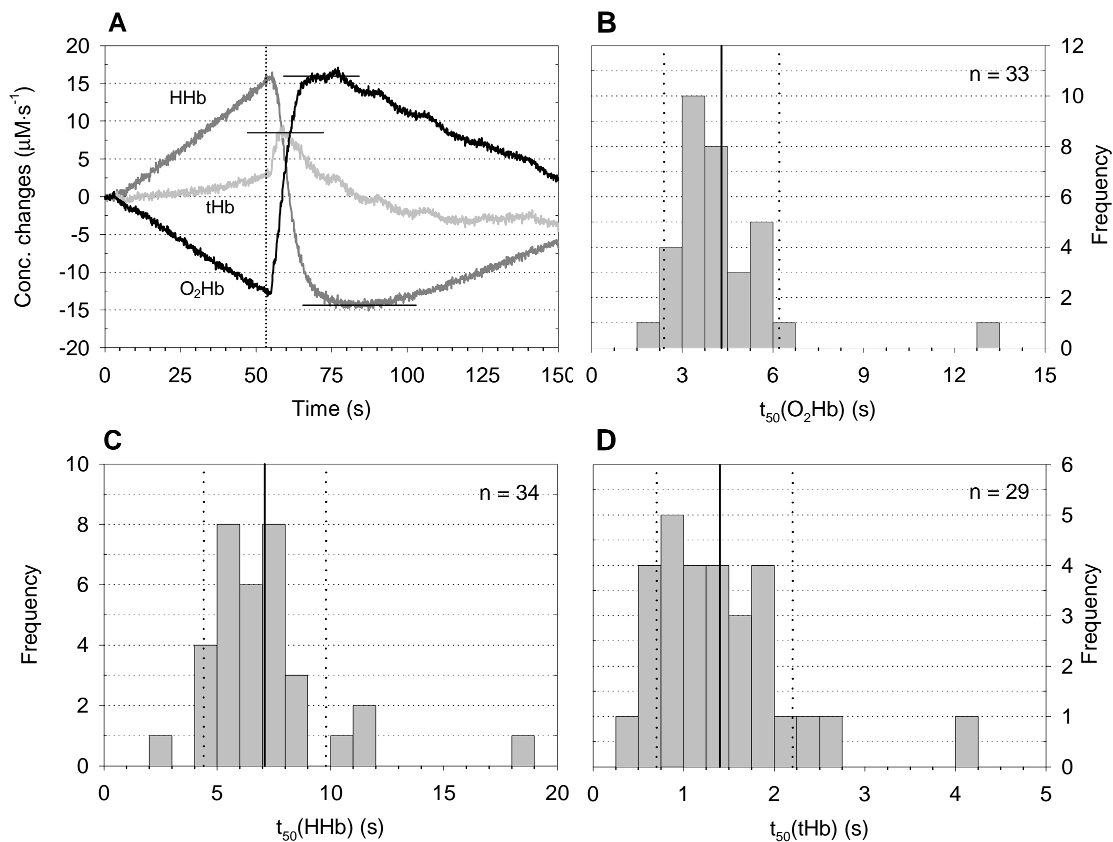

Fig. 7 A) Calculation of half-recovery time (t_{50}) in the flexor digitorum superficialis muscle, after cessation of arterial occlusion at rest. Distribution (bars), mean (straight line), and SD (dotted lines) for half-recovery time of B) oxyhaemoglobin (O_2Hb), C) deoxyhaemoglobin (HHb), and D) *total haemoglobin (tHb).*

We have found that in the forearm, a distinct hyperaemic response after cessation of occlusion or exercise is practically always present thereby leaving the first method as the only legitimate approach for calculation of half-recovery time in the forearm. In Chapter 5, we calculated recovery times using a mono-exponential curve fit because hyperaemic response was small or absent in the leg. As for the cause of the differences between arm and leg measurements as well as for the differences and physiological relevance of both approaches, more research is necessary. Whether half-recovery time is dependent [Chance et al. 1992] or independent [McCully et al. 1994a] of work intensity should also be addressed in future research.

Reoxygenation rate

Methods

Another variable that can be calculated in relation to recovery from arterial occlusion or exercise is the rate of O_2Hb reoxygenation. Whereas the half-recovery is a function of time, the reoxygenation rate reflects the velocity at which the recovery starts off after release of exercise or ischemia.

The reoxygenation rate (ΔO_2Hb, in $\mu M \cdot s^{-1}$) was calculated as the rate of increase in O_2Hb during the initial 3 s after cessation of occlusion and/or exercise. This variable reflects the initial inflow of O_2Hb over a fixed time period and is, therefore, not influenced by the presence or absence of a hyperaemic response. Whereas the recovery time (or half-recovery time) includes all processes for total recovery of vascular O_2Hb, muscular O_2Mb as well as the continued oxygen consumption during recovery, the reoxygenation rate is thought to reflect the fast initial recovery rate at which primarily vascular components are restored. It is directly related to microvascular function and can, therefore, be a functional new variable in the investigation of disorders concerning O_2 delivery or in the discrimination between disorders of O_2 delivery and O_2 consumption.

The reoxygenation rate might, similar to the half-recovery time, be influenced by the duration of the preceding arterial occlusion, being 45 s in our study. However, since the reoxygenation rate reflects the initial fast component of the recovery, the influence is probably less than that of the (half-)recovery time. Nevertheless, this issue remains to be investigated in future research.

Literature

No data concerning this variable is available from the literature, possibly due to the high sample frequency required for this variable. We have chosen this variable because it describes the fast component of recovery after cessation of exercise or occlusion, thus reflecting mainly vascular components and not the perfusion of oxygen or the

replenishment of the oxygen deficit. We have used this variable in the investigation of dermatomyositis, a muscle disorder characterised by complement mediated necrosis, resulting in ischemia and hypoperfusion, as is described in Chapter 9.

Data

The reoxygenation rate of O_2Hb was calculated in 34 healthy subjects after cessation of arterial occlusion at rest (Table 1). The within-subject variability derived from repeated measurements was tested in 6 subjects over several days and was reasonably good (23.4%) while the distribution of individual values was wide (Fig. 8). The recovery rate at rest as well as following various exercise intensities was also used to investigate the effect of treatment in dermatomyositis.

CONCLUSION

In this chapter we have combined all resting data gathered over the last few years. The purpose of combining these datasets was to enlarge the subject pool and thus obtaining information about normal range and biological variability of the various NIRS variables. Moreover, we have tried to give an overview of the published data concerning the various quantitative NIRS variables.

As for the most commonly used variable, oxygen consumption calculated from arterial occlusion proved reproducible and was in good agreement with the literature, both compared with other NIRS studies as well as to more established methods. The other commonly used variable; blood flow, was also reproducible, but the variability between and within subjects was larger than that for oxygen consumption. This has also been reported in other NIRS studies and in several other flow methods.

Quantitative measurement of venous oxygen saturation from venous occlusion has been proposed as a variable of special interest in the neonatology. However, calculation of venous oxygen saturation in our healthy adult subjects ranged from 12-100% in normal resting conditions and it is, therefore, our opinion that this variable can not be accurately measured by NIRS.

Half-recovery time of NIRS signals has, so far, only scarcely been used in the investigation of health versus peripheral vascular disease and for the comparison of trained versus untrained subjects. In this chapter we showed that the within-subject variability of repeated measurements was good for both half-recovery of oxyhaemoglobin as well as deoxyhaemoglobin. The variability of half-recovery of hyperaemic blood volume changes (tHb) after exercise and/or occlusion was much larger and, therefore, probably lacks clinical relevance.

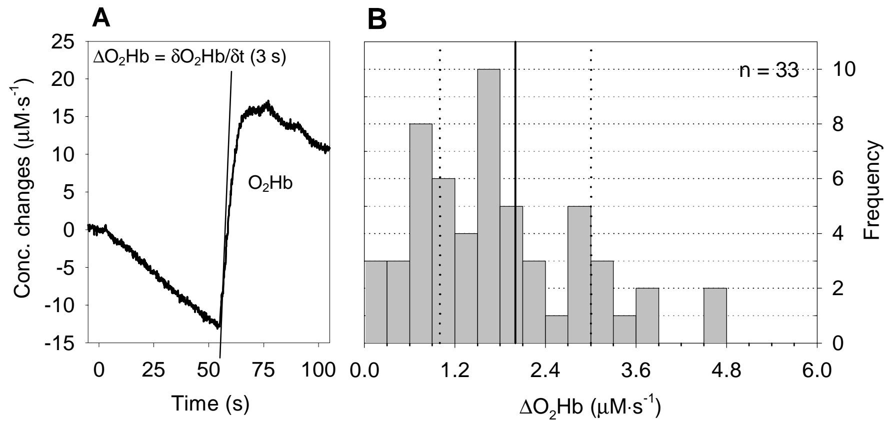

Fig. 8 Rate of reoxygenation (ΔO_2Hb) in the flexor digitorum superficialis muscle, calculated immediately after cessation of arterial occlusion at rest. A) Concentration changes of oxyhaemoglobin (O_2Hb). B) Distribution, mean (straight line), and SD (dotted lines) for the individual values.

Finally, the reoxygenation rate is a new variable representing the fast initial recovery rate at which primarily vascular components are restored and is, therefore, directly related to microvascular function. This in contrast to the half-recovery time, which includes all processes for recovery of vascular and muscular oxygen content as well. Whether the reoxygenation rate has sufficient clinical relevance has to be proven in future research. As described in this thesis, this variable proved functional in the successful monitoring of the effect of medication, corresponding to the clinical findings.

PART 2

CLINICAL APPLICATION

7

Clinical application of quantitative *in vivo* near-infrared spectroscopy in neuromuscular disorders

NIRS IN NEUROMUSCULAR METABOLIC DISORDERS

In the previous chapters, we showed that NIRS allows the noninvasive measurement of local oxygenation, blood flow, and oxygen consumption. This is of great importance for enhancing the knowledge of normal physiological responses, but can also be of great benefit in the investigation and understanding of pathological situations of impaired O_2 delivery or O_2 consumption. Impaired O_2 delivery not only occurs under hypoxic conditions, as is present e.g. at high altitude, but can also be due to pulmonary abnormalities, heart failure, or peripheral vascular disease. Impaired O_2 extraction can result from diffusion problems, metabolic enzyme deficiencies, or mitochondrial defects.

In the past few years, NIRS has shown to be a sensitive tool in the discrimination between normal and pathological states of the muscle. For instance, using NIRS, abnormal oxygenation due to insufficient delivery has been found in patients with heart failure [Belardinelli et al. 1995, Mancini et al. 1994a, Matsui et al. 1995, Wilson et al. 1989] and peripheral vascular disease [Cheatle et al. 1991, Komiyama et al. 1994, Kooijman et al. 1997, McCully et al. 1994b]. NIRS was also used to characterise patients with metabolic myopathies, in which abnormalities in oxygenation pattern are related to O_2 extraction instead of O_2 delivery [Abe et al. 1997, Bank et al. 1994, Bank et al. 1995, Gellerich et al. 1998, Wariar et al. 2000] (Table 1).

The application of NIRS in metabolic and mitochondrial myopathies was pioneered in 1994 by Bank & Chance during low-speed treadmill exercise [Bank et al. 1994]. The major finding of this study was an impaired oxygen utilisation in patients with various metabolic myopathies. Instead of deoxygenation during exercise as seen in the controls, an increase in oxygenation was found in a patient with cytochrome c oxidase deficiency, a mitochondrial myopathy. Cytochrome c oxidase deficiency is thought to limit the capacity of the mitochondria to generate ATP due to a defect in the electron transport chain. Similar results as compared with the patient with mitochondrial myopathy were found in patients with other defects in the energy generating pathways (Table 1). As, for instance, in myophosphorylase deficiency, or McArdle's disease, where utilisation of muscle glycogen is blocked, and in phosphofructokinase deficiency where utilisation of both glycogen and glucose are blocked. Both groups showed a transient deoxygenation, followed by oxygenation. The two patients with carnitine palmitoyl transferase deficiency, which impairs the incorporation and beta-oxidation of long-chain fatty acids, had a normal deoxygenation in response to treadmill exercise [Bank et al. 1994]. Similar data were described in another paper of Bank & Chance [Bank et al. 1995] with addition of a second patient with cytochrome c oxidase deficiency.

This work was followed by a study of another group, monitoring oxygenation and blood volume patterns during bicycle exercise in 4 patients with mitochondrial myopathies [Abe et al. 1997]. Three patients with chronic progressive external ophthalmoplegia (CPEO) and one patient with mitochondrial myopathy, encephalopathy,

Table 1. Near-infrared spectroscopy in metabolic/mitochondrial myopathies.

