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研究課題名（和文）細動脈血管壁の力学的仕事量とエネルギー消費に関する研究

研究課題名（英文）Energetics in arterioles during nitric oxide dependent and independent vasodilation

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研究成果の概要（和文）：
Krogh の研究以来１世紀近く、生命活動を維持する上で最も重要な物質である酸素は、拡散により毛細血管から組織へ供給されると信じられてきたが、本研究により毛細血管の前に位置する細動脈においても既に血中酸素濃度は低下しており、かつ細動脈壁で大きな酸素濃度勾配が存在することが明らかになった。これは、毛細血管のみが唯一酸素供給の場ではなく、細動脈も組織への酸素供給源として機能している可能性を示唆するものである。しかし、この細動脈での血中酸素濃度の低下を、組織への酸素拡散のみで説明しようとすると、細動脈血管壁内での酸素拡散係数が自由拡散係数よりも大きくなるという物理的矛盾が生じる。我々は、細動脈レベルでの血中酸素濃度の低下に、細動脈の仕事に伴う血管壁の酸素消費が影響しているのではないかという仮説を提唱し、この仮説を実験的に証明するため、微小循環領域での酸素分圧を実測、得られたデータを基に細動脈血管壁での酸素消費率を求め、酸素濃度勾配形成と細動脈血管壁での仕事量の関連を検討した。血管内外の酸素濃度勾配より求めた骨格筋細動脈血管壁での酸素消費率は、下流側細動脈より上流側で、さらには血管拡張時（血管平滑筋弛緩時）より通常時の方が高値を示し、血管平滑筋の仕事量に依存した。また得られた値は、これまでの *in vitro* 実験系による報告値より遙かに大きく、細動脈での酸素濃度勾配の形成に強く関与していることが分かった。さらに運動生理や病態生理との関連について内皮細胞由来の血管拡張物質である一酸化窒素（NO）の効果を調べた結果、運動時の NO 依存性血管拡張は、血管壁自身の酸素消費を低下させ効率的な組織への酸素供給を可能とする半面、NO 産生能低下時の血管収縮は、血管壁での酸素消費を増加させ組織の低酸素化を招く危険性のあることがわかった。
研究成果の概要（英文）：
The objective of this study was to evaluate whether the nitric oxide (NO) would decrease vessel wall oxygen consumption by decreasing the mechanical work of vascular smooth muscle. The oxygen consumption rate (QO_2) of arteriolar walls in rat cremaster muscle was determined *in vivo* during NO dependent and -independent vasodilation based on the intra- and perivascular oxygen tension (PO_2) measured by phosphorescence quenching technique. NO dependent vasodilation was induced by increased NO production due to increased blood flow, while NO independent vasodilation was induced by topical administration of papaverine. The energy efficiency was evaluated by the variable ratio of wall tension to QO_2 between normal and vasodilated conditions. NO dependent and -independent dilation increased arteriolar diameters by 13% and 17%, respectively. Vascular wall QO_2 decreased significantly during both dilations. There was no significant difference between the energy efficiency during NO dependent and -independent vasodilation, suggesting the decrease in vascular wall QO_2 produced by NO to be related to a decrease in the mechanical work of vascular smooth muscle.

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１．研究開始当初の背景

心臓から送り出された血液は、動脈−毛細血管（微小血管）−静脈を通り、再び心臓に戻る。一般的な血液循環はこのマクロな血液の流れをイメージするが、本来の循環の目的は、毛細血管領域で営まれる組織への栄養の補給と老廃物の回収にある。特に組織への酸素供給は最重要課題であり、微小循環はそれに最適な構築を持っている。A. Krogh の酸素輸送に関する理論的研究が発表されて以来、生命活動を維持する上で最も重要な物質である酸素は、組織の代謝率に応じて消費されながら拡散により毛細血管から組織へ供給されると信じられている。よって生理的状態にある骨格筋組織の毛細血管分布は、酸素拡散に関する物理的特性により支配されるため、体重 30g のマウスも 5000kg のゾウもほぼ同等の幾何学的特徴を有している。しかし、最近の我々の研究では、毛細血管の前に位置する細動脈においても既に血中酸素濃度は低下しており、かつ細動脈壁を挟んだ血管内外で大きな酸素濃度勾配が存在することが明らかになってきた。これらの結果は、毛細血管のみが唯一酸素供給の場ではなく、細動脈も組織への酸素供給源として機能している可能性を示唆するものである。しかし、この細動脈での血中酸素濃度の低下を、組織への酸素拡散のみで説明しようとすると、細動脈血管壁内での酸素拡散係数が自由拡散係数よりも大きくなるという物理的矛盾が生じる。一方、細動脈は、収縮弛緩を繰り返し組織への血流調節と全身の血圧調節のため常に仕事を行っている。特に運動時には安静時の 20 倍以上の血流増加を可能とする骨格筋細動脈の仕事量の多さは容易に想像できる。組織が仕事をするためには当然、安静時より多くの酸素を必要とするが、これまでの血管壁の酸素消費に関する研究は、全て摘出血管を対象とした in vitro 実験系により行われている。このような非生理的状態で得られた血管壁の酸素消費率は、運動時の骨格筋酸素消費率に比べ２オーダー低く、安静時の骨格筋酸素消費率とほぼ同等であるため、骨格筋内に存在する細動脈血管壁の容積を考慮すると組織への酸素供給過程では血管壁の酸素消費は無視できる量である。事実、これまでの酸素輸送に関する研究で、血管壁の酸素消費に注目したものは皆無であった。

