DOI: 10.1111/micc.12784

Microcirculation WILEY

ABSTRACT

# Abstracts from the 47th Annual Meeting of Japanese Society for Microcirculation

March 19th-20th, 2022 Online Conference, Japan
President: Takanori Yasu
Department of Cardiovascular Medicine and Nephrology,
Dokkyo Medical University Nikko Medical Center
632 Takatoku, Nikko, Tochigi 321-2593, Japan
Email: jsm_jimukyoku@js-micro.com
**Correspondence**
Kazuto Masamoto Ph.D[1,2]
[1]Journal Committee, Japanese Society for Microcirculation
[2]Center for Neuroscience and Biomedical Engineering, University of Electro-Communications, 1-5-1 Chofugaoka, Chofu, Tokyo 182-8585, Japan
Email: masamoto@mce.uec.ac.jp

Keynote Lecture | **KL: The Japanese society for microcirculation: Origin and future**

Hidekazu Suzuki
*Division of Gastroenterology and Hepatology, Department of Internal Medicine, Tokai University School of Medicine, Kanagawa, Japan*

On August, 1971, Dr. Masaharu Tsuchiya established "Gathering of Microcirculation Researchers" in consultation with founders, Dr. Takehiko Azuma, Dr. Makishige Asano, Dr. Yoshio Mishima, and Dr. Ryu Nakayama. This workshop was a forum for exchanging opinions on the microcirculatory system in various parts of the body, whether basic or clinical. Held from the first "Gathering of Microcirculation Researchers" (February, 1976) to 26th, during that time, Tokyo International Symposium on Microcirculation (July, 1981, at Sasagawa Hall), and Symposium on INTRAVITAL OBSERVATION OF ORGAN MICROCIRCULATION (June, 1983, at Tokai Alumni Association) were held. Before that, in 1977, Dr. Tsuchiya was appointed as a professor at Keio University, and was quickly recognized in the fields of microcirculation to establish a study group and further developed into an academic society. This study group developed into the "Japanese Society for Microcirculation" on February 16, 1985. Then, Prof. Tsuchiya, as a President, held the 4th World Congress for Microcirculation (July, 1987, Keio Plaza Hotel) in Tokyo with 440 foreign delegates from 27 countries and 500 domestic delegates. Without Prof. Tsuchiya, we can't talk about the Japanese Society for Microcirculation today. After Prof. Tsuchiya passed away, Prof. Hiromasa Ishii was appointed as the second President in 2001, and then Prof. Makoto Suematsu was appointed as the third President in 2008 and held the 10th World Microcirculation Society in Kyoto (Kyoto International Conference Center) with 240 delegates from overseas (30 countries) and 164 delegates from Japan. On March 26, 2016, I was appointed as the fourth president of the Japanese Society for Microcirculation. Now, life science research in the coming era is an area that covers a broad range from basic to clinical, such as microcirculatory science. In the field of gastroenterology, the vascular pattern of microvessels has been adopted for endoscopic diagnosis, and the momentum is approaching histopathological diagnosis, and the era of automatic diagnosis has come with the application of artificial intelligence. This alone makes remarkable progress in research. For this purpose, the activities of this academic society are extremely important, encompassing many interdisciplinary areas, and between researchers in Japan as well as abroad. It is necessary to foster the next generation while promoting exchanges and joint research. It is also important to carry on new academic challenges positively and promptly while inheriting the brilliant flow established by seniors and respecting tradition. The 12th World Congress for Microcirculation was scheduled to be held in September 2022 under the chairman of Professor Jing-Yan Han of Peking University, who is also a director of the Japan Microcirculation Society. However, due to an unprecedented COVID-19 pandemic, it was postponed to September 20-24 in 2023. I hope that a face-to-face international conference will be held in Beijing in September 2023, and that all those who study microcirculation will meet.

Presidential Lecture | **PL: Another role of leukocyte: Mobile renin-angiotensin system modulator in microcirculation**

Takanori Yasu
*Department of Cardiovascular Medicine and Nephrology, Dokkyo Medical University Nikko Medical Center, Tochigi, Japan*

Levels of triglycerides and free fatty acids (FFAs) are elevated in patients with diabetes and may contribute to endothelial dysfunction through renin-angiotensin system (RAS) activation and oxidative stress. We propose that the enhanced production of angiotensin II by FFA in mononuclear and polymorphonuclear cells causes activation of leukocytes that consequently impairs endothelial function. RAS in leukocytes may regulate the leukocyte-vasculature interaction as the mobile RAS in humans. The present study explored how

systemic FFA loading affected myocardial microcirculation during hyperemia via RAS. Eight healthy men received candesartan, perindopril, or a placebo for two days in a double-blind crossover design, and then myocardial microcirculation during hyperemia induced by a 2-h infusion of lipid/heparin was assessed using dipyridamole stress-myocardial contrast echocardiography (MCE). Leukocyte activity and hemorheology were also assessed ex vivo using a microchannel flow analyzer, serum levels of oxidative stress markers, and IκB-α expression in mononuclear cells. Serum FFA elevation by the infusion of lipid/heparin significantly decreased myocardial capillary blood velocity and myocardial blood flow during hyperemia. Both candesartan and perindopril significantly prevented the FFA-induced decrease in capillary blood velocity and myocardial blood flow during hyperemia. Systemic FFA loading also caused an increase in the number of adherent leukocytes and prolonged the whole blood passage time. These effects were blocked completely by candesartan and partially by perindopril. Both agents prevented the FFA-induced enhancement of oxidative stress and IκB-α degradation in mononuclear cells.