Authors	n	Patients
Bank et al. 1994	1	Cytochrome c oxidase deficiency
	3	Myophosphorylase deficiency (McArdle)
	3	Phosphofructokinase deficiency
	2	Carnitine palmitoyl transferase deficiency
Bank et al. 1995	2	Cytochrome c oxidase deficiency
	3	Myophosphorylase deficiency (McArdle)
	3	Phosphofructokinase deficiency
Abe et al. 1997	3	Chronic progressive external ophthalmoplegia (CPEO)
	1	Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS)
Gellerich et al. 1998	1	Exercise induced myalgia
	1	Proximal weakness + pseudohypertrophy
	1	External ophthalmoplegia
Wariar et al. 2000	1	Coenzyme Q_{10} deficiency

lactic acidosis, and strokelike episodes (MELAS) were tested on a bicycle ergometer during 15 minutes of cycle exercise at 15 Watt. Although changes in oxygenation were less pronounced than those in the controls, all four patients showed a deoxygenation and no oxygenation in response to cycling at low-intensity work [Abe et al. 1997]. This study showed that the paradoxical oxygenation during exercise found in mitochondrial and metabolic myopathies, as described by Bank & Chance [Bank et al. 1995],[Bank et al. 1994] is not uniformly present in all mitochondrial myopathies.

In our own study as described in Chapter 8 [Van Beekvelt et al. 1999], we found no paradoxical oxygenation and a normal decrease in blood volume in three out of five CPEO patients studied during sustained isometric handgrip exercise. However, the other two showed no response to exercise at all, but it is our opinion that this was most likely due to the confounding effect of adipose tissue thickness as these two subjects had the highest value for adipose tissue thickness of the group (7.7 and 8.0 mm, respectively). With the increase of adipose tissue thickness, NIRS measurement of oxygenation is less determined by muscle tissue and more by adipose tissue and oxygenation is, therefore, less influenced by exercise (Chapter 4). Since no data about adipose tissue thickness are available in the aforementioned studies, it remains unclear whether the paradoxical results found in metabolic and mitochondrial myopathies are differences based on the metabolic defect or on adipose tissue thickness.

Instead of exercise at one exercise intensity, Gellerich et al. [Gellerich et al. 1998] used an incremental exercise test to investigate three subjects with suspected muscle disease (Table 1) and twelve healthy non-athletes. Patients and controls performed an intermittent exercise protocol on a bicycle ergometer with periods of 1.5 min of cycling followed by 3 min of rest. The workload was set to 20 W and increased by each subsequent period until the maximum workload was reached. This study showed that oxygenation instead of deoxygenation during exercise was not a typical feature of metabolic or mitochondrial myopathies, but could also be present in healthy subjects. The load dependent changes in oxygenation of healthy subjects showed that, at workloads below 75 W, a steady state level of tissue oxygenation was preceded by a short period of increased oxygenation. This relative oxygenation that was evident at low work intensities was called the "warm-up" effect and was observed in all controls, but one. With increasing workload the amount of oxygenation decreased and shifted to deoxygenation at sufficiently high workload.

Concerning the myopathic patients that were investigated in this study (Table 1), the patient with exercise-induced myalgia was within the limits of the controls and this was in agreement with her maximal workload and the mitochondrial function as investigated biochemically. The patient with proximal weakness and pseudohypertrophy of the calves showed no warm-up effect (= oxygenation) at all and had abnormal deoxygenation at low workloads. In the patient with external ophthalmoplegia, hearing loss, mental impairment, and increased muscle fatigability on exercise, the initial oxygenation was abnormally high and did not shift to deoxygenation at all. Both latter patients showed less activity of complex I and cytochrome c oxidase as well as lower respiration rates.

As might be concluded from these studies, the clinical features measured by NIRS are not crisp and clear yet since the abnormalities described are not uniform and not present in all individual patients. Moreover, the number of patients investigated has been invariably low. Therefore, we have chosen for a quantitative NIRS approach instead of a *qualitative* approach and measured oxygen consumption and blood flow at rest and during exercise in patients with neuromuscular disorders and, in particular, mitochondrial myopathies.

In this thesis, we have investigated a homogeneous group of chronic progressive external ophthalmoplegia (CPEO), a mitochondrial myopathy, and measured oxygen consumption and blood flow at rest as well as during low intensity isometric handgrip exercise (Chapter 8). Chapter 9 describes the measurement of oxygen consumption and the rate of reoxygenation in a patient with dermatomyositis, performing rhythmic isometric handgrip exercise at a broad range of exercise intensities. Furthermore, we investigated muscle oxygenation in a large group of Friedreich ataxia patients during aerobic and ischemic isometric calf exercise (Chapter 5).

MITOCHONDRIAL MYOPATHIES

Mitochondrial myopathies are neuromuscular disorders caused by functional or structural abnormal mitochondria. The mitochondria are considered as the "power plants" of the cell and provide roughly 90% of the energy that is needed for the functionality of the body. Mitochondrial ATP production occurs by means of oxidative phosphorylation, a complex system consisting of many proteins that are clustered in five multiprotein complexes (Fig. 1). When mitochondrial function is impaired, as in mitochondrial myopathies, ATP production will be impaired. This becomes especially evident in tissues that have a high energy turnover such as brain, kidney, and muscle tissue. Defects in the oxidative phosphorylation system result, therefore, in devastating, mainly multisystem, diseases [Smeitink et al. 2001].

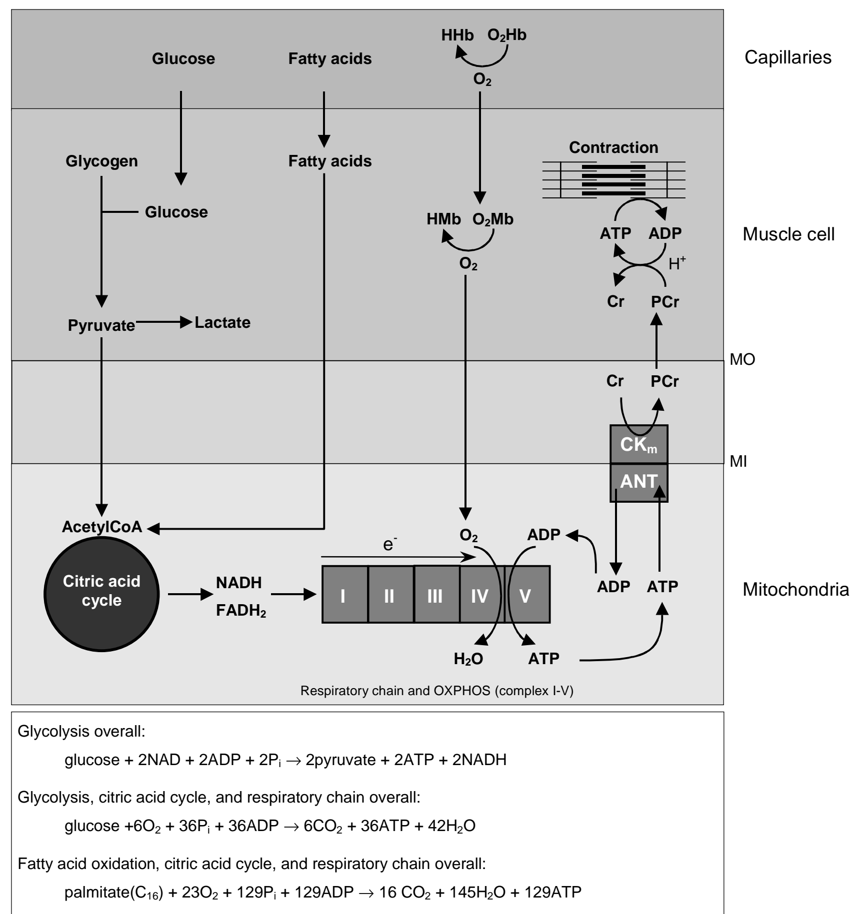

Fig. 1. Strongly simplified scheme of the pathway of oxidative phosphorylation (OXPHOS) from blood born substrates to adenosine triphosphate (ATP). The respiratory chain is presented by the roman-numbered squares, existing of five complexes. Electrons are transported from complex I to complex IV where they are adopted by O_2 to form H_2O. ATP then, is formed from adenosine diphosphate (ADP) by complex V, (ATPsynthase). Subsequently, phosphocreatine (PCr) can be resynthesised from creatine (Cr) by the adenine nucleotide translocator (ANT) and mitochondrial creatine kinase (CK_m). MO; mitochondrial outer membrane, MI; mitochondrial inner membrane.

Neuromuscular disorders

Chronic Progressive External Ophthalmoplegia (CPEO):

CPEO is the most frequent form of adult mitochondrial myopathies. Ptosis and external ophthalmoplegia are usually the first clinical signs because mitochondrial volume of the extraocular muscles is several times greater than that of other skeletal muscle. Bindoff et al. 1997: in addition to ophthalmoplegia, most patients have mild proximal myopathy. Patients might have pigmentary retinopathy, ataxia, sensory neural deafness, mild pyramidal or extrapyramidal features. The onset might be as late as the fifth or sixth decade. Muscle biopsy shows cytochrome oxidase (COX) negative fibres and ragged red fibres (RRF). Mitochondrial DNA (mtDNA) analysis will show a single large scale rearrangement in approximately 50% (sporadic cases). In a significant proportion of the other patients, point mutations of mtDNA can be defined.

Dermatomyositis:

Dermatomyositis is an inflammatory muscle disease with the identifying clinical features of erythema of the skin in combination with muscle weakness and pain (Engel et al. 1990). It is a multisystem disorder that can occur at any age from infancy to late adult onset. Apart from skin and muscle, dermatomyositis is associated with many other organs and systems (e.g. heart and lungs) as well as with malignancy (Hill et al. 2001). The cause of dermatomyositis is still unknown, but a strong indication for autoimmune mechanisms exists (Dalakas 1998). Dermatomyositis is characterised by complement mediated capillary necrosis, resulting in ischemia and hypoperfusion. The pathological features consist of a decreased number of capillaries per muscle fibre and necrosis of single muscle fibres or clusters of fibres at the periphery of the fasciculi (Engel et al. 1990). Although the mechanism is not completely understood, it is thought that the muscle fibre injury could be secondary to ischemia caused by the decrease in capillary density. Muscle fibre regeneration and an increased number of capillaries were shown after intravenous immune globulin treatment (Dalakas et al. 1993), but corticosteroids are still considered to be the first line of therapy.

Friedreich ataxia:

Friedreich ataxia (FRDA) is a progressive degeneration of the dorsal root ganglia, spinocerebellar tracts, corticospinal tracts and Purkinje cells of the cerebellum. It is characterised by a gradual loss of coordination, usually the earliest and most prominent characteristic of the disease. Increasing impairment of balance and movement eventually lead to the loss of the ability to walk. It is also associated with cardiomyopathy. Friedreich Ataxia is an autosomal recessive neurodegenerative disorder characterised by an expanded (GAA)n trinucleotide repeat in intron 1 of the frataxin gene on chromosome 9q13 (Campuzano et al. 1996). It is the most common hereditary ataxia occurring with a frequency of 1 in 50 000 in the Caucasian population. Frataxin is associated with the inner membrane of the mitochondria, and mutations in the frataxin gene are thought to lead to impaired mitochondrial function. The actual function of Frataxin remains to be elucidated.

Although mitochondrial myopathies are phenotypically and genetically heterogeneous disorders, they share exercise intolerance, undue fatigue and lactic acidosis during low- to moderate-intensity work [Taivassalo et al. 1998]. Other clinical features that reflect the multi-system nature of the disorder include heart failure or rhythm disturbances, dementia, movement disorders, stroke-like episodes, deafness, blindness, vomiting, and seizures, but these are not uniformly presented. Although mitochondrial myopathies are rare, they may mimic a large number of other disorders because of this highly variable clinical expression. As a consequence, they are frequently considered in differential diagnoses [Poulton 1998].

The diagnostic confirmation of mitochondrial myopathies is difficult due to the aspecific clinical symptoms, and requires investigation of muscle biopsy samples for histology, enzyme studies, and molecular genetics. However, muscle biopsies are invasive and expensive, and a definite diagnostic confirmation is not always achieved. As for noninvasive methods, phosphorus magnetic resonance spectroscopy (^{31}P-MRS) is sometimes used in neuromuscular disorders, but is expensive and its availability is limited.

