博士（人間科学）学位論文

Heterogeneities in skeletal muscle perfusion and metabolism and their physiological roles

（酸素供給および利用の不均一性とその生理学的役割）

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GENERAL ABSTRACT

Skeletal muscle perfusion and metabolism are essentially heterogeneous, not only within different muscle groups but also within a single muscle at rest and during exercise. To more clearly understand perfusion and metabolism in an exercising muscle and their relationship, it is necessary to detect their regional differences i.e., their heterogeneity. Furthermore, physiological roles of heterogeneous functions have not been fully understood. The purpose of this dissertation was to evaluate heterogeneities of skeletal muscle perfusion and metabolism and to examine their physiological role during exercise. Four studies were performed to achieve this purpose. The first two studies evaluate regional differences in blood flow and oxygen consumption of resting and recovering muscle using positron emission tomography. It was demonstrated that a systematic difference between proximal and distal regions in the quadriceps femoris muscle both rest and recovery phase. The next two studies examine a physiological significance in their heterogeneities between proximal and distal portion of the quadriceps muscle, judging from exercise pressor reflex. It was demonstrated that distal portion of the muscle makes a greater contribution to the pressor response than does the proximal portion. The results of these experiments indicated that blood flow and oxygen consumption in the quadriceps femoris muscle are heterogeneous at rest, during exercise, and during recovery from exercise: the differences is systematic between proximal and distal regions. The heterogeneities during exercise links to systemic cardiovascular regulation: the distal portion of the muscle makes a greater contribution to the pressor response than does the proximal portion.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1. Review related literatures

1-1. INTRODUCTION

Blood flow to skeletal muscle has been measured by several techniques (Radegran, 1999). In contrast, metabolic demand e.g., O_2 consumption, in skeletal muscle has been measured only on the basis of the Fick principle. However, concerns about hemodynamic measures and the possible problems involved in measuring arterio-venous differences in O_2 levels have been recently reviewed (Van Hall et al., 1998). Values calculated by venous blood

gas, in particular, contain unnecessary information, such as that for skin and non-active muscle at rest but not at maximal exercise (Augsti et al., 1994). Furthermore, recent studies have reported that techniques based on the Fick principle do not detect local changes during exercise (Boushel et al., 1998; Van Beekvelt et al., 2001). Moreover, blood flow and metabolic demand are essentially heterogeneous, not only within different organs but also within a single muscle at rest and during exercise. Therefore, to more clearly understand perfusion and metabolism in an exercising muscle and their relationship, it is necessary to detect their regional differences i.e., their heterogeneity.

Recently developed advanced methodologies, especially imaging modalities such as near-infrared spectroscopy (NIRS), magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), and positron emission tomography (PET), provide more information than conventional methodologies in measuring local and regional functions in the human body. These modalities can detect heterogeneous physiological functions *in vivo*. This paper describes the characteristics of these methodologies in measuring heterogeneous functions *in vivo*, and provides the evidence of heterogeneous functions with a focus on human muscles. Then, the physiological roles of heterogeneous functions will be discussed.

1-2. METHODS USED TO MEASURE HETEROGENEOUS PHYSIOLOGICAL FUNCTIONS IN HUMANS

The characteristics of four typical imaging modalities, NIRS, MRI, MRS, and PET are described. **Table 1-1** summarizes the comparative characteristics of each modality. Because

there are some advantages and disadvantages in each device, their individual values should be interpreted carefully in a suitable experimental model.

Table 1-1. *Methodologies to detect heterogeneous skeletal muscle perfusion and metabolism during exercise*

Modality	Invasiveness	Time-resolution	Spatial-resolution	Quantification	Available exercise mode and exercise intensity
NIRS (Mutichannel)	Noninvasive	High (msec)	Low only superficial	Relative change without ICG/Occ.	High intensity with no restriction
MRI/MRS	Noninvasive	Relatively high (sec)	Relatively high (cm)	Relatively quantitative	Low intensity with restricted by apparatus design
PET	Invasive catheter insertion radiation exposure	Low (min)	High (mm)	Quantitative	Low intensity with restricted by apparatus design

NIRS, near-infrared spectroscopy; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; PET, positron emission tomography. ICG, idocyanine green; Occ., arterial or venous occlusion.

1-2-1. Near-infrared spectroscopy (NIRS)

NIRS is used to monitor oxygenation status, which reflects the balance of O_2 utilization and O_2 supply in exercising muscles (see reviews by Hamaoka et al., 2003; McCully and Hamaoka, 2000; Quaresima et al., 2003). Earlier studies used single-source detector pairs to evaluate one area of muscle, but recently multiple-source detector pairs have been used evaluate regional differences in oxygenation status. This paper focuses on a multi-channel NIRS device. An advantage of NIRS is that it is portable and relatively inexpensive, with high temporal resolution (up to 10 Hz). A disadvantage of NIRS is that quantitative measurements require the use of an indocyanine green infusion technique (Boushel et al., 2000), and venous (Homma et al., 1996) or arterial occlusion (Hamaoka et al. 1996). In NIRS, the light travels in a shallow arc into the tissue to a penetration depth of about one-half the separation distance (McCully and Hamaoka, 2000). Because the available light-emitter-detector distances reported by most previous studies are 30~40 mm, deep areas of tissue (below 15~20 mm) could not be detected.

1-2-2. Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS)

Magnetic resonance imaging (MRI) and spectroscopy (MRS) have been extensively used to measure muscle structure and muscle metabolism, respectively. MRI provides high-resolution accurate information about muscle structure that is useful for mapping muscle recruitment patterns, assessed by transverse relaxation time (T_2) during exercise. Recently, MRI has been used to evaluate muscle blood flow with an arterial spin labeling method, which allows the measurement of both spatially and temporally resolved blood flow in vivo (Frank et al., 1999). MRS provides information on the chemical composition of the tissue. Depending on the nucleus observed, MRS allows the observation of high-energy phosphates (^{31}P-MRS; Heerschap et al., 1999), glycogen (^{13}C-MRS; Price and Gore, 1998), and intra/extramuscular lipids (^{1}H-MRS; Rico-Sanz et al., 1998). Importantly, ^{31}P chemical shift imaging, allows the acquisition of comparable phosphorus spectra from multiple voxels simultaneously. Because phosphocreatine (PCr) depletion is directly proportional to ATP hydrolysis, its relative depletion can be used as an index of muscle O_2 uptake. Using MRS and MRI, oxidative metabolism, muscle perfusion, and recruitment patterns can be visualized non-invasively with relatively high temporal resolution, as reported by Richardson et al. (2001). However, they are limited in the type exercise mode that can be studied. Furthermore, the equipment and running costs are very high.

1-2-3. Positron emission tomography (PET)

PET is potentially an attractive technology for use in exercise physiology research because it allows quantitative visualization of not only hemodynamic parameters but also physiological functions, such as glucose uptake (Ohmori et al., 2000), protein synthesis (Fischman et al., 1998), and receptor function (Mizuno et al., 2004b). In the premier and most impressive study, Fujimoto et al. (1996) showed whole-body glucose mapping after running exercise, using PET and ^{18}F fluoro-deoxyglucose. After this study, PET received a lot of attention in the disciplines of exercise and sports physiology. PET has been used for cardiac and skeletal muscle physiology from the mid-1990s, especially in the Turku PET Centre in Finland (Nuutila and Kalliokoski, 2000). It has the great advantage over conventional methodologies that it to measures regional physiological functions *in vivo*, and has provided many significant findings. PET is also very sensitive compared with NIRS, MRI, and MRS. A disadvantages of this apparatus is its low temporal resolution when measuring hemodynamic parameters compared with that of conventional techniques. For instance, the calculation of blood flow requires at least 240 s (Ruotsalainen et al., 1997). Moreover, like MRI/MRS techniques, it is limited in the type and intensity of exercise that can be studied because movement often causes artifactual noise. The equipment and running costs are very high, and the technique is invasive because it involves the use of short-lived radioisotopes.

1-3. EVIDENCE FOR HETEROGENEITY IN PERFUSION AND METABOLISM IN HUMAN SKELETAL MUSCLE

Evidence of cross- (**Table 1-2**) and longitudinal-sectional (**Table 1-3**) heterogeneity in skeletal muscle perfusion and metabolism at rest, during exercise, and during recovery from exercise will be described in this section, with a focus on human studies performed with imaging modalities such as NIRS, MRI/MRS, and PET.

Table 1-2. *Evidence of heterogeneous physiological functions in cross sections from human studies*

Authors	Region	Modality	Situation	Type of Exercise	Intensity	Variables	Findings
Quaresima et al. ('01a)	Calf	NIRS	during exercise	dynamic PF	33% MVC	SO_2	MG < SOL = TA
Vandenborne et al. ('93)	Calf	MRS	during exercise	planter flexion	maximum	pH	LG = MG < SOL
Allen et al. ('97)	Calf	MRS	during exercise	planter flexion	20/30/40% MV(	PCr	LG = MG < SOL
Torricelli et al. ('04)	Calf	NIRS	during exercise	planter flexion	50% MVC	SO_2	LG < MG
Kalliokoski et al. ('00)	QF	PET	rest			blood flow	VM = VI > VL = RF
			during exercise	intermittent isometric KE	18% MVC	blood flow	VM = VI > VL = RF
						relative dispersion	VM = VI > VL = RF
Shinohara et al. ('99)	QF	NIRS	during exercise	dynamic KE	incremental	oxygenation	VM < VL < RF
Azuma et al. ('01)	QF	NIRS	during exercise	exhaustive dynamic KE	30% MVC	ΔSo_2	VL > RF
Richardosn et al. ('98)	QF	MRI	(during) exercise	dynamic KE	90% maximun	ΔT_2	VM = VL = VI < RF
				bicycle	90 % maximum	ΔT_2	VM = VI = VL = RF

QF, *m.* quadriceps femoris; PF, plantar flexion; KE, knee extension; NIRS, near-infrared spectroscopy; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; PET, positron emission tomography; MVC, maximal voluntary contraction; SO_2, muscle oxygen saturation; PCr, phosphocreatine; T_2, transverse relaxation time; MG, *m.* medial head of gastrocnemius; TA, *m.* tibialis anterior; SOL, *m.* soleus; LG, *m.* lateral head of gastrocnemius; VM, *m.* vastus medialis; VI, *m.* vastus intermedius; *m.* vastus lateralis; RF, *m.* rectus femoris. < and > mean significant difference, and = means non-significant.

1-3-1. Cross sections

Blood flow and metabolic demand differ considerably within a synergetic muscle group, and heterogeneous metabolic functions have been observed even within a single muscle, in the flexor digitorum profundus muscle (Jeneson et al., 1992) and the tibialis anterior muscle (Houtman et al., 2001) during exercise. In the calf muscles, the soleus muscle does not seem to be a metabolically active region compared with the gastrocnemius muscle, independent of the

exercise mode and exercise intensity, whereas each synergetic muscle is activated during plantar flexion exercise (**Figure 1-1**). Furthermore, the lateral head of the gastrocnemius seems more activated than the medial head of the gastrocnemius during relatively high-intensity exercise. In contrast, there is little evidence available of heterogeneous blood flow in the calf muscle (Ament et al., 1998).

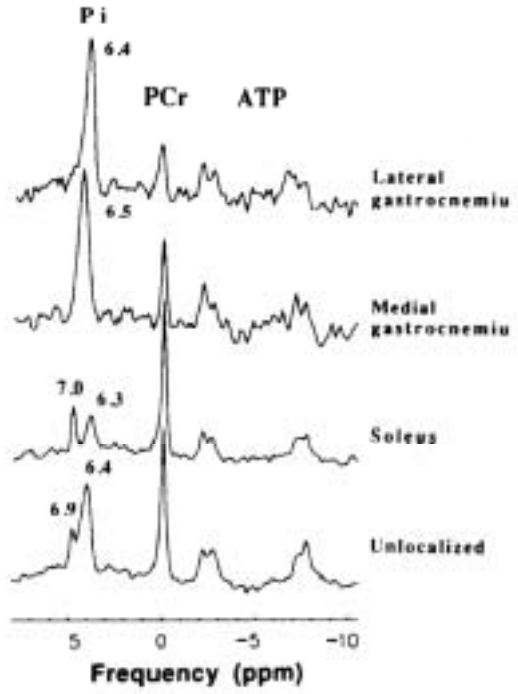

Figure 1-1. Muscle metabolism assessed by ^{31}P-MRS spectra during steady-state planter flexion is remarkably heterogeneous within the synergetic muscle (Vandenborne et al., 1993).

The quadriceps femoris muscle group (QF) also has a non-uniform O_2 supply and metabolic demand at rest and during exercise. From the visual impression of blood flow derived from PET images by Kalliokoski et al. (2000), the deeper region has a higher and more homogeneous blood flow than the more superficial region of QF at rest and during exercise (**Figure 1-2**). The rectus femoris muscle (RF), in particular, appears to have more characteristic functions within each head of the QF. That is the RF receives a lower blood flow that is less

deoxygenated during exercise compared with the other head of the QF, despite its higher activation according to MRI. Further evidence is required to clarify the nature of the heterogeneity in the QF in terms of the exercise mode and exercise intensity. Interaction between cross and longitudinal sectional heterogeneity (as noted below) is also required.

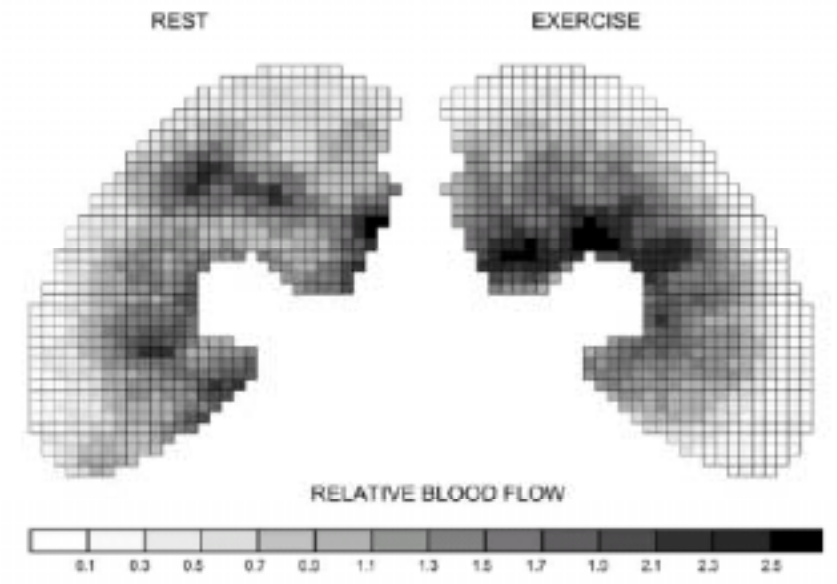

Figure 1-2. Pixel-by-pixel PET image of relative blood flow (divided by the mean blood flow of the whole quadriceps femoris) in resting (*right*) and exercising (*left*) quadriceps femoris muscles. Relative blood flow is highest in the deeper area of the exercising muscle (Kalliokoski et al., 2000).

1-3-2. Longitudinal sections

Evidence of heterogeneity in longitudinal sections seems to be less than that observed in cross sections (**Table 1-3**). Furthermore, evidence of heterogeneity in longitudinal sections has been evaluated only by NIRS device. An evaluation using other imaging modalities is needed to clarify heterogeneity in longitudinal sections. O_2 supply and metabolic demand are essentially different in the proximal and distal portions of muscles at rest, during exercise, and

Table 1-3. *Evidence of heterogeneous physiological functions in longitudinal sections from human studies*

Authors	Region	Modality	Situation	Type of Exercise	Intensity	Variables	Differences
Wolf et al. ('03)	Calf	NIRS	rest			blood flow, VO2	Pro > Dis
Miura et al. ('01;'04)	MG	NIRS	during exercise	dynamic heel-up	50% BW	ΔSaturation,	Pro < Dis
						Δblood volume	Pro < Dis
			recovery			Δblood volume	Pro < Dis
Quaresima et al. ('01b)	VL	NIRS	rest			VO_2	Pro > Dis
			during exercise	intermittent static KE	MVC	VO_2	Pro > Dis
Quaresima et al. ('04)	VL	NIRS	rest			blood flow, VO2	Pro < Dis
			end of exercise	static KE	MVC	blood flow	Pro > Dis

MG, *m.* medial head of gastrocnemius; VL, *m.* vastus lateralis; NIRS, near-infrared spectroscopy.

KE, knee extension; BW, body weight; MVC, maximum voluntary contraction; VO_2, oxygen consumption; Pro, proximal; Dis, distal. < and > mean significant difference, and = means non-significant.

during recovery from exercise (**Figure 1-3)**, independent of the muscle group. Basically, blood flow and O_2 consumption are lower in the distal portion than in the proximal portion at rest. Consequently, the relative changes in each appear larger in the distal portion than in the proximal portion, although the absolute values in the distal portion might be lower compared with proximal one. Likewise, the recovery rate is lower (i.e., the changes is greater) in the distal portion than in the proximal portion during recovery from exercise.

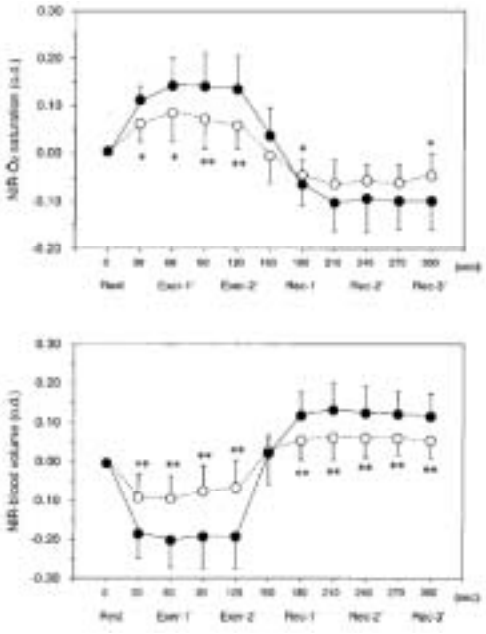

Figure 1-3. Changes in O_2 saturation (*top*) and blood volume (*bottom*) measured by NIRS are greater in the distal (*closed*) than in the proximal (*open*) portion of the gastrocnemius muscle during heel-up exercise and during recovery from exercise. (Miura et al., 2001)

1-4. MECHANISMS UNDERLYING HETEROGENEITIES IN SKELETAL MUSCLE PERFUSION AND METABOLISM

Using imaging modalities to evaluate muscle blood flow and muscle metabolism, several line of evidence have been derived from in human data, as described above. However, few studies are yet now available that address the mechanisms underlying these heterogeneities in humans. Some potential mechanisms will be considered in this section, with focus on animal studies.

