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Title: Transcutaneous carbon dioxide attenuates impaired oxidative capacity in skeletal muscle in hyperglycemia

Running title: Effects of CO2 therapy on muscle oxidative capacity

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Name	*Affiliations*
Tomohiro Matsumoto	1. Rehabilitation Sciences, Kobe University Graduate School of Health Sciences, Kobe, Japan
Dr. Masayuki Tanaka	1. Physical Therapy, Osaka University of Human Sciences, Osaka, Japan
Dr. Ryosuke Nakanishi	1. Rehabilitation, Kobe international University, kobe, Japan
Miho Takuwa	1. Rehabilitation Sciences, Kobe University Graduate School of Health Sciences, Kobe, Japan
Takumi Hirabayashi	1. Rehabilitation Sciences, Kobe University Graduate School of Health Sciences, Kobe, Japan
Kohei Ono	1. Rehabilitation Sciences, Kobe University Graduate School of Health Sciences, Kobe, Japan
Takuya Ikeji	1. Rehabilitation Sciences, Kobe University Graduate School of Health Sciences, Kobe, Japan
Dr. Noriaki Maeshige	1. Rehabilitation Sciences, Kobe University Graduate School of Health Sciences, Kobe, Japan
Prof. Yoshitada Sakai	1. Rehabilitation Medicine, Kobe University Graduate School of Medicine, Kobe, Japan
Prof. Toshihiro Akisue	1. Rehabilitation Sciences, Kobe University Graduate School of Health Sciences, Kobe, Japan
Dr. Hiroyo Kondo	1. Food Science and Nutrition, Nagoya Women's University, Nagoya, Japan
Prof. Akihiko Ishihara	1. Cell Biology and Life Science, Kyoto University, Kyoto, Japan
Prof. Hidemi Fujino	1. Kobe University, Kobe, Japan

Corresponding author: Prof. Hidemi Fujino <fujino@phoenix.kobe-u.ac.jp>

Abstract

Hyperglycemia impairs oxidative capacity in skeletal muscle. Muscle oxidative capacity is regulated by PGC-1α. Transcutaneous carbon dioxide (CO2) enhances PGC-1α in skeletal muscle. Therefore, the aim of this study was to clarify the effects of CO2 therapy on muscle oxidative capacity impaired by streptozotocin (STZ)-induced hyperglycemia. Eight-week-old male Wistar rats were randomly divided into 4 groups: control, CO2 treatment, STZ-induced hyperglycemia, and STZ-induced hyperglycemia treated with CO2 treatment groups. STZ-induced hyperglycemia resulted in a decrease of muscle oxidative capacity and the expression levels of PGC-1α and COX-

4. Application of Transcutaneous CO2 attenuated the decrease in muscle oxidative capacity and the expression levels of PGC-1α and COX-4, and enhanced the expression levels of eNOS. These results indicate that transcutaneous CO2 improve the impaired muscle oxidative capacity via an enhancement of eNOS and PGC-1α-related signaling in the skeletal muscle of hyperglycemia.

Keywords: carbon dioxide; muscle oxidative capacity; hyperglycemia

Tables:
Tab. 1 - download

Transcutaneous carbon dioxide attenuates impaired oxidative capacity in skeletal muscle in hyperglycemia model

Tomohiro Matsumoto[1], Masayuki Tanaka[2], Ryosuke Nakanishi[3], Miho Takuwa[1], Takumi Hirabayashi[1], Kohei Ono[1], Takuya Ikeji[1], Noriaki Maeshige[1], Yoshitada Sakai[4], Toshihiro Akisue[1], Hiroyo Kondo[5], Akihiko Ishihara[6], and Hidemi Fujino[1]

[1]*Department of Rehabilitation Science, Kobe University Graduate School of Health Sciences, 7-10-2 Tomogaoka, Suma-ku, Kobe-shi, Hyogo 654-0142, Japan.*

[2]*Department of Physical Therapy, Faculty of Human Sciences, Osaka University of Human Sciences, 1-4-1 Shojaku, Settsu-shi, Osaka 566-8501, Japan.*

[3]*Department of Rehabilitation, Kobe international University, 9-1-6 Kouyouchounaka, Higashinada-ku, Hyogo 658-0032, Japan.*

[4]*Division of Rehabilitation Medicine, Kobe University Graduate School of Medicine,*

650-0017 Kobe, Japan.

[5]*Department of Food Science and Nutrition, Nagoya Women's University, Nagoya, 4-21 Shioji-cho, Mizuho-ku, Nagoya-shi, Aichi 467-8611, Japan.*

[6]*Laboratory of Cell Biology and Life Science, Graduate School of Human and Environmental Studies, Kyoto University, Yoshida-nihonmatsu-cho, Sakyo-ku, Kyoto-shi, Kyoto 606-8501, Japan.*

E-mail for the first author

E-mail: tomohiro19900804@gmail.com

Running title: Effects of CO_2 therapy on muscle oxidative capacity

Address for correspondence: Hidemi Fujino, Ph.D.

Professor, Department of Rehabilitation Science, Kobe University Graduate School of Health

Sciences, 7-10-2 Tomogaoka, Suma-Ku, Kobe 654-0142, Japan.