２．研究の目的

前述の細動脈レベルでの血中酸素濃度の低下に、細動脈からの酸素拡散に加え、この細動脈の仕事に伴う血管壁の酸素消費が強く影響しているのではないかと考えている。本研究ではこの仮説の妥当性を検証するため、機能的状態にある細動脈血管壁の仕事量と酸素消費の関係を明らかにすることを第一の目的とするが、将来的には物質交換のために効率的にデザインされた循環システムの合目的性を酸素ダイナミクスの面から示したい。

３．研究の方法

以下の方法で研究を遂行した。

微小循環酸素分圧の光学的計測法の整備：本研究ではラット cremaster 筋の直径 100μm 程度の細動脈から 10μm程度の前毛細血管細動脈までを対象として血管壁での酸素消費率の定量化を試みる。酸素分圧の測定には、励起後の消光過程が生体内でのプローブ濃度に依存しない燐光消光法を用い、酸素感受性色素 Pd-porphyrin のリン光寿命の変化より求める。当研究室には微小循環での酸素分圧計測用の時間分解型レーザー顕微鏡を現有しており、本システムを基に血管内から組織にかけて血管壁を挟んだ酸素分圧を連続的に測定できる空間分解能を有するシステムを整備する。

微小循環酸素拡散モデルの開発：Krogh モデルを基本に、本研究で対象とするラット cremaster 筋の解剖学的および血行力学的特徴を加えた独自のモデルを開発する。実測情報に基づき開発した酸素拡散モデルと血管壁を介した酸素分圧分布の実測値を基に、血管壁の酸素消費率を同定するシミュレーションを行う。

動物実験による微小循環酸素分圧の計測：麻酔下のラット cremaster 筋を対象に、小動脈、細動脈、毛細血管、細静脈血管の内部（血中）と外壁周辺部位における酸素分圧分布の計測を行う。細動脈血管長軸および円周方向の酸素分圧勾配から、血管平滑筋の緊張度の違いによる細動脈酸素レベルの低下の差を比較する。

細動脈血管壁における酸素消費率の推定：安静時および血管拡張時の血管内外の酸素分

圧実測値を基に、新たに開発した微小循環酸素拡散モデルを用い、細動脈血管壁での酸素消費率の同定を行う。同一個体において、上流側細動脈から毛細血管の直前の細動脈まで、分岐回数と血管径による血管壁酸素消費率の違いを明らかにする。各血管部位での酸素消費率の違いと、血管内圧および血管壁の内皮／平滑筋の容積比を基に、各部位における細動脈平滑筋の酸素消費率を求め、その仕事量を推定する。

微小循環酸素拡散モデルの妥当性検討：本研究で開発した酸素拡散モデルと各細動脈血管壁の酸素消費率を用い、各レベルの細動脈血管内外の酸素分圧値を求め、実測酸素分圧値と比較することにより、モデルの妥当性を検討する。研究期間後半は、運動時を含めた生理的状況下や、高血圧・老化等の病態モデルによるデータ収集を行い、得られた血管壁での酸素消費率を基に、細動脈機能と組織への酸素供給能に関する統合的な知識を提供する。以上の研究成果に基づき、細動脈レベルでの血中酸素濃度の低下と細動脈の仕事に伴う血管壁の酸素消費の関係、さらには細動脈機能の変化に伴う血管壁での酸素消費が組織への酸素供給効率に及ぼす影響を総合的に判定し、細動脈機能と組織への酸素供給能に関する統合的な知識を提供する。