In conclusion, both candesartan and perindopril can prevent FFA-induced myocardial microcirculatory dysfunction during hyperemia via modulation of leukocyte activation and microvascular endothelial function.

## Special Lecture 1 | SL-1: Pancreatic digestive enzymes and autodigestion as cause of cell dysfunction, disease and organ failure

Geert W. Schmid-Schönbein

*Department of Bioengineering, Center for Autodigestion Innovation, University of California San Diego, CA, USA*

Digestive enzymes, synthesized in the pancreas as fundamental requirement for food digestion, are usually compartmentalized within the lumen of the intestine by the mucin/epithelial barrier. They are highly concentrated, relatively non-specific, fully activated enzymes, and daily degrade large volumes of biopolymers from diverse food sources. The intestinal barrier is permeable to small molecular weight molecules, such as degradation products from food, but usually impermeable to the larger molecular weight digestive enzymes (>19kDa). An increasing body of evidence shows, however, that the mucin/epithelial barrier can be disturbed by a multitude of mechanisms, including but not limited to hypoperfusion of the intestine, the presence of unbound free fatty acids or bacterial products. Once the mucin barrier is breached, digestive enzymes, such the serine proteases, are carried into the epithelium and proteolytically degrade interepithelial adhesion molecules (e.g., E-cadherin), which, in turn, further opens the intestinal barrier. Digestive enzymes can escape into the lamina propria of the villi, the intestinal lymphatics and venules, the peritoneum, and appear in the systemic circulation and other organs. Blockade of digestive enzymes by endogenous inhibitors is frequently insufficient to fully block their activity. In the circulation active digestive enzymes degrade plasma proteins, proteolytically clip membrane receptors and thereby compromise the function of these receptors, a process that can reach organ failure. We will discuss the current evidence supporting this autodigestion process by digestive enzymes in the cases of type II diabetes and metabolic disease, heart failure, sepsis, and hemorrhagic shock. Intervention against digestive enzymes by enteral blockade in post-surgical patients is currently in double-blind randomized clinical trials. Autodigestion may be a fundamental process in nature that accompanies digestion. Its investigation is still at an early stage and its full involvement in human diseases and death remain to be elucidated.

**Acknowledgement**: I wish to sincerely acknowledge the distinguished members of the Japanese Microcirculatory Society and the wonderful colleagues with whom I had the privilege to collaborate and to benefit from over years of interactions.

## Symposium 1 | S1-2: Carbon monoxide as a novel therapeutic agent: Discussion of microcirculation research findings

Tomohisa Takagi[1,2]; Kazuhiro Katada[1]; Kazuhiko Uchiyama[1]; Yuji Naito[3,*]

[1]*Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan*; [2]*Department for Medical Innovation and Translational Medical Science, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan*; [3]*Department of Human Immunology and Nutrition Science, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan*

Carbon monoxide (CO), an invisible, chemically inert, colorless, and odorless gas, is a major product of the incomplete combustion of carbon and carbon-containing compounds. CO is endogenously generated by heme oxygenase (HO) because of heme degradation and contributes to many physiological and pathophysiological processes, including anti-inflammatory activity. Using microcirculation research techniques, we demonstrated that CO produced from HO-1 exerts protective effects against intestinal ischemia-reperfusion injury through its anti-inflammatory action[1]. Exogenous CO has long been considered a highly toxic gas owing to its ability to bind hemoglobin with a much higher affinity than that of oxygen. However, exogenous CO also exerts cytoprotective and anti-inflammatory effects[2-4]. In particular, its potent anti-inflammatory action in the intestinal mucosa was revealed through the inhibition of Th1 and Th17 response. Based on these findings, we are developing new therapeutic agents based on CO for various inflammatory diseases. In this symposium, I would like to introduce our recent data, efforts and accomplishments in this regard.

1, Katada K, Takagi T, Uchiyama K, Naito Y. *J Gastroenterol Hepatol*. 2015;30(Suppl 1):46–52.

2, Takagi T, Naito Y, et al. *Nitric Oxide*. 2021;107:19–30.

3, Takagi T, Naito Y, et al. *J Clin Biochem Nutr*. 2018;63(3):169–174.

4, Takagi T, Naito Y, et al. *Free Radic Res*. 2018;52(11–12):1328–1335.