E-mail: fujino@phoenix.kobe-u.ac.jp

Abstract

Hyperglycemia impairs oxidative capacity in skeletal muscle. Muscle oxidative capacity is regulated by peroxisome proliferator-activated receptor-γ co-activator-1α (PGC-1α). Transcutaneous carbon dioxide (CO_2) enhances PGC-1α expression in skeletal muscle. Therefore, the aim of this study was to clarify the effects of CO_2 therapy on muscle oxidative capacity impaired by streptozotocin (STZ)-induced hyperglycemia. Eight-week-old male Wistar rats were randomly divided into 4 groups: control, CO_2 treatment, STZ-induced hyperglycemia, and STZ-induced hyperglycemia treated with CO_2. STZ-induced hyperglycemia resulted in a decrease of muscle oxidative capacity and decreased PGC-1α and cytochrome c oxidase subunit 4 (COX-4) expression levels; while, application of transcutaneous CO_2 attenuated this effect, and enhanced the expression levels of endothelial nitric oxide synthesis (eNOS). These results indicate that transcutaneous CO_2 improves impaired muscle oxidative capacity via enhancement of eNOS and PGC-1α-related signaling in the skeletal muscle of rats with hyperglycemia.

Key words: carbon dioxide, muscle oxidative capacity, hyperglycemia

Abbreviations

PGC-1α, peroxisome proliferator-activated receptor-γ co-activator-1α; CO_2, carbon dioxide; eNOS, endothelial nitric oxide synthesis; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; cGMP, cyclic guanosine monophosphate; SIRT1, sirtuin1; COX-4, cytochrome c oxidase subunit 4; STZ, streptozotocin; CON, control; CS, citrate synthase; PBST, phosphate-buffered saline with 0.1% Tween 20.

Introduction

Hyperglycemia induces widespread tissue dysfunction and deleterious complications (Blake and Trounce 2014). Especially, hyperglycemia impairs not only muscle protein synthesis but also oxidative capacity in the skeletal muscle (Py et al. 2002; Frier et al. 2008; Fortes et al. 2015; Ono et al. 2015). Muscle oxidative capacity is an important factor determining exercise capacity (Adams and Schuler 2011). It is critically regulated by mitochondrial function represented by adenosine triphosphate synthesis through the tricarboxylic acid cycle. Muscle oxidative capacity depends on mitochondrial enzymatic activity and biogenesis (Short et al. 2003; White and Schenk 2012), both of which are decreased by hyperglycemia in diabetes (Patti et al. 2003; Boushel et al. 2007; Fujimaki and Kuwabara 2017; Wang et al. 2018), leading to the decrement of exercise capacity. Therefore, attenuation of hyperglycemia-induced impairment of muscle oxidative capacity is important to maintain exercise capacity.

Peroxisome proliferator-activated receptor-γ co-activator-1α (PGC-1α) is known as a

master regulator of oxidative capacity in the skeletal muscle (Wende et al. 2005; Calvo et al. 2008; Wenz et al. 2009; Tadaishi et al. 2011), and regulates mitochondrial enzymatic activity and biogenesis (Ventura-Clapier et al. 2008). Indeed, in a previous study, PGC-1α transgenic mice showed an increase in muscle oxidative capacity (Lin et al. 2002) In addition, endurance exercise induced an increase in muscle oxidative capacity via an increase in PGC-1α expression (Russell et al. 2003; Geng et al. 2010). These reports strongly suggest that PGC-1α plays a key role in enhancing muscle oxidative capacity. On the other hand, a decrease in PGC-1α expression has been shown to lower muscle oxidative capacity. (Leone et al. 2005; Vainshtein et al. 2015). It has been reported that low muscle oxidative capacity in diabetes is associated with decreased PGC-1α expression (Nagatomo et al. 2011 2011; Wang et al. 2018). Therefore, it would be beneficial to attenuate the decrease in PGC-1α expression in order to suppress the decline of muscle oxidative capacity due to hyperglycemia.

Physical exercise is a principal method to improve low muscle oxidative capacity in diabetes (Lumb 2014). However, it is physically difficult for some diabetic patients due to

their complications and exercise intolerance. Therefore, it is necessary to develop an alternative treatment, which is effective even for diabetic patients with exercise intolerance. Carbon dioxide (CO_2) therapy has long been used in Europe as an effective treatment for cardiac disease and skin lesions (Riggs 1960; Goodman et al. 1975; Wells 1999). Exposure to CO_2 elevates blood flow and microcirculation in many tissues as well as partially increases O_2 pressure in the local tissues, a phenomenon known as the Bohr effect (Riggs 1960; Wells 1999; Jensen 2004; Izumi et al. 2015). Also, it is well known that CO_2 therapy induces peripheral vasodilation, thereby increasing tissue blood flow (Hartmann et al. 1997; Sakai et al. 2011). The transfer of CO_2 across the skin might have beneficial local vasomotor effects without causing systemic hemodynamic modifications (Savin et al. 1995). In addition, the effects of CO_2-enriched water on subcutaneous microcirculation are regulated by peripheral vasodilation, which results from increased parasympathetic and decreased sympathetic nerve activity (Toriyama et al. 2002). Together, these reports indicate that CO_2 therapy has a positive impact on microcirculation. A blood flow-induced mechanical factor enhances the

expression level of endothelial nitric oxide synthesis (eNOS) in vascular endothelial cells (Harrison et al. 1996; Fleming and Busse 2003). eNOS is one of three NOS isozymes, which plays a major role in many physiological functions, such as regulating vascular tone (Huang et al. 1995; Duplain et al. 2001) and insulin sensitivity (Vincent et al. 2003). Additionally, nitric oxide synthesized by eNOS can increase PGC-1α protein expression in skeletal muscle via activation of cyclic guanosine monophosphate (cGMP) and consequently promote mitochondrial biogenesis and function (Le Gouill et al. 2007; Ventura-Clapier et al. 2008; Nisoli et al. 2004, 2003; Lira et al. 2010). On the other hand, it has been reported that application of CO_2 therapy up-regulates eNOS and cGMP expression in skeletal muscle via an increase in blood flow (Irie et al. 2005; Izumi et al. 2015). Moreover, the expression of positive regulators of oxidative capacity, including PGC-1α and sirtuin1 (SIRT1) is enhanced by transcutaneous application of CO_2 therapy (Oe et al. 2011). These results raise the possibility that transcutaneous CO_2 might enhance PGC-1α expression via increase in blood flow-induced eNOS signaling. Therefore, we hypothesized that application of transcutaneous