４．研究成果

研究成果を以下に記す。

NO synthesized by the endothelium of small vessels not only controls vascular tone, but also regulates tissue oxygen consumption. Our recent study demonstrated that inhibition of NO synthesis increases oxygen consumption of arteriolar walls, while enhancement of flow-induced NO release decreases it [1]. On the other hand, oxygen consumption by vascular walls in vivo arterioles depends on vascular tone; vascular smooth muscle contraction significantly increases oxygen consumption, whereas vascular smooth muscle relaxation decreases it [2]. These findings raise the question of whether NO modulation of oxygen consumption has a direct effect on cell respiration or is a result of change in the mechanical work of vascular walls. To answer the question, we determined the energy efficiency of vascular walls in skeletal muscle arterioles in vivo during NO dependent and -independent vasodilation. The energy efficiency was calculated by changes in circumferential wall tension and vascular wall oxygen consumption rates before and during vasodilation.

Experiments were performed using male Wistar rats weighing 150 to 200 g. All animal procedures were approved by the University of Tokyo Animal Care and Use Committee. Animals were anesthetized with urethane, and a tracheotomy was performed to facilitate spontaneous breathing. The cremaster muscle was spread out in a bath chamber to observe microcirculation. General observations of microcirculation and in vivo PO_2 measurements were performed by an intravital laser microscope combined with the oxygen-dependent quenching of phosphorescence decay technique [3]. Intra- and perivascular PO_2 measurements were made at first order (1A) arterioles having a diameter of approximately 100 μm. QO_2 of vascular walls was calculated by employing a modified Krogh capillary-tissue model for an arteriolar wall, as previously described [2]. Assuming that the arteriole is cylindrical and has an outer radius and an internal radius of R_o and R_i, respectively, the oxygen consumption rate per unit tissue volume per unit of time in its wall (QO_2: $mlO_2/s/g$) can be expressed as:

$$QO_2 = (PO_{2in} - PO_{2peri})\,(4\alpha_t D_t)/[2R_o^2 \ln(R_o/R_i) - (R_o^2 - R_i^2)]$$

where PO_{2peri} and PO_{2in} represent the PO_2 values measured in the vicinity of the outer surface of the arteriolar wall and within the arteriole, respectively. α_t and D_t represent oxygen solubility and oxygen diffusivity, respectively, in the arteriolar wall, for which values of 3.0×10^{-5} ml/g/mmHg and 1.5×10^{-5} cm^2/s, respectively, were used. Therefore, QO_2 of the vessel wall was determined by utilizing the measured intra- and perivascular PO_2 values of the arteriole.

After intra- and perivascular PO_2 measurements of 1A arterioles under normal conditions, the PO_2 measurements during NO dependent or independent vasodilation were once again performed at the same sites. NO dependent vasodilation was induced by the flow-induced NO release from ECs, in which the mechanical occlusion of one branch of arteriolar bifurcation causes an increase in blood flow in the unoccluded branch. NO independent vasodilation was induced by topical administration of papaverine (10^{-4} mol/l) to the muscle surface. As mechanical energy would be required to maintain the vessel diameter against blood pressure, the energy efficiency of the vessel wall was determined from the total amount of mechanical work needed to change vessels from a vasodilated to a normal state and its energy cost. This value was defined as the ratio of changes in circumferential wall stress and oxygen consumption rate in the arteriole from vasodilated to normal state [$\Delta T/\Delta QO_2$].

All data are reported as means ± SD. Data within each group were analyzed by analysis of variance for repeated measurements (ANOVA). Differences between groups were determined using a *t*-test with the Bonferroni correction. Differences with a P-value of < 0.05 were considered statistically significant.

Nine rats were used to study the response to NO dependent vasodilation, and six rats to study NO independent vasodilation. Systemic arterial PO_2, PCO_2, and pH were measured with a blood analysis system in samples from the carotid arteries after performing the microvascular PO_2 measurements. In the NO dependent vasodilation study, arterial PO_2 averaged 89.7 ± 6.0 mmHg, and arterial PCO_2 and pH averaged 48.8 ± 8.7 mmHg and 7.33 ± 0.05, respectively. On the other hand, for the study of NO independent vasodilation, the arterial PO_2 was 97.8 ± 10.5 mmHg, the arterial PCO_2 46.1 ± 8.2 mmHg and pH 7.31 ± 0.05. Mean arterial blood pressure averaged 77.6 ± 7.1 mmHg.