1-4-1. Muscle fiber type and capillary density

The most likely explanation for heterogeneous skeletal muscle perfusion is the number of capillaries and the muscle recruitment associated with the composition of the muscle fiber type (Armstrong and Laughlin, 1983; Laughlin and Armstrong, 1982). Animal studies clearly demonstrated that red muscle receives higher blood flow than white muscle at rest and during exercise. Some anatomical studies have shown that within a single muscle, the superficial and distal portion haves a lower proportion of slow oxidative fibers than the deep and proximal portions (Laughlin and Armstrong, 1982; DeRuiter et al., 1996; Torrella et al., 2000; Wang and Kernell, 2000). Although few data are available for humans, deeper areas have a lower proportion of type I fibers compared with superficial areas in the vastus lateralis and vastus medialis (Johnson et al., 1973). Likewise, Lexell et al. (1983) showed in longitudinal section, a slightly lower proportion of slow oxidative fibers in the distal than in the proximal portion in the

vastus lateralis muscle. These reports support the existence of heterogeneous perfusion in both cross and longitudinal sections of human muscle, as indicated in **Tables 1-2** and **1-3**.

Muscle fiber type and capillary density are also involved in heterogeneous metabolism. The difference in oxidative capacity between slow-twitch fibers and fast-twitch fibers is the results of the greater muscle mitochondrial density in the subsarcolemmal area of the slow oxidative fibers (Philippi and Sillau, 1994). O_2 consumption (Sullivan and Pittman, 1984) and O_2 diffusion coefficients (Ellsworth and Pittman, 1984) in resting muscle depend on muscle fiber type and capillary density. Because intramuscular PO_2 is not dependent on muscle fiber type or capillary density (Greenbaum et al., 1997), heterogeneous O_2 consumption in resting muscle can be explained by the passive diffusion associated with O_2 delivery. In contrast, PO_2 decreases more (below critical PO_2) in fast-twitch fibers than in slow-twitch fibers during muscle stimulation (Behnke et al., 2003). Consequently, the lower O_2 consumption in fast-twitch fibers is caused by the inhibition of O_2 utilization compared with that in slow-twitch fibers.

1-4-2. Intramuscular pressure

Another possible explanation of heterogeneous muscle perfusion and metabolism, especially in longitudinal sections, is related to mechanical factors induced by intramuscular pressure within a single muscle. Ameredes and Provenzano (1997) demonstrated greater intramuscular pressure in the distal than in the proximal portion of a muscle during exercise. Consequently, blood flow is lower in the distal portion than in the proximal portion, as describe above (**Table 1-3**). Based on this theory, Miura et al. (2004) showed that regional differences in

oxygenation status are consistent with regional differences in muscle architecture, which are related to regional differences in intramuscular pressure. More specifically, larger fascicle angles and longer fascicle lengths are associated with decreased blood volume and decreased O_2 saturation.

In animal and human studies, blood flow within a muscle is also heterogeneous between the superficial and deep portions. Deep areas have higher blood flow than superficial areas (**Figure 1-2**). However, intramuscular pressure is higher in the superficial area than in the deep areas during exercise (Sejersted et al., 1984; Ameredes and Provenzano, 1997). In human lower leg muscles, intramuscular pressure in the soleus muscle is greater than that in the tibialis anterior muscle during walking and running (Ballard et al., 1998). However, O_2 supply seems to be higher in the soleus muscle than in the tibialis anterior muscle during exercise in rats (Laughlin and Armstrong, 1982). It is difficult to explain cross-sectional heterogeneity of blood flow by intramuscular pressure only. Interactions between fiber type and intramuscular pressure are required. Because blood flow is also temporally heterogeneous between the contraction and relaxation phase of dynamic contraction (Walloe and Wesche, 1988), temporal heterogeneity must also be considered.

1-4-3. Others

Kalliokoski et al. (2003c) suggested that the vascular branching pattern evaluated with PET does not explain the differences in perfusion heterogeneity between endurance-trained and untrained men, even during exercise. Therefore, vascular structure might not completely explain the heterogeneous blood flow in human.

Sympathetic nerves are involved in regulating muscle blood flow during exercise (Peterson et al., 1988). However, Iversen and Nicolaysen (1990) showed that, when the sympathetic vasomotor nerves are blocked, the perfusion pattern is not affected in rabbit muscle. In contrast, muscle metabolism is affected by efferent neural regulation (e.g., central command). The energy costs of force development are lower during voluntary exercise than during electrically induced exercise, at submaximal exercise intensity (Ratkevicius et al., 1998). To the authors' knowledge, it is yet known whether the sympathetic outflow to a single muscle is uniform. Taken together, these data suggest that further investigations are required to clarify the association between neural regulation and heterogeneous physiological functions.

1-5. PHYSIOLOGICAL ROLE OF HETEROGENEITY

O_2 uptake is greatly affected by O_2 supply to the tissue. In a model study, heterogeneous blood flow impaired O_2 extraction by peripheral tissues (Piiper and Haab, 1991; Walley, 1996). This is confirmed by data from a human study (**Figure 1-4**; Kalliokoski et al., 2004a). As the basic concept deduced from these studies, a homogeneous O_2 supply is better for exercising muscle because it enhances O_2 extraction.

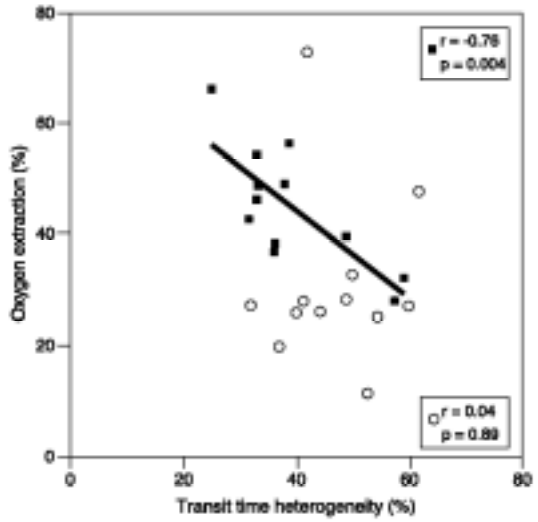

Figure 1-4. Homogenous blood transit time (as an index of blood flow velocity) enhances O_2 extraction in an exercising muscle (*filled boxes*) but not in a resting muscle (*open circles*), as assessed by PET (Kalliokoski et al., 2004a)

1-5-1. Are heterogeneities in perfusion and metabolism matched or mismatched?

O_2 demand in exercising muscle increases in parallel with exercise intensity. Blood flow and O_2 supply to skeletal muscle increase simultaneously to meet the metabolic demand when measured in the whole body (Savard et al., 1989) or in an individual limb (Anderson & Saltin, 1985). Moreover, at the microvascular level, there is a direct coupling between blood flow in the capillaries and the energy metabolism induced by electrical stimulation of a skeletal muscle (Berg et al., 1997; Murrant and Sarelius, 2000).

Are heterogeneous O_2 supply and metabolic demand in exercising muscles spatially matched? Using microspheres, Iversen and Nicolaysen (1991) demonstrated that regional blood flow within single skeletal rabbit muscle is not strongly linked to regional glucose uptake (r = 0.51), a marker of metabolic activity, either at rest or during electrical stimulation. Similar to this finding but in a human MRI study, Richardson et al. (2001) showed that there is no relationship between PCr depletion and exercise-induced blood flow during plantar flexion exercise (**Figure 1-5**). They suggested that the mismatch in $\dot{Q}/\dot{V}O_2$ may play a significant role in determining O_2 transport and utilization during exercise.

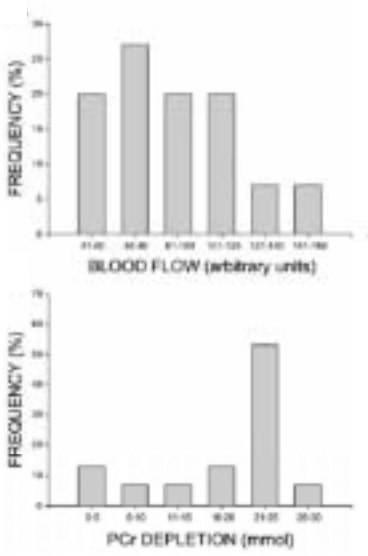

Figure 1-5. Local blood flow measured by MRI with arterial spin labeling methods (*top*) and local PCr depletion measured by ^{31}P-MRS (*bottom*) in the gastrocnemius muscle do not match during submaximal plantar flexion exercise (Richardson et al., 2001).

In contrast to both these findings, other controversial results have been reported. For instance, Kalliokoski et al. (2004b) suggested that muscle blood flow and O_2 uptake, measured by PET in the individual heads of the QF, are significantly correlated during light exercise (**Figure 1-6**). Similarly, Toussaint et al. (1996) demonstrated by ^{31}P-MRS that during recovery from ischemic exercise, PCr recovery is associated with the perfusion rate. They concluded that ATP synthesis is related to blood flow and thereby to O_2 availability in human skeletal muscle. The discrepancies between these reports might be explained by differences in the size of the regions of interest (ROIs). Matched phenomena were observed when ROIs were in muscle with the same function, whereas mismatches were observed when analyses were done on a pixel-by-pixel (or boxel-by-boxel) basis. Muscle perfusion and metabolic demand might be coupled at a functional level, but cannot be randomly assigned in a point-by-point fashion.

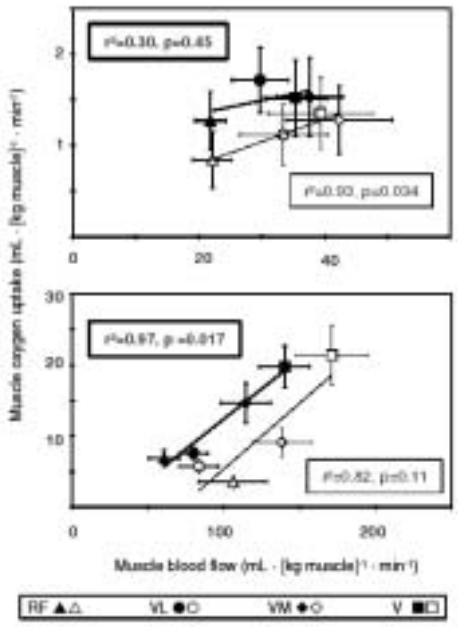

Figure 1-6. Relationship between blood flow and oxygen uptake at rest (*top*) and during exercise (*bottom*) measured by PET in the quadriceps femoris muscle. Blood flow and oxygen uptake are tightly coupled during submaximal knee extension exercise but not rest (Kalliokoski et al. 2004b).

1-5-2. Exercise mode and exercise intensity

Exercise mode. When heterogeneous blood flow is compared between intermittent and continuous static exercise, muscle perfusion is increased and O_2 extraction reduced during continuous exercise relative to that in intermittent static exercise at the same workload. These differences are associated with increased perfusion heterogeneity within the QF during continuous static exercise (Kalliokoski et al., 2003b). Furthermore, Laaksonen et al. (2003) demonstrated with PET that dynamic exercise causes higher and less-heterogeneous blood flow in exercising QF compared with intermittent isometric exercise at the same exercise intensity. They speculated that the higher blood flow might be due to a more effective muscle pump; less heterogeneous blood flow might be caused by more uniform recruitment of different muscle parts. Taken together, daily rhythmical and dynamic exercise (i.e., walking and running) is an efficient type of exercise in terms of the O_2 extraction associated with heterogeneous perfusion.

Exercise intensity. Because of the methodological limitations of PET described above, NIRS and MRS/MRI are more suitable devices with which to evaluate the effects of exercise intensity on heterogeneous functions. Boushel et al. (1998) indicated that at lower exercise intensity, the arterio-venous difference method could not detect the small changes that are detectable by NIRS. Muscle deoxygenation in the vastus lateralis muscle seems more heterogeneous at lower exercise work rates (Kime et al., 2004). Although heterogeneity in synergetic muscles (**Table 1-2**) seems to be unaffected by exercise intensity, skeletal muscle perfusion and metabolism in longitudinal sections (**Table 1-3**) are altered from heterogeneous to homogeneous as a function of exercise intensity.

1-5-3. Physical training and aging

Physical training. Exercise training results in a change in the distribution of blood flow within and among muscles (Armstrong and Laughlin, 1984). In human study, Kalliokoski et al. (2001) showed that endurance-trained subjects have a higher O_2 extraction fraction in the exercising muscles than do untrained subjects, which could potentially be associated with longer blood transit times and more homogeneous perfusion. Furthermore, endurance training does not alter the perfusion distribution between muscles, but it decreases perfusion heterogeneity within muscles (Kalliokoski et al., 2003a).

Aging. As mentioned above, muscle fiber type and capillary density are possible mechanism inducing heterogeneous O_2 supply and metabolic demand. Fiber type distribution becomes more homogeneous with aging in human (Larsson et al., 1978). Based on this phenomenon, aging might make physiological functions more homogeneous. A recent study suggested that the heterogeneous muscle deoxygenation observed in young adults during moderate and heavy exercises was diminished in older adults (DuManoir et al., 2004). Furthermore, more rapid muscle deoxygenation was observed during heavy exercise in older adults than young adults. Interestingly, physical training and aging might induce similar effects on blood flow distribution, whereas they are antithetical factor for human fitness. Clarifying whether these changes are caused by similar or differential processes should help identify the physiological roles of heterogeneous functions.

1-5-4. Links to systemic responses

CHAPTER 1. REVIEW RELATED LITERATURES

Exercise stimulates the cardiovascular response by two neural mechanisms: central command (see review by Matsukawa, 2001) and the exercise pressor reflex (see review by Hayashi, 2003). One chemical stimulus that evokes the exercise pressor reflex is a metabolic error signal resulting from a mismatch between metabolic demand and O_2 supply in an exercising muscle (Hanna et al., 2002). Based on this concept, the exercise pressor reflex should be inhomogeneous within an exercising muscle because of the heterogeneous O_2 supply and metabolic demand. However, to our knowledge, it is not clear whether there are regional differences in the exercise pressor reflex within an exercising muscle in humans.

The O_2 uptake kinetics at the onset of exercise is an interesting field for future study, as a topic of sports and exercise physiology (see review by Koga et al., 2003). Whipp et al. (2002) expressed concern that when O_2 uptake kinetics are evaluated, the mean response of a group of muscles or even a particular muscle is likely to mask important regional variation. It has been frequently showed that prior heavy exercise facilitates pulmonary O_2 kinetics at the onset of subsequent heavy exercise. As one possible explanation of this phenomenon, the less homogeneous muscle perfusion and metabolism induced by prior exercise may consequently accelerate pulmonary O_2 uptake kinetics during subsequent exercise. To the authors' knowledge, the relationship between pulmonary O_2 uptake and inhomogeneous muscle O_2 consumption in humans has yet to be clarified because of the methodological limitations of the currently available imaging modalities. One device has high temporal and low spatial resolution, whereas the other device has low time and high spatial resolution. NIRS and MRS/MRI seem to be appropriate tools with which to address this issue.

1-6. SUMMARY

The nature of heterogeneous functions in humans was reviewed, with a focus on perfusion and metabolism, and an assessment made of some available methodologies. Some possible mechanisms for and perspectives on these heterogeneities were discussed.

To date, conventional techniques that measure O_2 supply and metabolism in exercising muscle based on the Fick principle have been used extensively. However, recently developed methodologies reveal that approximate physiological functions do not seem to be homogeneous within either individual limbs or single muscles. Based on this background, it is important to measure time course and/or exercise intensity-dependent response as well as their spatial distribution in human skeletal muscle when evaluating cardiorespiratory parameters during exercise. Although there is some evidence that physical training and aging alter the extent of heterogeneity in physiological functions, the responsible mechanisms are not clear. Clarification of the nature of heterogeneous hemodynamic and metabolic functions will also be useful for clinical applications and in the rehabilitation of arteriosclerosis obliterans. Further studies are required, using advanced and conventional methodologies, both to confirm these data with descriptive evidences and to evaluate the physiological significance of heterogeneous functions.

Chapter 2. Rationale and specific aims

Firstly, evidence of heterogeneity in longitudinal sections seems to be less than that observed in cross sections Furthermore, evidence of heterogeneity in longitudinal sections has been evaluated only by NIRS device (see Chapter 1, **Table 1-2** and **3**). Taken together, further evaluation using other imaging modalities is needed to clarify heterogeneity in longitudinal sections.

Secondary, to date physiological roles of heterogeneous functions have not been fully understood, although much descriptive evidence is available. More specifically, it is remains unclear how heterogeneous responses links to systemic responses during exercise.

Therefore, the purpose of this dissertation was to evaluate heterogeneity in skeletal muscle perfusion and metabolism in longitudinal section (Chapter 3 and 4), and to examine physiological role of their heterogeneities during exercise (Chapter 5 and 6).