### S1-3: Enhanced interference microscopy for hemodynamics and rheology in the microcirculation model at regulated temperatures

Yuji Kikuchi

*Kikuchi Microtechnology Laboratory Co. Ltd, Ibaraki, Japan*

The present author and colleagues reported "Reduced deformability of erythrocytes exposed to hypercapnia" in 1979 (*EXPERIENTIA* 35, 343), which now appears to be a most relevant mechanism for the "Association of red blood cell distribution width with mortality risk in hospitalized adults with SARS-CoV-2 infection" (*JAMA Network* Open 2020). In this report, I will review the very long distance from the above paper and "An analysis of water movement between myocardial tissue and capillary blood during reactive hyperemia" (*JJP* 29, 1-13,1979) to the present report, or, from the symposium on recent developments in microcirculation research (1981) to the present symposium (2022); we might be able to elucidate why red blood cell distribution width (RCD) is associated with elevated risk for morbidity and mortality in a wide range of diseases.

### Symposium 2 | S2-1: In vivo observation of subendocardial and subepicardial coronary microcirculation of the beating canine heart

Toyotaka Yada

*Department of Medical Engineering, Kawasaki University of Medical Welfare, Okayama, Japan*

Endocardial vulnerability to ischemia is well known and is associated with a coronary flow reserve that is lower in the endocardium than in the epicardium. The conventional explanations for this difference in coronary flow reserve have invoked the differences in intramyocardial pressures in different layers of myocardium. However, because of the technical difficulty involved in the direct observation of subendocardial microvessels, to date no one has examined that there might be regional differences in vascular reactivity. It is difficult to observe the subendocardial microvessels without fluorescent emission in the left ventricle. The aim of the present study was to evaluate the image of subendocardial arterioles directly by an infrared fluorescence microscope. The experimental design was approved by the animal research committee of Kawasaki Medical School. We have developed an infrared fluorescence microscope in observing subendocardial microvessels with a high vision camera by attaching an object lens to laparoscope with indocyanine green (ICG) of fluorescent dye. In open-chest anesthetized dogs, the sheathed needle probe with a doughnut-shaped balloon and a microtube for flushing away the intervening blood was introduced into the left ventricle through an incision in the left atrial appendage via the mitral valve. Subendocardial arterioles with ICG were visualized with an infrared fluorescence microscope with a doughnut-shaped balloon to avoid compression of the vessels placed on subendocardial arterioles. In conclusion, using a developed microscope system, we were able to observe the subendocardial vessels in diastole and systole. Infrared fluorescence microscope is useful for in vivo investigation of subendocardial arterioles.

### S2-3: Development of functional in vivo imaging in kidney using multiphoton laser microscope

Kengo Kidokoro

*Department of nephrology and hypertension, Kawasaki Medical School, Okayama, Japan*

Recent advances in bioimaging technology have allowed visualization of various biological phenomena in vivo given their powerful capability to clarify renal physiology and pathophysiology. We have established an in vivo imaging technique that can directly visualize renal hemodynamics and glomerular permeability using multiphoton laser microscopy (MPM) (*Microcirculation*, 2010). This technique was performed to study the progressive mechanism of chronic kidney disease, especially due to diabetic kidney disease (DKD), and to evaluate the effects of various drugs on renal hemodynamics and renal function. Recently, we reported the renal protective mechanisms of the sodium–glucose co-transporter-2 inhibitor (SGLT2i), with a focus on the glomerular hemodynamic effects and tubuloglomerular feedback (TGF) using in vivo MPM imaging techniques in mice with diabetes. The adenosine/adenosine A1 receptor pathway played a pivotal role in the regulation of glomerular filtration rate via TGF mechanisms in DKD (*Circulation*, 2019). In addition to kidney imaging, methods for functional imaging techniques for several other organs have been developed. We have reported on the effect of angiotensin II and angiotensin type 1 receptor blocker (ARB) on pancreatic islet microcirculation and insulin secretion (*Microcirculation*, 2013). Angiotensin II significantly induced blood vessel contraction in the pancreatic islets in a dose-dependent manner, resulting in decreased glucose-stimulated insulin secretion. In contrast, ARB induced vasodilation of blood vessel in the islet and improved glucose-stimulated insulin secretion. Therefore, in vivo imaging can visualize various biological activities and contribute to the further elucidation of the pathophysiology of various diseases. The experimental protocols were approved by the Ethics Review Committee for Animal Experimentation at Kawasaki Medical School, Kurashiki, Japan.

### Symposium 3 | S3-1: Elucidation of the role of hemodynamic factors in the formation and growth of cerebral aneurysms for the development of therapeutic drugs

Shunichi Fukuda

*Department of Neurosurgery, National Hospital Organization Kyoto Medical Center, Kyoto, Japan*

**Purpose**: Cerebral aneurysms, the main cause of subarachnoid hemorrhage, can only be treated surgically, and there exist no therapeutic

drugs. We are working on the development of therapeutic agents for cerebral aneurysms based on the hypothesis that hemodynamic stress in vascular endothelial cells triggers aneurysm development.