Changes in vessel diameters during vasodilation.

The maximum values of internal diameter during the occlusion period (60 seconds) and after application of papaverine were defined as the diameter values during NO dependent and independent vasodilation. The individual internal diameter changes of arterioles for each preparation and mean percent changes before and during NO dependent and independent vasodilation are shown in Fig. 1. NO dependent and independent dilation increased diameters by 13%, and 17%, respectively, relative to the values under normal conditions. The diameter values during NO dependent and independent dilation were both significantly higher than the values before dilation. Based on these vessel diameter data, the changes in circumferential wall tension in the arteriole under normal conditions and during vasodilation were calculated utilizing the Laplace law. Fig. 2 shows the circumferential wall stress increment in the arteriole before and during NO dependent or independent vasodilation (ΔT: dyn/cm^2). The ΔT during NO independent vasodilation was greater than that during the NO dependent vasodilation, since ΔT depends on changes in vessel diameter.

Intra- and perivascular PO_2 and vascular wall QO_2.

The changes in the average values of intra- and perivascular PO_2 in arterioles before and during NO dependent and independent vasodilation are shown in Fig. 3. Intravascular PO_2 values of the arterioles under all conditions were significantly lower than the systemic arterial PO_2 value. Perivascular PO_2 values of the arterioles during NO dependent and independent

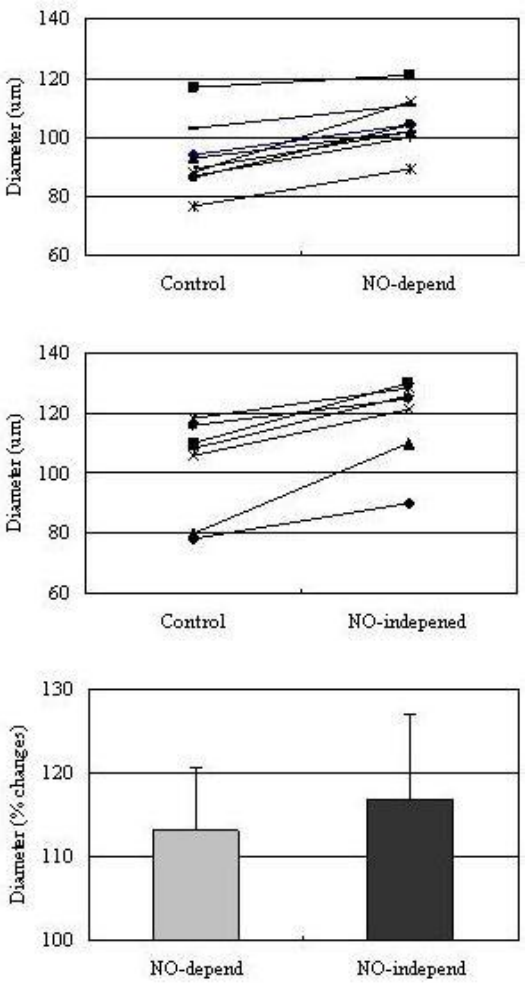

Figure 1 Individual changes in internal diameters of arterioles for each preparation before and during NO dependent and independent vasodilation (top and middle), and their mean relative changes (bottom).

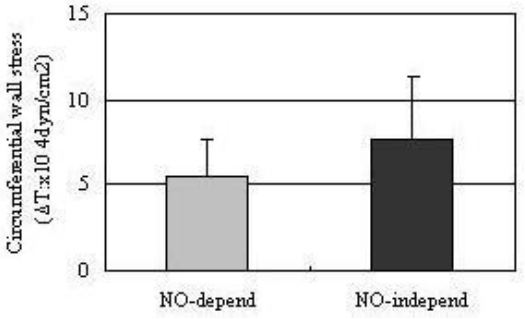

Figure 2 The circumferential wall stress increment in the arteriole before and during NO dependent and independent vasodilation (ΔT: x10^{4}dyn/cm^2).

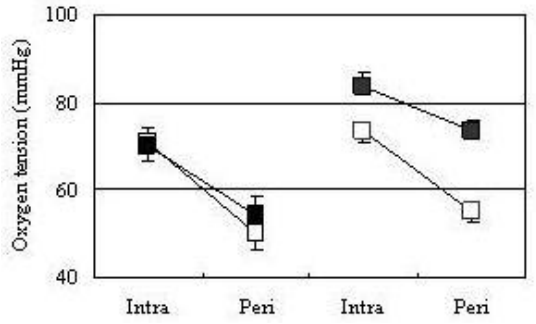

Figure 3 The changes in intra- and perivascular PO_2 values of arterioles before (□) and during (■) NO dependent (left) and independent (right) vasodilation.