Chapter 3. Regional differences in blood flow and oxygen consumption in resting muscle and their relationship during recovery from exhaustive exercise

3-1. INTRODUCTION

Blood flow and oxygen supply to skeletal muscle increase in parallel with oxygen demand and exercise intensity when measured in the whole body (Savard et al., 1989) and in an individual limb (Andersen and Saltin, 1985; Richardson et al., 1995). Moreover, at the microvascular level, there is a direct coupling between blood flow in the capillaries and energy metabolism induced by electrical stimulation of a skeletal muscle (Berg et al., 1995). In contrast

to the relationship between blood flow and oxygen consumption during exercise, previous studies (Bangsbo and Hellsten, 1998; Bangsbo et al., 1990; Morganroth et al, 1975) have suggested that oxygen supply is not directly regulated by oxygen demand in the skeletal muscle during recovery from exercise, based on a difference in the time course of both variables after exercise.

Concerns about hemodynamic measures and possible problems involved in measuring arterio-venous difference in oxygen levels have been reviewed (Van Hall et al., 1998). In addition, a recent study reported that the technique based on Fick's principle could not detect regional changes in the forearm muscles during a handgrip exercise (Boushel et al., 1998; Van Beekbelt et al., 2001). Because absolute changes in blood flow and oxygen consumption are smaller during recovery after exercise than during exercise, it is possible that conventional techniques cannot detect small changes during recovery. Moreover, blood flow is heterogeneous in the skeletal muscle of animals (Laughlin and Armstrong, 1982; Piiper et al., 1985) and humans (Kalliokoski et al., 2000; Kalliokoski et al., 2001).

To more clearly understand the relationship between blood flow and oxygen demand in skeletal muscle during and after exercise, it is necessary to first identify factors such as differences in regional distribution of blood flow during and after exercise, technical problems in measuring hemodynamics, and heterogeneous recovery in a uniform muscle. To date, the regional distribution of oxygen supply and demand within a limb and/or muscles has not been measured during recovery from exercise.

Recently developed advanced methodologies, especially imaging modalities such as magnetic resonance imaging and positron emission tomography (PET), provide more

information than conventional methodologies to measure local and regional functions in the human body. PET provides a non-invasive imaging technique to assess various biochemical and physiological functions in living tissues, and can quantify hemodynamic variables in the skeletal muscle with a higher spatial resolution than conventional techniques. The purpose of this study was to evaluate regional differences in hemodynamic parameters within an exercised muscle, and to examine their relationship during recovery from exercise. Muscle blood flow, oxygen consumption, oxygen extraction fraction, and blood volume were measured in skeletal muscle by PET before and after one-legged exhaustive cycling exercise.

3-2. METHODS

Subjects

Five healthy males participated in this study (age 20─24 yr; height 168─178 cm; weight 62─79 kg). The subjects were fully informed of the purpose, nature, and potential risks of the experiments, and gave their written informed consent to participate in this study. The Ethics Committee of the Tokyo Metropolitan Institute of Gerontology approved the study protocol.

Experimental procedures

The subjects were instructed to abstain from drinking alcohol and caffeine, and to avoid heavy exercise for 24 h before the study. They were fasted overnight (> 12 h) before the experiment. The experimental protocol is summarized in **Figure 3-1**. After 30 min supine rest, a catheter was inserted into a radial artery to withdraw blood for measuring plasma radioactivity,

percentage of oxygen saturation (*%Sat*), and hemoglobin content (*Hb*). The subjects were positioned supine to place the femoral regions of both legs in the PET scanner. Eight minutes of transmission scanning was first performed to correct photon attenuation, and then emission scans were performed to measure muscle blood flow, oxygen uptake, and blood volume using inhalation of $[^{15}O]CO_2$, $[^{15}O]O_2$, and $[^{15}O]CO$, respectively. After the PET scans, subjects performed one-legged cycling exercise on a cycle ergometer (model 232C50, Combi, Tokyo, Japan) in a seated position. The right leg exercised while the left leg remained stationary on the ergometer frame. The pedaling rate was set at 80 revolutions per min (rpm) and was controlled by a metronome. Exercise intensity was set at 30 watts (W) for the first 3 min, followed by increments of 6 W every minute until exhaustion. Exhaustion was defined as the point at which subjects were unable to maintain the pedaling rate. The subjects were familiarized with this exercise protocol in advance. Ten minutes after the end of exercise, the second PET measurement was performed using the same procedure as that used before exercise.

To prevent misalignment between before and after exercise PET images, the PET camera was carefully positioned using a laser marker equipped in the PET camera. The subject's legs were immobilized with a plastic cover to prevent displacement of legs during the PET scan.

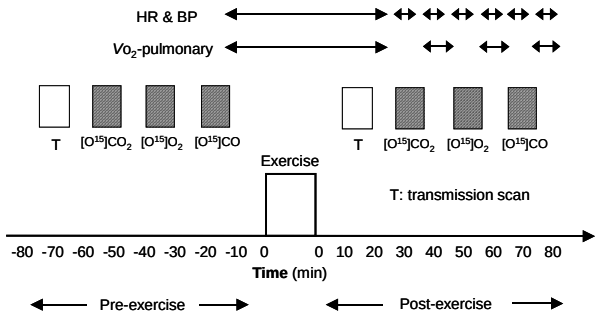

Figure 3-1. Experimental protocol. HR, heart rate; BP, blood pressure; $\dot{V}o_2$-pulmonary, pulmonary oxygen uptake.

Measurements of physiological functions

Systemic parameters: Heart rate (HR) and blood pressure (BP) were measured simultaneously with an automatic blood pressure analyzer (EBP300, Minato Medical Science Co., Tokyo, Japan) applied to the upper part of the left arm. Pulmonary oxygen uptake ($\dot{V}o_2$-pulmonary) was measured with an automatic gas analyzer (MG360, Minato Medical Science Co., Tokyo, Japan) and an automatic respiration monitor (RM300, Minato Medical Science Co., Tokyo, Japan). HR and $\dot{V}o_2$-pulmonary were monitored every 30 s at rest and during exercise. During recovery from exercise, HR and BP were recorded every 10 min, and $\dot{V}o_2$-pulmonary was recorded 10, 20, 40, 60, and 80 min after exercise. Time constants (τ, min) of the recovery rate of HR and $\dot{V}o_2$-pulmonary were calculated by fitting a single exponential curve extending from peak values to pre-exercise values in equation (*1*), which represented a modified model for the off-transient (Rossiter et al., 2002):

$$Y(t) = P - X \cdot (1 - exp^{-t/\tau}) \text{----------} (1)$$

where Y is each parameter after exercise, P is the peak value obtained during exercise (*i.e.*, exhaustion), X is the difference between peak and pre-exercise values, and τ is defined as the rate of recovery.

PET parameters: All radioactive gases were synthesized with a small cyclotron (CYPRIS-370, Sumitomo Heavy Industries, Ltd., Tokyo, Japan). The PET camera (HEADTOME-V (Shimadzu Co., Kyoto, Japan) had an axial field of view of 200 mm, consisting of four-ring detectors, providing a set of 32-slice images at center-to-center intervals of 6.25 mm with an image spatial resolution of 4.2 mm full-width at half maximum, and an axial resolution of 4.5

mm full-width at half maximum. The subjects received radioactive gases that were diluted with room air ($[^{15}O]CO_2$: 0.75/100; $[^{15}O]O_2$ and $[^{15}O]CO$: 0.15/100, v/v). Arterial blood was continuously withdrawn at a flow rate of 3 $ml \cdot min^{-1}$, and the radioactivity was measured using an on-line radioactivity detector system (ß-counter, Shimadzu Co., Kyoto, Japan). Arterial blood was sampled 0, 1, 3, 5, 7, and 8.5 min after gas inhalation, and radioactivity was measured with a well-type γ-counter (BSS, Shimadzu Co., Kyoto, Japan) to calibrate the count from the ß-counter. All data were corrected for dead time, decay, dispersion, and photon attenuation before data analysis.

Calculation of blood flow: The subjects underwent simultaneously a 4-min continuous inhalation of $[^{15}O]CO_2$ containing 1500 $MBq \cdot ml^{-1}$, and an 8-min static PET scan. The blood flow ($ml \cdot 100g^{-1} \cdot min^{-1}$) value was calculated using an autoradiographic method (Ruotsalainen et al., 1997) from equation (*2*):

$$C(t) = f \cdot \int_0^t Ca(\tau)d\tau - \frac{f}{p} \cdot \int_0^t C(\tau)d\tau \quad \text{---------- (2)}$$

where *C* and *Ca* indicate the radioactivity in tissue and arterial plasma, respectively. The parameter *f* denotes blood flow, and *p* is the partition coefficient of water in the skeletal muscle, which was considered as a constant and fixed a value of 0.95 (Ruotsalainen et al., 1997).

Calculation of oxygen extraction fraction and oxygen uptake: The subjects underwent simultaneously an 8.5-min continuous inhalation of $[^{15}O]O_2$ containing 2000 $MBq \cdot ml^{-1}$, and an 8.5-min static emission scan. The oxygen extraction fraction (OEF) was calculated using equation (*3*) (Hollden et al., 1988; Iida et al., 1996):

$$C = \frac{OEF \cdot Ao + f \cdot Aw}{f / p} \quad \text{----------} (3)$$

where *C*, *Ao*, and *Aw* are the radioactivity in tissue, $[^{15}O]O_2$ in arterial blood, and its metabolite $[^{15}O]H_2O$ in arterial blood, respectively. $[O_2]a$ denotes the oxygen content in the arterial blood, and was calculated using equation (*4*):

$$[O_2]a = 1.39 \cdot \%Sat \cdot Hb. \text{----------} (4)$$

The oxygen uptake ($ml \cdot 100g^{-1} \cdot min^{-1}$) was calculated using equation (*5*):

$$oxygen\ uptake = OEF \cdot f \cdot [O_2]a. \text{----------} (5)$$

Calculation of blood volume: The subjects received a 4-min continuous inhalation of $[^{15}O]CO$ mixed room air containing 2000 $MBq \cdot ml^{-1}$, and then a 6-min static PET scan was performed following a 2-min room air inhalation. The blood volume ($ml \cdot 100g^{-1}$) value was calculated from equation (*6*) (Raitakari et al., 1995):

$$blood\ volume = \frac{Aco}{Tco} \cdot \frac{1}{r} \text{----------} (6)$$

where *Aco*, *Tco*, and *r* represent the radioactivity of $[^{15}O]CO$ in arterial blood, in tissue, and the tissue-to-large vessel hematocrit ratio, respectively. The value of *r* applied was 0.91 (Raitakari et al., 1995).

Regions of interest: The center of the 200-mm axial view of the PET camera was positioned at a point equivalent to 50% of the length between the greater trochanter and the knee joint. The five slices were selected for further investigation included the center of the axial field of view (middle); 31 mm (proximal-31 mm), and 62 mm (proximal-62 mm) from the center in the

proximal direction; and 31 mm (distal-31 mm), and 62 mm (distal-62 mm) from the center in the distal direction. Regions of interest (ROIs) were placed over the quadriceps femoris muscles in both thighs on the PET images with a reference to the images of the transmission scan. In selecting the ROIs, large vessels appearing as hot spots were carefully avoided, and sufficient distance was maintained between the body surface and bone. Consequently, the total volumes of ROIs were as follows (mean ± SD): for proximal-62 mm, 40.8 ± 4.5 cm^3; for proximal-31 mm, 40.5 ± 6.1 cm^3; for middle, 34.9 ± 5.3 cm^3; for distal-31 mm, 30.3 ± 4.5 cm^3; and for distal-62 mm, 27.9 ± 4.8 cm^3. Identical ROIs were applied for data both before and after exercise.

Statistical analysis

All data are presented as mean ± SE. For systemic parameters, one-way analysis of variance (ANOVA) for repeated measurements followed by Scheffe's post hoc test was performed to test differences between pre- and post-exercise values. Student's unpaired *t*-test was performed to test differences between time constants for HR and $\dot{V}O_2$-pulmonary. To test portion-dependency for PET parameters, the Kendall's rank correlation coefficient was applied. For PET parameters in pre- and post-exercise, two-way ANOVA (*Time* & *Leg*) for repeated measurements was performed to test the effect of exercise in each portion. The factor *Time* and *Leg* indicates comparisons between pre- and post-exercise, and between non-exercised and exercised leg, respectively. When an F-test was significant, pairwise comparison was performed using Scheffe's post hoc test. Linear relationships between parameters were tested with Pearson's correlation coefficients. Values of $P < 0.05$ were considered statistically significant.

3-3. RESULTS

Systemic parameters

The parameters measured at exhaustion are shown in **Table 3-1**, and the time courses of systemic parameters after exercise are shown in **Figure 3-2**.

Table 3-1. *Parameters in one-legged cycling exercise at exhaustion*

	Peak HR, beats/min	Peak Pulmonary Vo_2, ml/min	Peak Work Rate, W	Time to Exhaustion, min
Subject 1	153	2230	96	14.1
Subject 2	178	2636	126	18.6
Subject 3	189	3205	138	20.1
Subject 4	194	2682	162	24.1
Subject 5	194	2252	126	18.2
Mean	181	2601	130	19.0
SD	17	398	24	3.6

HR, heart rate; Vo_2, O_2 uptake.

HR was significantly elevated for 20 min after exercise, but there were no significant changes in BP. $\dot{V}o_2$-pulmonary was elevated for 10 min after exercise. The recovery rate τ of HR was significantly slower than that of $\dot{V}o_2$-pulmonary (**Figure 3-2A** and **3-2C**): 11.1 ± 1.0 vs. 3.4 ± 0.2 min ($P < 0.01$). There were no significant differences in all systemic parameters between 30 and 80 min after exhaustive exercise, when the PET scans were performed. These results indicate that steady state was achieved during the PET scan, which requires constant physiological function in the target organs.

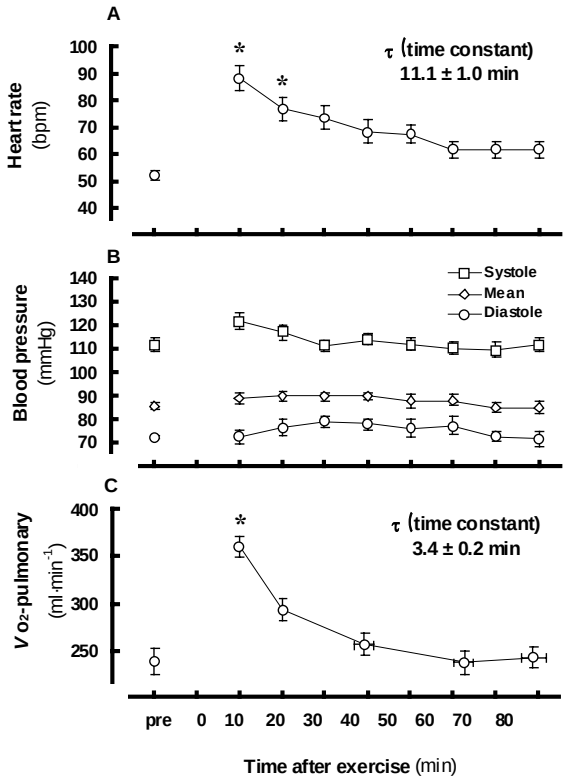

Figure 3-2. Changes in systemic parameters after exercise. A: heart rate. bpm, Beats, min. B: blood pressure. C. pulmonary $\dot{V}o_2$. *Significant difference from preexercise values. $P < 0.05$.

PET parameters

Examples of parametric images (subject 2) of hemodynamic parameters are shown in **Figure 3-3**. Although systemic parameters reached to steady state, remarkable differences can be found between exercise and non-exercised legs in the PET images. Some anatomical structures are apparent in the PET images.

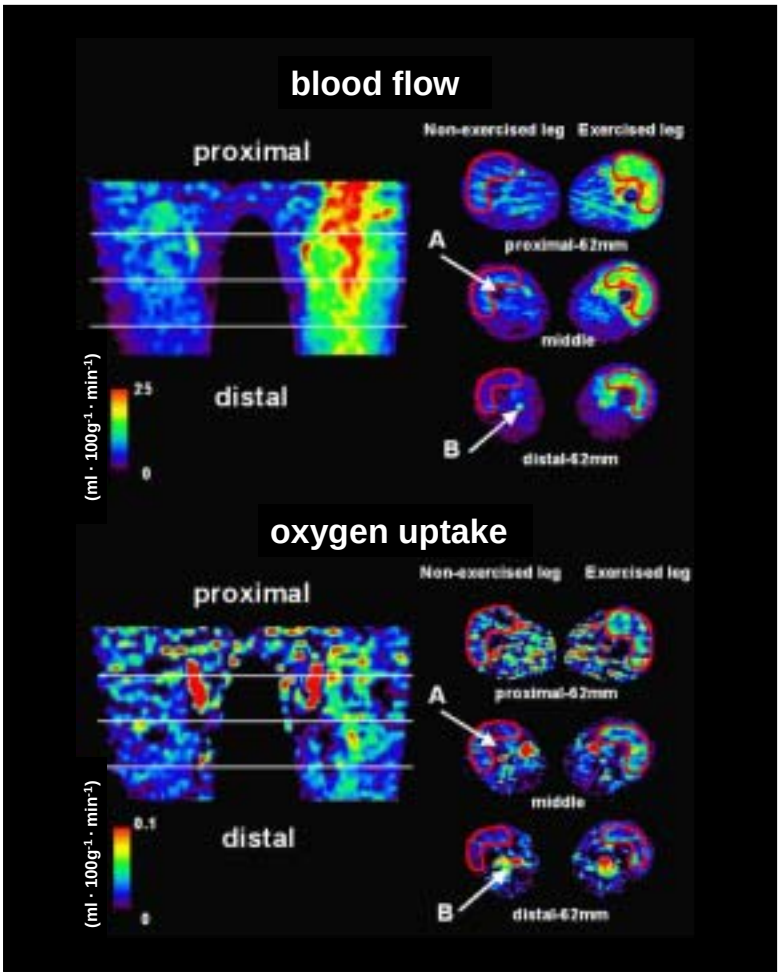

Figure 3-3. Examples of PET images of blood flow (*upper*), oxygen uptake (*bottom*) in one subject (subject no.2). *Left* panels are the coronal images (immediately above the femur), and *right* panels are the transaxial images at the proximal-62 mm (*top*), middle (*middle*), and distal-62 mm (*bottom*) portions. The right leg is the exercised leg, and the left leg is the non-exercised leg. Regions of interest are represented by red lines. White arrows indicate anatomical structures: A = femur; B = large vessel.