CO_2 therapy attenuates the impaired muscle oxidative capacity in diabetes via up-regulation of eNOS and PGC-1α signaling. In the present study, we investigated the effect of CO_2 therapy on muscle oxidative enzymatic activity and protein expression of eNOS, PGC-1α, and cytochrome c oxidase subunit 4 (COX-4) using type 1 diabetes rodent model generated by a single injection of streptozotocin (STZ), a compound that displays a preferential toxicity toward pancreatic β-cells.

Materials and Methods

Animals

Eight-week-old male Wistar rats (Japan SLC, Shizuoka, Japan) were used. These animals were randomly divided into 4 groups: control (CON/CO_2 (–); n = 5), CO_2 treatment (CON/CO_2 (+); n = 5), STZ-induced diabetes (STZ/CO_2 (–); n = 5), and STZ-induced diabetes treated with CO_2 (STZ/CO_2 (+); n = 5). All animals were housed at a temperature of 22 ± 2 °C with 12/12 h light/dark cycle and provided standard rodent chow and water *ad libitum*. Diabetes was induced by a single intravenous injection of 50 mg/kg STZ (Wako, Osaka, Japan) dissolved in citrate buffer. The blood glucose levels were measured 2 days after injection, and animals with blood glucose levels more than 250mg/dL were used as a model for diabetes. Rats in both the STZ groups were injected with STZ, and the rats in both CON groups were injected with the same volume of citrate buffer. This study was approved by the Institutional Animal Care and Use Committee and carried out according to the Kobe University Animal Experimentation Regulations. All experiments were conducted in

accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Transcutaneous CO_2 therapy

All animals were anesthetized with isoflurane (Wako, Osaka, Japan), and the hair on their hind limbs were shaved. CO_2 hydrogel, which enhances transcutaneous CO_2 absorption (NeoChemir Inc Kobe, Japan) as previously described (Oe et al. 2011), was applied on their hind limbs without anesthesia. The CO_2 adaptor was attached to the limbs and sealed. In the CON/CO_2 (+) and STZ/CO_2 (+) groups, 100% CO_2 gas (Mizushima Sanso, Kobe, Japan) was administered into the adaptor for 30 min, as previously described (Oe et al. 2011). This treatment was started from 5 days after injection of STZ and performed 5 times a week for 8 weeks.

Fasting blood glucose

After a fasting period of 12 h, the blood samples were obtained from the caudal vein. The blood glucose levels were measured using a portable blood glucose analyzer (Glutest Neo Super; Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, Japan) and monitored every 2 weeks.

Surgical procedure

After 8 weeks, rats were anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*). The soleus muscle was removed and weighed, and then the muscle tissue was rapidly frozen using isopentane cooled in dry ice and stored at –80 °C until further biochemical analysis.

Citrate synthase (CS) activity

The activity of CS, a key mitochondrial enzyme in the tricarboxylic acid cycle, is used as an indicator of oxidative capacity of the skeletal muscle. The sample was homogenized in 10 mM Tris (pH 7.4), 175 mM KCl, and 2 mM EDTA. The homogenates were frozen, thawed thrice, and then centrifuged at 15,000 *g* for 10 min at 4 °C. The supernatants were collected

and used for measuring the CS activity by Srere's method (Srere 1969). Briefly, supernatants were reacted with 5 mM oxaloacetate acid after addition of 100 mM Tris (pH 7.4), 3 mM acetyl-CoA, and 1 mM 5,5' -dithiobis [2-nitrobenzoric acid], and the absorbance was measured at 412 nm for 5 min.

Western blotting

Portions (approximately 10 mg) of each soleus muscle were homogenized in RIPA lysis buffer containing 1 mM Na_3VO_4, 1 mM NaF, and protease inhibitor cocktail (1:100, P8340; Sigma Chemicals, Perth, WA, USA). Total supernatant protein concentrations were determined according to Bradford method using a protein assay kit (Bradford 1976) (Bio-Rad Laboratories, Hercules, CA, USA) before loading onto either 7.5 or 15% sodium dodecyl sulfate-polyacrylamide gels. Proteins were blotted onto polyvinylidene difluoride membranes, which were then blocked for 1 h with 5% skimmed milk in phosphate-buffered saline with 0.1% Tween 20 (PBST). Membranes were incubated with antibodies against PGC-1α (1:200

in PBST, sc-13067; Santa Cruz Biotechnology, Santa Cruz, CA, USA), COX-4 (1:1000 in PBST, #4850; Cell Signaling Technology), or eNOS (1:1000 in PBST, #5880; Cell Signaling Technology) overnight at 4 °C and then incubated in a solution with horseradish peroxidase-conjugated anti-mouse or rabbit secondary antibody (1:1000 in PBST; GE, Healthcare, Waukesha, WI, USA) for 1 h. Proteins were detected using EzWestLumi Plus kit (ATTO, Tokyo, Japan). Finally, images were analyzed with an LAS-1000 (Fujifilm, Tokyo, Japan) using a chemiluminescent image analyzer and quantified using the Multi-Gauge Image Analysis Software program (Fujifilm) against a relative concentration of GAPDH (1:1000 in PBST, #97166; Cell Signaling Technology) as an internal control.