**Methods**: We investigated the role of P2X4 purinoceptor, which is involved in flow-sensitive mechanisms in vascular endothelial cells, in the development of cerebral aneurysms by using the aneurysm-induced animal model. We also prospectively enrolled human patients with unruptured cerebral aneurysms, observed them for 3 years with cerebrovascular 3D imaging and carotid artery echocardiography, and compared the hemodynamic environments of the growth group with those of the non-growth group using computational fluid dynamics (CFD) technique.

**Results**: The incidence of cerebral aneurysms in aneurysm-induced P2X4 $^{(-/-)}$ mice was significantly lower than that in P2X4 $^{(+/+)}$ mice, and the incidence of cerebral aneurysms in aneurysm-induced rats treated with a P2X4 inhibitor, paroxetine was significantly lower than that in the non-treated group. Paroxetine treatment also significantly suppressed aneurysm growth. In the clinical study of human unruptured cerebral aneurysms, 461 patients were enrolled, and during the 3-year observation period, 38 aneurysms enlarged and 209 ones did not. In the CFD analysis, the magnitude of wall shear stress and transWSS, a metrics for shear stress disturbance, was significantly increased in the enlarged group, especially in the aneurysm neck.

**Conclusions**: The cerebral aneurysm development is thought to progress by inducing inflammatory factors in the vessel wall triggered by increased local hemodynamic stress in the cerebral artery. Paroxetine may be a potential clinical remedy for cerebral aneurysms, since it has been used safely in humans as an antidepressant. We are currently conducting a retrospective study to evaluate the aneurysm growth rate in patients with unruptured cerebral aneurysms treated with paroxetine.

## S3-2: Structural and functional plasticity of cerebral microcirculation

Kazuto Masamoto
*Center for Neuroscience and Biomedical Engineering, University of Electro-Communications, Tokyo, Japan*

Cerebral blood flow (CBF) decreases with age in the elderly. However, little is known about age-associated changes in cerebral microvasculature. A pre-clinical study showed that arterioles are more susceptible to the decline of age-dependent vascular density than venules. The penetrating arteriole, which is responsible for certain functional territories plays a bottleneck role in determining parenchymal blood supply and in draining clearance in brain tissue. Recent studies have also focused on the function of pre-capillary arterioles as a bottleneck for maintaining capillary perfusion. Cerebral capillaries account for more than 50% of parenchymal microcirculation and are supposed to respond to neuronal demands by controlling the distribution of blood cells. In brain diseases, like cerebral amyloid angiopathy and stroke, arterial vasomotion is suppressed. Additionally, the capillary perfusion is blocked by the white blood cell stall. It is estimated that approximately 0.12% of cortical capillaries are subject to the white blood cell stall per day. The transient cessation of capillary blood flow ultimately leads to a loss of capillary networks and thus an age-dependent decrease of CBF. Detailed spatial and temporal analysis of dynamic fluctuations in capillary flow and diameters has shown that capillary flow fluctuations are subject to a number of branches in capillary networks, regardless of capillary diameter changes. Therefore, to keep the blood supply well balanced across the capillary networks in the brain, it can be beneficial to minimize the resistance of the cerebral capillary flow. This would include maintaining lower plasma viscosity and cell density (to prevent aggregation) as well as maintaining deformable red blood cells and normal interactions with endothelial surface layers.

## Free paper | F-04: Estimation of luminal pressure of the initial lymphatics during tissue movement with special reference to bubble formation in decompression sickness

Fumitaka Ikomi
*Division of Medical Experiment, Maritime Self-Defense Force Undersea Medical Center, Saitama, Japan*

Decompression sickness is caused by bubbles formed in the body. Metabolically inactive gases, such as nitrogen, are accumulated in body fluid under pressure. During and after decompression, these gases occasionally form small bubbles in the body. Decompression sickness is characterized by various symptoms including joint pain, skin rashes, cutis marmorata, lymph edema, dyspnea, circulatory shock, paralysis and other neuronal disorders. These skin lesions are possible to be induced by local bubbles formed in the tissues as well as in the blood vessels. Bubbles in the blood vessels are mostly observed in the venous blood rather than the arterial blood. Partial pressures of metabolically inactive gases are usually higher in tissue fluid than in venous blood during and after decompression. Furthermore, tissue fluid pressure in the skin is lower than venous pressure. When lymph formation occurs in the skin, tissue fluid is sucked into the initial lymphatics according to tissue movement. Thus, bubbles are possible to be formed easier in the initial lymphatics than in the veins. It has not been clarified, however, that situation of pressure changes in the initial lymphatics during tissue movement. By using a viscoelastic model of the tissue, we previously suggested that passive expansion of the skin causes filling of the initial lymphatics in a certain area following the equation of $V(t) = 0.17\{1 - \exp(-t/10)\}$, where $V(t)$ is increasing volume of the initial lymphatics in microliter $t$ second after expansion (*Microcirculation* 2020;27:e12606). From the model, it is derived that larger amount of expansion, higher frequency of the movement and larger resistance of tissue fluid flow make initial lymphatic pressure lower. These situations may contribute to form bubbles in the initial lymphatics in the skin.