Before exercise, muscle blood flow and oxygen uptake decreased significantly in the direction from the proximal to the distal portions (**Figure 3-4A**). There were no significant differences in oxygen extraction fraction or in muscle blood volume among the portions measured. In contrast,

during recovery from exercise, the gradient in muscle blood flow and oxygen uptake diminished (**Figure 3-4B**).

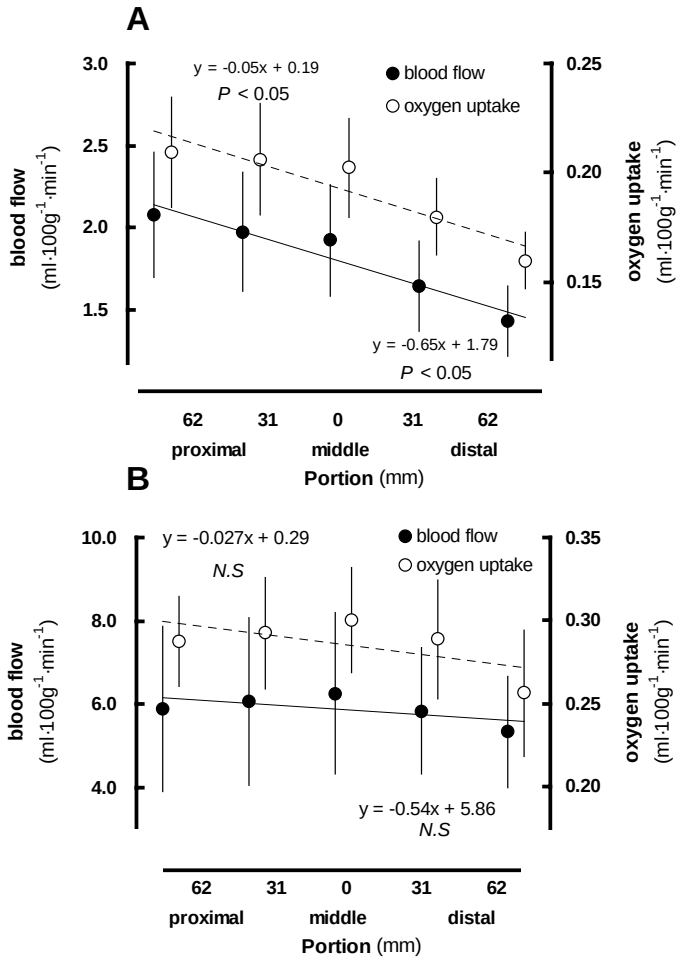

Figure 3-4. Portion differences in blood flow (open circles) and oxygen uptake (closed circles) in quadriceps femoris muscle in pre-exercise (A) and post-exercise (B). Solid and dotted lines represented regression lines of blood flow and oxygen uptake, respectively.

The changes in PET parameters pre- and post-exercise are summarized in **Table 3-2**. There were significant interactions, which indicated an effect of exercise on muscle blood flow in each portion, at distal-31 mm and distal-62 mm. Similarly, there were significant interactions in muscle oxygen uptake in the middle and both distal portions. In contrast, there was no

significant interaction in oxygen extraction fraction in any portion. These results suggest a difference in recovery rates across the muscle.

Table 3-2. Changes of PET parameters before and after exhaustive exercise

	Nonexercised leg		Exercised leg		Comparison Factors		
	Pre	Post	Pre	Post	Time	Leg	Interaction
			Blood flow, ml 100g^{-1} min^{-1}				
Proximal-62 mm	2.0 ± 0.5	2.2 ± 0.9	2.1 ± 0.6	5.9 ± 2.0			
Proximal-31 mm	1.9 ± 0.5	2.3 ± 0.9	2.0 ± 0.6	6.1 ± 2.0			
Middle	1.9 ± 0.5	2.1 ± 0.7	1.9 ± 0.5	6.2 ± 1.9			
Distal-31 mm	1.6 ± 0.4	1.8 ± 0.6	1.6 ± 0.4	5.8 ± 1.5	0.025	0.035	0.036
Dsital-62 mm	1.4 ± 0.3	1.5 ± 0.5	1.4 ± 0.3	5.3 ± 1.3	0.018	0.020	0.021
			Oxygen extraction fraction				
Proximal-62 mm	0.56 ± 0.03	0.47 ± 0.05	0.56 ± 0.05	0.37 ± 0.10	0.032		
Proximal-31 mm	0.55 ± 0.03	0.51 ± 0.07	0.59 ± 0.04	0.37 ± 0.10			
Middle	0.59 ± 0.06	0.54 ± 0.06	0.57 ± 0.05	0.37 ± 0.11			
Distal-31 mm	0.61 ± 0.05	0.59 ± 0.07	0.60 ± 0.05	0.36 ± 0.12			
Dsital-62 mm	0.64 ± 0.05	0.56 ± 0.08	0.60 ± 0.07	0.37 ± 0.12			
			Vo_2, ml 100g^{-1} min^{-1}				
Proximal-62 mm	0.21 ± 0.04	0.16 ± 0.03	0.21 ± 0.04	0.29 ± 0.03			
Proximal-31 mm	0.20 ± 0.04	0.17 ± 0.02	0.21 ± 0.04	0.29 ± 0.03			
Middle	0.21 ± 0.03	0.18 ± 0.02	0.20 ± 0.03	0.30 ± 0.03			0.049
Distal-31 mm	0.18 ± 0.02	0.17 ± 0.02	0.18 ± 0.03	0.29 ± 0.04		0.048	0.036
Dsital-62 mm	0.17 ± 0.02	0.13 ± 0.02	0.15 ± 0.02	0.26 ± 0.04		0.036	0.011

Proximal-62 and proximal-31, 62 mm and 31 mm, respectively, from the center in the proximal direction; middle, center of the axial field; distal-62 and distal-31, 62 mm and 31 mm, respectively, from the center in the distal direction. Pre and Post, before and after exercise, respectively. The factors Time and Leg indicates multiple comparisons (Scheffe's post hoc test) between pre- and postexercise and between nonexercised and exercised leg, respectively. Significance only as indicated; otherwise, no significance was found.

The relationship between muscle blood flow and oxygen uptake is shown in **Figure 3-5**. Significant correlations emerged when inter-subject averages of normalized muscle blood flow and oxygen uptake by those pre-exercise values were plotted ($r = 0.963$, $P < 0.01$).

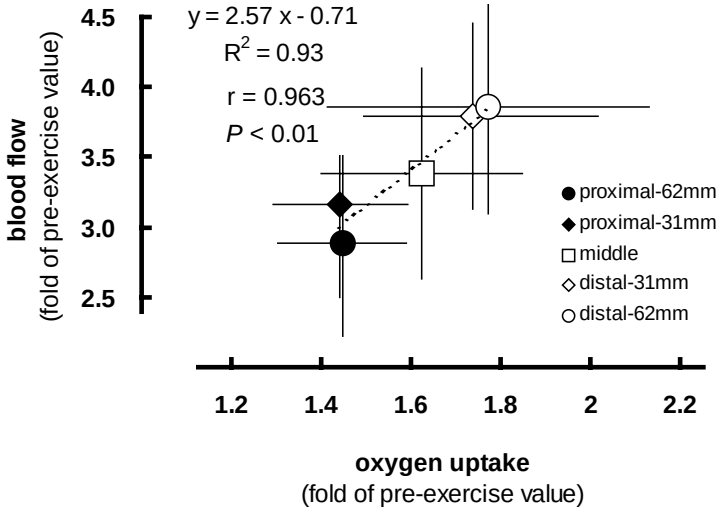

Figure 3-5. Relationship between muscle oxygen uptake (x-axis) and blood flow (y-axis) after exhaustive exercise. Values are normalized to the pre-exercise values. Closed symbols (circle and diamond) are proximal portions (proximal–31 and –62 mm, respectively); open symbols (circle and diamond) are distal portions (distal–31 and –62 mm, respectively), and open square is the middle portion (middle).

3-4. DISCUSSION

The purpose of this study was to quantify, by PET, regional difference in blood flow and oxygen consumption in an exercised muscle, and to clarify their relationship after exhaustive exercise.

PET for exercise physiology

PET is potentially an attractive technology for use in exercise physiology research as it enables quantitative visualization of regional hemodynamic parameters. Previous studies have shown high correlations between PET parameters and muscle blood flow as measured by venous occlusive plethysmography (Raitakari et al., 1996) and oxygen extraction fraction using

Fick's principle (Nuutila et al., 2000). The hemodynamic data obtained in the present study were consistent with those reported in previous PET studies (Raitakari et al., 1996; Nuutila et al., 2000). Resting muscle blood flow in the present study ranged from 0.8 to 4.4 $ml \cdot 100g^{-1} \cdot min^{-1}$ (average: 1.8 ± 0.1 $ml \cdot 100g^{-1} \cdot min^{-1}$) and was comparable to values reported by Raitakari et al. (1996) (range from 1.1 to 7.5 $ml \cdot 100g^{-1} \cdot min^{-1}$; average 3.1 ± 1.7 $ml \cdot 100g^{-1} \cdot min^{-1}$). Likewise, resting muscle oxygen consumption in this study (0.19 ± 0.1 $ml \cdot 100g^{-1} \cdot min^{-1}$) was consistent with that measured by Nuutila et al. (2000) (0.23 ± 0.1 $ml \cdot 100g^{-1} \cdot min^{-1}$).

PET has a low time resolution compared with conventional techniques to measure hemodynamic parameters; this point should be considered when studying events post-exercise because these hemodynamic parameters change rapidly after exercise. In the present experimental protocol, PET measurements started 30 min after exhaustive exercise, when it is expected hemodynamics have reached a steady state following the dynamic changes immediately after exercise. No significant change in any systemic parameter was observed from 30 to 80 min after exercise (**Figure 3-2**), suggesting that the hemodynamic variables achieved a steady state within 30 min after exercise.

During PET measurements, the subjects remained in a rested state to insure consistency in the measurement duration of the hemodynamics. To minimize the effects of any pre-conditions and the duration of the PET measurements, which may have influenced the muscle contraction while subjects maintained posture, the subjects' legs were fixed with a plastic cover. In addition, pre-exercise measurements of blood flow began after 30 min of rest in the spinal position.

Regional difference in blood flow and oxygen uptake at rest

Before exercise, the muscle blood flow and oxygen uptake decreased significantly in the direction from the proximal to the distal (**Figure 3-4A**). Use of an ultrasound/Doppler technique in humans has shown that resting blood flow and velocity are lower within the popliteal artery than within the superficial femoral artery (Holland et al., 1998). Decreases in blood flow in the present study, therefore, can be attributed to differences in the distance from the heart between the proximal and distal portions of the muscle. Another possible explanation may relate to the relationship between regional blood flow in a resting muscle and the percentage content of slow oxidative fiber, as reported in studies on animals (Laughlin and Armstrong, 1982). Within a single muscle, the distal portion has a lower percentage of slow oxidative fibers than in the proximal portion (Torrela et al., 2000; Wang and Kernell, 2000). In a study on human muscle, Lexell et al. (1983) showed a lower percentage of slow oxidative fibers in the distal than in the proximal portion in *m.* vastus lateralis. These previous reports have suggested the existence of a difference in muscle blood flow between the proximal and distal portions within a single muscle. In addition, in an *in vitro* study, Sullivan and Pittman (1984) indicated that oxygen consumption in the skeletal muscle depended upon the muscle fiber type and the capillary density.

Regional difference in the extent of recovery in exercised muscle

Greig et al. (1985) showed that nearly all muscle fibers are recruited at an exercise intensity eliciting 100% $\dot{V}o_2$ max. In our protocol, all subjects exercised until exhaustion, which we assume would have recruited nearly all muscle fibers in *m.* quadriceps femoris. There were

no significant interactions of PET parameters in proximal portions of the muscle, although distal portions showed significant interactions of blood flow and oxygen uptake (**Table 3-2**). Both blood flow and oxygen uptake significantly increased in all portions investigated after exercise, and the increment became larger in the direction from the proximal to distal portions (**Figure 3-5**). These results suggest the presence of regional differences in the extent of recovery from exercise in an exercised muscle, and are consistent with a previous study (Miura et al., 2001).

One possible interpretation of these regional differences relates to the effects of intramuscular pressure within single muscle. Ameredes and Provenzano (1997) showed greater intramuscular pressure in the distal than in the proximal portion of a muscle. Higher intramuscular pressure would inhibit circulation during muscle contraction, which might be compensated during recovery after exercise. An alternative explanation is that regional differences may arise because of the presence of non-uniform muscle fiber type, as mentioned above. In the present study, there were differences in the regional parameters between different portions of the muscle pre-exercise (**Figure 3-4A**), which might reflect lower oxidative capacity in the distal compared with the proximal portion, resulting in decreasing recovery rates in the distal portion. However we did not measure muscle fiber type, and further investigation is required to any relationship to muscle fiber type in the human.

Spatial relationship between oxygen uptake and blood flow during recovery from exercise

Previous studies reported a mismatch between blood flow and oxygen consumption during recovery after exercise (Bangsbo and Hellsten, 1998; Bangsbo et al., 1990; Morganroth et al, 1975). The time courses of HR and $\dot{V}O_2$-pulmonary observed in the present study are

consistent with previous reports (**Figure 3-2A** and **3-2B**). BP remained unchanged, and the time constant of $\dot{V}O_2$-pulmonary was one-third that of HR. In addition, the PET data support measures of systemic parameters. Because there was a significant *Time* effect of oxygen extraction fraction in the most proximal portions ($P = 0.032$, **Table 3-2**), oxygen extraction was lower after than before exercise. These results support previous reports (Bangsbo and Hellsten, 1998; Bangsbo et al., 1990; Morganroth et al, 1975), and demonstrate that excess perfusion may occur during recovery from exercise.

With regional blood flow distribution, however, PET showed a high correlation between muscle blood flow and oxygen uptake, demonstrating a tight coupling between oxygen supply and demand during recovery from exercise (**Figure 3-5**). The inconsistency between systemic and regional PET parameters can be explained by differences in spatial resolution of hemodynamic measurements. PET was able to detect the coupling between hemodynamic variables, which might disappear with excess perfusion during recovery; this might be difficult to detect using measurement at the whole body and limb level. Previous studies (Bangsbo and Hellsten, 1998; Bangsbo et al., 1990; Morganroth et al, 1975) that reported a 'mismatch' between oxygen supply and oxygen demand used a technique based on Fick's principle, which is regarded as the "gold standard" measure of hemodynamics in skeletal muscle. However, two recent studies (Boushel et al., 1998; VanBeekvelt et al., 2001) demonstrated that this technique is not able to quantify local changes as well as near-infrared spectroscopy techniques. In addition, Berg et al. (1997) showed a direct coupling between blood flow and energy metabolism induced by electrical stimulation in a micro-vascular system. Similarly, our data show a correlation

between blood flow and oxygen consumption, indicating a consistent relationship between these hemodynamic variables during both exercise and post-exercise recovery.

3-5. CONCLUSION

We found a significant gradient in blood flow and oxygen uptake in resting muscle, decreasing in the direction from proximal to distal portion; the magnitude of this gradient diminished after exhaustive exercise in recovering muscle. Consequently, during recovery from exercise, there was a significant positive relationship between changes in blood flow and oxygen consumption in the exercised muscle; this increment became larger in the direction from proximal to distal portions. These results suggest that there is a systematic difference between proximal and distal regions in the quadriceps femoris muscle. The present study also demonstrates that, when evaluating cardiorespiratory parameters during and after exercise, it is important to measure both the time-course and spatial changes in human skeletal muscle.

Chapter 4. Regional differences in blood volume and blood transit time in resting skeletal muscle

4-1. INTRODUCTION

Skeletal muscle blood flow is heterogeneous in muscle groups and in single muscle in rats (Laughlin and Armstrong, 1982) and in humans (Kalliokoski et al., 2000; Kalliokoski et al., 2001; Mizuno et al., 2003a). This heterogeneity may arise from regional differences in capillary density or muscle fiber type (Laughlin and Armstrong, 1982). We have previously used positron emission tomography (PET) to visualize regional differences in blood flow in human resting

skeletal muscle, and have recently reported that blood flow decreases from proximal to distal potions within the quadriceps muscle (Mizuno et al., 2003a). We propose that this regional difference in blood flow may result from differences in skeletal muscle fiber type or distance from the heart within the thigh muscles. The purpose of this study was to clarify the reason for these regional differences in blood flow. To address this issue, we used PET to measure muscle blood volume (as an index of vascular distribution) and blood transit time (as an index of blood flow velocity) in human resting muscle.

4-2. METHODS

Subjects

Five healthy male subjects participated in this study; their mean ± SD age was 23.6 ± 1.8 yr; mean height 173.6 ± 4.0 cm; and mean weight 66.7 ± 9.5 kg. Written informed consent was obtained after the purpose, nature, and potential risks of the experiments were explained to the subjects. The Ethics Committee of the Tokyo Metropolitan Institute of Gerontology approved the study protocol.

Experimental protocol

Subjects were instructed to avoid heavy exercise before the study, and had fasted overnight for at least 12 hours. After each subject rested for 30 min in a supine position, a catheter was inserted into a radial artery to withdraw blood to measure the radioactivity of the plasma, and blood oxygen saturation and hemoglobin content. The subjects were then positioned supinely in a PET scanner with their legs were fixed with a plastic cover to prevent

any abrupt movement. The femoral regions of both legs were located in the 200 mm field of view, and the center of view was positioned at a point midway between the trochanter major and the knee joint. A transmission scan was performed for 8 min to correct for photon attenuation. Emission scans were then performed to measure regional muscle blood flow and blood volume after subjects inhaled [^{15}O]CO_2 and [^{15}O]CO, respectively. Before and after the PET scans, the systemic parameters of heart rate, blood pressure, and pulmonary oxygen uptake ($\dot{V}o_2$-pulmonary) were measured. The average of two measurements was recorded and analyzed.