Statistical analysis

All data are presented as mean ± standard error of mean (SEM). The differences were assessed by two-way analysis of variance (ANOVA) followed by Tukey's post hoc test. All data of time-dependent changes of blood glucose levels were assessed by two-way repeated

measured ANOVA followed by Tukey's post hoc test. Results were deemed statistically significant at $p < 0.05$.

Results

Body mass and soleus muscle mass

There was no significant difference in body mass and muscle mass between the STZ/CO_2 (–) and STZ/CO_2 (+), and the CON/CO_2 (–) and CON/CO_2 (+) groups, respectively. The mean body mass and soleus muscle mass were significantly decreased due to induction of hyperglycemia (8 weeks) (Table 1).

Fasting blood glucose

Figure 1 shows the time-dependent change of fasting blood glucose levels for 8 weeks. There was no significant difference in blood glucose levels between the CON/CO_2 (–) and CON/CO_2 (+) groups. The blood glucose levels were significantly higher in both STZ groups compared to those in both CON groups, and lower in STZ/CO_2 (+) group compared to those in STZ/CO_2 (–) group at a point in 4, 6, and 8 weeks after the start of the experiment.

CS activity

There was no significant difference in CS activity between both the CON groups (Figure 2). CS activity was significantly lower in STZ/CO_2 (–) group than that in CON/CO_2 (–) group, and higher in STZ/CO_2 (+) group than that in STZ/CO_2 (–) group.

Protein expression levels of PGC-1α, COX-4, and eNOS

Representative images of western blots for PGC-1α, COX-4, and eNOS expression in the soleus muscle are shown in Figure 3. There were no significant differences in the protein content of PGC-1α, COX-4, eNOS between both the CON groups. The protein level of eNOS was significantly higher in the STZ/CO_2 (+) group than that in the STZ/CO_2 (–) group. The protein levels of PGC-1α and COX-4 were significantly lower in the STZ/CO_2 (–) group than those in the CON/CO_2 (–) group, but significantly higher in the STZ/CO_2 (+) group than those in the STZ/CO_2 (–) group.

Discussion

The novel finding of the present study was that application of transcutaneous CO_2 therapy attenuated the decrease in CS activity in the skeletal muscle of rats with STZ-induced hyperglycemia. Furthermore, the protein expression levels of eNOS, PGC-1, and COX-4 were higher in the STZ/CO_2 (+) group compared with those in the STZ/CO_2 (–) group. These observations indicated that application of transcutaneous CO_2 to rats with STZ-induced diabetes improved the impaired muscle oxidative capacity via enhancement of eNOS and PGC-1α-related signaling in hyperglycemic skeletal muscle.

Many studies have reported that PGC-1α is an important regulator of oxidative capacity in skeletal muscle (Zechner et al. 2010; Tadaishi et al. 2011; Kang et al. 2012). In the present study, the activity of CS, an indicator of oxidative capacity, and expression of COX-4, an enzyme of the mitochondrial respiratory chain, in the skeletal muscle were decreased in rats with STZ-induced hyperglycemia (Figure 2), which is consistent with previous reports (Py et al. 2002; Roberts-Wilson et al. 2010; Padrão et al. 2012, Wang et al. 2018). Additionally, the

expression level of PGC-1α in the STZ/CO_2 (–) group was significantly decreased compared with that in the CON/CO_2 (–) group (Figure 3). Thus, the hyperglycemia-related decline in skeletal muscle oxidative capacity could be due to the down-regulation of PGC-1α.

It has been reported that shear stress associated with an increase in blood flow increases the expression level of eNOS (Yang et al. 2013), which can also be achieved by administration of α_1-adrenergic receptor antagonist prazosin, an inducer of vasodilation (Baum et al. 2004), and exercise (Lloyd et al. 2001; Vassilakopoulos et al. 2003; Egginton 2009; Lee-Young et al. 2010). These reports suggest that blood flow appears to be a strong modulator of eNOS levels. On the other hand, Izumi et al. (2015) showed that CO_2 therapy promotes blood flow in the subcutaneous tissues, and up-regulates the expression of eNOS in the hind limb of ischemic rats. Kindig et al. (1998) showed that STZ-induced hyperglycemia in rat results in a decrease in the proportion of capillaries in the skeletal muscle, due to which the blood flow within the skeletal muscle may be impaired. In the present study, an increase in the expression level of eNOS was observed in the STZ/CO_2 (+) group, but not in the

CON/CO_2 (+) group. Our results, combined with previous findings, suggest the possibility that CO_2 therapy might influence eNOS expression only under conditions of reduced blood flow. Therefore, the increased eNOS expression in the STZ/CO_2 (+) group might be associated with enhanced blood flow within the skeletal muscle, consistent with a previous report showing the positive effect of CO_2 therapy in a hind limb ischemia model.