### F-05: Acute hypotension induced by thigh cuff release and cerebral oxygenation changes

Atsuhiro Tsubaki[1,*]; Danni Qu[2]; Hajime Tamiya[1]; Kazuki Hotta[1]

[1] *Institute for Human Movement and Medical Sciences, Niigata University of Health and Welfare, Niigata, Japan*; [2] *Graduate School of Health and Welfare, Niigata University of Health and Welfare, Niigata, Japan*

Normal brain function depends on stable cerebral blood flow and cerebral autoregulation. Cerebral blood flow is maintained even when blood pressure fluctuates. However, microcirculation in the cortex is not well understood when blood pressure drops rapidly. The purpose of this study was to determine oxyhemoglobin ($O_2$Hb) changes, the indicator of cortical blood flow changes, during hypotension induced by thigh cuff release. Ten healthy students participated in this study. They were seated in a recumbent position in a quiet room. The cuffs of digital tourniquets were placed on both thighs and inflated to 250 mmHg for 5 min after 5 min rest, followed by deflation for 5 min. Right (R-) and left (L-) prefrontal cortex (PFC) $O_2$Hb levels were measured using a multi-channel near-infrared spectroscopy system (LABNIRS; Shimadzu Co). $O_2$Hb levels for each area were measured. Beat-to-beat mean arterial pressure (MAP) was recorded by volume clamping the finger pulse with a finger photoplethysmograph (Finometer; Finapres Medical Systems) on the left middle finger. $O_2$Hb and MAP were averaged 1-sec epoch throughout the experiment. This study was approved by the Ethics Committee of Niigata University of Health and Welfare (18649-210618). The decrease in MAP after the cuff release was 31.3 ± 5.6 mmHg. The decrease in $O_2$Hb levels in the L-PFC (0.196 ± 0.088 mM·cm) was larger than that in R-PFC (0.165 ± 0.072 mM·cm) ($p < .05$). These results suggest that the effect of hypotension induced by thigh cuff release on cortical blood flow might be different between cortical regions.

### F-06: Effects of pemafibrate on hemorheology and leukocyte activity in patients with hypertriglyceridemia associated with type 2 diabetes and/or metabolic syndrome

Tomohiro Iwakura[1,2]; Tomoe Takahashi[1]; Atsuhiko Kawabe[1]; Takushi Sugiyama[1]; Takanori Yasu[1,*]

[1] *Department of Cardiovascular Medicine and Nephrology, Dokkyo Medical University, Nikko Medical Center, Tochigi Japan*; [2] *Department of Cardiovascular Surgery, Sakakibara Heart Institute, Tokyo, Japan*

**Background**: Persistently high serum levels of triglyceride (TG) and free fatty acid (FFA), which are commonly observed in metabolic syndrome and type 2 diabetes mellitus, are residual risk factors after statin therapy for atherosclerotic cardiovascular disease (ASCVD). The elevation of plasma FFA levels after lipid/heparin infusion results in endothelial and microvascular dysfunction in healthy subjects. However, the effects of lowering serum TG and FFA on microcirculation are unclear. Pemafibrate, an orally active, a novel selective peroxisome proliferator-activated receptor α modulator (SPPARMα), is used for treatment of hypertriglyceridemia in clinical setting. The aim of this study is to explore the effects of pemafibrate on hemorheology, leukocyte activation, and plasma levels of FFA.

**Method**: The study design was a single-center, prospective, observational study with propensity score analysis. Study patients were aged 20 years or older, type 2 diabetes mellitus (HbA1c 6%–10%) and/or metabolic syndrome associated with fasting TG ≥150 mg/dl within 2 weeks, and whole blood transit time (corrected) >45 s on microarray channel flow analyzer (MCFAN). Finally, 50 patients who were newly prescribed pemafibrate 0.2 mg/day p.o., and propensity score matched 50 patients who were not prescribed pemafibrate were included. Blood were sampled to assess whole blood transit time as a hemorheological parameter, and leukocyte activity by MCFAN, plasma level of FFA, and dROM as a parameter of oxidative stress at the baseline, 8 and 16 weeks after the start of the study.

**Statistics and Analysis**: Patients in the pemafibrate free group matched for age, sex, and other confounding factors were compared by propensity score analysis with patients sampled by the same protocol control group. Comparisons before and after treatment were analyzed by analysis of variance for continuous variables and $\chi^2$ test for nominal variables.

**Results and Conclusion**: The results will be opened in the coming JSM annual meeting.

The authors attest they are in compliance with human studies committees of the Dokkyo Medical University, Nikko Medical Center, and Drug Administration guidelines, including patient consent where appropriate.