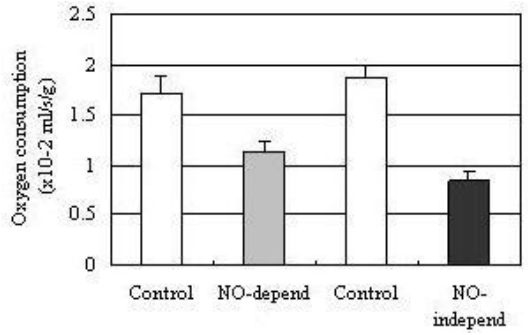

Figure 4 The vessel wall oxygen consumption rates in arterioles before and during NO dependent and independent vasodilation.

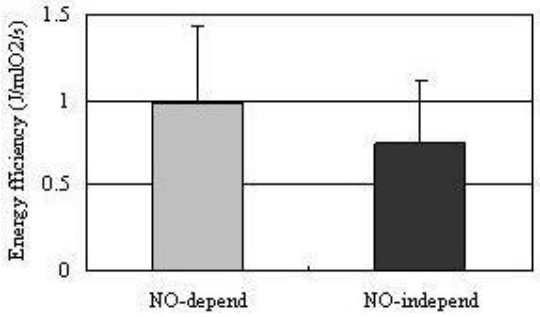

Figure 5 The vessel wall energy efficiencies during NO dependent and independent vasodilation..

vasodilation were significantly higher than control. The average values of vessel wall QO_2 in arterioles before and during NO dependent and independent vasodilation calculated from the individual intra- and perivascular PO_2 data are shown in Fig. 4. The QO_2 of arteriolar walls during NO dependent and independent vasodilation was significantly lower than control, however, vessel wall QO_2 during NO independent vasodilation was significantly lower than that during NO dependent vasodilation. To evaluate the participation of NO in vessel wall oxygen consumption, the energy efficiencies of vessel walls during NO dependent and independent vasodilation were calculated (Fig. 5). There was no significant difference in vessel wall energy efficiency during NO dependent versus independent vasodilation, suggesting that vessel wall oxygen consumption depends on the amount of mechanical work performed by vessel walls.

The principal finding of the present study is that the energy efficiencies of vessel walls in arterioles during NO dependent and independent vasodilation have no significant difference, and the decrease in vessel wall oxygen consumption produced by NO depends on the reduced mechanical work of vascular smooth muscle.

Vascular wall oxygen consumption rates obtained in this study were 1-2 orders of magnitude higher than reported values of vascular cells measured from isolated vascular segments. High oxygen consumption by functional arterioles has been demonstrated by many groups [4]. These discrepancies from in vitro data may be explained by the differences of experimental environments. Since most tissue segment studies were conducted under static conditions, in the absence of physiological function, they were under lower metabolic activity.

The QO_2 of arteriolar walls was calculated based on the measured intra- and perivascular PO_2 values before and during NO dependent and independent vasodilation. NO dependent vasodilation was performed by the parallel arteriolar bifurcation occlusion method [5], in which increased NO release by vascular endothelial cells was induced by an increase in blood flow through an unoccluded arteriole. Intravascular pressure within an unoccluded arteriole may rise during occlusion, but the effect of the increase in pressure on vasodilation is negligible. Most of the vasodilation was induced by the increase in flow-dependent NO release, since arteriolar diameter increased only 2.5% during occlusion using L-NAME or L-NAME plus indomethacin (data not shown), whereas it increased 13% during occlusion. The 2.5% increase in diameter may have been caused by intravascular pressure changes.

In conclusion, NO dependent and independent vasodilation both decreased vessel wall oxygen consumption in arterioles. There was no significant difference in energy efficiencies of vessel walls between NO dependent and independent vasodilation. It appears that NO decreases vessel wall oxygen consumption by decreasing vascular smooth muscle mechanical work.