eNOS is a key factor for the enhancement of muscle oxidative capacity via up-regulation of PGC-1α expression. In a previous study, application of CO_2 to a hind limb ischemia model enhanced eNOS expression in the skeletal muscle (Irie et al. 2005; Izumi et al. 2015). Additionally, application of transcutaneous CO_2 to sedentary rats for 12 weeks increased the mRNA level of PGC-1α and SIRT1 and mitochondria number (Oe et al. 2011). Our results showed an increase in the protein expression of PGC1α as well as eNOS by application of transcutaneous CO_2 therapy to rats with STZ-induced hyperglycemia. On the other hand, application of transcutaneous CO_2 had no influence on the protein expression levels of PGC-1α and eNOS in the CON/CO_2 (+) group. This result suggested that the

increase in expression of PGC-1α in the STZ/CO_2 (+) group was mediated by increased blood flow and resultant up-regulation of eNOS. Therefore, the effects of transcutaneous CO_2 on muscle oxidative capacity in hyperglycemic rats could be involved in the up-regulation of PGC-1α thorough an increase in eNOS expression.

In the present study, application of transcutaneous CO_2 decreased the fasting blood glucose levels in rats with STZ-induced hyperglycemia. It has been reported that an increase in PGC-1α expression improves impaired glucose metabolism (Puigserver 2005). Here, the expression level of PGC-1α was increased in the STZ/CO_2 (+) group compared to that in the STZ/CO_2 (–) group. Hence, our results suggest that application of transcutaneous CO_2 can improve hyperglycemia via increase of glucose metabolism mediated by increased PGC-1α expression.

In conclusion, this study demonstrates a novel effect of transcutaneous CO_2 on the impaired muscle oxidative capacity of rats with STZ-induced hyperglycemia. Application of transcutaneous CO_2 improved hyperglycemia-related decline in muscle oxidative capacity, as

shown by an increase in CS activity and increased expression levels of COX4 and PGC-1α, which contributed to the amelioration of hyperglycemia. These results indicate that transcutaneous CO_2 therapy can be used to improve hyperglycemia-induced muscle metabolic dysfunction.

Acknowledgment

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

References

Adams V, Anker SD, Schuler G (2011): Muscle metabolism and exercise capacity in cachexia. Curr. Pharm. Des. **35**, 3838–3845

Baum O, Da Silva-Azevedo L, Willerding G, Wöckel A, Planitzer G, Gossrau R, Pries AR, Zakrzewicz A (2004): Endothelial NOS is main mediator for shear stress-dependent angiogenesis in skeletal muscle after prazosin administration. Am. J. Physiol. Heart Circ. Physiol. **287**, H2300–H2308

Blake R, Trounce IA (2014): Mitochondrial dysfunction and complications associated with diabetes. Biochim. Biophys. Acta. **1840**, 1404–1412

Boushel R, Gnaiger E, Schjerling P, Skovbro M, Kraunsøe R, Dela F (2007): Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. Diabetologia **50**, 790–796

Bradford MM (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**,

248–254

Calvo JA, Daniels TG, Wang X, Paul A, Lin J, Spiegelman BM, Stevenson SC, Rangwala SM (2008): Muscle-specific expression of PPARgamma coactivator-1alpha improves exercise performance and increases peak oxygen uptake. J. Appl. Physiol. **104**, 1304–1312

Duplain H, Burcelin R, Sartori C, Cook S, Egli M, Lepori M, Vollenweider P, Pedrazzini T, Nicod P, Thorens B, Scherrer U (2001): Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. Circulation **104**, 342–345

Egginton S (2009): Invited review: activity-induced angiogenesis. Pflugers Arch. **457**, 963–977

Fleming I, Busse R (2003): Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. Am. J. Physiol. Integr. Comp. Physiol. **284**, R1–R12

Fortes MA, Pinheiro, CH, Guimarães-Ferreira L, Vitzel KF, Vasconcelos DA, Curi R (2015): Overload-induced skeletal muscle hypertrophy is not impaired in STZ-diabetic rats.

Physiol. Rep. **3**, e12457

Frier BC, Noble EG, Locke M (2008): Diabetes-induced atrophy is associated with a muscle-specific alteration in NF-κB activation and expression. Cell Stress Chaperones **13**, 287–296

Fujimaki S, Kuwabara T (2017): Diabetes-induced dysfunction of mitochondria and stem cells in skeletal muscle and the nervous system. Int. J. Mol. Sci. **18**

Geng T, Li P, Okutsu M, Yin X, Kwek J, Zhang M, Yan Z (2010): PGC-1α plays a functional role in exercise-induced mitochondrial biogenesis and angiogenesis but not fiber-type transformation in mouse skeletal muscle. Am. J. Physiol. Cell Physiol. **298**, C572–C579

Goodman M, Moore GW, Matsuda G (1975): Darwinian evolution in the genealogy of haemoglobin. Nature **253**, 603–608

Harrison DG, Sayegh H, Ohara Y, Inoue N, Venema RC (1996): Regulation of expression of the endothelial cell nitric oxide synthase. Clin. Exp. Pharmacol. Physiol. **23**, 251–255

Hartmann BR, Bassenge E, Pittler M (1997): Effect of carbon dioxide-enriched water and

fresh water on the cutaneous microcirculation and oxygen tension in the skin of the foot. Angiology **48**, 337–343

Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC (1995): Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature **377**, 239–242

Irie H, Tatsumi T, Takamiya M, Zen K, Takahashi T, Azuma A, Tateishi K, Nomura T, Hayashi H, Nakajima N, Okigaki M, Matsubara H (2005): Carbon dioxide-rich water bathing enhances collateral blood flow in ischemic hindlimb via mobilization of endothelial progenitor cells and activation of NO-cGMP system. Circulation **111**, 1523–1529