### Applicants' Presentation for Young Investigator Award | Y-01: Microcirculatory responses during voluntary cycle ergometer exercise with whole-body neuromuscular electrical stimulation

Kaori Ochiai[1]; Yuma Tamura[1]; Masato Terashima[1]; Takanori Yasu[2,*]

[1] *Department of Rehabilitation, Dokkyo Medical University Nikko Medical Center, Tochigi, Japan*; [2] *Department of Cardiovascular medicine and Nephrology, Dokkyo Medical University Nikko Medical Center, Tochigi, Japan*

The purpose of this study was to explore the efficacy and safety of hybrid exercise combining cycle ergometer exercise and WB-NMES, and to examine the effects of hybrid exercise on microcirculation dynamics and metabolism. The protocol of this study was approved by the Ethics Committee of the Nikko Medical Center of Dokkyo Medical University (Approval No.: Nikko 20-020). The Whole blood passage time for the ex vivo microcirculatory evaluation, sublingual microcirculation and nail epithelial capillaries for the in vivo microcirculatory assessments were compared between cycle ergometer exercise and hybrid exercise at maximum load. No arrhythmias were detected during the hybrid exercise and 20 min recovery, and although blood fluidity was transiently exacerbated just after both exercise programs, in vivo parameters in the sublingual and nailfold

microcirculation remained unchanged. Even with the same workload as the cycle ergometer exercise, the oxygen uptake during the hybrid exercise remained higher than during the cycle ergometer exercise alone ($p < .05$, $r = .79$, power = .81). Both the hybrid and voluntary cycle ergometer exercises transiently exacerbated blood fluidity ex vivo; however, microvascular flow was not adversely affected in vivo.

### Y-02: Multidrug resistance protein 1 dependent extracellular release of glutathione induces cardiomyocyte ferroptosis after ischemia–reperfusion

Genki Ichihara[1]; Yoshinori Katsumata[1]; Yuki Sugiura[2]; Motoaki Sano[1]; Makoto Suematsu[2]; Keiichi Fukuda[1,*]

[1] *Department of Cardiology, Keio University School of Medicine, Tokyo, Japan*; [2]*Department of Biochemistry, Keio University School of Medicine, Tokyo, Japan*

**Background:** Cardiomyocytes consume large amounts of oxygen, and their membrane components are rich in polyunsaturated fatty acids (PUFA), which are easily oxidized. These facts suggest the existence of an efficient lipid redox system in the heart, and the failure of the redox system during myocardial ischemia (MI) and reperfusion (IR) may cause cell death (ferroptosis) due to lipid peroxidation, although the much remains unclear.

**Objective:** To identify the time phase during IR when the myocardial reduction system is disrupted and subsequently ferroptosis is induced, and to construct an intervention method to inhibit ferroptosis.

**Methods and Results:** Initially, in order to monitor the changes in redox metabolism induced during IR over time, we performed metabolomic analysis of continuously collected myocardial interstitial fluid using an in vivo microdialysis method, and found that glutathione was significantly released into the extracellular space after IR. Furthermore, in the in vitro anoxia-reoxygenation model, glutathione release into the extracellular space and the accompanying decrease in intracellular glutathione concentration were also observed. This extracellular glutathione release was prevented by inhibition of glutathione transporters, mainly multidrug resistance protein 1 (MRP-1). In addition, administration of MRP-1 inhibitors reduced intracellular reactive oxygen species levels and lipid peroxidation, resulting in reduced cell death in vitro. Next, induction of ferroptosis was assessed in vivo using endogenous oxidized phosphatidylcholine as a marker, and multiple phospholipid peroxide levels increased 6 h after IR. Furthermore, prior in vivo administration of ferrostatin-1, a lipophilic radical scavenging antioxidant, successfully prevented IR injury. Ferrostatin-1 exerted a protective effect even when administered 3 h after reperfusion. Finally, intravenous administration of MRP-1 inhibitor or glutathione significantly reduced IR injury in vivo.

**Conclusion:** During IR, glutathione was continuously released mainly in an MRP-1-dependent manner, and induced ferroptosis. The inhibition of glutathione release and ferroptosis mitigates myocardial cell death in MI/IR.

This study was approved by the ethics committee of animal in our institute.

### Y-03: Cortical oxygenation changes during high-intensity interval exercise versus moderate-intensity continuous exercise

Danni Qu[1]; Kazuki Hotta[2]; Hajime Tamiya[2]; Atsuhiro Tsubaki[2,*]

[1] *Graduate School of Health and Welfare, Niigata University of Health and Welfare, Niigata, Japan*; [2]*Institute for Human Movement and Medical Sciences, Niigata University of Health and Welfare, Niigata, Japan*