Exercise tests provide a valuable tool to detect patients with a suspected mitochondrial myopathies since these patients share the characteristics of exercise intolerance, undue fatigue and lactic acidosis during low- to moderate intensity work. However, exercise can be potentially hazardous in these patients, due to associated cardiac conduction abnormalities reported e.g. in chronic progressive external ophthalmoplegia (CPEO) [Bindoff et al. 1997, Poulton 1998]. Apart from that, a high percentage of patients is incapable to perform whole-body exercise because they are severely affected or simply too young. The development of a noninvasive, widely available screening test that is capable to detect patients with mitochondrial myopathies is, therefore, of utmost importance. As mentioned before, NIRS could be a potential tool in the development of such a test as it monitors oxygenation directly in the muscle and is noninvasive, inexpensive, and easy applicable.

In this thesis, we have investigated whether NIRS is able to discriminate mitochondrial myopathies from normal muscle by measuring muscle O_2 consumption and forearm blood flow in a group of patients with chronic progressive external ophthalmoplegia (CPEO) and comparing them with healthy controls (Chapter 8). Using NIRS and ^{31}P-MRS as complementary methods, we investigated patients with Friedreich ataxia (FRDA), a different form of a mitochondrial cytopathy, in order to monitor changes in both oxidative and glycolytic kinetics during exercise and recovery (Chapter 5). Another clinical application of NIRS in neuromuscular metabolic disorders is the measurement of variables based on O_2 delivery instead of O_2 consumption. Microvascular reoxygenation after exercise was measured in dermatomyositis, a muscle disorder that is characterised by complement mediated capillary necrosis, resulting in ischemia and hypoperfusion (Chapter 9).

8

Quantitative Near Infrared Spectroscopy Discriminates between Mitochondrial Myopathies and Normal Muscle

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SUMMARY

Five patients with chronic progressive external ophthalmoplegia (CPEO) and 27 healthy controls were examined by near-infrared spectroscopy (NIRS) for the noninvasive and direct quantitative measurement of muscle oxygen consumption and forearm blood flow. NIRS measurements were obtained at rest and during static isometric handgrip exercise at 10% of the maximum voluntary contraction (MVC) force. A significantly decreased oxygen consumption at rest as well as during exercise was found in patients with CPEO. Our results suggest that NIRS is able to discriminate between CPEO patients and healthy controls, which makes NIRS a valuable tool in the diagnostic workup of patients suspected to have a mitochondrial myopathy.

INTRODUCTION

Although mitochondrial myopathies are phenotypically and genetically heterogeneous disorders, they share exercise intolerance, undue fatigue, and lactic acidosis during low- to moderate-intensity work [Taivassalo et al. 1998]. As a result of these common characteristics, exercise tests are used to evaluate metabolic and especially mitochondrial myopathies [Argov et al. 1997, Haller et al. 1990].

Even apart from the very young and the severely affected patients who are incapable of performing exercise, exercise tests can be potentially hazardous in patients with mitochondrial myopathies because of associated cardiac involvement [Bindoff et al. 1997, Poulton 1998]. Nevertheless, patients with mitochondrial myopathies have almost exclusively been tested during whole-body exercise. The isolated muscle *in vivo* has been monitored by magnetic resonance spectroscopy (MRS) [Argov 1998], but no muscle studies have directly and quantitatively examined oxygen uptake and oxygen delivery in resting and exercising muscle of patients with mitochondrial myopathies.

The aim of the present study was, therefore, to investigate whether near-infrared spectroscopy (NIRS), a direct, noninvasive method of tissue oximetry, could discriminate between a group of patients with chronic progressive external ophthalmoplegia (CPEO) and healthy controls. Furthermore, our goal was to develop a test that is functional in the wide range of patients suspected of having a mitochondrial myopathy.

PATIENTS AND METHODS

We studied a group of 5 patients with varying clinical severity of sporadic CPEO diagnosed according to established criteria (Table) [Bindoff et al. 1997]. CPEO patients were compared with 27 healthy controls. As described previously in detail [Van Beekvelt et al. 1998], patients and controls were measured at rest as well as during static isometric handgrip exercise at a workload equal to 10% of the subject's maximum voluntary contraction (MVC) force. NIRS was used for the quantitative measurement of oxygen consumption and forearm blood flow.

NIRS is a noninvasive optical method for continuous monitoring of oxygenation and haemodynamics in tissue. It is based on the relative tissue transparency to light in the near-infrared region, and on the oxygen-dependent absorption changes of haemoglobin and myoglobin. Using a modified Lambert-Beer law, in which a physical path-length is incorporated to account for light scattering, it is now possible to calculate quantitative values for oxygen consumption and blood flow in skeletal muscle [Ferrari et al. 1997].

NIRS measurements were obtained with a continuous wave near-infrared spectrophotometer (Oxymon, Biomedical Engineering Department, University of Nijmegen, NL). Using this spectrophotometer that generates light at 905, 850, and 770 nm [Van der Sluijs et al. 1998], it is possible to differentiate between oxyhaemoglobin/

Table. Clinical characteristics of patients with sporadic chronic progressive external ophthalmoplegia

No	Sex/age (yr)	MVC (N)	Disease duration (yr)	Fatigue[a]	Prox. Muscle Strength (MRC)	Modified Rankin scale[b]	Muscle Biopsy RRF[c]	Serum lactate[d] (μmol/L)
1	M/54	132	28	-	4/5	2	+	1520
2	M/19	262	3	+	5/5	2	++	1586
3	F/36	168	4	-	5/5	2	++	1288
4	F/42	153	22	+++	3/5	3	++	844
5	F/54	100	27	++	3/5	3	+	2660

[a] : - = not present; + = mild; ++ = moderate; +++ = severe
[b] : Modified Rankin scale described by Swieten et al. [van Swieten et al. 1988]
[c] : RRF = ragged red fibres; + = present; ++ = abundant
[d] : Normal values: < 1700 μmol/L
M = male; F = female; MVC = maximal voluntary contraction force; MRC = medical research council

myoglobin (O_2Hb/O_2Mb) and deoxyhaemoglobin/myoglobin (HHb/HMb). The sum of O_2Hb and HHb reflects the total amount of haemoglobin/myoglobin (tHb/tMb). Quantitative NIRS values for oxygen consumption were calculated by evaluating the rate of decrease in [O_2Hb] during arterial occlusion (Fig.1A). Forearm blood flow was calculated from NIRS data by evaluating the rate of increase in [tHb] during venous occlusion [Van Beekvelt et al. 1998] (Fig. 1B). The optical fibres were placed on top of the flexor digitorum superficialis muscle with an interoptode distance of 35 mm. The sample time was 0.1 s and all data were displayed in real time and stored on disk for analysis.

The CPEO patients were compared with healthy controls using Student's *t*-test. A paired *t*-test was used for differences between rest and exercise within each group. A *P* value less than 0.05 was considered to be statistically significant. All results are reported as mean ± SE.

RESULTS

The maximum voluntary contraction force (MVC) was 163 ± 61 Newton (N) for the CPEO group and 286 ± 62 N for the control group. We found a significantly lower oxygen consumption at rest (P = 0.02) in combination with a significantly higher blood flow (P = 0.002) in the CPEO group (respectively 0.063 ± 0.027 $mlO_2{\cdot}min^{-1}{\cdot}100g^{-1}$ and 1.344 ± 0.264 $ml{\cdot}min^{-1}{\cdot}100ml^{-1}$) as compared with the control group (0.106 ± 0.006 $mlO_2{\cdot}min^{-1}{\cdot}100g^{-1}$ and 0.719 ± 0.063 $ml{\cdot}min^{-1}{\cdot}100ml^{-1}$, respectively) (Fig. 2A). This difference between CPEO and controls, concerning the low oxygen consumption in

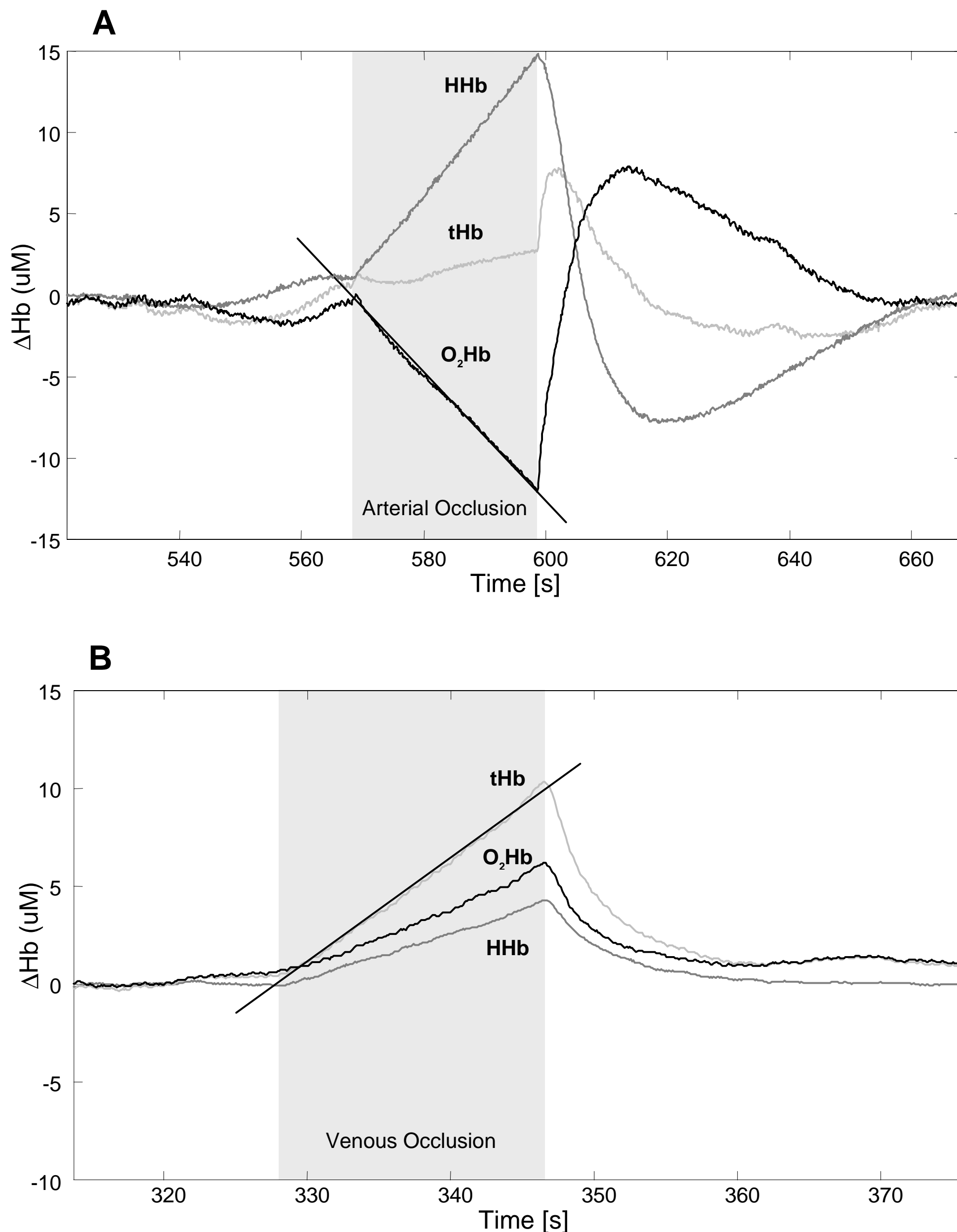

Fig. 1. Representative raw NIRS data as displayed real time on screen. Oxyhemoglobin (O_2Hb), deoxyhaemoglobin (HHb), and the sum of O_2Hb and HHb, reflecting total haemoglobin (tHb), are shown during A) arterial occlusion (240 mmHg) and B) venous occlusion (50 mmHg). Oxygen consumption is calculated during arterial occlusion as the rate of decrease in O_2Hb indicated by the straight line in Fig. A. Forearm blood flow is calculated during venous occlusion as the rate of increase in tHb indicated by the straight line in Fig. B.

combination with high blood flow, was even more pronounced during low-intensity exercise at 10% MVC (Fig. 2B). During exercise, oxygen consumption was 0.262 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ (± 0.107) in the CPEO group as compared with 0.570 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ (± 0.052) in the control group (P = 0.03). Forearm blood flow in the CPEO group was 2.422 $ml \cdot min^{-1} \cdot 100ml^{-1}$ (± 0.702) as compared with 0.984 $ml \cdot min^{-1} \cdot 100ml^{-1}$ (± 0.090) in the control group (P = 0.003).