Izumi Y, Yamaguchi T, Yamazaki T, Yamashita N, Nakamura Y, Shiota M, Tanaka M, Sano S, Osada-Oka M, Shimada K, Wanibuchi H, Miura K, Yoshiyama M, Iwao H (2015): Percutaneous carbon dioxide treatment using a gas mist generator enhances the collateral blood flow in the ischemic hindlimb. J. Atheroscler. Thromb. **22**, 38–51

Jensen FB (2004): Red blood cell pH, the Bohr effect, and other oxygenation-linked phenomena in blood O_2 and CO_2 transport. Acta Physiol. Scand. **182**, 215–227

Kang C, Li Ji L (2012): Role of PGC-1α signaling in skeletal muscle health and disease. Ann. N. Y. Acad. Sci. **1271**, 110–117

Kindig CA, Sexton WL, Fedde MR, Poole DC (1998): Skeletal muscle microcirculatory structure and hemodynamics in diabetes. Respir. Physiol. **111**, 163–175

Lee-Young RS, Ayala JE, Hunley CF, James FD, Bracy DP, Kang L, Wasserman DH (2010): Endothelial nitric oxide synthase is central to skeletal muscle metabolic regulation and enzymatic signaling during exercise in vivo. Am. J. Physiol. Regul. Integr. Comp. Physiol. **298**, R1399–R1408

Le Gouill E, Jimenez M, Binnert C, Jayet PY, Thalmann S, Nicod P, Scherrer U, Vollenweider P (2007): Endothelial nitric oxide synthase (eNOS) knockout mice have defective mitochondrial beta-oxidation. Diabetes **56**, 2690–2696

Leone TC, Lehman JJ, Finck BN, Schaeffer PJ, Wende AR, Boudina S, Courtois M, Wozniak

DF, Sambandam N, Bernal-Mizrachi C, Chen Z, Holloszy JO, Medeiros DM, Schmidt RE, Saffitz JE, Abel ED, Semenkovich CF, Kelly DP (2005): PGC-1α deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. PLoS Biol. **3**, e101

Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, Spiegelman BM (2002): Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. Nature **418**, 797–801

Lira VA, Brown DL, Lira AK, Kavazis AN, Soltow QA, Zeanah EH, Criswell DS (2010): Nitric oxide and AMPK cooperatively regulate PGC-1α in skeletal muscle cells. J. Physiol. **588**, 3551–3566

Lloyd PG, Yang HT, Terjung RL (2001): Arteriogenesis and angiogenesis in rat ischemic hindlimb: role of nitric oxide. Am. J. Physiol. Heart Circ. Physiol. **281**, H2528–H2538

Lumb A (2014): Diabetes and Exercise. Clin. Med (Lond). **14**, 673–676

Nagatomo F, Fujino H, Kondo H, Gu N, Takeda I, Ishioka N, Tsuda K, Ishihara A (2011):

PGC-1α mRNA level and oxidative capacity of the plantaris muscle in rats with metabolic syndrome, hypertension, and type 2 diabetes. Acta Histochem. Cytochem. **44**, 73–80

Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C, Bracale R, Valerio A, Francolini M, Moncada S, Carruba MO (2003): Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. Science **299**, 896–899

Nisoli E, Falcone S, Tonello C, Cozzi V, Palomba L, Fiorani M, Pisconti A, Brunelli S, Cardile A, Francolini M, Cantoni O, Carruba MO, Moncada S, Clementi E (2004): Mitochondrial biogenesis by NO yields functionally active mitochondria in mammals. Proc. Natl. Acad. Sci. U. S. A. **101**, 16507–16512.

Oe K, Ueha T, Sakai Y, Niikura T, Lee SY, Koh A, Hasegawa T, Tanaka M, Miwa M, Kurosaka M (2011): The effect of transcutaneous application of carbon dioxide (CO_2) on skeletal muscle. Biochem. Biophys. Res. Commun. **407**, 148–152.

Ono T, Takada S, Kinugawa S, Tsutsui H (2015): Curcumin ameliorates skeletal muscle

atrophy in type 1 diabetic mice by inhibiting protein ubiquitination. Exp. Physiol. **100**, 1052–1063

Padrão AI, Carvalho T, Vitorino R, Alves RM, Caseiro A, Duarte JA, Ferreira R, Amado F (2012): Impaired protein quality control system underlies mitochondrial dysfunction in skeletal muscle of streptozotocin-induced diabetic rats. Biochim. Biophys. Acta. **1822**, 1189–1197

Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, Landaker EJ, Goldfine AB, Mun E, DeFronzo R, Finlayson J, Kahn CR, Mandarino LJ (2003): Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proc. Natl. Acad. Sci. U.S.A. **100**, 8466–8471

Puigserver P (2005): Tissue-specific regulation of metabolic pathways through the transcriptional coactivator PGC1-α. Int. J. Obes (Lond). **29**, S5–S9

Py G, Lambert K, Milhavet O, Eydoux N, Préfaut C, Mercier J (2002): Effects of

streptozotocin-induced diabetes on markers of skeletal muscle metabolism and monocarboxylate transporter 1 to monocarboxylate transporter 4 transporters. Metabolism. **51**, 807–813

Riggs A (1960): The nature and significance of the Bohr effect in mammalian hemoglobins. J. Gen. Physiol. **43**, 737–752