Measurements

PET measurement: The [^{15}O]CO_2 and [^{15}O]CO gases were produced by a small cyclotron (CYPRIS-370, Sumitomo Heavy Industries, Ltd., Tokyo, Japan). The PET measurements were performed using a HEADTOME-V scanner (Shimadzu Co., Kyoto, Japan), which acquires 32 slices at a center-to-center interval of 6.25 mm, with transverse resolution of 4.2 mm at full-width of half maximum and axial resolution of 4.5 mm full-width of half maximum. Arterial blood was continuously withdrawn at a flow rate of 3 mL min^{-1}, and the radioactivity was measured using an on-line radioactivity detector system (ß-counter, Shimadzu Co., Kyoto, Japan). Arterial blood was sampled 0, 1, 3, 5, 7, and 8.5 min after gas inhalation, and radioactivity was measured with a well-type τ-counter (BSS, Shimadzu Co., Kyoto, Japan) to calibrate the count from the ß-counter. All data were corrected for dead time, decay, dispersion, and photon attenuation before data analysis.

Blood flow: The subjects underwent simultaneously a 4-min continuous inhalation of [^{15}O]CO_2 containing 1500 MBq mL^{-1}, and an 8-min static PET scan. The blood flow (mL 100g^{-1} min^{-1}) value was calculated using an autoradiographic method from equation (*1*) as previously described (Mizuno et al., 2003a)

$$C(t) = f \int_0^t Ca(\tau)d\tau - \frac{f}{p}\int_0^t C(\tau)d\tau \quad \text{----------(1)}$$

where *C* and *Ca* indicate the radioactivity in tissue and arterial plasma, respectively. The parameter *f* denotes blood flow, and *p* is the partition coefficient of water in the skeletal muscle, which was considered a constant and fixed at a value of 0.95 (Ruotsalainen et al., 1997). The calculation for muscle blood flow is based on the radioactive atoms of oxygen was transferred from [^{15}O]CO_2 to [^{15}O]H_2O in the pulmonary capillary blood, which becomes distributed throughout the total volume of blood in the body.

Blood volume: The subjects received a 4-min continuous inhalation of [^{15}O]CO mixed room air containing 2000 MBq mL^{-1}, and then a 6-min static PET scan was performed following a 2-min room air inhalation. The blood volume (mL 100g^{-1}) value was calculated from equation (*2*), as previously described (Mizuno et al., 2003a)

$$blood\ volume = \frac{Aco}{Tco} \cdot \frac{1}{R} \text{----------} (2)$$

where *Aco* and *Cco*, represent the radioactivity of [^{15}O]CO in arterial blood and in tissue, respectively and *R* represents the tissue-to-large vessel hematocrit ratio. The applied value of *R* was 0.91 (Raitakari et al., 1995). The calculation for muscle blood volume is based on the strong affinity of CO for red blood cells to form carbohemoglobin, which becomes distributed throughout the total volume of blood in the body.

Blood transit time: The blood transit time in muscle was calculated by dividing blood volume by blood flow (Kallikoski et al., 2001).

Regions of interest: The regions of interest were placed on the quadriceps femoris muscle group of both thighs in five of a total of 32 slices acquired. The slices included the centers of the greater trochanter and knee joint, and 31 mm and 62 mm from these centers in both the proximal and distal directions. The regions of interest were delineated with references to typical magnetic resonance images and to individual images of the transmission scan to avoid large vessels (**Figure 4-1**). To identify the quadriceps muscle at each portion, we also referenced to the PET images after exhaustive exercise from the same subjects (Mizuno et al., 2003a)

Systemic parameters: The heart rate and blood pressure were measured simultaneously using an automatic blood pressure analyzer (EBP300, Minato Medical Science Co., Tokyo, Japan) on the left upper arm before and after the PET scans. The $\dot{V}o_2$-pulmonary value was also measured using an automatic gas analyzer (MG360, Minato Medical Science Co., Tokyo, Japan) and an automatic respiration monitor (RM300, Minato Medical Science Co., Tokyo, Japan). The average of two measurements was recorded and analyzed.

Statistics

The PET parameters included data from both legs (n = 10 for each position in five subjects). For each parameter, a nonparametric test for regression, Kendall's robust line-fit method, was performed on the five positions. Kendall's rank correlation coefficient was applied to test for significance. *P* values < 0.05 were considered statistically significant.

4-3. RESULTS

Systemic parameters

Table 4-1 shows the systemic parameters determined during this study. There was no remarkable difference in systemic parameters between subjects.

Table 4-1. Heart rate, blood pressure, and pulmonary oxygen uptake of five subjects.

	Heart rate	Blood pressure (mmHg)			Vo_2-
	(bpm)	MBP	SBP	DBP	(ml min^{-1})
Subject 1	48	86	114	72	233.6
Subject 2	50	87	118	71	222.5
Subject 3	54	89	117	74	250.7
Subject 4	57	82	104	71	281.8
Subject 5	53	83	105	73	204.3
mean	52.2	85.4	111.6	72.2	238.6
SE	1.6	1.3	3.1	0.7	13.2

The systemic parameters were measured before and after PET scans. Values are expressed as the average of two measurements. MBP, mean blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; and $\dot{V}o_2$-pulmonary, pulmonary oxygen uptake.

PET parameters

Figure 4-1 shows typical PET images of muscle blood volume. **Figure 4-2** summarizes the portion-dependency of the hemodynamic parameters. As previously reported by us, blood flow decreased in the direction from the proximal to the distal portions (e.g., from 2.0 ± 0.5 to 1.4 ± 0.3 mL 100 g^{-1} min^{-1}) (Mizuno et al., 2003a). Blood volume was not significantly different between the five portions of muscle (**Figure 4-2A**). Consequently, the tendency of blood transit time values increase from proximal to distal portions was significant (**Figure 4-2B**).

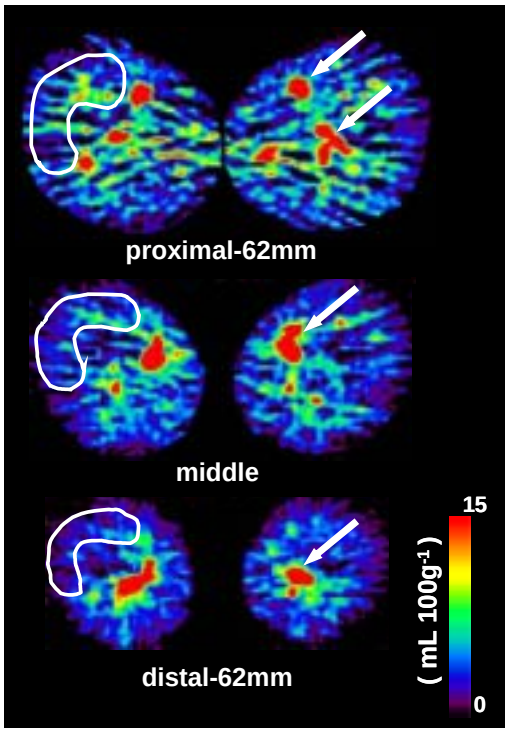

Figure 4-1. Typical PET images of muscle blood volume. Panels represent the transverse images at the proximal–62 mm (top), middle (middle), and distal–62 mm (bottom) portions. Regions of interest are indicated by white lines on the left legs. White arrows on the right legs indicate large blood vessels.

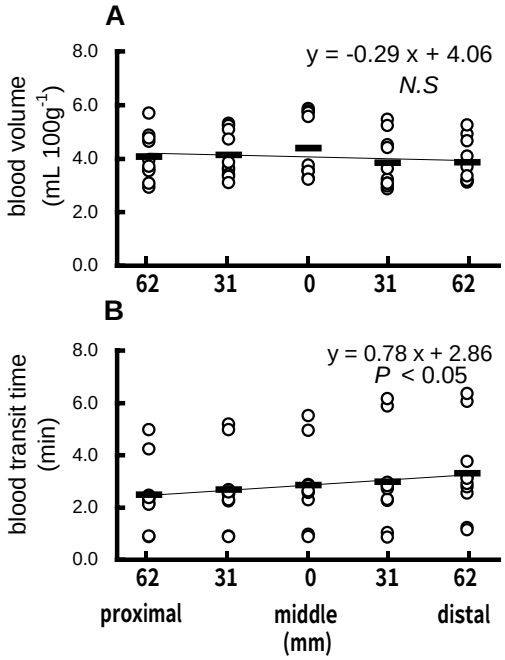

Figure 4-2. Position-dependency of the hemodynamic parameters measured by PET in five different portions of the quadriceps femoris muscle group. A, blood volume; B, blood transit time. Ten sets of data for each portion were obtained from the left and right legs of the five subjects. Horizontal bars indicate the mean values in each portion.

4-4. DISCUSSION

We previously reported that blood flow significantly decreases from the proximal to distal portions in resting quadriceps muscle (e.g., from 2.0 ± 0.5 to 1.4 ± 0.3 mL 100 g^{-1} min^{-1}) (Mizuno et al., 2003a). We performed the present study to identify the mechanism(s) responsible for this regional variation in blood flow within a resting skeletal muscle.

Blood transit time significantly related to portions of the muscle, increasing in linear fashion from proximal to distal portions (**Figure 4-2B**). Considering the lack of difference in arteriole and/or capillary structure of the muscle, this may reflect decreasing blood flow velocity in the muscle as a function of the distance from the heart. Using an ultrasound/Doppler technique to measure blood flow velocity in large vessels of the lower extremity in humans, Holland et al. (1998) reported that the velocity in the popliteal artery was lower than that in the femoral artery. Thus, the decline in blood flow in a lengthwise direction in the resting quadriceps muscle, which we previously reported (Mizuno et al., 2003a), most likely reflects the decline in the blood flow velocity with distance from the heart.

A previous animal study reported a relationship between regional blood flow and the percentage content of slow oxidative (SO)-type fibers in resting muscle (Laughlin and Armstrong, 1982). In animal studies, the distal portion of skeletal muscle has a lower percentage of SO-type fibers than the proximal portion (Torrella et al., 2000; Wang and Kernell, 2000). Regional differences in blood flow may reflect regional differences in the number of arterioles and/or capillaries, which may depend on the muscle fiber types (Laughlin and Armstrong, 1982). If the muscle fiber type distribution differed in a lengthwise direction, we would expect to find differences in blood volume, which represents arteriole and/or capillary density, among the

different portions of muscle. In our current study, however, there was no significant difference in blood volume between different regions of the muscle. One possible explanation is that not all the capillaries are open at rest (Honig et al., 1980). Alternative explanation is that human have fairly mixed fiber types in the most muscles (Johnson et al., 1973). Likewise, PET cannot evaluate blood volume with distinguishing between arterial and venous blood. Therefore, venous blood volume affect on the values of muscle blood volume in the resting state when most of the blood volume can be in the veins (Rowell, 1993). Thus, difference in blood flow within resting muscle cannot be explained only by regional differences in blood volume.

In our current study, the subjects continued to rest during PET measurement, so that the hemodynamic variables remained constant (**Table 4-1**). To minimize any effects of unwanted muscle contraction during PET measurements, the subjects' legs were fixed with a plastic cover. Subjects also rested in a supine position for 30 min before beginning the PET measurements.

4-5. CONCLUSION

We used PET to study hemodynamic variables (blood volume and blood transit time) in the human resting quadriceps femoris muscle. We found a position dependency in the blood transit time in a lengthwise direction, but not in blood volume. The blood transit time significantly related to portions of the muscle, increasing in linear fashion from proximal to distal portions, whereas blood volume remained constant among the portions. We conclude that the blood flow velocity slows from proximal portion to distal portions within a given muscle despite no regional changes in blood volume.

Chapter 5. Inflection points of cardiovascular responses and oxygenation are correlated in the distal but not the proximal portions of muscle during incremental exercise

5-1. INTRODUCTION

Exercise stimulates the cardiovascular response by two neural mechanisms: central command (Eldridge et al., 1985) and the exercise pressor reflex (Mitchell, 1985; Victor et al., 1989). The exercise pressor reflex is a feedback mechanism that acts via afferent nerves, which arise from the peripheral system and are capable of sensing chemical and mechanical stimuli (Kaufman and Rybicki, 1987). One such chemical stimulus is a metabolic error signal resulting

from the mismatch between metabolic demand and oxygen supply in exercising muscle (Hanna et al., 2002).

Oxygen supply is heterogeneous in the skeletal muscle of animals (Laughlin and Armstrong, 1982; Piiper et al., 1985) and humans (Kalliokoski et al., 2000; Mizuno et al., 2003a) at rest and during exercise. This heterogeneity in blood supply between proximal and distal portions (Mizuno et al., 2003a; Piiper et al., 1985) within a given muscle is remarkable. Within a given muscle, intramuscular pressure is greater in the distal portion than in the proximal portion, and this difference in pressure is a major cause of heterogeneity in regional blood flow (Ameredes and Provenzano, 1997). Differences in the muscle architecture can affect intramuscular pressure, and higher pressure inhibits circulation during muscle contraction (Miura et al., 2004). Consequently, oxygenation status, which reflects the balance of oxygen utilization and oxygen supply, can differ between proximal and distal portions of a human muscle. For example, near-infrared spectroscopy (NIRS) has shown that deoxygenation occurs to a greater extent in the distal portion than in the proximal portion of the medial gastrocnemius muscle (Miura et al., 2001; Miura et al., 2004).

Cardiovascular variables do not always exhibit a simple linear pattern during incremental exercise (Kagaya, 2003; Riley et al., 1997). Several physiological variables exhibit threshold phenomena during incremental exercise; the anaerobic threshold is a prime example. Grassi et al. (1999) reported that the onset of muscle deoxygenation, determined with NIRS as the inflection point of muscle oxygenation, is highly correlated with the onset of blood lactate accumulation during incremental exercise on a cycle ergometer. However, it is unclear whether there is a regional difference in the inflection point of muscle oxygenation within a given muscle,

nor is the relationship between the inflection point of cardiovascular variables and muscle oxygenation completely understood.

The first objective of this investigation was to examine whether there is a regional difference (i.e., proximal vs. distal) in the inflection point of oxygenation within a given muscle during incremental exercise. The primary goal was to test the hypothesis that there is a regional difference in the exercise pressor reflex within a given muscle by examining whether the inflection point of cardiovascular responses and oxygenation are correlated in either or both the proximal and distal portions. We hypothesized that the inflection point of the cardiovascular response might be correlated with that of the distal but not the proximal portion of an exercising muscle.

5-2. METHODS

Subjects

Seven men participated in this investigation; their mean age was 24.6 ± 3.8 (SD) yr, height 171.9 ± 4.6 cm, and weight 67.5 ± 11.0 kg. The subjects were fully informed of the purpose, nature, and potential risks of the experiments, and gave their written informed consent to participate in this study. This study was approved by the local ethics committee and all work was performed in accordance with the Declaration of Helsinki.

Experimental protocol

Static knee extension was employed as the exercise in this study. Subjects were positioned supinely with the knee joint flexed to 45º and remained in a supine position during

testing and recovery. Maximal torque during static knee extension was determined in the right leg using the Cybex® isokinetic dynamometer (Lumex Inc., Ronkonkoma, NY). The average of three attempts was taken as the subject's maximal torque.

We used the modified incremental exercise test originally reported by Kagaya (10). Exercise consisted of incremental 30 s static knee extension exercises, each separated by 30 s of recovery in a supine position. The initial load was 5% of MVC, and the load was increased by 5% MVC every 60 s until exhaustion. Subjects performed incremental static knee extensions to an average of 59.3% of MVC (SD 7.9%, range 55–75%). Subjects monitored their exerted torque with an oscilloscope (DCS7020, Kenwood, Tokyo, Japan).

Measurements

Heart rate (HR) was determined using standard ECG leads (OEC-8108, Nihon Kohden, Tokyo, Japan). Arterial blood pressure was measured with a finger cuff (2300 Finapres, Ohmeda Inc., Englewood, CO). The monitoring finger cuff was placed around the middle finger of the left hand. Averaged values were calculated from HR and mean arterial blood pressure (MAP) values obtained during the final 5 s of each 30 s exercise interval.

Calf blood flow in the nonexercising leg was measured by venous occlusion plethysmography using a mercury-in-silastic strain gauge (EC-5R, Hokanson Inc., Bellevue, WA). The left leg was maintained in a horizontal position at heart level. The strain gauge was placed around the largest area of the left calf. A pneumatic cuff placed around the thigh was inflated to 60 mmHg to measure calf blood flow during the final 5 s of each 30 s exercise interval. As an indicator of sympathetic nerve activity (Hansen et al., 1994; Ray and Mark,

1995; Saito et al., 1990; Seals, 1989), calf vascular resistance (CVR) in the nonexercising leg was calculated by dividing MAP by calf blood flow.