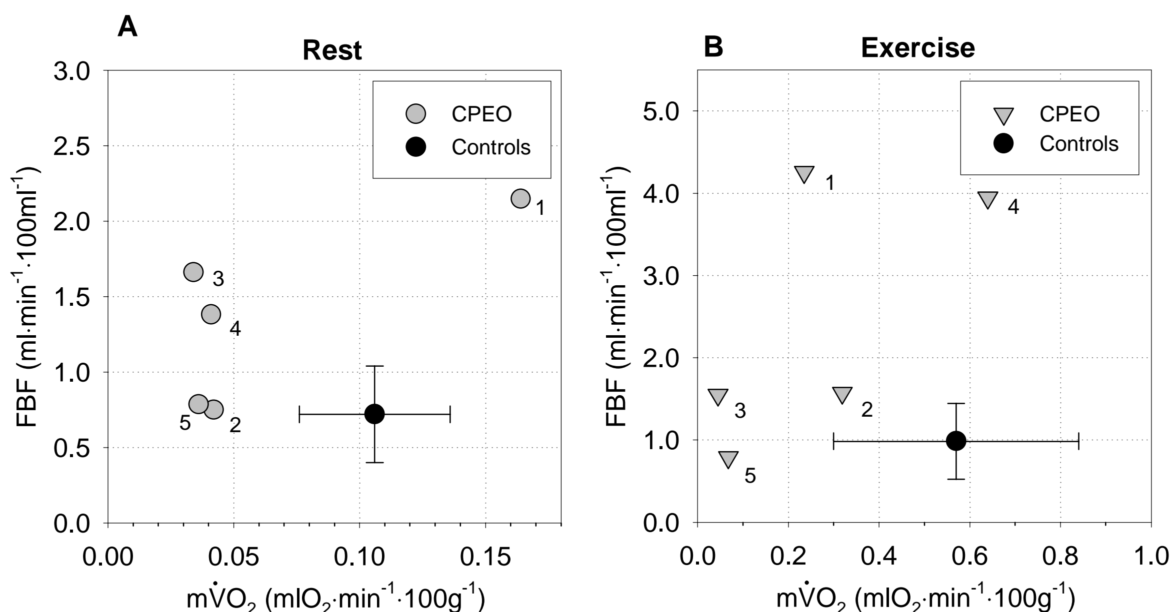

Fig. 2. Near infrared spectroscopy values for muscle oxygen consumption ($m\dot{V}O_2$) and forearm blood flow (FBF) in patients with chronic progressive external ophthalmoplegia (CPEO) and healthy control subjects A) at rest and B) during static isometric handgrip exercise at 10% of the subject's maximum voluntary contraction force (MVC). Individual values of $m\dot{V}O_2$ and FBF are shown for the patients with CPEO. Mean ± **SD** *in both directions are shown for the control group.*

DISCUSSION

Mitochondrial myopathies are highly variable in clinical expression and may mimic a large number of other disorders. As a consequence, they are frequently considered in differential diagnoses, but are rarely confirmed [Poulton 1998]. The diagnostic confirmation of mitochondrial myopathies requires definite investigations by enzyme studies in muscle biopsy samples, muscle histology, phosphorus magnetic resonance spectroscopy (^{31}P-MRS), and molecular genetic techniques. Molecular genetics identify only known defects, sensitivity of muscle histology is limited, and routine use of ^{31}P-MRS is restricted by its limited availability [Argov et al. 1997].

Exercise tests provide a valuable tool to detect patients with a suspected mitochondrial myopathy, as these patients share the characteristics of exercise intolerance, undue fatigue, and lactic acidosis during low- to moderate-intensity work. However, exercise can be potentially hazardous in these patients, as a result of associated cardiac conduction abnormalities reported, for example, in CPEO [Bindoff et al. 1997, Poulton 1998]. Apart from that, there are patients who are incapable to perform exercise because they are severely affected or simply too young. This is why the development of a noninvasive widely available screening test that is functional in the range of patients suspected of having mitochondrial myopathies is of utmost importance.

In the present study, NIRS was used for the noninvasive direct examination of muscle oxygen consumption and forearm blood flow in patients with CPEO. Static isometric handgrip exercise was chosen to include all patients independent of disease status. We found a decreased oxygen consumption in the CPEO group compared with controls, in combination with an increased forearm blood flow present in both low-intensity exercise and at rest. Our NIRS data on oxygen consumption during exercise are compatible with those of previous reports [Abe et al. 1997, Bank et al. 1994] and support the hypothesis of a hyperkinetic circulatory response to exercise in mitochondrial myopathies [Haller 1994]. Furthermore, our data show that this upregulated flow, compared with that in controls, is also present at rest (see Fig.). This mismatch between O_2 delivery and consumption might function as a compensatory mechanism for the impaired oxygen consumption in CPEO muscle.

The present data on decreased oxygen consumption in CPEO as measured by NIRS during low-intensity work as well as at rest suggest that NIRS can serve as a highly valuable and promising tool in the diagnostic workup of patients with mitochondrial myopathies of varying severity. Because NIRS is able to measure within the muscle, it provides unique information about local tissue oxygenation and might reveal new facts about the process of mitochondrial myopathies.

POSTSCRIPTUM:

NEAR-INFRARED SPECTROSCOPY IN CPEO: ADIPOSE TISSUE THICKNESS CONFOUNDS DECREASED MUSCLE OXYGEN CONSUMPTION

Published as *Letter* by Van Beekvelt MCP, Van Engelen BGM, Wevers RA, and Colier WNJM in *Ann Neurol* 51(2): 273-274, 2002

Near-infrared spectroscopy (NIRS) is a noninvasive optical method for continuous monitoring of oxygen consumption and haemodynamics in tissue (e.g. muscle tissue). Bank and Chance [Bank et al. 1994] were the first to use NIRS for the investigation of metabolic myopathies. They found specific abnormalities in a variety of metabolic myopathies, indicating abnormal oxygen utilisation. Several other investigators followed, including our group [Van Beekvelt et al. 1999], and reported similar abnormalities in mitochondrial myopathies as compared with normal muscle.

However, NIRS is still a relatively new technique, and methodological constraints have not yet been fully exploited. We have investigated several methodological aspects of *in vivo* NIRS measurements, and one of these concerned the influence of adipose tissue thickness (ATT) on *in vivo* NIRS measurements. From this study it became clear that *in vivo* NIRS measurement of quantitative muscle oxygen consumption ($m\dot{V}O_2$) was

confounded by the thickness of the subcutaneous fat layer separating the light fibres from muscle tissue [Van Beekvelt et al. 2001a]. Resting $m\dot{V}O_2$ in the 10 leanest subjects was twice as high as $m\dot{V}O_2$ in the 10 subjects with the highest ATT (r = -0.70, $P \leq 0.01$).

In the light of these new data, we reinvestigated our previous published data on patients with chronic progressive external ophthalmoplegia (CPEO) by incorporation of skinfold thickness, measured between the NIRS optodes using a skinfold caliper, in order to compare CPEO patients with the large subject pool that we have described previously [Van Beekvelt et al. 2001a].

The results of this additional analysis led to the conclusion that the major part of the difference found between CPEO patients and their controls [Van Beekvelt et al. 1999] can be explained by the confounding effect of ATT. As can be seen in the Figure, four out of five CPEO patients had a high ATT while the majority of the controls (77%) had an ATT less than 5.0 mm. Although $m\dot{V}O_2$ in the patients was low, it was not low enough to discriminate the individual patients from the controls, since their values were still within the 95% confidence interval.

The present results, obtained by combining our previously reported CPEO study [Van Beekvelt et al. 1999] with our recently published data on ATT [Van Beekvelt et al. 2001a], underscore the high significance of incorporating ATT into all future NIRS studies. Providing information about ATT is absolutely necessary in comparisons of groups, or data from literature. Moreover, because measurement of skinfold thickness is easy and fast, it should become a standard routine in every *in vivo* NIRS measurement.

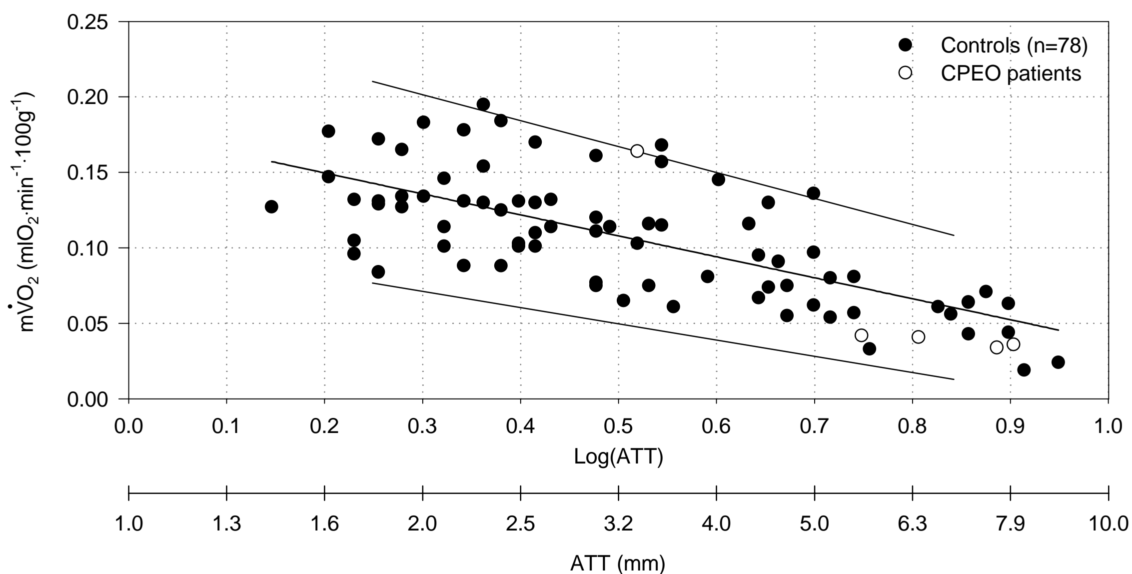

Fig. Correlation between log(ATT) and NIRS muscle oxygen consumption ($m\dot{V}O_2$) at rest, measured in 78 healthy controls and 5 CPEO patients. The straight lines represent regression line and 95% confidential interval.

9

Muscle tissue oxygenation as a functional tool in the follow-up of dermatomyositis

Van Beekvelt MCP, Colier WNJM, Wevers RA, Van Engelen BGM

J Neurol Neurosurg Psychiatry, in press

SUMMARY

Near-infrared spectroscopy (NIRS) is a direct, noninvasive optical method for the measurement of local oxygenation and haemodynamics in muscle tissue. Although it has recently been used for diagnosis of metabolic myopathies, it has until now never been used in inflammatory myopathies. Because dermatomyositis is a muscle disorder characterised by complement mediated capillary necrosis, resulting in ischemia and hypoperfusion, we have used NIRS to study the effect of corticosteroid treatment in dermatomyositis. Clinical improvement correlated with NIRS, showing an increase in local muscle oxygen consumption and reoxygenation rate, indicating normalisation of microvascular function. This study shows that NIRS is able to detect therapy effect; direct, noninvasively, and with relative ease.

The pathological features of dermatomyositis are characterised by a decreased number of capillaries per muscle fibre and necrosis of single muscle fibres or clusters of fibres at the periphery of the fasciculi [Engel et al. 1990]. Although the mechanism is not completely understood, it is thought that the muscle fibre injury could be secondary to ischemia caused by the decrease in capillary density.

Muscle fibre regeneration and an increased number of capillaries were shown after intravenous immune globulin treatment [Dalakas et al. 1993], but corticosteroids are still considered to be the first line of therapy. In clinical setting, the effect of therapy is mainly determined by muscle strength and creatine kinase (CK) levels. Direct measurement of capillary and muscle fibre status can only be acquired by repeated muscle biopsies, but apart from the fact that muscle biopsies are invasive, they reflect a static representation of muscle tissue at a fixed time point and a certain localisation (selection bias).

This is the first time that near-infrared spectroscopy (NIRS), a noninvasive optical method for the measurement of oxygenation and haemodynamics in muscle tissue, was used to study the effect of therapy in a patient fulfilling the clinical and histological criteria for definite dermatomyositis [Dalakas 1991]. A young woman from Aruba, aged 24 years, presented with subacute erythema of the facial skin and severe proximal muscle weakness (arm muscles: Mean Medical Research Council (MRC) grade 3, leg muscles: MRC 2). Serum CK levels were slightly increased (220 U/L). 5 weeks after the start of symptoms, treatment with corticosteroids was started at our department at a dose of 60 mg/day (for 6 weeks). Subsequently, the dose was tapered. CK levels decreased and muscle strength increased (arm muscles: MRC 4, leg muscles: MRC 3) in week 12.