Roberts-Wilson TK, Reddy RN, Bailey JL, Zheng B, Ordas R, Gooch JL, Price SR (2010): Calcineurin signaling and PGC-1α expression are suppressed during muscle atrophy due to diabetes. Biochim. Biophys. Acta. **1803**, 960–967

Russell AP, Feilchenfeldt J, Schreiber S, Praz M, Crettenand A, Gobelet C, Meier CA, Bell DR, Kralli A, Giacobino JP, Dériaz O (2003): Endurance training in humans leads to fiber type-specific increases in levels of peroxisome proliferator-activated receptor-γ coactivator-1 and peroxisome proliferator-activated receptor-α in skeletal muscle. Diabetes **52**, 2874–2881

Sakai Y, Miwa M, Oe K, Ueha T, Koh A, Niikura T, Iwakura T, Lee SY, Tanaka M,

Kurosaka M (2011): A novel system for transcutaneous application of carbon dioxide causing an "artificial Bohr effect" in the human body. PLoS One **6**, e24137

Savin E, Bailliart O, Bonnin P, Bedu M, Cheynel J, Coudert J, Martineaud JP (1995): Vasomotor effects of transcutaneous CO_2 in stage II peripheral occlusive arterial disease. Angiology **46**, 785–791

Short KR, Vittone JL, Bigelow ML, Proctor DN, Rizza RA, Coenen-Schimke JM, Nair KS (2003): Impact of aerobic exercise training on age-related changes in insulin sensitivity and muscle oxidative capacity. Diabetes **52**, 1888–1896

Srere PA (1969): Citrate synthase. Meth. Enzymol. **13**, 3–11

Tadaishi M, Miura S, Kai Y, Kano Y, Oishi Y, Ezaki O (2011): Skeletal muscle-specific expression of PGC-1α-b, an exercise-responsive isoform, increases exercise capacity and peak oxygen uptake. PLoS One **6**, e28290

Toriyama T, Kumada Y, Matsubara T, Murata A, Ogino A, Hayashi H, Nakashima H, Takahashi H, Matsuo H, Kawahara H (2002): Effect of artificial carbon dioxide foot

bathing on critical limb ischemia (Fontaine IV) in peripheral arterial disease patients. Int. Angiol. **21**, 367–373

Vainshtein A, Desjardins EM, Armani A, Sandri M, Hood DA (2015): PGC-1α modulates denervation-induced mitophagy in skeletal muscle. Skelet. Muscle **5**, 9

Vassilakopoulos T, Deckman G, Kebbewar M, Rallis G, Harfouche R, Hussain SN (2003): Regulation of nitric oxide production in limb and ventilatory muscles during chronic exercise training. Am. J. Physiol. Lung Cell Mol. Physiol. **284**, L452–L457

Ventura-Clapier R, Garnier A, Veksler V (2008): Transcriptional control of mitochondrial biogenesis: the central role of PGC-1α. Cardiovasc. Res. **79**, 208–217

Vincent MA, Barrett EJ, Lindner JR, Clark MG, Rattigan S (2003): Inhibiting NOS blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. Am. J. Physiol. Endocrinol. Metab. **285**, E123–E129

Wang D, Sun H, Song G, Yang Y, Zou X, Han P, Li S (2018): Resveratrol improves muscle atrophy by modulating mitochondrial quality control in STZ-induced diabetic mice. Mol.

Nutr. Food Res. **62**, e1700941

Wells RM (1999): Evolution of haemoglobin function: molecular adaptations to environment. Clin. Exp. Pharmacol. Physiol. **26**, 591–595

Wende AR, Huss JM, Schaeffer PJ, Gigue V, Kelly DP (2005): PGC-1α coactivates PDK4 gene expression via the orphan nuclear receptor ERRα: a mechanism for transcriptional control of muscle glucose metabolism. Mol. Cell. Biol. **25**, 10684–10694

Wenz T, Rossi SG, Rotundo RL, Spiegelman BM, Moraes CT (2009): Inceased muscle PGC-1α expression protects from sarcopenia and metabolic disease during aging. Proc. Natl. Acad. Sci. U S A. **106**, 20405–20410

White AT, Schenk S (2012): NAD^+/NADH and skeletal muscle mitochondrial adaptations to exercise. Am. J. Physiol. Endocrinol. Metab. **303**, E308–E321

Yang B, Rizzo Y (2013): Shear stress activates eNOS at the endothelial apical surface through β1 containing integrins and caveolae. Cell. Mol. Bioeng. **6**, 346–354

Zechner C, Lai L, Zechner JF, Geng T, Yan Z, Rumsey JW, Collia D, Chen Z, Wozniak DF,

Leone TC, Kelly DP (2010): Total skeletal muscle PGC-1 deficiency uncouples mitochondrial derangements from fiber type determination and insulin sensitivity. Cell Metab. **12**, 633–642

Figure legends

Figure 1. Time-dependent effects of STZ and transcutaneous CO_2 on fasting blood glucose levels (two-way repeated measured ANOVA, main effects: time, $p < 0.05$; group, $p < 0.05$; interaction, $p < 0.05$). Values are presented as mean ± SEM. *, †, and ‡ significantly different from CON, CO_2, and STZ, respectively, at $p < 0.05$.

Figure 2. CS activity in the soleus muscle (two-way ANOVA, main effects: STZ, $p < 0.05$; CO_2, n.s.; interaction, $p < 0.05$). Values are presented as mean ± SEM. * and † significantly different from CON with same intervention and CO_2 (–) vs CO_2 (+), respectively, at $p < 0.05$.