The effects of high-intensity interval exercise (HIIE) on exercise tolerance and preventing exacerbations equate with those of moderate-intensity continuous exercise (MICE) in patients with cardiac disease. The MICE can increase cortical oxygenation; however, the effects of HIIE are unknown. This study aimed to compare the cortical oxygenation during HIIE versus MICE. Thirty right-handed sedentary student participants performed HIIE and MICE in a random order. The HIIE consisted of high-intensity at 80% $VO_{2peak}$ for 3-min and low-intensity exercise at 40% $VO_{2peak}$ for 3-min repeated 4 times. The MICE consisted of moderate intensity exercise at 60% $VO_{2peak}$ for 24-min. Oxygenated hemoglobin ($O_2Hb$) levels were measured at right (R-) and left (L-) prefrontal cortex (PFC), premotor cortex (PMC), supplementary motor area (SMA), and primary motor cortex (M1) using a multi-channel near-infrared spectroscopy system (LABNIRS; Shimadzu Co). The oxygenated hemoglobin ($O_2Hb$) levels during exercise were integrated and compared between HIIT and MICE. The study was approved by the Ethics Committee of Niigata University of Health and Welfare (18656-210618). The $O_2Hb$ of HIIE (57.1 ± 14.6 mM·cm·sec) was significantly higher than that of MICE (18.3 ± 8.8 mM·cm·sec) in the M1 ($p < .05$). In contrast, there were no significant intergroup differences in $O_2Hb$ in the L-PFC (40.1 ± 13.2 vs. 42.6 ± 9.5 mM·cm·sec), R-PFC (35.3 ± 12.6 vs. 46.9 ± 8.8 mM·cm·sec), L-PMC (33.9 ± 6.4 vs. 31.0 ± 7.1 mM·cm·sec), R-PMC (32.9 ± 9.0 vs. 38.6 ± 10.0 mM·cm·sec), or SMA (40.6 ± 10.9 vs. 25.6 ± 9.8 mM·cm·sec). These results suggested that HIIE induces greater cortical oxygenation in the M1 than MICE did even though the same workload.

## Y-06: Transcapillary $PO_2$ gradients in contracting muscles of diabetes

Ren Takamizawa[1]; Naoki Hitosugi[1]; Hajime Tamiya[2]; Atsuhiro Tsubaki[2]; Kazuki Hotta[2,*]

[1] *Field of Physical Therapy, Graduate School of Niigata University of Health and Welfare, Niigata, Japan*; [2] *Institute for Human Movement and Medical Sciences, Niigata University of Health and Welfare, Niigata, Japan*

Diabetes impairs microvascular function and induces morphological changes in skeletal muscle microvasculature. Transcapillary $O_2$ gradients are necessary to drive blood-myocytes. We tested our hypothesis that the diabetes reduces skeletal muscle $PO_2$ gradients ($PO_{2mv}$ – $PO_{2is}$, microvascular and interstitial $PO_2$, respectively) from rest to contractions. Adult male Wistar rats ($n$ = 12, 6–8 weeks old, 224.8 ± 40.0 g) were divided into diabetic (DIA: streptozotocin i.p.) and sham (saline i.p.) groups. Eight weeks later, the $pO_{2mv}$ and $PO_{2is}$ of the extensor digitorum longus (EDL) muscle were measured at rest and during muscle contraction using the phosphorescence quenching method. Muscle contraction was elicited by electrical stimulation (1 Hz, 6 V, 180 s). As compared to sham group, resting $PO_{2mv}$ and the amplitude of the decrease in $PO_{2mv}$ with contraction was higher in DIA ($p$ < .05, respectively). From rest to muscle contractions, there was no significant difference in $PO_{2is}$ between DIA and sham groups. The pressure gradient between $PO_{2mv}$ and $PO_{2is}$ at rest was higher in the DIA compared to the sham. These results suggested that driving force for oxygen transfer was higher from rest to muscle contractions in diabetic rats compared to sham rats. The high driving force for oxygen transfer may be a compensatory effect of the morphological or functional changes in skeletal muscle microvasculature associated with diabetes.

## Y-07: Perivascular flow dynamics revealed with two-photon microscopy

Marie Tanaka; Itsuki Hasegawa; Yoko Hirayoshi; Shinobu Minatani; Akitoshi Takeda; Yoshiaki Itoh*

*Department of Neurology, Osaka City University, Osaka, Japan*

**Background**: Perivascular flow in the brain, also known as glymphatic system, may function as a washout mechanism of large molecules, including amyloid beta in Alzheimer's disease. Flow dynamics of perivascular space was evaluated with live imaging system using two-photon microscopy.

**Methods**: Under isoflurane anesthesia, a closed cranial window was installed in a Tie2-GFP mouse (287 Sato/J). TRITC-labeled dextran solution of 100 μM was applied over the cortex and dynamic images of perivascular space were obtained repeatedly. To differentiate convective flow from diffusion, different size of dextran was used, that is, 4.4 kD (n = 19), 40 kD ($n$ = 13) and 155 kD ($n$ = 5). Furthermore, combined solution of FITC-dextran and HiLyte Fluor647 β-amyloid (1–40) (5315.4 g/mol) was administered over the cortex in C57BL/6 mice ($n$ = 20) to compare the dynamics of different type of molecules. The study was approved by the Animal Ethics Committee.