Tissue oxygenation was measured by NIRS just prior to medication and again after 3 and 7 weeks of treatment. Localisation of the light fibres, on top of the flexor digitorum superficialis muscle, was the same for all measurements. NIRS muscle oxygen consumption ($m\dot{V}O_2$) was calculated during arterial occlusion as previously described [Van Beekvelt et al. 2001b]. Reoxygenation rate (ΔO_2Hb) was determined as the rate of initial increase in O_2Hb measured over 3 seconds immediately after cessation of arterial occlusion. Both $m\dot{V}O_2$ and ΔO_2Hb were calculated at rest and following rhythmic isometric handgrip exercise at various work intensities (Fig. 1). Each exercise session consisted of 1 min exercise at a contraction rate of $30 \cdot min^{-1}$ (50% duty cycle) immediately followed by 45 s of arterial occlusion for calculation of $m\dot{V}O_2$ and ΔO_2Hb. Whereas $m\dot{V}O_2$ is a measure for mitochondrial function at a certain workload and is dependent on the vascular capacity of oxygen delivery, ΔO_2Hb reflects the initial recovery rate at which deoxygenated haemoglobin/myoglobin are resaturated [Chance et al. 1992] and is, therefore, directly related to microvascular function. All measurements were performed at the same absolute work intensities.

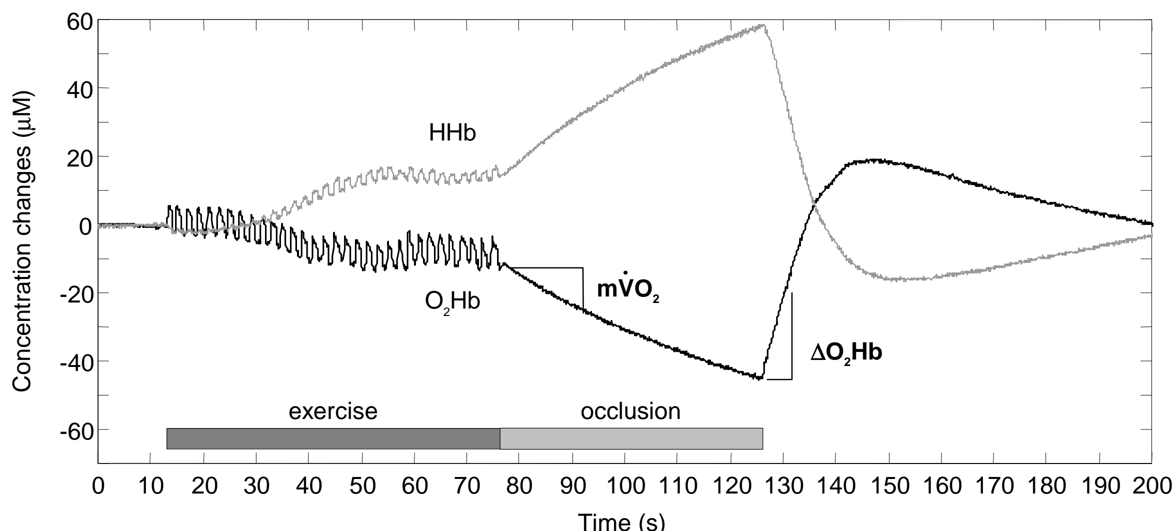

Fig. 1 NIRS oxyhaemoglobin (O_2Hb) and deoxyhaemoglobin (HHb) signal during exercise and occlusion. Muscle oxygen consumption ($m\dot{V}O_2$) is calculated from the initial rate of linear increase in HHb during arterial occlusion. Reoxygenation rate (ΔO_2Hb) is determined as the rate of increase in O_2Hb over the initial 3 s after cessation of arterial occlusion.

Fig. 2 shows the effect of therapy measured noninvasively and with relative ease by NIRS during corticosteroid treatment in the patient with severe dermatomyositis. Prior to medication, resting $m\dot{V}O_2$ in the patient was slightly higher as compared with healthy controls (0.19 vs. 0.14 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$, respectively). However, $m\dot{V}O_2$ during exercise was roughly 60% lower than that of the controls over the whole range of exercise intensities (Fig. 2A). After 3 weeks of therapy, $m\dot{V}O_2$ had already markedly increased. After 7 weeks of therapy, $m\dot{V}O_2$ had increased even further and was now only 25% lower than that of the controls and within the normal range at several work intensities. Serum CK levels were normalised while muscle strength had increased. ΔO_2Hb (Fig. 2B) showed similar results with slow recovery rates prior to medication and an increase over all workloads after both 3 and 7 weeks of medication. ΔO_2Hb after 7 wks of medication even exceeded normal values.

Since NIRS measures local oxygenation and haemodynamics within muscle, it can give direct insight in the working microvascular system. ΔO_2Hb increased during therapy, indicating an increase in capillary function. As a result of the increased capillary function and a possible regeneration of muscle fibres, muscular oxygen availability increased, enhancing oxidative capacity as reflected by the increase in local muscle oxygen consumption.

Although a muscle biopsy will remain indispensable for the diagnosis of dermatomyositis, NIRS is an interesting and noninvasive tool in monitoring therapy

effect. While both serum CK levels and muscle strength are indirect measures and muscle biopsy samples reflect a static fingerprint of the muscle, NIRS directly measures local microvascular and mitochondrial function in the intact and working physiological setting.

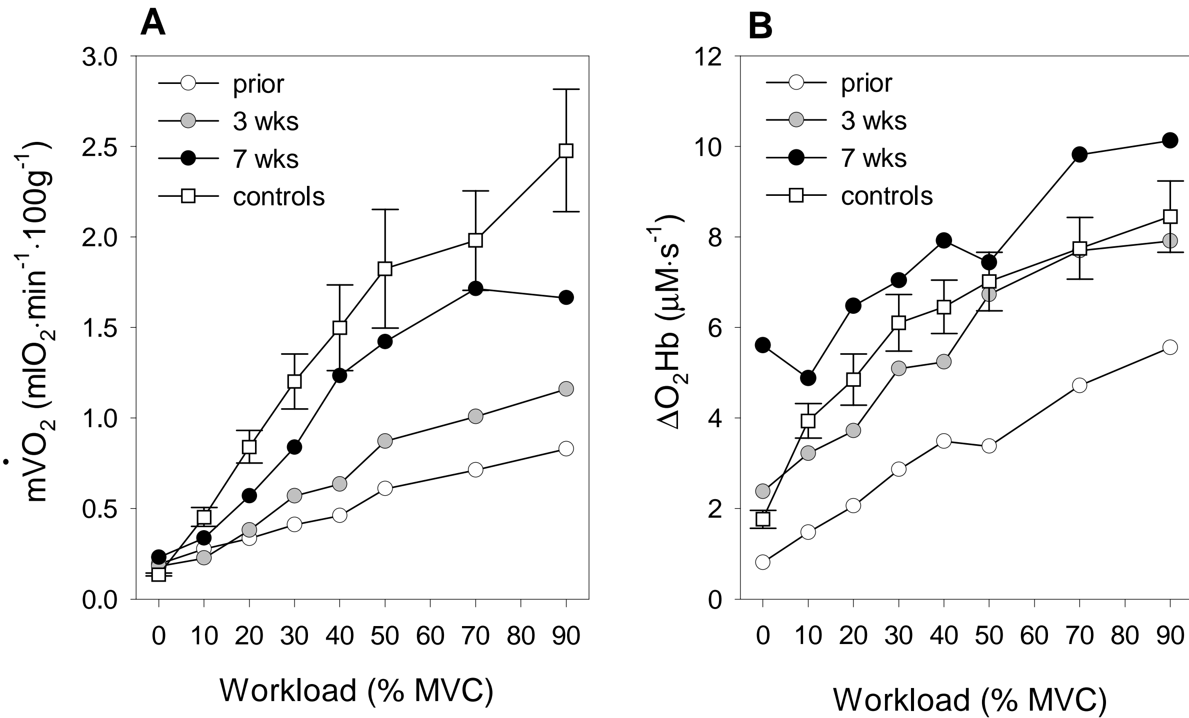

Fig. 2 Effect of therapy in a patient with severe dermatomyositis on A) muscle oxygen consumption ($m\dot{V}O_2$) and B) reoxygenation rate (ΔO_2Hb) measured noninvasively by NIRS at rest and following exercise at various levels of the maximum voluntary contraction force (MVC). Mean ± SD values are shown for the controls

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Summary and future perspectives

Outline

Thesis objectives
Methodological considerations
- *Nature of signal*
- *Path-length factor*
- *Haemoglobin vs. myoglobin*
- *Skinfold thickness*
- *Venous vs. arterial occlusion method*
- *Increase tHb during arterial occlusion*

Clinical considerations
- *Sensitivity/specificity*
- *Quantitative measurement*
- *Clinical applicability*

Future perspectives
- *Bio-energetic characterisation*
- *Effect of therapy*
- *Multi-channel NIRS*
- *Pathophysiology of myopathies*

THESIS OBJECTIVES

The aim of this thesis was twofold. The *first objective* was to investigate methodological aspects necessary for the legitimate use of NIRS in the investigation of normal and pathological states of *in vivo* muscle metabolism. This goal was pursued in the experiments described in Chapters 2 - 5. The first step that we took was an attempt to validate quantitative NIRS measurements for muscle oxygen consumption ($m\dot{V}O_2$) and forearm blood flow (FBF) by comparison with the well-established Fick method, combining blood gas analysis, tissue oximetry, and strain-gauge plethysmography (Chapter 2). However, direct comparison proved impossible since both methods have different measurement sites. Whereas Fick measures regional changes in the total limb, NIRS measures local changes, directly in the muscle. Consequently, NIRS can detect local differences based on muscle activity that are not detectable by the Fick method.

Subsequently, we investigated the reproducibility of NIRS $m\dot{V}O_2$ at rest as well as during exercise and proved that $m\dot{V}O_2$ was reproducible over several days, both at rest as well as during exercise (Chapter 3). The reproducibility of NIRS $m\dot{V}O_2$ and FBF within one test session proved reliable as well (Chapter 2 and 4).

Another important methodological aspect studied, was based on the optical characteristics of the NIRS technique. Propagation of the light within the tissue is complex as it travels through multiple layers of the inhomogeneous medium. Individual differences in optical properties are, therefore, likely to affect NIRS measurements. We thus studied the influence of adipose tissue thickness (ATT) on *in vivo* NIRS measurements in a large group of healthy subjects and found that ATT has a substantial confounding effect on quantitative *in vivo* NIRS measurements (Chapter 4). These findings underscore the high significance of incorporating ATT in all future NIRS studies.

Furthermore, we studied muscle metabolism by the complementary use of NIRS and phosphorus magnetic resonance spectroscopy (^{31}P-MRS) which is nowadays considered to be the "gold standard" of noninvasive measurement of human muscle metabolism (Chapter 5). Although this method can provide indirect information about oxidative metabolism, it requires ischemic exercise to do so. NIRS provides direct measurement of oxygen consumption, obtained at rest, and during aerobic and ischemic exercise as well. The combination of ^{31}P-MRS and NIRS enabled simultaneous measurement of both the kinetics of high energy phosphates and oxidative processes of muscle metabolism within the same muscle volume of interest, and showed that depletion of oxygen during ischemic exercise might take substantially longer than is commonly assumed. This study indicates the high potential of combining both methods in the investigation of muscle metabolism in physiological as well as pathological situations.

Finally, we have combined all resting values of our healthy controls in order to obtain information about normal range and biological variability of the different quantitative variables that can be derived from *in vivo* NIRS measurements (Chapter 6).

With the aforementioned studies we clarified some important methodological aspects of *in vivo* NIRS measurements. NIRS has shown to be a reproducible method for the investigation of local oxygenation and haemodynamics in muscle tissue. It is able to detect local differences in muscle activity, is applicable at rest as well as under various exercise conditions, and has a high potential in the complementary use with ^{31}P-MRS, all as long as the confounding factor of ATT is taken into close consideration.