To provide an indicator of muscle oxygenation, the change in optical density (OD) of oxygenated hemoglobin (O_2Hb) was measured at 1 Hz by near-infrared continuous wave spectroscopy (NIRcws) using a commercially available device (OM-200; Shimadzu Co., Kyoto, Japan) in NIRcws mode. One NIRS probe was placed on the proximal portion and one NIRS probe on the distal portion of the vastus lateralis muscle; the portions of the muscle were determined by measuring 25% (proximal) and 75% (distal) of the length between the greater trochanter and the knee joint in the exercising leg. The light emitter–detector distance of the device was 40 mm. This apparatus uses three wavelengths of near-infrared light to measure changes in OD in O_2Hb and deoxygenated hemoglobin (HHb). O_2Hb and HHb were calculated according to the following equations:

$$\Delta[O_2Hb] = -1.631 \cdot \Delta Abs_{780} + 0.683 \cdot \Delta Abs_{805} + 1.605 \cdot \Delta Abs_{830}$$

$$\Delta[HHb] = 1.970 \cdot \Delta Abs_{780} - 0.314 \cdot \Delta Abs_{805} - 1.195 \cdot \Delta Abs_{830}$$

where ΔAbs_{780}, ΔAbs_{805}, ΔAbs_{830} are the absorbance changes at the near-infrared light wavelengths of 780 nm, 805 nm, and 830 nm, respectively. The equations had been experimentally determined by the manufacturer. The changes in OD for total hemoglobin (total Hb) were calculated by summing the changes in OD of O_2Hb and HHb. $\Delta[O_2Hb]$ and $\Delta[HHb]$ were calculated with respect to an initial arbitrarily set value equal to zero and expressed in arbitrary units. The sum of the two variables, $\Delta[O_2Hb + HHb]$, reflects changes in the "total Hb volume" in the muscle region of interest, whereas the difference between the two variables,

$\Delta[O_2Hb - HHb]$, reflects an "oxygenation index". Average $\Delta[O_2Hb]$, $\Delta[HHb]$, $\Delta[O_2Hb + HHb]$, and $\Delta[O_2Hb - HHb]$ values were calculated during the final 5 s of each 30 s exercise interval.

Myoelectric activity was detected using surface electromyography (EMG), and recorded using bipolar 5 mm diameter Ag/AgCl electrodes with an interelectrode distance of 40 mm. The EMG electrodes were placed on the same muscle portions used to measure oxygenation. Signals were amplified by a bioelectric amplifier (AB-621G; Nihon-Kohden, Tokyo, Japan), and the EMG for each muscular contraction was integrated using an EMG integrator (Maclab; ADInstruments, Castle Hill, Australia). Maximum integrated EMG (iEMG) was calculated by averaging the three maximum values obtained during the maximum knee extension test before the exercise session. The iEMG value of each contraction was normalized to the maximum value. Averaged iEMG values were calculated during the final 5 s of each 30 s exercise interval.

Calculation of inflection points

Inflection points for MAP, CVR, and $\Delta[O_2Hb - HHb]$ were determined by iteratively fitting different combinations of two linear regressions to contiguous experimental points obtained during incremental exercise and by evaluating which combination yielded the lowest sum of squared residuals (Grassi et al., 1999). This analysis did not use resting values for each variable. Although the number of averaged beats differed by 5–8 beats at each workload, we calculated average values of MAP and HR in the final 5 s of each workload to match sampling time with other parameters such as NIRS, calf blood flow, and iEMG.

On a day other than the day of the exercise session, an ultrasonic apparatus (SSD-1000, Aloka, Tokyo, Japan) was used to measure thigh fat thickness in the same muscle portions used to measure oxygenation. Thigh fat thickness did not differ significantly between proximal (4.0 ± 0.4 mm) and distal (3.4 ± 0.4 mm) portions (mean ± SE).

Statistical analysis

All data are represented as mean ± SE. For regional parameters, a two-way ANOVA, with load and portions as main effects, was used to determine significant differences. If the *F*-test was significant, pairwise comparisons were performed using Scheffe's *post hoc* test. The student's unpaired *t*-test was used to test differences between inflection points of oxygenation in the proximal and distal portions of the vastus lateralis muscle. Simple linear regression analysis was used to determine the relationship between the inflection points of cardiovascular responses and muscle oxygenation. Values of $P < 0.05$ were considered significant.

5-3. RESULTS

Figure 5-1 summarizes the changes in cardiovascular and regional variables. HR, MAP, and CVR significantly increased with increasing workload (**Figures 5-1*A***, **5-1*B***, and **5-1*C***). $\Delta[O_2Hb - HHb]$ and iEMG changed significantly with increasing workload (**Figures 5-1*E*** and **5-1*F***). In contrast, $\Delta[O_2Hb + HHb]$ did not change significantly with increasing workload (**Figure 5-1*D***). There were significant differences between muscle portions in $\Delta[O_2Hb + HHb]$ and $\Delta[O_2Hb - HHb]$, but not in normalized iEMG; no significant interactions were observed for any variable.

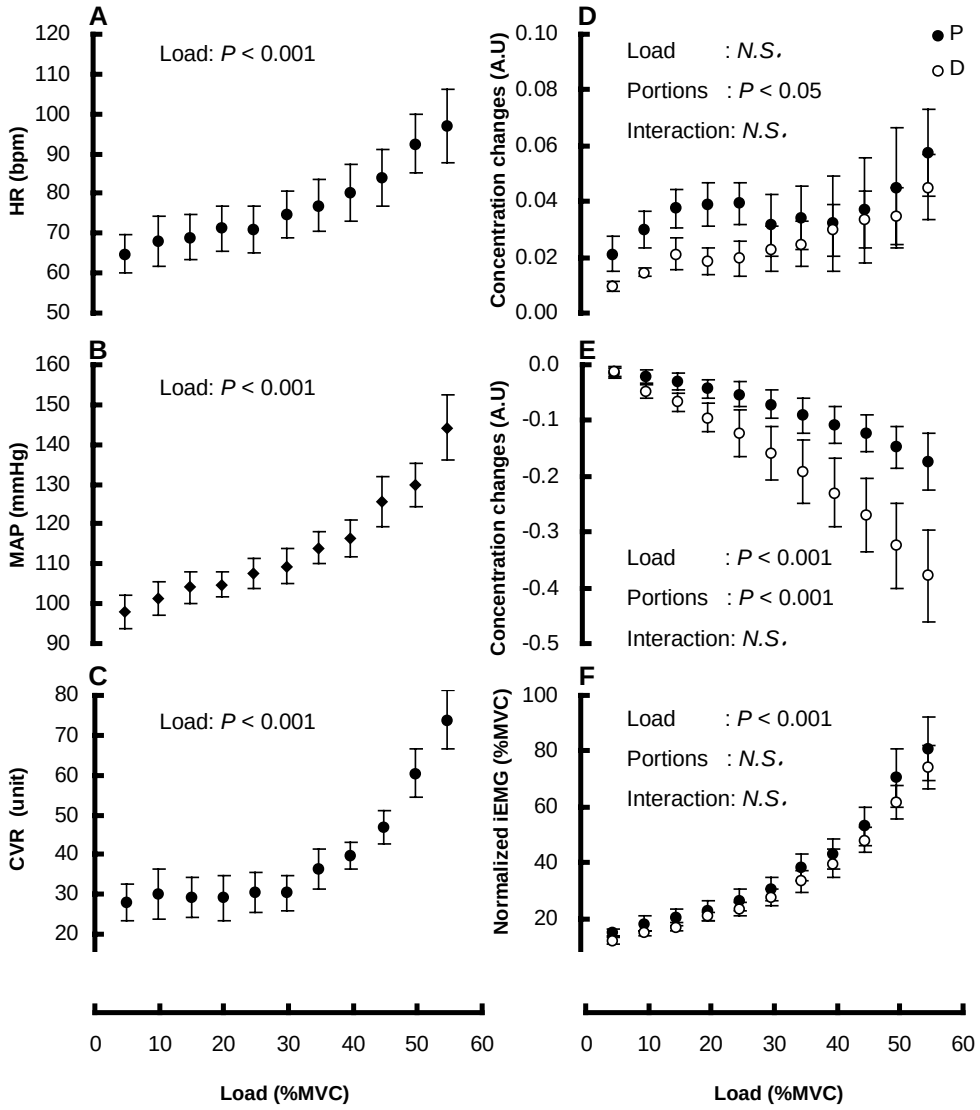

Figure 5-1. Summary data (n =7) showing changes in cardiovascular responses and regional variables during incremental exercise. *A:* heart rate (HR). *B:* mean arterial pressure (MAP). *C:* calf vascular resistance (CVR). *D:* $\Delta[O_2Hb + HHb]$ (total Hb volume). *E:* $\Delta[O_2Hb - HHb]$ (oxygenation index). *F:* normalized integrated electromyogram (normalized iEMG). P, proximal portion (●); D, distal portion (○). All variables are shown for exercise up to 55% MVC, which all subjects completed (see Methods) except for the CVR for one subject (subjects 4) who did not complete the final increment at 55% MVC (see Results).

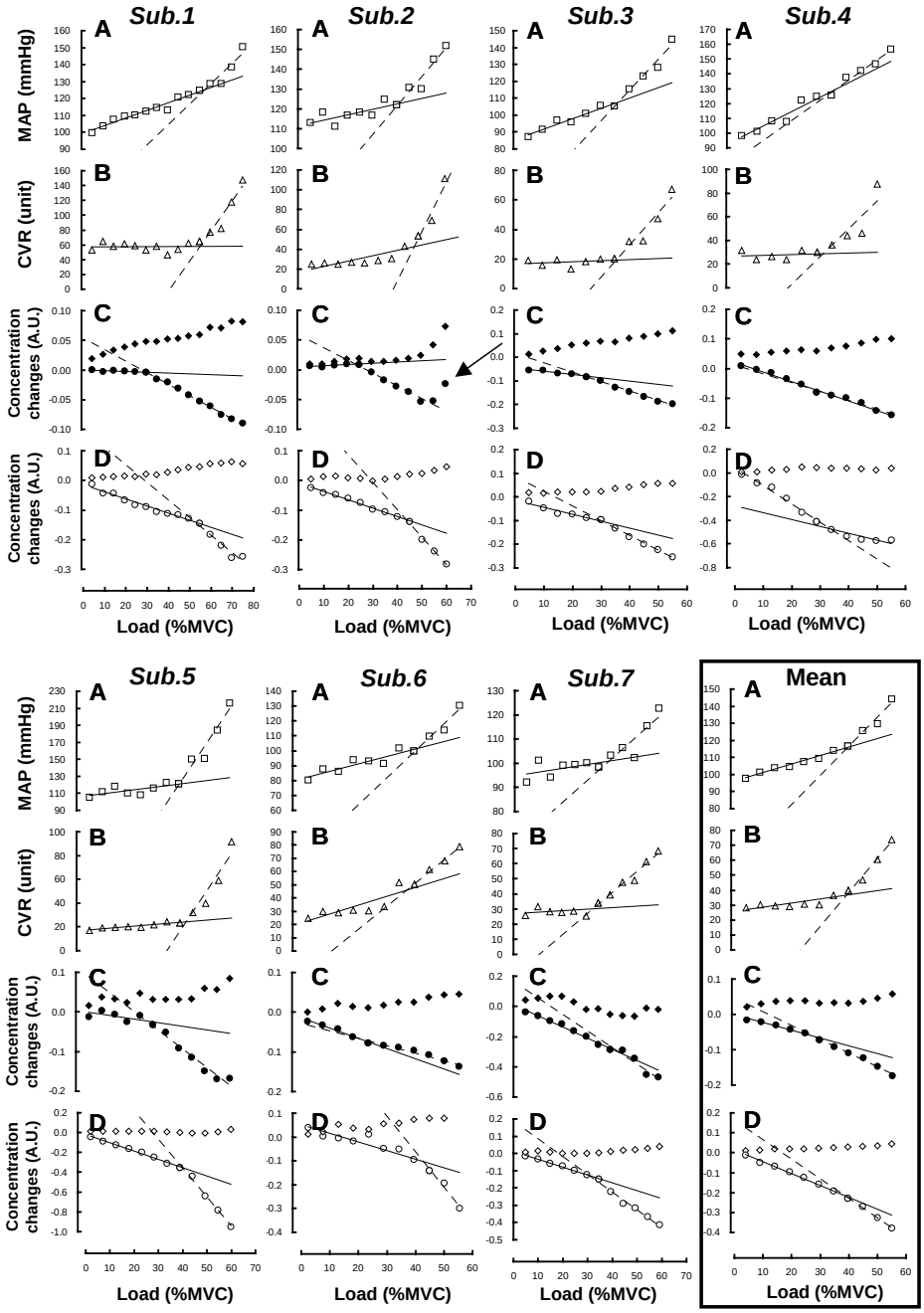

Figure 5-2. Individual data (*Subjects 1–7*) and the group mean (n = 7) during incremental static knee extension exercise. *A:* mean arterial pressure (MAP). *B:* calf vascular resistance (CVR). *C:* concentration changes of $\Delta[O_2Hb + HHb]$ (total Hb volume, ◆) and $\Delta[O_2Hb - HHb]$ (oxygenation index, ●) in the proximal portion of the vastus lateralis muscle. *D:* concentration changes of $\Delta[O_2Hb + HHb]$ (total Hb volume, ◇) and $\Delta[O_2Hb - HHb]$ (oxygenation index, ○) in the distal portion of the vastus lateralis muscle. A change in slope (inflection point) in these variables was identified by calculating the intersection of two linear regression equations representing lower (solid lines) and higher (dotted lines) exercise workloads. In the data for *Subject 2*, the arrow indicates an individual value that was not included in the linear regression analysis. In the data for *Subject 4*, the value for CVR at 55% MVC was missing. The group mean of completed incremental exercise was 59.3% MVC (SD 7.9%, range 55–75% MVC).

Figures 5-2 shows individual data and the group mean for variables MAP, CVR, $\Delta[O_2Hb + HHb]$, and $\Delta[O_2Hb - HHb]$. Inflection points of these variables were identified by calculating the intersection of two linear regression equations; in these figures, the solid and dotted lines represent the regression fits of the shallow and steeper slopes that occur across a range of workloads. One subject (*subject 2*) showed a leveling off in $\Delta[O_2Hb - HHb]$ in the proximal portion at the highest exercise workload. This value was not included in the regression analysis. We were unable to measure calf blood flow and to obtained data for CVR at 55% MVC one subject (subjects 4); however the inflection point of CVR was clearly demonstrated. All variables exhibited similar patterns of change, except for $\Delta[O_2Hb - HHb]$ in *subject 4*. The inflection points of each parameter are clearly evident in both the individual and mean responses.

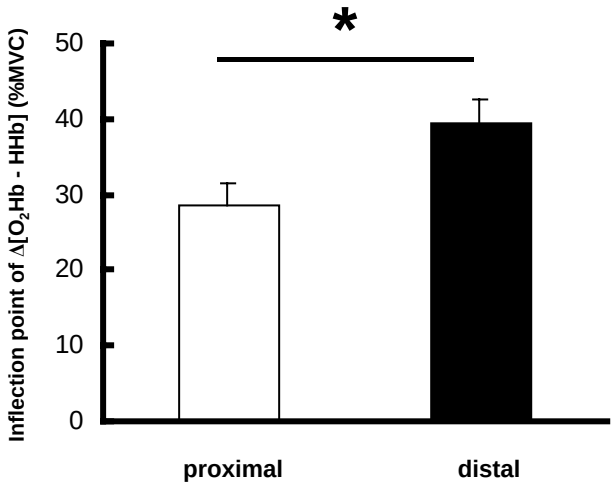

Figure 5-3. Comparison of the mean inflection point of $\Delta[O_2Hb - HHb]$ (oxygenation index) between the proximal and distal portions of the vastus lateralis muscle during incremental static exercise. The data were calculated for individual subjects (n = 7). Open bar, proximal portion; solid bar, distal portion. * Significant difference, $P < 0.05$.

Figure 5-3 shows the values for the inflection point of $\Delta[O_2Hb - HHb]$ in both proximal and distal portions during incremental exercise. The inflection point in the proximal portion was significantly lower than that in the distal portion (28.5 ± 3.0 vs. 39.5 ± 3.0% MVC, respectively, $P < 0.05$); that is, the inflection point occurred at a lower percentage of MVC in the proximal portion than in the distal portion. In addition, in the distal portion the inflection points for MAP and CVR occurred at similar percentages of MVC (MAP, 41.2 ± 2.4 % MVC; CVR, 39.8 ± 3.1% MVC).

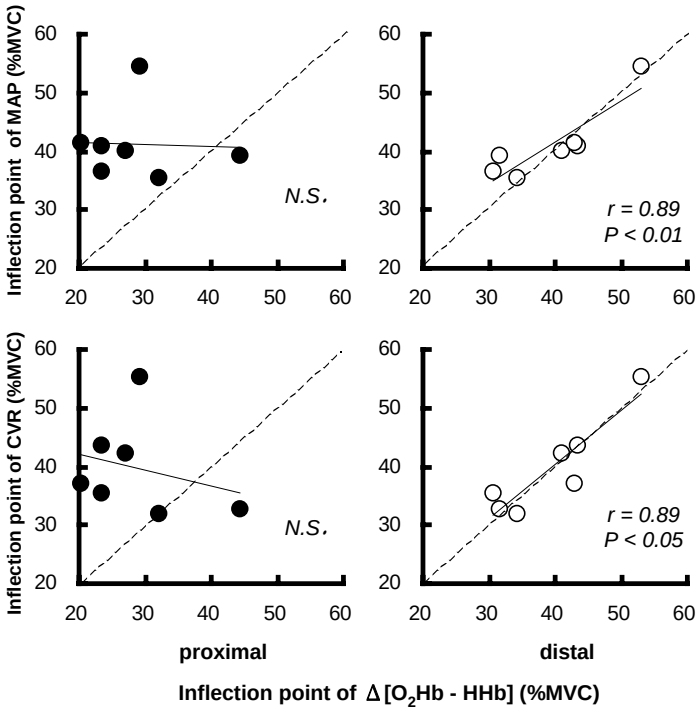

Figure 5-4. Relationship between inflection points of the cardiovascular responses and $\Delta[O_2Hb - HHb]$ (oxygenation index). Mean arterial pressure (MAP), *top* panels; calf vascular resistance (CVR), *bottom* panels, and $\Delta[O_2Hb - HHb]$ in the proximal portion (*left* panels) and distal portion (*right* panels). Dotted lines represent identical lines.

Figure 5-4 shows the relationship between the inflection points of cardiovascular variables and $\Delta[O_2Hb - HHb]$. The inflection point of MAP was significantly correlated with

that of $\Delta[O_2Hb - HHb]$ in the distal portion ($r = 0.89$, $P < 0.01$), but not in the proximal portion ($r = 0.05$, ns). Similarly, the inflection point of CVR was significantly correlated with that of $\Delta[O_2Hb - HHb]$ in the distal portion ($r = 0.89$, $P < 0.05$), but not in the proximal portion ($r = 0.07$, ns).