**Results**: Time dependent increase in small size dextran was observed along the penetrating arteries and veins as well as in the brain parenchyma. Diffusion of the dextran through the parenchyma was observed from the surface down to 150 μm deep. Larger size dextran was dominantly found in the perivascular space. The flow of dextran was most rapid in the first 30 min and became slow and steady later. The flow was often observed in the entire circumference of the vessels but localized flow was also found. Furthermore, double luminal flow suggestive of intra/extra vessel wall flow was also observed in larger vessels. Transportation of amyloid β was identical to that of large size dextran.

**Conclusion**: Perivascular space may function as transportation of membrane-impermeant molecules. The flow is quite slow once it is stabilized. The flow may be circumferential, localized or double luminal based on the size of the vessel, location and animal.

## Y-08: Effect of sepsis on microvascular oxygen pressure in rat diaphragm

Kazuki Hotta*; Hajime Tamiya; Atsuhiro Tsubaki

*Department of Physical Therapy, Niigata University of Health and Welfare, Niigata, Japan*

**Background**: Sepsis is life-threatening disease. During recovery from sepsis, atrophy of the diaphragm can cause respiratory dysfunction. Skeletal muscle microvascular perfusion is reported to be impaired in a rat model of normotensive sepsis, which could cause fatigue during muscle contractions. However, the effect of sepsis on microvascular oxygen dynamics in diaphragm is unknown. The aim of this study is to test the hypothesis that sepsis reduces microvascular oxygen pressure ($pO_{2mv}$) of diaphragm during contractions.

**Method**: Cecal ligation and puncture (CLP) model was used as septic model in this study. Male Wistar rats ($n$ = 13, 3–5 months old) were randomly assigned to CLP and sham groups. Diaphragm was exposed under mechanical ventilator control. Electrodes were placed on the exposed diaphragm to induce 3-min of muscle contractions (10 V, 2 ms, 2 Hz). Phosphorescence quenching techniques were used to measure $pO_{2mv}$ in rat diaphragm.

**Results**: Baseline $pO_{2mv}$ was significantly lower in sepsis than sham group (15.7 ± 1.7 vs. 12.0 ± 1.0 mmHg, sham vs. CLP, $p$ < .01). At the onset of diaphragm contractions, $pO_{2mv}$ decreased in both groups. The $pO_{2mv}$ nadir was not different between groups. After reaching the nadir, the $pO_{2mv}$ gradually increased until the end of contraction in sham group; however, it kept low value in CLP group. The $pO_{2mv}$ at the end of contraction was significantly lower in CLP than sham group (12.3 ± 3.6 vs. 5.3 ± 2.3 mmHg, sham vs. CLP, $p$ < .01).

**Conclusion**: CLP-induced sepsis decreased microvascular oxygen pressure in rat diaphragm.

## Y-11: Skeletal muscle interstitial oxygen partial pressure from rest to contractions in the rat cecal ligation and puncture model

Naoki Hitosugi[1]; Kazuki Hotta[2]; Ren Takamizawa[1]; Hajime Tamiya[2]; Atsuhiro Tsubaki[2,*]

[1] *Graduate School of Health and Welfare, Niigata University of Health and Welfare, Niigata, Japan*; [2]*Department of Physical Therapy, Niigata University of Health and Welfare, Niigata, Japan*

**Background**: Multiorgan failure in sepsis induces microcirculatory disturbances in the organs. In septic rat induced by cecal ligation and puncture (CLP), capillary blood flow was limited in skeletal muscle, which may impair oxygen delivery-to-utilization matching. However, skeletal muscle oxygen delivery-to-utilization matching from rest to contractions is unknown. The aim of this study is to test the hypothesis that interstitial $PO_2$ ($PO_{2is}$) is limited from rest to contraction in septic model rats.

**Method**: Male Sprague-Dawley rats ($n$ = 16, 2–3 months old) were randomly divided into CLP and SHAM groups. The left spinotrapezius muscle $PO_{2is}$ was measured via phosphorescence quenching during muscle contractions induced by electrical stimulation (1 Hz, 2 ms, 6 V for 180 s).

**Results**: No group difference were found for the resting $PO_{2is}$ (CLP 13.0 ± 1.9 vs. SHAM 14.9 ± 2.5 mmHg, $p > .05$). However, the nadir $PO_{2is}$ during the muscle contractions was lower in CLP than SHAM (CLP 2.0 ± 1.1 vs. SHAM 5.7 ± 3.6 mmHg, $p < .05$). In the CLP, the time constant of oxygen dynamics at the onset of contraction was faster compared with SHAM (CLP 6.1 ± 1.7 vs. SHAM 10.6 ± 5.1 s, $p < .05$).

**Conclusion**: In the rat model of sepsis, the spinotrapezius $PO_{2is}$ during muscle contractions was lower, and the response was faster compared to SHAM. These results may reflect a sepsis-induced mismatch between muscle oxygen supply and demand during exercise.