The *second objective* was to investigate the potential applicability of NIRS in neuromuscular disorders, in order to develop a noninvasive screeningstest that is functional in a wide range of patients suspected to have a metabolic/mitochondrial myopathy. We started by testing whether NIRS is able to distinguish patients with a proven mitochondrial defect (chronic progressive external ophthalmoplegia (CPEO)) from a group of healthy controls (Chapter 8) and found that oxygen consumption was decreased in the patient group while blood flow was increased. This was in agreement with the mismatch between O_2 delivery and O_2 utilisation that was previously described in literature [Haller 1994]. However, the function of a screeningstest is to discriminate individual patients from healthy controls and although both groups could be discriminated from each other, only three out of five individual patients were outside the 95% confidence interval. Moreover, when these results were combined, later on, with the results concerning the confounding effect of adipose tissue thickness on *in vivo* NIRS measurements (Chapter 4), it turned out that a substantial part of the difference between patients and controls had to be ascribed to the difference in adipose tissue thickness. Although most patients still had low consumption rates, they were now within the 95% confidence interval of the controls (Chapter 8 postscriptum).

Monitoring of the disease status over time or the effect of training or therapy is another possibility for *in vivo* NIRS measurements. The advantage of such a longitudinal design is that the patient can be his/her own control, thereby avoiding items like between-subject variability and confounding factors like ATT. We have used NIRS to study the effect of medication in dermatomyositis, an inflammatory myopathy that is characterised by a microvasculopathy, and were able to detect improvement of $m\dot{V}O_2$ and haemodynamics after three and seven weeks of medication, corresponding to the clinical findings (Chapter 9).

In the investigation of patients with neuromuscular disorders NIRS has shown that it can discriminate a group of patients with mitochondrial defects from healthy controls. Although these patients had proven mitochondrial defects, it is not known whether these defects directly affect mitochondrial O_2 consumption or mitochondrial ATP formation. NIRS was not sensitive enough to discriminate individual CPEO patients from controls,

but might do so for patients with other metabolic/mitochondrial myopathies where mitochondrial O_2 consumption or oxidative ATP-production is more severely affected such as in MELAS or Leigh syndrome.

The use of NIRS as a noninvasive tool for monitoring of therapy effects proved successful in dermatomyositis. Treatment is well established for this muscle disorder, but this is not the case for most other neuromuscular disorders. Apart from trials with vitamin supplements and substances like creatine and carnitine, there is as yet no treatment for mitochondrial myopathies.

METHODOLOGICAL CONSIDERATIONS

NIRS has proven to be an easy and valuable tool, applicable in the investigation of normal physiological as well as pathological situations. Due to the direct and local measurement of muscle tissue at rest and during exercise it can contribute in future research to clarify the gaps of *in vivo* human physiology and pathology.

NIRS has a number of significant advantages. It is noninvasive and measures oxygenation and haemodynamics directly in the muscle. The light intensity is very low and, therefore, harmless. It has a high sample frequency, up to 50 Hz, which enables measurement of fast components, for example, at the transition from rest to exercise and visa versa. Furthermore, it is relatively inexpensive, easy to apply, portable, and applicable at the bedside.

The disadvantage of NIRS is, in the first place, that it measures relative changes in concentrations instead of the absolute amounts of oxy- and deoxyhaemoglobin/myoglobin, but future technical developments can overcome this problem. Other items, like the nature of the signal, differential path-length factor, distinction between haemoglobin and myoglobin, confounding effect of ATT, are more or less considered as disadvantages of the technique although the relevance of some of those can be discussed.

Nature of signal

The maximum measurement depth of NIRS is thought to be roughly half the distance between source and detector [Cui et al. 1991, Homma et al. 1996b]. For an interoptode distance of 35 mm the light will, therefore, penetrate approximately 18 mm into the tissue where it is either scattered or absorbed. Most of the detected light will travel through muscle tissue, if the skinfold is not too thick. However, it is not completely clear from which vascular compartment the detected photons emerge. Currently, it is thought that NIRS measures changes in tissue oxygenation at the level of small blood vessels, capillaries, and intracellular sites of oxygen uptake [Chance et al. 1992, Hampson et al. 1988, Homma et al. 1996a, Mancini et al. 1994b] based on the fact that the photons are

almost completely absorbed in the larger arteries and veins due to the high concentration of erythrocytes, and thus haemoglobin [Chance et al. 1992, Mancini et al. 1994b]. However, a fundamental base for this hypothesis is still missing.

Path-length factor

Quantitation of changes in oxy- and deoxyhaemoglobin/myoglobin requires incorporation of a "differential path-length factor" (DPF) to account for scattering of the light within the tissue. The DPF for skeletal muscle has been measured by several investigators under different conditions and using different instrumentation [Delpy et al. 1988, Duncan et al. 1995, Essenpreis et al. 1993, Ferrari et al. 1992, van der Zee et al. 1992]. The average values found for DPF in the human forearm lie between 3.59 and 4.57. In this thesis, we have used a fixed value of 4.0 since direct measurement of the individual DPF is impossible using continuous-wave spectrophotometers. This way, however, correction for inter-individual differences in the scattering of light is not possible and might increase the between-subject variability. However, an important factor in determining the individual DPF is the adipose tissue thickness (ATT). As we have shown, ATT confounds *in vivo* NIRS measurements and a correction factor was provided for calculation of $m\dot{V}O_2$ and FBF (Chapter 4). If future research can reveal more information about the relationship between ATT and DPF it becomes possible to make a more accurate assumption of the individual DPF and incorporate this information directly in the algorithm.

Haemoglobin versus myoglobin

NIRS is unable to distinguish between changes in oxyhaemoglobin (O_2Hb) and oxymyoglobin (O_2Mb) or in deoxyhaemoglobin (HHb) and deoxymyoglobin (HMb) due to identical absorption spectra of Hb and Mb. Although there is no consensus yet about whether the NIRS signal originates from Hb [Seiyama et al. 1988, Wang et al. 1990] or Mb [Mole et al. 1999, Tran et al. 1999], this does not affect the results as presented in this thesis since we have focussed on the amount of O_2 consumed independently whether it came from Hb or Mb.

Skinfold thickness

As shown in Chapter 4, ATT substantially confounds the measurement of muscle oxygenation and haemodynamics using NIRS. The clinical relevance of this knowledge is described in Chapter 8 where a substantial part of the difference in oxygen consumption and forearm blood flow that was found between patients and controls had to be ascribed to the difference in adipose tissue thickness. These results underscore the high

significance of incorporating ATT into all future NIRS studies. Providing information about ATT is absolutely necessary to compare groups or data from literature. Moreover, because measurement of skinfold thickness is easy and fast, it should become a standard routine in every *in vivo* NIRS measurement.

Venous versus arterial occlusion method

In the past it has been suggested that the venous occlusion method is to be preferred over the arterial occlusion method because venous occlusion is less inconvenient for the subject, the recovery is much faster, and $m\dot{V}O_2$ and flow can be measured simultaneously [De Blasi et al. 1997, Homma et al. 1996a]. However, the venous occlusion is also more prone to always occurring variations in flow within the arm due to changes in blood pressure and local vasoreactivity whereas these influences are negligible during arterial occlusion based on the closed compartment, temporarily cut off from centrally mediated variations. As we showed in this thesis, $m\dot{V}O_2$ from venous occlusion appeared to be unreliable when repeated several times within one session (Chapter 2). In another study (Chapter 4), a coefficient of variation of 30.4% was found for the within-subject variability of resting $m\dot{V}O_2$ during venous occlusion while the coefficient of variation was much lower (16.2%) for the arterial occlusion method. Therefore, it is our opinion that the arterial occlusion method is to be preferred over the venous occlusion method for calculation of $m\dot{V}O_2$.

Increase tHb during arterial occlusion

Calculation of $m\dot{V}O_2$ using the arterial occlusion method can be derived from the rate of decrease in O_2Hb and from the rate of decrease in Hb_{diff}. When changes in O_2Hb and HHb are identical, but in opposite direction, then $m\dot{V}O_2$ calculated from O_2Hb and Hb_{diff} will also be identical. However, this can only be achieved when blood volume stays constant during the occlusion, and this is not always the case. An increase in tHb has been reported in several studies [Cheatle et al. 1991, Colier et al. 1995, Kooijman et al. 1997, Kragelj et al. 2000] and is addressed to a redistribution of blood during the occlusion. Although both methods are used in the literature, it is not clear whether they give similar results. As described in Chapter 2, we calculated $m\dot{V}O_2$ at various work intensities from both the rate of decrease in O_2Hb and Hb_{diff} and found a significant difference between both methods due to changes in tHb. This difference was negligible at low intensities, but became more pronounced during high intensity work. The increase in tHb results in a discrepancy between $m\dot{V}O_2$ calculated from O_2Hb and that calculated from Hb_{diff}. Since it is unknown whether the increase in blood volume originates from the arterial or venous side and, therefore, whether the extra volume contains mostly

O_2Hb or HHb, it remains unclear if the true consumption is reflected by the decrease in O_2Hb or by the increase in HHb and Hb_{diff} will give, when blood volume is not constant, either an underestimation (O_2Hb) or overestimation (HHb) of $m\dot{V}O_2$. However, it is our opinion that as long as the origin of the extra blood volume is not identified, Hb_{diff} is the best choice for calculation of $m\dot{V}O_2$ since choosing the wrong variable (O_2Hb or HHb) will increase the measurement error.

CLINICAL CONSIDERATIONS

Sensitivity/specificity

One of the objectives of this thesis was to investigate the possibilities of NIRS for the development of a noninvasive screeningstest that would be functional in a wide range of patients suspected to have a mitochondrial myopathy. Although NIRS was able to discriminate a homogeneous group of CPEO patients from healthy controls, it could not distinguish the individual patients. These patients had proven mitochondrial defects, but it is not known whether these defects directly affect O_2 consumption or mitochondrial ATP formation. When used in other metabolic/mitochondrial myopathies where O_2 consumption and/or oxidative ATP-production is more directly or more severely affected, NIRS might be sensitive enough to distinguish also at the individual level.

On the other hand, our CPEO patients were tested during low intensity work and it is, therefore, possible that the demand on muscle metabolism was simply not enough to provoke a difference between patients and controls. It might also be worthwhile to focus on those muscles that are most severely affected, although this approach may encounter practical limitations since it indicates many different measurement sites due to the heterogeneous clinical expression.

It is our opinion that, although the expenses will be many times higher, combination of NIRS with phosphorus magnetic resonance spectroscopy will have a high potential in the establishment of a noninvasive screeningstest. Combining the two methods enables simultaneous measurement of the kinetics of high-energy phosphates and oxidative processes within the same muscle volume of interest, and can, therefore, give a more complete view on muscle metabolism in health and disease. Since more aspects of muscle metabolism can be measured simultaneously and within the same muscle, it is likely that this combination will also lead to more specificity as is now possible by NIRS alone.

Quantitative measurement

NIRS is an elegant noninvasive technique providing relevant information about O_2 delivery and consumption, all the more since direct noninvasive measurement of mitochondrial function is not possible. Quantitative NIRS measurement of O_2

consumption and blood flow has proven to be a reliable method that can detect local differences between muscles at different activity levels. Moreover, we only focussed on oxygen consumption and blood flow measurements in the presented patient-studie of Chapter 8 while other quantitative variables can be determined as well (Chapter 6). As, for instance, is described in Chapter 9 where the reoxygenation rate is used to monitor the effect of medication in a patient with dermatomyositis, a muscle disorder characterised by capillary vasculitis. Combining several variables can possibly give new insights and might lead to a bio-energetic characterisation of the different disorders.

Clinical applicability

From the above mentioned aspects it is clear that NIRS is not yet ready for clinical application in the form of a screeningstest. For the NIRS measurements themselves, it can be said that although the application is relatively easy, familiarisation with the technique is necessary. A substantial amount of experience is required for data analysis although the software has improved substantially over the years. However, techniques like phosphorus magnetic resonance spectroscopy and echo doppler are even far more complicated in both application as well as analysis, but even so established as clinical technique.

Since the measurements themselves have no risk, take a relatively small time span, and are patient friendly, it is preferable to test more patients and more different defects in order to obtain a better view of oxygenation and haemodynamics in patients with neuromuscular disorders.

FUTURE PERSPECTIVES

Bio-energetic characterisation

In this thesis, NIRS was used to measure muscle oxygen consumption and blood flow in several myopathies. NIRS was mainly used during low-intensity work but can also be applied during higher work intensities, as presented in Chapter 3 and 9. This way, differences in consumption, flow, or recovery time might become more pronounced between patients and controls. Moreover, we have mainly focussed on oxygen consumption while several other variables can be measured by NIRS, thus extending the picture of muscle oxygenation and haemodynamics in metabolic or mitochondrial disorders. On the other hand, there are many other myopathies of which some directly affect O_2 consumption and the ATP production by a defect in the respiratory chain. More research is needed in order to fully judge the applicability of NIRS in the development of a noninvasive screeningstest. Various patient groups, different exercise protocols, and more NIRS variables need to be investigated.