5-4. DISCUSSION

The two main purposes of this study were to quantify regional differences in the inflection point of muscle oxygenation and to clarify their relationship with systemic cardiovascular responses.

Physiological factors that might have affected the NIRS signal

NIRS is used to monitor oxygenation status in exercising muscles (Grassi et al., 1999; Miura et al., 2001; Miura et al., 2004). The subcutaneous fat layer can strongly influence the NIRS signal (McCully and Hamaoka, 2000). A previous review by McCully and Hamaoka (2000) showed that in NIRS the light travels in a shallow arc to a penetration depth of about one-half the separation distance into the tissue. Thus, as the light emitter–detector distance of the device used in the present study was 40 mm, we estimate that the penetration depth was ~20 mm. The thickness of adipose tissue where NIRS measurements were performed did not differ significantly between portions (4.0 ± 0.4 mm in the proximal and 3.4 ± 0.4 mm in the distal portions; see Methods). Thus, the NIRS signal reflected the metabolic changes occurring mainly in the muscle tissue in both muscle portions. Similarly, the fat layer would not have affected the inflection point of the oxygenation index.

Regional differences in changes in total Hb volume and oxygenation between portions

Because the normalized iEMG did not differ between portions during incremental exercise (**Figure 5-1*F***), we expected that muscular electrical activity would also be similar in the two portions. However, deoxygenation during incremental exercise was greater in the distal portion than in the proximal portion (**Figure 5-1*E***). This result confirms data from previous studies by Miura et al. (2000; Miura et al., 2004), who showed that regional differences in oxygenation status are consistent with regional differences in muscle architecture, which are related to regional differences in intramuscular pressure. Ameredes and Provenzano (1997) showed that intramuscular pressure is greater in the distal portion than in the proximal portion of a muscle. Higher intramuscular pressure would inhibit circulation during muscle contraction, which might affect the inflection point of muscle deoxygenation. An alternative explanation is that, within a single muscle, the proximal portion contains a higher percentage of slow oxidative fibers than does the distal portion (Torrella et al., 2000; Wang and Kernell, 2000). Oxidative fibers have a higher aerobic capacity and capillary density (Laughlin et al., 1982), so that the proximal portion might have a higher aerobic capacity and capillary density than the distal portion. It is possible that during incremental exercise, deoxygenation occurs to a lesser extent in the proximal portion because of the higher blood flow and oxygen supply to this portion than in the distal portion.

The total Hb volume in the proximal portion was higher than in the distal portion during exercise; this finding supports the data from Miura et al. (2001; Miura et al., 2004). However we observed no significant changes with increasing workload in both muscle portions

(**Figure 5-1*D***). Although this result contrasts with data from previous studies (Miura et al., 2001; Miura et al., 2004), this discrepancy can be explained by differences in body position (e.g., standing vs. supine) or the type of muscle contraction (e.g., dynamic vs. static) between our study and previous studies (Miura et al., 2001; Miura et al., 2004). During static muscle contraction, as used in our study, intramuscular pressure inhibits arterial inflow to and venous outflow from the exercising muscle preventing any change in total Hb volume. In contrast, rhythmic muscle contraction as used by Miura et al. (2001; Miura et al., 2004) elicits arterial back-flow and increases venous blood flow via the muscle pump; consequently, total Hb volume decreased in both portion of the exercising muscle. Furthermore, hydrostatic pressure and sympathetic nerve activity, which are greater in standing than in supine position, might have promoted a decrease in total Hb volume in the previous study (Miura et al., 2001; Miura et al., 2004).

Relationships between the inflection points of cardiovascular variables and muscle oxygenation

The inflection point of the oxygenation index was tightly coupled to that of cardiovascular variables in the distal portion, but not in the proximal portion (**Figure 5-4**). The exercise pressor reflex is a feedback mechanism that acts via afferent nerves arising from the peripheral system, which are capable of sensing chemical and mechanical stimuli. Intramuscular pressure increases linearly with increasing exerted torque during isometric muscle contraction (Ballard et al., 1998; Sjersted et al., 1989). In our study, muscle mechanical stimuli might have increased in both portions independent of the inflection point. In contrast, one chemical stimulus is a metabolic error signal resulting from the mismatch between metabolic demand and oxygen

supply in exercising muscle (Hanna et al., 2002). Evaluation of the muscle oxygenation index by NIRS reflects the balance between oxygen utilization and oxygen delivery. Grassi et al. (1999) showed that the inflection point of muscle deoxygenation is coupled with that of blood lactate accumulation. During muscle contraction, sympathetic nerve discharge evoked by the exercise pressor reflex is associated with the accumulation of lactic acid (Ettinger et al., 1991; Kaufman and Hayes, 2002), and a decrease in muscle pH (Sinoway et al., 1989; Systrom et al., 1990; Victor et al., 1988), which were enhanced by muscle anaerobic metabolism.

As mentioned above, one possible explanation for regional differences in sensing the exercise pressor reflex might relate to differences in the longitudinal distribution of skeletal muscle fiber type between proximal and distal portions (Torrella et al., 2000; Wang and Kernell, 2000). Wilson et al. (1995) showed that the fiber type of the contacting muscle influences the magnitude of the pressor response to static contraction; that is, static muscle contractions elicit a larger pressor reflex in predominately glycolytic muscle than in primarily oxidative muscle. An alternative explanation is that the distribution or number of afferent nerves may differ between proximal and distal portions within a muscle. Kumazawa and Mizumura (1977) reported that muscular polymodal receptors are more frequently found in the head, the tail, and the edge of dog muscle. Further neurophysiological investigation is needed to identify the distribution of afferent nerves in human muscle.

The exercise pressor reflex is affected by muscle mass (Iellamo et al., 1999), and is greater when arising from forelimb as opposed to hindlimb muscles (Hayashi et al, 2001; Saito, 1995). To date, it is unclear whether there are regional differences in the exercise pressor reflex within an exercising muscle. However our present data suggest that the metabolic error signal

resulting from the mismatch between metabolic demand and oxygen supply in exercising muscle is not homogenous, and consequently, the exercise pressor reflex is not uniform within an exercising muscle.

Regional differences in the inflection point of the muscle oxygenation index within a given muscle

As mentioned above, several factors such as intramuscular pressure (Amerdes and Provenzano, 1997), muscle architecture (Miura et al., 2004), and muscle fiber type (Wilson et al., 1995) may explain regional differences in muscle oxygenation. Considering data from previous reports, we hypothesize that the inflection point of muscle oxygenation would occur in the distal portion before the proximal portion. Surprisingly, our data indicated the opposite, that the inflection point occurred earlier in the proximal portion (**Figure 5-2** and **5-3**). A possible explanation is that an additional inflection point in the proximal portion might appear at higher workload (> 80% MVC). We speculate that the inflection point of the oxygenation index might have a physiological function only under severe metabolic conditions, because there was an approximate two-fold difference in oxygenation level among the portions at the inflection point (see **Figure 5-2**). Although a reverse slope change in oxygenation index (i.e., from steeper to more shallow) was observed only in *subject 4*, the inflection point of the oxygenation index was correlated with both cardiovascular responses (**Figure 5-2**). This finding might suggest that the inflection point of oxygenation index indicates an onset of critical oxygenation level at which aerobic metabolism becomes inhibited. Therefore the critical oxygenation level might be important in interpreting the physiological processes reflected in the inflection point of the

oxygenation index. However, because of methodological limitation of the NIRS device, we could measure only relative and not absolute (i.e., quantitative) changes in muscle oxygenation. Thus, we cannot completely explain the precise physiological processes reflected in the inflection point of muscle oxygenation in exercising muscle. Further investigation is needed to clarify the reasons for the regional differences in the inflection points between muscle portions while considering the relationship between the critical level of muscle oxygenation and the pressor response.

5-5. CONCLUSION

Our data show that the inflection point of muscle oxygenation occurred at a different workload in proximal and distal portions of the vastus lateralis muscle. The inflection point of muscle oxygenation was highly correlated with the inflection point of cardiovascular responses during incremental exercise in the distal but not in the proximal portion. Our data also suggest that the distal portion of the vastus lateralis muscle makes a greater contribution to the pressor response than does the proximal portion.

Chapter 6. Effects of regional cooling on cardiovascular responses during sustained exercise

6-1. INTRODUCTION

Exercise elevates the cardiovascular response by two neural mechanisms: central command (Eldridge et al., 1985) and the exercise pressor reflex (Mitchell, 1985; Kaufman and Rybicki, 1987; Victor et al., 1989). The exercise pressor reflex acts via afferent nerves, which arise from the peripheral system and are capable of sensing thermal, chemical, and mechanical stimuli.

CHAPTER 6. REGIONAL DIFFERENCES IN PERIFERAL REFLEX

The exercise pressor reflex is affected by exercising muscle mass (Mitchell et al., 1980; Iellamo et al., 1999). In contrast, Hayashi et al. (2001) reported the opposite findings in an animal study: when the ratio between tension development and muscle mass was similar in the forelimb and hindlimb, the exercise pressor reflex arising from the forelimb was greater than that arising from the hindlimb. In humans, muscle sympathetic nerve activity (MSNA) is greater when arising from the upper limb than from the lower limb, regardless of the muscle mass (Saito, 1995). Thus, the region involved in sensing chemical and mechanical stimuli alters the magnitude of sympathetic outflow evoked by the exercise pressor reflex. However, to our knowledge, it is not clear whether there are regional differences in the exercise pressor reflex within an exercising muscle in humans.

The purpose of this study was to examine whether there are regional differences in the exercise pressor reflex within a given muscle. To evaluate this question, we applied a regional cooling model (Ray et al., 1997). Ray and colleagues (Ray and Gracey, 1997; Ray et al., 1997) previously reported that muscle temperature affects the discharge of MSNA, and that muscle cooling delays the activation of the muscle metaboreflex during exercise. We measured calf vascular resistance as an index of sympathetic nerve activity evoked by the exercise pressor reflex, and compared calf vascular resistance during static knee extension exercise with regional cooling of the proximal and distal portions of the femoral muscle groups. We presumed that a differential response in vascular resistance might occur depending on which region of the muscle was stimulated.

6-2. METHODS

Subjects.

Nine men participated in this investigation; their mean (± SD) age was 24.6 ± 3.1 yr, height 169.9 ± 3.9 cm, and weight 64.7 ± 5.9 kg. The subjects were fully informed of the purpose, nature, and potential risks of the experiments, and gave their written informed consent to participate in this study. This study was approved by our local ethics committee and all work conformed to the Declaration of Helsinki.

Procedures

Exercise. Static knee extension was used as the exercise in this study. Subjects were positioned supinely with the knee joint flexed to 45º. On a different day before the experimental sessions, maximal torque generated during static knee extension was determined in the right leg using the Cybex® dynamometer (Lumex Inc., Ronkonkoma, NY, USA). The average of three attempts was taken as the subject's maximal torque. For subsequent exercise tests, the load was set at 30% of maximal torque (25.2 ± 2.7 Nm). Subjects monitored their exerted torque with an oscilloscope (DCS7020, Kenwood, Tokyo, Japan). Two minutes before exercise, a pneumatic cuff placed around the upper thigh was inflated to 250 mmHg to induce muscle ischaemia, which was continued during 2 min of static knee extension exercise. The laboratory temperature was maintained at 22–25ºC.

Trials. The subjects performed three trials, each on a separate day, consisting of a control trial (C-trial, without cooling), a trial in which the proximal thigh was cooled (P-trial), and a trial in which the distal thigh was cooled (D-trial). The portions of the muscle to be cooled

were determined by measuring 25% (proximal) and 75% (distal) of the length between the greater trochanter and the knee joint in the exercising leg. The specific region was cooled with an ice pack (10 cm × 15 cm) applied for 30 min before and during each P-trial and D-trial. Trial order was randomly assigned, with at least 24 h between sessions.

Measurements

Heart rate (HR) was determined using standard ECG leads (CEC-8108, Nihon Kohden, Tokyo, Japan). Blood pressure was measured with a finger cuff (2300 Finapres, Ohmeda Inc., Englewood, CO, USA). The monitoring finger cuff was placed around the middle finger of the left hand. Baseline values were obtained by averaging for 2 min before exercise. HR and mean arterial pressure (MAP) were calculated every 30 s during exercise.

Calf blood flow (CBF) in the nonexercising leg was measured by venous occlusion plethysmography using a mercury-in-silastic strain gauge (EC-5R, Hokanson Inc., Bellevue, WA, USA). The left leg was maintained in a horizontal position at heart level. The strain gauge was placed around the largest area of the left calf. A pneumatic cuff placed around the thigh was inflated to 60 mmHg to measure CBF at 30 s intervals throughout the experiment. Calf vascular resistance (CVR) in the nonexercising leg was calculated by dividing mean blood pressure by CBF.

Before exercise, thigh skin temperatures were measured in the proximal and distal portions every 10 min (Model 6510, Mallinckrodt Inc., Hazelwood, MO, USA). During exercise, skin temperatures were monitored at 1 min intervals. On a day other than the day of the exercise test, thigh fat thickness was measured in the portions that underwent cooling using an

ultrasonic apparatus (SSD-1000, Aloka, Tokyo, Japan). There was no significant difference in thigh fat thickness between proximal and distal portions (3.3 ± 0.5 and 3.7 ± 0.8 mm, respectively, $P > 0.05$).

Statistics

All data are represented as means ± SE. A two-way analysis of variance (ANOVA) for repeated measures, with time and trial as main effects, was employed to determine significant differences. If the *F*-test was significant, pairwise comparisons were performed using Bonferroni's post-hoc test. Values of $P < 0.05$ were considered statistically significant.

6-3. RESULTS

Skin temperature

Changes in thigh skin temperature in each trial are summarized in **Figure 6-1**. No change was observed between proximal and distal portions in the C-trial (averaged 32.7 ± 0.1ºC vs. 32.0 ± 0.1ºC, respectively; **Figure 6-1A**). In contrast, in both the P- and D-trials, skin temperature in the cooled portion decreased significantly below that of the opposite portion. Immediately before exercise, skin temperature was 16.5 ± 0.6ºC in the P-trial and 17.4 ± 0.8ºC in the D-trial (**Figure 6-1B and -6C**). At the end of exercise, proximal skin temperature in the P-trial was significantly lower than distal skin temperature in the D-trial (13.7 ± 0.6ºC and 16.4 ± 0.8ºC, respectively; $P < 0.05$).

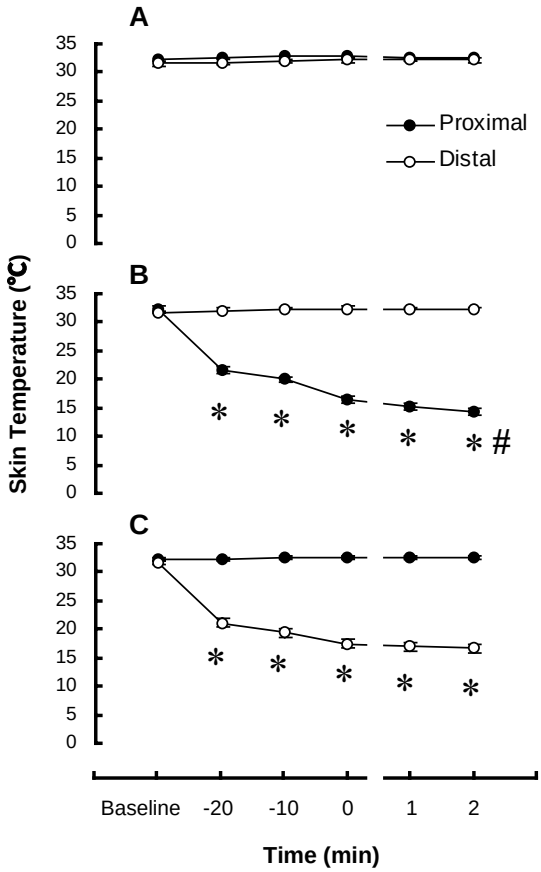

Figure 6-1. Changes in skin temperature during baseline, rest, and exercise in the C-trial (A), P-trial (B), and D-trial (C). Closed and open circles indicate skin temperature in the proximal and distal portions, respectively. *Significant difference vs. no cooling portion in each trial ($P < 0.05$). #Significant difference vs. skin temperature in the distal portion of the D-trial ($P < 0.05$).

Cardiovascular response

Changes in HR and MAP are shown in **Figure 6-2**. Immediately before exercise, neither HR nor MAP differed significantly between trials. HR and MAP increased significantly during exercise ($P < 0.001$) in both trials, but there were no significant differences between trials.

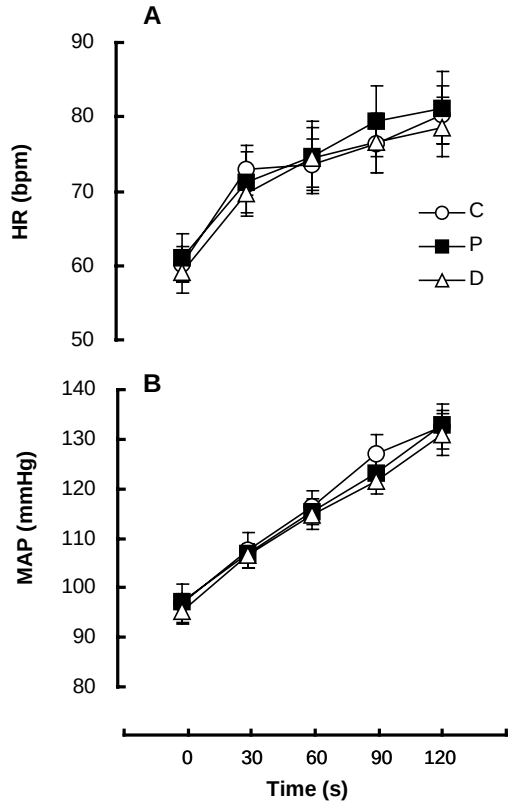

Figure 6-2. Changes in heart rate (A) and mean arterial pressure (B) during exercise in each trial. Open circles indicate the C-trial, closed squares indicate the P-trial and open triangles indicate the D-trial.