REFERENCES
[1] Shibata M, Ichioka S, Kamiya A. Nitric oxide modulates oxygen consumption by arteriolar walls in rat skeletal muscle. Am J Physiol Heart Circ Physiol 289: H2673-H2679, 2005.
[2] Shibata M, Ichioka S, Kamiya A. Estimating oxygen consumption rates of arteriolar walls under physiological conditions in rat skeletal muscle. Am J Physiol Heart Circ Physiol 289: H295-H300, 2005.
[3] Shibata M, Ichioka S, Ando J, Kamiya A. Microvascular and interstitial pO_2 measurements in rat skeletal muscle by phosphorescence quenching. J Appl Physiol 91: 321-327, 2001.
[4] Tsai AG, Johnson PC, Intaglietta M. Oxygen gradients in the microcirculation. Physiol Rev 83: 933-963, 2003.
[5] McGahren ED, Dora KA, Damon DN, Duling BR. A test of the role of flow-dependent dilation in arteriolar responses to occlusion. Am J Physiol Heart Circ Physiol 272: 714 – 721, 1997.

５．主な発表論文等

〔雑誌論文〕（計８件）

① Shibata M, Ohura N, Ichioka S: Oxygen consumption by arteriolar wall during enhancement and inhibition of nitric oxide synthesis. IFMBE Proceedings 25: 125-128, 2009
② Shibata M, Ohura N, Ichioka S: Oxygen dynamics around arterioles in skeletal muscle. Clinical Hemorheology and Microcirculation. 42: 226, 2009
③Shibata M, Yamakoshi T, Yamakoshi K: Physiological role of nitric oxide in oxygen consumption by arteriolar wall. Conf Proc IEEE Eng Med Biol Soc: 1389-1392, 2008
④Ichioka S, Ando T, Shibata M, Sekiya N, Nakatsuka T: Oxygen consumption of keloids and hypertrophic scars. Ann Plast Surg 60: 194-197, 2008
⑤Ichioka S, Watanabe H, Sekiya N, Shibata M, Nakatsuka T: A technique to visualize wound bed microcirculation and the acute effect of negative pressure. Wound Repair Regen 16:460-465, 2008
⑥Nakagami G, Sanada H, Matsui N, Kitagawa A, Yokogawa H, Sekiya N, Ichioka S, Sugama J, Shibata M: Effect of vibration on skin blood flow in an in vivo microcirculatory model. BioScience Trends 1: 161-166, 2008
⑦Shibata M, Ohura N, Sekiya N, Ichioka S, Yamakoshi T, Yamakoshi K, Kamiya A: Compact capillaroscopy for human skin using CCD video-probe. Microvascular Reviews and Communications 1: 4-7, 2007
⑧Ichioka S, Sekiya N, Shibata M, Nakatsuka T: Alpha V beta 3 (αvβ3) integrin inhibition reduces leukocyte-endothelium interaction in a pressure-induced reperfusion model. Wound Rep Reg 15: 572-576, 2007

〔学会発表〕（計８件）

① Shibata M, Ohura N, Ichioka S: Oxygen consumption by arteriolar wall during enhancement and inhibition of nitric oxide synthesis. World Congress on Medical Physics and Biomedical Engineering. Munich, Sep. 2009
② Shibata M, Ohura N, Ichioka S: Oxygen dynamics around arterioles in skeletal muscle. 5th International Conference on Clinical Hemorheology. St. Moritz, Jul. 2009
③ Shibata M, Yamakoshi T, Yamakoshi K: Oxygen consumption by vascular wall in skeletal muscle arterioles under physiological conditions. 8th IEEE international Conference on BIBE, Athens, Oct. 2008
④ Shibata M, Yamakoshi T, Yamakoshi K: Energetics in arterioles during nitric oxide dependent and independent vasodilation. 30th IEEE international Conference on EBM, Vancouver, Aug. 2008
⑤ Shibata M: Oxygen dynamics around arterioles, 36th Annual ISOTT conference, Sapporo, Aug. 2008
⑥ Shibata M, Kawarada A: Application of phosphorescence quenching technique to in vivo microvascular oxygen measurements. 22nd SICE Symposium on Biomedical and Physiological Engineering, Harbin, Jan. 2008
⑦ Shibata M, Yamakoshi T, Yamakoshi K: Energetics in arterioles during nitric oxide dependent and independent vasodilation. 29th IEEE international Conference on EBM, Lyon, Aug. 2007
⑧Shibata M, Ohura N, Sekiya N, Ichioka S, Kamiya A: Nitric oxide contribution to vascular wall oxygen consumption in arterioles. 9th World Conference for Microcirculation, Milwaukee, Aug. 2007

〔図書〕（計１件）

柴田他　（共著）：からだと酸素の事典, 朝倉書店,2009

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