Another future perspective of NIRS measurements in neuromuscular disorders can, in our opinion, be found in the combination of NIRS with other noninvasive techniques like phosphorus magnetic resonance spectroscopy, echo Doppler, and EMG. With these techniques combined simultaneously, the picture of local muscle metabolism becomes almost complete, providing an extended and noninvasive view on muscle physiology in health and disease. With the additional information it might very well be possible to clarify the physiological impairment in neuromuscular disorders and to develop a bio-energetic characterisation of the different defects.

Effect of therapy

This thesis showed that application of NIRS might be successful in the monitoring of treatment or therapy effect. Considering the fact that with such a longitudinal measurement design, some of the general problems of NIRS are avoided (e.g. inter-subject variability and confounding effect of adipose tissue thickness), this approach seems promising in the follow-up of neuromuscular disorders. Although there is no treatment that is generally effective for mitochondrial myopathies, recent studies showed that aerobic exercise might be beneficial in mitochondrial myopathies [Taivassalo et al. 1996, Taivassalo et al. 1998, Taivassalo et al. 2001]. Aerobic exercise is not thought to reduce the mitochondrial defect, but might reverse the chronic deconditioning that accompanies the primary mitochondrial defect. More research is needed to investigate the underlying mechanisms of this beneficial effect of aerobic training.

Multichannel NIRS

Although the localised measurement of NIRS has the great advantage of measuring directly in the muscle, the magnitude of the measured volume is limited (about 3 cm^3) and information about heterogeneity can not be obtained using a single channel spectrophotometer. This raises the question whether the measured value is representative for the whole muscle. Especially in large muscles like the quadriceps or gastrocnemius muscle, this might not be the case. In healthy subjects, perfusion heterogeneity exists within regions of the same muscle and between various muscles. It was found that increases in muscle blood flow during exercise were directed to newly recruited muscle and not to increased perfusion of already engaged muscle [Ray et al. 1998]. These results are compatible with our own measurements using two separate NIRS spectrophotometers finding that flow and consumption were different between exercising muscles (Chapter 2).

Multi-channel NIRS measurements are nowadays available and have the great advantage of measuring a larger portion of the muscle or even amongst different muscles. With the spectrophotometer as used in this thesis it is now possible to extend the device

to up to 12 channels. This way, simultaneous oxygenation and haemodynamics can be obtained at twelve measurement sites, covering a skin area of 8 x 8 cm^2, and measured with an acquisition time of 0.1 s [Quaresima et al. 2001].

Pathophysiology of myopathies

Apart from more insight in regional blood flow distribution and its regulation during exercise in healthy humans, these spatial and temporal features of the heterogeneity of perfusion and metabolism will lead to better understanding of the pathophysiology of myopathies and might result in a different approach for therapy. Only recently, NIRS was used to demonstrate that the protective mechanism of nitric oxide in the regulation of blood flow within the exercising skeletal muscle, by modulating the vasoconstrictor response, is defective in children with Duchenne muscular dystrophy [Sander et al. 2000]. Lacking this regulation results in an enhanced sympathetic vasoconstriction, stressing muscle perfusion and thus leading to muscle ischemia. This study showed that progressive muscle fibrosis may be accelerated by exercise in some myopathies and, as a consequence, training in these diseases should be strictly limited to low-profile exercise.

SAMENVATTING

Near-infrared spectroscopy, ofwel nabij-infrarood spectroscopie, kortweg NIRS genoemd, is een optische techniek waarmee op niet-invasieve wijze informatie verkregen kan worden over de hoeveelheid aanwezige zuurstof (oxygenatie) en de bloedvoorziening van het weefsel. De techniek is gebaseerd op de relatieve transparantie van het weefsel voor infrarood licht en op de zuurstof-afhankelijke absorptieverschillen van hemoglobine en myoglobine. Wanneer rekening gehouden wordt met de verstrooiing van het licht in het weefsel, kan een kwantitatieve maat voor de zuurstofconsumptie en de doorbloeding berekend worden.

Zuurstof is van essentieel belang voor de energiehuishouding van het lichaam. Als op de weg van de longen naar het weefsel problemen ontstaan, dan zal dat onherroepelijk gevolgen hebben voor de energieproductie. Naast stoornissen in de longfunctie of de circulatie kunnen defecten in de zuurstofhuishouding ook perifeer gelegen zijn. In de mitochondriën is zuurstof essentieel voor de laatste stap in de ademhalingsketen en voor de oxydatieve fosforylering. Bij een stoornis in deze biochemische processen zal dus de energieproductie verminderd zijn. Echter, ook bij een defect in het traject voorafgaand aan de ademhalingsketen komt de energieproductie in gedrang. Aandoeningen in de spier waarbij primair sprake is van een defect ergens in het traject van substraat naar ATP noemt men een metabole myopathie. Is dit defect specifiek in de mitochondriën gelegen, dan spreekt men van een mitochondriële myopathie. De term myopathie geeft aan dat deze aandoeningen zich vaak als eerste manifesteren in spierweefsel.

Mitochondriële myopathiën worden veelal veroorzaakt door een gendefect. Het zijn zowel fenotypisch als genotypisch heterogene aandoeningen: de klinische expressie van patiënten met eenzelfde gendefect is zeer variabel, terwijl patiënten met hetzelfde klinische beeld weer verschillende genetische defecten kunnen hebben. Mitochondriële ziekten kunnen zich beperken tot de skeletspieren, maar vaak zijn ook andere organen met een hoge energiebehoefte aangedaan, zoals de hersenen, het hart, de nieren of de lever. De klachten waarmee deze patiënten bij de neuroloog komen zijn inspanningsintolerantie en abnormale spiervermoeidheid. Bij lichte tot matige inspanning wordt vaak een verhoogde lactaatconcentratie gevonden.

Door de grote variatie in klinische symptomen, lijkt het beeld vaak op dat van andere aandoeningen. Ze komen daarom nogal eens voor in de differentiaal diagnose van andere aandoeningen, maar worden zelden bevestigd. Als na het neurologisch onderzoek en het bloedonderzoek nog steeds gedacht wordt aan een mitochondriële myopathie, dan wordt een spierbiopt genomen. Op dit stukje spierweefsel worden vervolgens enzymstudies, histologisch onderzoek en moleculair genetische technieken uitgevoerd om tot een bevestiging van de diagnose te komen. Desondanks leveren deze technieken in veel gevallen nog steeds geen eenduidige diagnose op.

Inspanningstests blijken eveneens een belangrijke methode om patiënten waarbij het vermoeden bestaat van een mitochondriële myopathie op te sporen. De klinische symptomen die deze patiënten gemeenschappelijk hebben zijn immers inspannings-intolerantie, abnormale spiervermoeidheid en een verhoogde lactaatconcentratie tijdens inspanning. Inspanningstests kunnen echter ook gevaarlijk zijn voor deze groep, daar het niet om een lokale aandoening gaat, maar om een systemische aandoening waarbij in sommige gevallen ook het hart aangedaan kan zijn. Daarnaast is het voor sommige patiënten onmogelijk om een "whole-body" inspanningstest uit te voeren, omdat de ernst van de aandoening dat niet toelaat, of omdat het zeer jonge kinderen betreft.

Er bestaat dus behoefte aan een eenvoudige test met een lage cardiovasculaire belasting die de neuroloog een eerste indicatie kan geven over het al dan niet aanwezig zijn van een myopathie. Aangezien met NIRS op een niet-invasieve en relatief gemakkelijke wijze informatie te verkrijgen is over de oxygenatie van spierweefsel, is het idee ontstaan om NIRS toe te passen bij myopathiën.

ONDERZOEKSDOEL

Het doel van het onderzoek was tweeledig. ***Ten eerste*** was het de bedoeling een aantal belangrijke methodologische aspecten van NIRS te onderzoeken, nodig voor een legitiem gebruik van NIRS bij het onderzoeken van de energiehuishouding van de spier in zowel de normale fysiologische situatie als ook de pathologische situatie. Dit doel ligt ten grondslag aan de experimenten die beschreven zijn in Hoofdstuk 2-5. Allereerst is getracht de kwantitatieve NIRS metingen te valideren door de met NIRS verkregen zuurstofconsumptie en doorbloeding te vergelijken met die verkregen middels de algemeen geaccepteerde Fick-methode, waarbij bloedgasanalyse, weefseloxymetrie en plethysmografie gecombineerd worden (Hoofdstuk 2). Uit dit onderzoek bleek echter dat directe vergelijking van beide methoden niet mogelijk is, omdat de Fick-methode een regionale waarde voor arm of been meet terwijl NIRS dat heel lokaal en direct in de spier doet. Lokale veranderingen in zuurstofconsumptie of doorbloeding, bijvoorbeeld ten gevolge van verschillen in spieractiviteit, kunnen daarom alleen gemeten worden met NIRS, terwijl deze niet te onderscheiden zijn met de Fick-methode.

Daaropvolgend is de reproduceerbaarheid onderzocht van de met NIRS gemeten zuurstofconsumptie gemeten in rust en tijdens inspanning. Gemeten over een tijdsbestek van enkele dagen bleek de zuurstofconsumptie gemeten met NIRS reproduceerbaar, zowel in de rustsituatie als tijdens inspanning op verschillende belastingsniveaus (Hoofdstuk 3). Ook bleek de gemeten zuurstofconsumptie en doorbloeding met NIRS gemeten binnen één enkele testsessie betrouwbaar te reproduceren (Hoofdstuk 2 en 4).

Een ander belangrijk methodologisch aspect dat is onderzocht betreft de optische aard van de NIRS techniek. De weg die het licht in het weefsel aflegt alvorens weer opgevangen te worden door de detector is complex, vanwege de passage door

verschillende weefsels van het inhomogene medium. Individuele verschillen in de optische eigenschappen van deze weefsels zullen daarom hoogstwaarschijnlijk van invloed zijn op de NIRS metingen. Omdat de grootste individuele verschillen te verwachten zijn in de huidplooidikte, is de invloed van de huidplooidikte op de *in vivo* NIRS metingen onderzocht in een grote groep gezonde proefpersonen. Uit dit onderzoek bleek dat de huidplooidikte inderdaad een verstorende factor is voor *in vivo* NIRS metingen. Deze bevindingen onderstrepen de noodzaak om de huidplooidikte als extra variabele mee te nemen in toekomstige NIRS studies.

In een ander experiment is het spiermetabolisme onderzocht door gebruik te maken van de complementaire methoden NIRS en fosfor magnetic resonance spectroscopy (^{31}P-MRS), die momenteel wordt beschouwd als de "gouden standaard" voor de niet-invasieve meting van het spiermetabolisme (Hoofdstuk 5). Met ^{31}P-MRS kan, onder andere, op indirecte wijze informatie verkregen worden over de oxydatieve energie-levering, mits voorafgegaan door een periode van ischemische inspanning. Met NIRS kan de zuurstofconsumptie direct in de spier gemeten worden, zowel in rust alsook tijdens aërobe en ischemische inspanning. De combinatie van ^{31}P-MRS en NIRS maakt het mogelijk veranderingen in hoog-energetische fosfaten en oxydatieve processen gelijktijdig te bestuderen, waarbij beiden globaal in hetzelfde spiervolume meten. Dit hoofdstuk laat zien dat deze combinatie een meerwaarde oplevert, die in dit geval aantoont dat de depletie van zuurstof tijdens arteriële occlusie en ischemische inspanning substantieel langer op zich kan laten wachten dan algemeen wordt aangenomen.

Tenslotte zijn alle rustwaarden, gemeten in gezonde proefpersonen tijdens de verschillende experimenten, bij elkaar genomen om zodoende een beeld te krijgen van normaalwaarden en biologische variabiliteit van de verschillende kwantitatieve *in vivo* NIRS variabelen (Hoofdstuk 6).

Met de hierboven beschreven experimenten zijn een aantal belangrijke methodologische vraagstukken voor de toepassing van *in vivo* NIRS metingen behandeld: NIRS heeft laten zien op reproduceerbare wijze oxygenatie en bloedvoorziening in spierweefsel te kunnen meten; NIRS kan op lokaal niveau verschillen in spieractiviteit aantonen; NIRS is niet alleen toepasbaar in rust, maar ook tijdens inspanning op verschillende belastingsniveaus; en NIRS heeft in de combinatie met ^{31}P-MRS grote toegevoegde waarde bij de *in vivo* bestudering van het spiermetabolisme. Dit alles zolang de verstorende factor, de huidplooidikte, in acht wordt genomen.