Changes in CBF and CVR during exercise are shown in **Figure 6-3**. Immediately before exercise, neither CBF nor CVR differed significantly between trials. CBF decreased significantly ($P < 0.001$) in both trials; as a result, CVR increased significantly ($P < 0.001$) during exercise in both trials. Neither CBF nor CVR differed significantly between C- and P-trials at any time. In contrast, at 60 s of exercise, CBF in the D-trial (2.94 ± 0.23 $ml \cdot 100g^{-1} \cdot min^{-1}$) was significantly higher than in the C-trial (1.97 ± 0.22 $ml \cdot 100g^{-1} \cdot min^{-1}$, $P < 0.05$), but not in the P-trial (2.18 ± 0.34 $ml \cdot 100g^{-1} \cdot min^{-1}$, $P = 0.058$). CBF in the D-trial at 90 s of exercise (2.68 ± 0.24 $ml \cdot 100g^{-1} \cdot min^{-1}$) showed a tendency to be higher than in the C-trial (1.98 ± 0.26 $ml \cdot 100g^{-1} \cdot min^{-1}$, $P = 0.07$) and in the P-trial (1.81 ± 0.20 $ml \cdot 100g^{-1} \cdot min^{-1}$, $P =$

0.066). Consequently, at 60 and 90 s of exercise, CVR in the D-trial (40.7 ± 2.9 units at 60 s and 48.4 ± 4.4 units at 90 s) was significantly lower than in the C-trial (62.7 ± 4.5 units at 60 s, $P < 0.05$ and 71.5 ± 7.5 units at 90 s, $P < 0.05$) and P-trial (62.8 ± 8.3 units at 60 s, $P < 0.05$, and 73.0 ± 9.2 units at 90 s, $P < 0.05$).

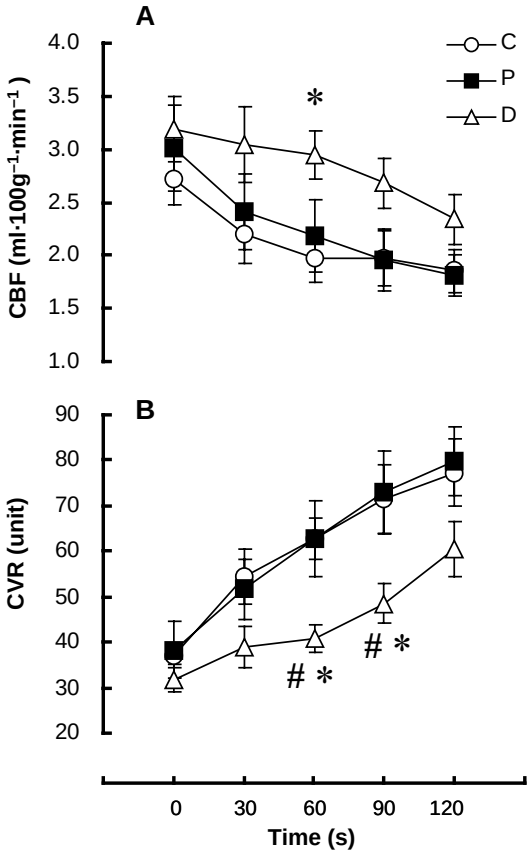

Figure 6-3. Changes in calf blood flow (A) and calf vascular resistance (B) during exercise in each trial. Open circles indicate the C-trial, closed squares indicate the P-trial and open triangles indicate the D-trial. *Significant difference vs. C-trial ($P < 0.05$); #Significant difference vs. P-trial ($P < 0.05$).

6-4. DISCUSSION

The purpose of this study was to determine whether there are regional differences in the exercise pressor reflex within an exercising muscle. To address this question, we examined

the effects of regional cooling on the pressor response during static exercise. The major finding was that cooling of the distal but not the proximal portion of the thigh attenuated an increase in vascular resistance during exercise.

A change in sympathetic outflow, which can be evaluated directly by recording MSNA, is closely matched to vascular resistance during exercise in humans (Seals, 1989; Saito et al., 1990). Attenuation of the increase in CVR during exercise can be explained by data from a previous study showing that muscle cooling delays the burst frequency of MSNA (Ray et al., 1997). However, we observed regional cooling effects on CVR in the D-trial but not in the P-trial during exercise (**Figure 6-3**). This is an important observation that may explain the mechanisms involved in the cardiovascular response when different regions of skeletal muscle are cooled.

One possible explanation for the attenuation of the increase in CVR might relate to differences in the longitudinal distribution of skeletal muscle fibre type between proximal and distal portions. Animal studies indicate a non-uniform distribution of skeletal muscle fibre type; for example, the percentage of type I fibre decreases in the direction from proximal to distal in hindlimb muscles (Torrella et al., 2000; Wang and Kernell, 2000). Wilson et al. (1995) showed that the fibre type of the contracting muscle influences the magnitude of the pressor response to a static contraction; that is, the pressor reflex to a static contraction is greater in a predominately glycolytic muscle than in a primarily oxidative muscle. Similarly, Sadamoto et al. (1992) reported that arterial blood pressure at the end of sustained handgrip exercise was positively correlated to the relative content of fast twitch fibres in the brachioradialis muscle.

CHAPTER 6. REGIONAL DIFFERENCES IN PERIFERAL REFLEX

Oxygen supply and consumption are heterogeneous in the skeletal muscle, and are related to the distribution of skeletal muscle fibre types between proximal and distal portions in animals (Laughlin and Armstrong, 1982; Piiper et al., 1985) and humans (Kalliokoski et al., 2000; Miura et al., 2001; Mizuno et al., 2003a) at rest and during and after exercise. Proximal and distal portions within a given muscle display heterogeneity in blood flow (Piiper et al., 1985; Mizuno et al., 2003a), oxygen uptake (Mizuno et al., 2003a), and oxygenation (Miura et al., 2001). We suggest that the metabolic error signal evoked by the exercise pressor response resulting from the mismatch between metabolic demand and oxygen supply in exercising muscle (Hanna et al., 2002) is not homogenous within an exercising muscle. In our present study, however, we employed an ischaemic knee extension exercise, using the methods reported by Ray et al. (1997), to eliminate changes in blood flow induced by muscle cooling and to counteract the heterogeneous blood flow within the muscle. It is unlikely that attenuation of CVR in the D-trial could be explained by the metabolic error caused by differences in oxygen supply between trials.

An alternative explanation is that the distribution or number of afferent nerves differs between proximal and distal portions within a muscle. Muscle temperature directly affects the discharge rate of muscle afferent nerves; decreasing muscle temperature attenuates the discharge frequency of chemically sensitive muscle afferents (Hertel et al., 1976; Kumazawa and Mizumura, 1977). If our current data resulted from the effects of decreasing temperature on afferent nerves, it could be speculated that the distal portion has more afferent nerves than does the proximal portion. Kumazawa and Mizumura (1977) reported that muscular polymodal receptors were more frequently found in the head, the tail, and the edges of dog muscle. Further

neurophysiological investigation is needed to identify the distribution of afferent nerves in human muscle. In contrast, decreasing muscle temperature alters muscle metabolism during isometric exercise in humans (Edwards et al., 1972), and it is unclear whether muscle cooling directly affects muscle afferent sensitivity or indirectly affects muscle metabolism.

Local skin cooling directly stimulates both cutaneous thermal receptors and nociceptors, which elicit an increase in sympathetic nerve activity (Kregel et al., 1992). We cannot completely exclude the possibility that the thermal stimulus could have operated in both cooling trials. However, we found no significant difference between trials in any pre-exercise variables despite decreased skin temperature (**Figures 6-2** and **6-3**). Moreover, we observed regional cooling effects on CVR during exercise in the D-trial but not the P-trial, whereas skin temperature decreased significantly in both regions (**Figure 6-1**). These results argue against a role of cutaneous thermal receptors, and we believe it is unlikely that stimulation of cutaneous thermal receptors was responsible for the attenuation of CVR during exercise.

The exercise pressor reflex arises in response to both mechanical and chemical stimuli (Mitchell, 1985; Kaufman and Rybicki, 1987; Victor et al., 1989). If regional cooling affected the muscle mechanoreflex, we should have observed attenuation of CVR at the onset of and during the entire bout of exercise in the D-trial. However, we found no difference in CVR between trials at both 30 s and 120 s of exercise in the D-trial. Because of regional differences in the mechanical stimulus, intramuscular pressure in the proximal portion is higher than in the distal portion within a muscle (Amerdes and Provenzano, 1997), and it is unlikely that regional cooling directly alters intramuscular pressure.

We observed no significant effect of regional cooling on heart rate and blood pressure measured at rest and during exercise (**Figure 6-2**), whereas cooling attenuated the increase in CVR during exercise. These findings are consistent with the study by Ray et al. (1997), which also found no effect on heart rate or arterial blood pressure with attenuation of MSNA by local cooling. It is possible that sympathetic outflow to other vascular beds may have increased to compensate for the decrease in CVR. Alternatively, the attenuation in CVR elicited by regional cooling may not have been of sufficient magnitude to change arterial pressure.

There are several methodological limitations of our study. We did not measure muscle temperature and thus could not determine the extent to which regional cooling might have decreased muscle temperature. However, the decrease in skin temperature caused by regional cooling in our study (average range 32.4 ± 0.2–15.5 ±0.5ºC) was similar to that previously reported by Ray et al. (1997). Because there was no significant difference in thigh fat thickness in the cooled portions, we assume that heat conductivity was similar between these portions. A previous study by Gonzalez-Alonso et al. (2000) using magnetic resonance imaging showed similar knee extensor muscle mass between proximal and distal portions. Thus, we believe it unlikely that in our study the cooling effect was different in the two regions of the muscle exposed to cooling. In addition, muscle and skin temperatures change in parallel during cold-water immersion (Kregel et al., 1992). Further experiments are needed to measure cooling-induced changes in the muscle temperature to clarify this issue.

6-5. CONCLUSION

CHAPTER 6. REGIONAL DIFFERENCES IN PERIFERAL REFLEX

Regional cooling of the distal portion of the thigh attenuated the increase in vascular resistance during exercise; however, no effect was observed with proximal cooling. These findings suggest that stimulating different regions within a given muscle causes different degrees of calf vascular resistance evoked by the exercise pressor reflex during exercise.

Chapter 7. Limitations and Perspectives

Studies in this dissertation have several issues. Firstly, possible mechanisms underlying heterogeneities in skeletal muscle perfusion and metabolism have not been completely addressed. Using imaging modalities such as PET and NIRS to evaluate muscle blood flow and muscle metabolism, several line of evidence has been derived from in human

data, as described above (chapter 3, 4, and 5). However, the mechanisms underlying these heterogeneities in humans remain a matter of speculation. Further investigations are needed, with actual measuring skeletal muscle fiber type and intramuscular pressure, to clarify mechanisms underlying heterogeneities in skeletal muscle perfusion and metabolism in human.

Secondly, although studies in chapter 5 and 6 indicated the distal portion of the muscle makes a greater contribution to the pressor response than does the proximal portion, exact mechanisms have not been addressed yet. Although evaluation of the muscle oxygenation index by NIRS reflects the balance between oxygen utilization and oxygen delivery (chapter 5), local muscle metabolites, which cause exercise pressor reflex such as lactate and adenosine, could not be measured. Furthermore, regional cooling using chapter 6 would affect several physiological functions (e.g., local metabolism, activation of afferent nerve etc.). An alternative explanation for regional differences in pressor response is the number and/or sensitivity of afferent nerves may differ between proximal and distal portions within a muscle. Further neurophysiological investigation is needed to identify the distribution and sensitivity of afferent nerves in human muscle.

Based on methodological difficulties to measure heterogeneous functions in human study, evaluation of heterogeneous perfusion and metabolism is one of developing field in physiology. Although there is some evidence that physical training and aging alter the extent of heterogeneity in physiological functions, the responsible mechanisms are not clear. Further investigation will be required to establish the physiological function of heterogeneous skeletal muscle perfusion and metabolism.

Chapter 8. Summary and Conclusion

The purpose of this dissertation was to evaluate heterogeneity in skeletal muscle perfusion and metabolism in longitudinal section, and to examine physiological role of their heterogeneities during exercise.

CHAPTER 8. SUMMARY AND CONCLUSION

In chapter 3, regional differences in blood flow and oxygen consumption and their relationship in exercised muscle during recovery from exhaustive exercise were evaluated. Five healthy male subjects performed an exhaustive one-legged cycling exercise. Blood flow, oxygen uptake, and oxygen extraction were measured in the quadriceps femoris muscle before and after exercise using positron emission tomography (PET). Regions of interest included five areas of the muscle—two proximal, one central, and two distal—which were evenly spaced across the muscle. Before exercise, blood flow and oxygen consumption decreased significantly ($P < 0.05$) in the direction from the proximal to the distal portions; blood flow declined from 2.0 ± 0.5 to 1.4 ± 0.3 ml·100g^{-1}·min^{-1}, and oxygen consumption decreased from 0.21 ± 0.04 to 0.17 ± 0.02 ml·100g^{-1}·min^{-1}. In contrast, these gradients in blood flow and oxygen consumption diminished during recovery after exercise. Consequently, there was a positive relationship between changes in blood flow and oxygen consumption in an exercised muscle during recovery after exercise ($r = 0.963$, $P < 0.01$). These changes became larger in the direction from proximal to distal portions; blood flow increased from 2.9 ± 0.7 to 3.9 ±0.8, and oxygen consumption: from 1.4 ± 0.1 to 1.8 ±0.4 times resting values. These results suggest that hemodynamic variables are heterogeneous within a muscle both at rest and during recovery from exercise, and that there is a systematic difference in these variables in the direction from proximal to distal regions within the quadriceps femoris muscle.

In chapter 4, to clarify the reason for these regional differences in blood flow, regional differences in blood volume and transit time along the length of a resting skeletal muscle were evaluated. Five healthy male subjects participated in this study. Blood volume as an index of

vascular distribution, blood transit time as an index of blood flow velocity were measured in the resting quadriceps femoris muscle using positron emission tomography (PET). Regions of interest included five areas of the muscle—two proximal, one central, and two distal—which were evenly spaced across the muscle. The blood transit time significantly related to portions of the muscle, increasing in linear fashion from proximal to distal portions, whereas blood volume remained constant among the portions. These findings suggested the blood flow velocity slows from proximal portion to distal portions within resting muscle despite no regional changes in blood volume.

In chapter 5, to test whether there is a regional difference in the exercise pressor reflex within a given muscle, the relationship between the inflection points of cardiovascular responses and muscle oxygenation during exercise were examined. Seven subjects performed incremental exercise, which consisted of incremental 30 s static knee extensions, each separated by 30 s of recovery. The workload started at 5% maximum voluntary contraction (MVC) and increased by 5% MVC for each increment until exhaustion. Changes in the concentrations of oxygenated Hb and deoxygenated Hb were monitored in proximal and distal portions of the vastus lateralis muscle using NIRS. The inflection points of MAP, CVR, and the muscle deoxygenation index were calculated as the intersection point of two regression equations obtained at lower and higher workloads. The inflection point of muscle deoxygenation index differed significantly between proximal and distal portions (28.5 ± 3.0% vs. 39.5 ± 3.0% MVC, $P < 0.05$). Linear regression analysis showed significant correlations between the inflection point of muscle deoxygenation index in the distal portion and MAP ($r = 0.89$; $P < 0.01$) and CVR ($r = 0.89$; $P <$

0.05), but no significant relationship between the inflection point in the proximal portion and MAP or CVR. These data show that the inflection point of muscle deoxygenation differs between proximal and distal portions within the vastus lateralis muscle during incremental exercise, and suggest that the distal portion of the vastus lateralis muscle contributes more to the pressor response than does the proximal portion.

In chapter 6, to retest whether there are regional differences in the exercise pressor reflex within an exercising muscle, we evaluated the effect of regional cooling, which delays activation of the muscle metaboreflex, using vascular resistance as an index of sympathetic nerve activity in the non-exercised limb during exercise. Nine subjects performed 2 min of ischaemic isometric knee extension at 30% of maximal torque in three trials: without cooling (C-trial), with cooling of the proximal thigh portion (P-trial), and with cooling of the distal portion (D-trial). Heart rate, mean arterial pressure, calf blood flow in the non-exercised leg, and calf vascular resistance in the non-exercised leg were measured. In both cooling trials, regional cooling significantly decreased the skin temperature of the exposed portion, but had no effect on the opposite portion of the thigh. During exercise, heart rate and mean arterial pressure increased significantly, but were not affected by regional thigh cooling. In contrast, at 60 s of exercise, calf blood flow was significantly higher in the D-trial than in the C-trial (2.94 ± 0.23 vs. 1.97 ± 0.22 $ml \cdot 100g^{-1} \cdot min^{-1}$, $P < 0.05$). Consequently, calf vascular resistance was significantly lower in the D-trial than in the C- and P-trials at 60 and 90 s of exercise. These findings suggest that stimulating different regions within a given muscle causes a different magnitude of increase in calf vascular resistance evoked by the exercise pressor reflex during exercise.

CHAPTER 8. SUMMARY AND CONCLUSION

In chapter 7, some limitations of these studies and future perspectives in this field were provided. In conclusion, blood flow and oxygen consumption in the quadriceps femoris muscle are heterogeneous at rest, during exercise, and during recovery from exercise: the differences is systematic between proximal and distal regions. The heterogeneities during exercise links to systemic cardiovascular regulation: the distal portion of the muscle makes a greater contribution to the pressor response than does the proximal portion. Further studies are required, using advanced and conventional methodologies, both to confirm these data with descriptive evidences and to evaluate the physiological significance of heterogeneous functions.

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