Figure 3. Mean protein expression levels of (A) eNOS (two-way ANOVA, main effects: STZ, $p < 0.05$; CO_2, n.s.; interaction, n.s.), (B) PGC-1α (two-way ANOVA, main effects: STZ, $p < 0.05$; CO_2, n.s.; interaction, n.s.), and (C) COX-4 (two-way ANOVA, main effects: STZ, n.s.; CO_2, n.s.; interaction, n.s.) in the soleus muscles of each group. The data are expressed as a

fold change (a.u.) from the value of the CON group that is set to a value of 1. The levels of protein expression were normalized to GAPDH level. Values are presented as mean ± SEM. * and † significantly different from CON with same intervention and CO_2 (–) vs CO_2 (+), respectively, at $p < 0.05$.

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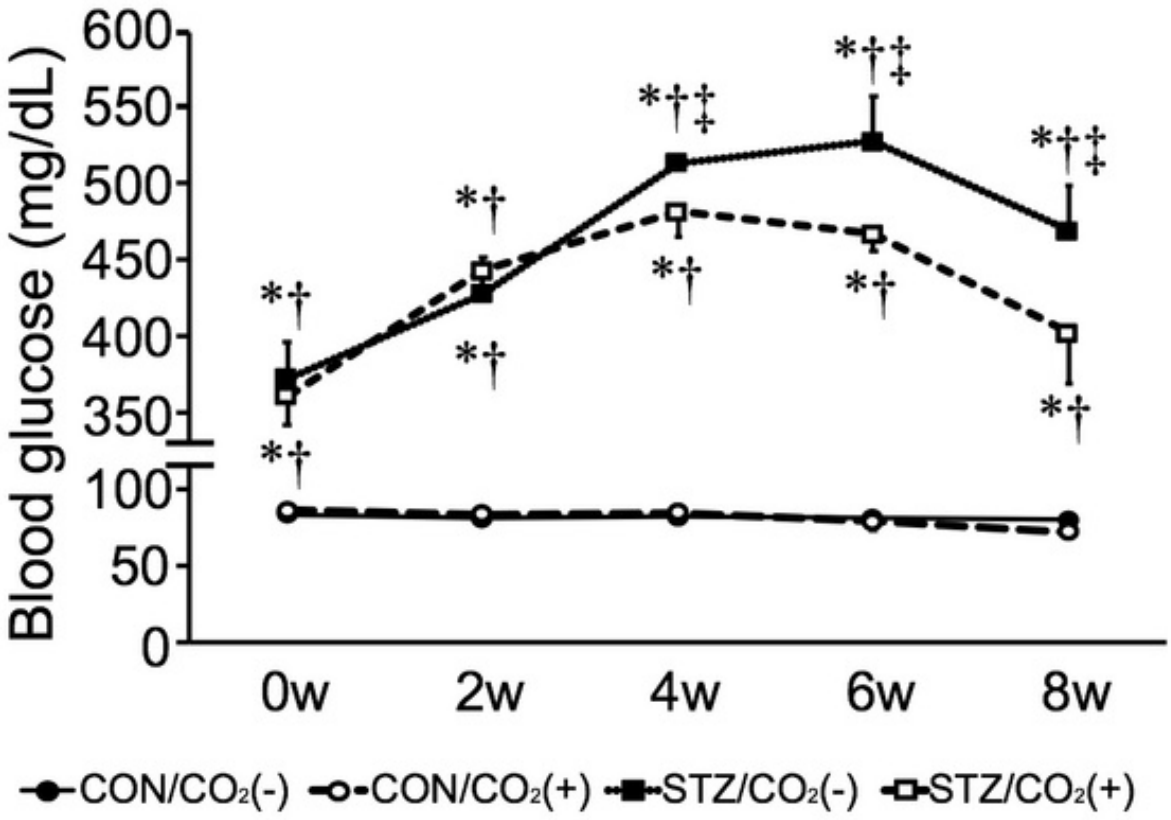

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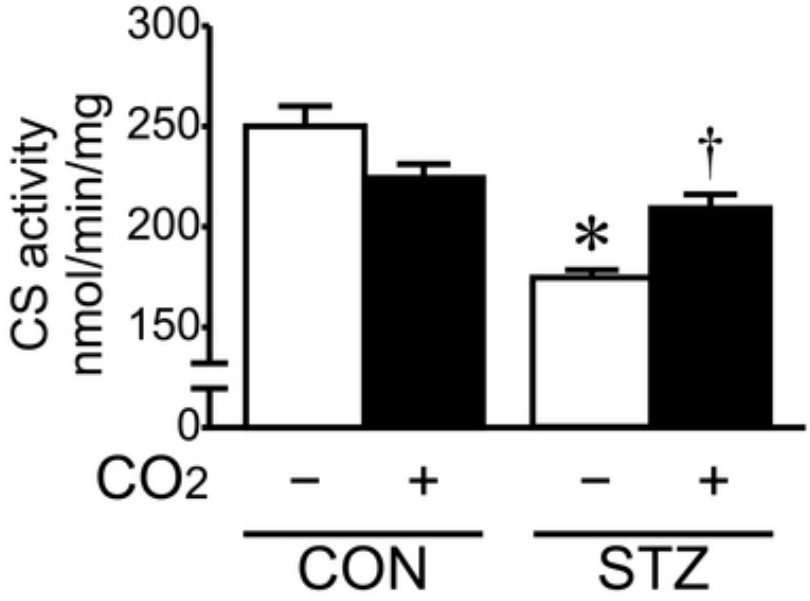

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