Het ***tweede doel*** van het onderzoek was het onderzoeken van de klinische toepasbaarheid van NIRS bij neuromusculaire aandoeningen, met als uiteindelijk doel de ontwikkeling van een niet-invasieve screeningstest voor patiënten waarbij het vermoeden bestaat van een metabole of mitochondriële spieraandoening. Als eerste is getest of het mogelijk is om met NIRS een onderscheid te maken tussen patiënten met een aangetoond mitochondriëel defect (chronic progressive external ophthalmoplegia (CPEO)) en gezonde proefpersonen (Hoofdstuk 8). De zuurstofconsumptie in de patiëntgroep bleek

lager dan die in de controlegroep, terwijl de doorbloeding juist hoger was. Deze bevinding is in overeenstemming met de disbalans tussen aanbod en consumptie van zuurstof zoals die bij sommige spieraandoeningen gevonden is [Haller 1994]. De functie van een screeningstest is echter om individuele patiënten te onderscheiden van gezonden. Hoewel beide groepen wel van elkaar te onderscheiden waren, vielen slechts drie van de vijf patiënten buiten het 95% betrouwbaarheidsinterval van de controlegroep. Bovendien bleek later, toen de data van dit onderzoek gecombineerd konden worden met de resultaten uit de Hoofdstuk 4, dat een groot gedeelte van het verschil tussen patiënten en controles toe te schrijven was aan de verstorende factor van de huidplooidikte. Alhoewel bij de meeste patiënten de zuurstofconsumptie nog steeds aan de lage kant was, lagen alle waarden nu binnen het 95% betrouwbaarheidsinterval van de controlegroep (Postscriptum Hoofdstuk 8).

Het volgen van het verloop van de ziekte in de tijd, of van het effect van medicatie of therapie, is een andere mogelijkheid voor het inzetten van *in vivo* NIRS metingen. Het voordeel van een dusdanig longitudinaal onderzoek is dat de patiënt zijn/haar eigen controle is, en daarmee zaken als inter-subject variabiliteit en verstorende factoren, zoals huidplooidikte, vermeden kunnen worden. In Hoofdstuk 9 is NIRS gebruikt om het effect van medicatie te bestuderen in dermatomyositis, een spieraandoening die gekarakteriseerd wordt door ontstekingsreacties op het niveau van de capillairen en waarbij de primaire beperking ligt bij de aanvoer van zuurstof en niet bij de consumptie. Na drie en na zeven weken medicatie liet NIRS een verbetering zien in zuurstofconsumptie en doorbloeding overeenkomstig de klinische bevindingen.

Voor wat betreft de toepassing van NIRS bij neuromusculaire aandoeningen heeft dit onderzoek laten zien dat CPEO patiënten als groep te onderscheiden zijn van gezonde personen, maar dat het onderscheidend vermogen op individueel niveau onvoldoende is. Hoewel de hier beschreven patiënten een bewezen mitochondriëel defect hadden, is het niet bekend of dit defect rechtstreeks van invloed is op de mitochondriële zuurstofconsumptie of energieproductie. Onderzoek bij andere metabole of mitochondriële spieraandoeningen waarbij de mitochondriële zuurstofconsumptie of energieproductie meer direct aangedaan is, zoals bij MELAS of het Leigh syndroom, zou een duidelijker beeld kunnen geven van de mogelijkheden van NIRS.

TOEKOMST PERSPECTIEVEN

Bio-energetische karakteristieken

In dit proefschrift is gebruik gemaakt van NIRS voor het meten van de zuurstofconsumptie en doorbloeding bij verschillende myopathiën. Hoewel de gebruikte protocollen voornamelijk bestonden uit inspanning met een lage intensiteit, kan NIRS ook worden toegepast bij hogere belastingsniveaus, zoals beschreven in Hoofdstuk 3 en

10. Door gebruik te maken van hogere belastingsniveaus zouden verschillen tussen patiënten en controles in zuurstofconsumptie, doorbloeding of hersteltijd beter tot uiting kunnen komen. Verder is in dit proefschrift de nadruk gelegd op het meten van de zuurstofconsumptie, maar zou een meer compleet beeld van oxygenatie en bloedvoorziening bij metabole of mitochondriële myopathiën verkregen kunnen worden door de verschillende met NIRS te meten variabelen te combineren. Daarnaast zijn slechts enkele myopathiën onderzocht. Vele andere vormen zouden gemeten kunnen worden, bijvoorbeeld de myopathiën die direct invloed hebben op de mitochondriële zuurstofconsumptie en ATP productie door een defect in de ademhalingsketen. Teneinde een duidelijk oordeel te kunnen vellen met betrekking tot de toepasbaarheid van NIRS bij de ontwikkeling van een niet-invasieve screeningstest is meer onderzoek nodig met uitbreiding naar verschillende patiëntgroepen, verschillende protocollen en meerdere NIRS variabelen.

Een andere optie voor de toepassing van NIRS bij myopathiën kan gezocht worden in de combinatie met andere niet-invasieve technieken zoals ^{31}P-MRS, echo doppler en EMG. De combinatie van deze technieken, simultaan toegepast, kan het fysiologisch plaatje van het spiermetabolisme op lokaal niveau aanzienlijk uitbreiden, en een niet-invasieve blik op de spierfysiologie in gezondheid en bij ziekte mogelijk maken. Deze extra informatie zou inzicht kunnen verschaffen in de fysiologische beperking bij metabole en/of mitochondriële myopathiën, en zodoende de kennis betreffende de bio-energetische karakteristieken van de verschillende defecten uitbreiden.

Therapie-effect

Dit proefschrift heeft laten zien dat NIRS veelbelovend is voor het volgen van het effect van medicatie/therapie. Het gebruik van een longitudinaal onderzoek, waarbij enkele van de algemene NIRS problemen omzeild worden (zoals de inter-subject variabiliteit en het verstorende effect van de huidplooidikte), lijkt uitermate geschikt voor het volgen van het verloop van de ziekte in de tijd. In tegenstelling tot de goede therapie mogelijkheden bij dermatomyositis, is dit helaas nog niet het geval bij de meeste mitochondriële aandoeningen. Afgezien van vitamine supplementen en substraten als creatine en carnitine is er voor de mitochondriële myopathiën nog geen algemene therapie beschikbaar. Recente studies hebben echter laten zien dat deze groep patiënten baat kan hebben bij aërobe training [Taivassalo et al. 1996, Taivassalo et al. 1998, Taivassalo et al. 2001]. Alhoewel aëroob trainen niet het mitochondriële defect zelf verminderd, kan zo wel het proces van chronische deconditionering doorbroken worden. De mechanismen die aan dit gunstige effect van aërobe training ten grondslag liggen zullen in de toekomst nader onderzocht moeten worden.

Meerkanaals NIRS

Alhoewel de lokale NIRS metingen het grote voordeel hebben dat direct in de spier gemeten kan worden, is het volume waarin daadwerkelijk gemeten wordt beperkt (ongeveer 3 cm^3) en is informatie over de heterogeniteit van het weefsel met een enkel-kanaals spectrofotometer niet meetbaar. Of de gemeten waarde representief is voor de gehele spier blijft dan ook de vraag. Vooral bij grote spieren, zoals de quadriceps en de gastrocnemius, zou dit wel eens een vertekend beeld kunnen geven, daar in gezonde personen een heterogene perfusie binnen regio's van eenzelfde spier en tussen spieren mogelijk is. Ook is beschreven dat de toename in doorbloeding, zoals die optreedt tijdens inspanning, toegeschreven kan worden aan nieuw gerekruteerd spierweefsel in plaats van een toename in de perfusie van het al actieve spierweefsel [Ray et al. 1998]. Deze bevindingen zijn verenigbaar met de metingen beschreven in Hoofdstuk 2, waarbij twee onafhankelijk opererende NIRS apparaten zijn gebruikt, en waaruit bleek dat doorbloeding en consumptie verschillend waren tussen verschillende spieren, afhankelijk van het activiteitsniveau van de spier.

Meerkanaals NIRS metingen behoren inmiddels ook tot de mogelijkheden, en hebben als groot voordeel dat een groter deel van de spier in de meting betrokken wordt of dat verschillende spieren tegelijk gemeten worden. De spectrofotometer zoals die in het onderzoek voor dit proefschrift gebruikt is, is op dit moment beschikbaar met een uitbreiding naar 12 kanalen. Met dit systeem kan de oxygenatie en bloedvoorziening op 12 plaatsen, binnen een grid van 8 x 8 cm^2, simultaan gemeten worden [Quaresima et al. 2001] .

Pathofysiologie van myopathiën

Naast het feit dat met een dergelijk meerkanaals NIRS systeem meer inzicht in de regionale doorbloeding en de regulatie daarvan verworven kan worden, kunnen spatiële en temporele eigenschappen van de heterogeniteit in perfusie en metabolisme leiden tot een beter begrip van de pathofysiologie van myopathiën, en zou daarmee eveneens kunnen leiden tot andere strategieën met betrekking tot therapie. Recentelijk is NIRS gebruikt om aan te tonen dat het beschermende mechanisme van NO (stikstof monoxide) bij de regulatie van de doorbloeding in de actieve spier (door modulatie van de vasoconstrictor respons) niet werkt bij kinderen met Duchenne spier dystrofie [Sander et al. 2000]. Het ontbreken van dit regelmechanisme resulteert in een toename van de sympathische vasoconstrictie, waarmee de perfusie van de spier in het gedrang komt en ischemie ontstaat. Deze studie toont daarmee aan dat inspanning, bij sommige myopathiën, progressieve spierfibrose zou kunnen versnellen, en dat daarom training bij deze ziekten absoluut beperkt moeten blijven tot zeer milde inspanning.

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PUBLICATIONS INCLUDED IN THIS THESIS

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CURRICULUM VITAE

Mireille van Beekvelt werd geboren op 13 juni 1969 aan de rand van het bos in Geldrop. Na een aanzienlijke hoeveelheid boven- en ondergrondse hutten gebouwd te hebben en een jaartje uitstel in de schakelklas werd op 13-jarige leeftijd, met frisse tegenzin, aan de serieuze scholing begonnen. Na een kort bezoek aan de HAVO haalde zij in 1986 met goed gevolg haar MAVO diploma aan de Albertus Magnus MAVO te Geldrop, alsnog gevolgd door haar HAVO diploma, behaald in 1988 aan het Strabrecht College te Geldrop. Aansluitend begon zij met de studie Fysiotherapie aan de Hogeschool Nijmegen en sloot ook deze opleiding met goed gevolg af in 1993. Uiteindelijk de smaak van het studeren te pakken hebbend begon zij daarop met de studie Bewegingswetenschappen. Haar wetenschappelijke stage liep zij op de afdeling Fysiologie met Prof. Dr. Berend Oeseburg aan het hoofd en onder begeleiding van Dr. Maria Hopman. Nog niet moe van alle leermomenten deed ze een extra stage in Kitchener/Waterloo in Ontario, Canada, onder begeleiding van Prof. Dr. Richard Hughson van de University of Waterloo alwaar zij de smaak voor het wetenschappelijk onderzoek te pakken kreeg. Na afronding van deze stage en het maximaal mogelijke uit de studiefinanciering te hebben gehaald behaalde zij het doctoraal diploma Bewegingswetenschappen in 1996. Van 1997 tot 2001 was ze werkzaam als promovenda op de afdeling Neurologie in samenwerking met de afdeling Fysiologie beide van het Universitair Medisch Centrum St. Radboud in Nijmegen. De resultaten van het onderzoek uitgevoerd in deze periode zijn beschreven in dit proefschrift. Vanaf april is zij als postdoc werkzaam bij de afdeling Klinische Neurofysiologie van het Universitair Medisch Centrum St. Radboud in Nijmegen.

CURRICULUM VITAE

Mireille van Beekvelt was born in Geldrop on June 13th 1969. She attended the Strabrecht College in Geldrop, where she obtained her HAVO diploma in 1988. Subsequently, she started her study Physiotherapy and graduated in 1993, followed by the study Biomedical Health Studies at the University of Nijmegen. She did her research project at the Department of Physiology (Prof. Dr. Berend Oeseburg) under supervision of Dr. Maria Hopman. An additional research project was done in Canada under supervision of Prof. Dr. Richard Hughson at the department of Kinesiology, University of Waterloo, Ontario, Canada. From 1997 to 2001 she was employed as a PhD student at the Department of Neurology in co-operation with the Department of Physiology at the University Medical Centre Nijmegen. The results of the research performed in this period are described in this thesis. Starting April 2002, she is employed at the Department of Neurology, University Medical Centre Nijmegen, as a postdoc researcher on a project using multichannel surface EMG on ion-channel pathology.

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