Title:

Effect of repeated forearm muscle cooling on the adaptation of skeletal muscle metabolism in humans

Authors:

Hitoshi Wakabayashi[1]*, Takayuki Nishimura[2], Titis Wijayanto[3], Shigeki Watanuki[4], Yutaka Tochihara[4]

1) Faculty of Engineering, Hokkaido University, Sapporo, Japan

2) Department of Public Health, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

3) Department of Mechanical and Industrial Engineering, Gadjah Mada University, Indonesia

4) Faculty of Design, Kyushu University, Fukuoka, Japan

Running head: cold adaptation in muscle metabolism

Corresponding author:

Hitoshi Wakabayashi, Ph.D.

Laboratory of Environmental Ergonomics, Faculty of Engineering, Hokkaido University

N13 W8, Kita-ku, Sapporo, Hokkaido, 060-8628, Japan

Tel&Fax: +81-11-706-6280

E-mail: wakabayashi@eng.hokudai.ac.jp

Abstract

This study aimed to investigate the effect of repeated cooling of forearm muscle on adaptation in skeletal muscle metabolism. It is hypothesized that repeated decreases of muscle temperature would increase the oxygen consumption in hypothermic skeletal muscle.

Sixteen healthy males participated in this study. Their right forearm muscles were locally cooled to 25°C by cooling pads attached to the skin. This local cooling was repeated eight times on separate days for eight participants (experimental group), whereas eight controls received no cold exposure. To evaluate adaptation in skeletal muscle metabolism, a local cooling test was conducted before and after the repeated cooling period. Change in oxy-hemoglobin content in the flexor digitorum at rest and during a 25-second isometric handgrip (10% maximal voluntary construction) was measured using near-infrared spectroscopy at every 2°C reduction in forearm muscle temperature. The arterial blood flow was occluded for 15 seconds by upper-arm cuff inflation at rest and during the isometric handgrip. The oxygen consumption in the flexor digitorum muscle was evaluated by a slope of the oxy-hemoglobin change during the arterial occlusion.

In the experimental group, resting oxygen consumption in skeletal muscle did not show any difference between pre- and post-intervention, whereas muscle oxygen consumption during the isometric handgrip was significantly higher post-intervention than pre-test from thermoneutral baseline to 31°C muscle temperature ($P<0.05$). This result indicated that repeated local muscle cooling might facilitate oxidative metabolism in the skeletal muscle.

In summary, skeletal muscle metabolism during submaximal isometric handgrip was facilitated after repeated local muscle cooling.

Key Words: hypothermic skeletal muscle; tissue oxygenation; non-shivering thermogenesis; local cold exposure; cold adaptation

Introduction

A number of researchers have studied the adaptation of non-shivering thermogenesis (NST) after repeated cold exposures in humans (Vybiral et al. 2000; van Marken Lichtenbelt and Daanen 2003; van Marken Lichtenbelt and Schrauwen 2011; van der Lans et al. 2013; Yoneshiro et al. 2013; Nishimura et al. 2015). Recently, brown adipose tissue (BAT) have been focused on as one of the major components of energy expenditure, since cold-induced activity of BAT in adult humans had been identified in studies using PET/CT scanning (Saito et al. 2009; van Marken Lichtenbelt et al. 2009). On the other hand, it is necessary to study the skeletal muscle's contribution to the adaptation of cold induced thermogenesis, concerning their large volume and wide distribution to human body.

A recent study reported increases of human BAT activity and NST after 10 consecutive days of mild cold exposure to 15-16°C in air for six hours a day, whereas no significant change was observed in skeletal muscle mitochondrial uncoupling in vitro (van der Lans et al. 2013). However, in the case of acute response to cold, a significant positive relationship has been reported previously between the increase of total daily energy expenditure during mild cold exposure (16°C in air for 48 hours) and the increase of mitochondrial uncoupling (state 4 respiration) of isolated human skeletal muscle biopsies taken after the cold exposure (Wijers et al. 2008; Wijers et al. 2011). Furthermore, recent studies reported an increase of skeletal muscle oxidative capacity in UCP1 knockout mice or BAT partial ablation mice after repeated cold exposure (Meyer et al. 2010; Mineo et al. 2012), that indicated the contribution of skeletal muscle to the increase of NST after chronic cold exposure. However, as was mentioned above, it was reported that there was no significant change in human skeletal muscle mitochondrial uncoupling after repeated mild cold exposure (van der Lans et al. 2013). Concerning these studies, it is suggested that mild cold exposure (defined here as cold stimulus which only decreases skin temperature and induces NST) would activate mitochondrial uncoupling in the skeletal muscle, but it is not cold enough to induce a significant increase in UCP activation after repeated

exposure to mild cold. The necessary condition for inducing metabolic adaptation in the skeletal muscle might be a repetition of severe enough cold stimulus which would decrease deep body temperature and/or initiate shivering. However, Cannon and Nedergaard (2004) insisted that the increase of muscle oxidative capacity observed after repeated cold exposure was a by-product of muscle training induced by repeated shivering. Thus, in this study, repeated local severe cold exposure was used as a thermal adaptation impulse for potentially inducing metabolic adaptation in the skeletal muscle. This method enables repetition of a strong cold stimulus locally in skeletal muscle without shivering. Increased muscle capillary density after repeated cold exposure was reported in rats which were exposed to 5°C in air for four weeks (Suzuki et al. 1997) and in human free-divers who routinely have been immersed into cold water for more than 20 years (Bae et al. 2003). This might be one potential mechanism to increase muscle oxidative capacity following repeated cold exposure. It is probably because of a decline of muscle temperature, which reduces enzyme activity as a result of the Q10 effect. To compensate for the reduction of the enzyme activity in each muscle fiber, more muscle fibers would be recruited and/or capillary density might be increased to satisfy an energy (oxygen) demand. Thus, reduction of muscle temperature might be an essential qualification to increase oxidative capacity after repeated cold exposure.

This study aimed to investigate the effect of a repeated severe local cold exposure (reduction of forearm muscle temperature) on metabolic adaptation of hypothermic skeletal muscle. Because the energy demand of resting muscle seems too small to be affected by the suppression of metabolism due to local cooling, submaximal isometric handgrip exercise conditioning was tested pre- and post-intervention. It is hypothesized that a repeated decrease of local skeletal muscle temperature will increase muscle-based thermogenesis in humans.

Methods

Participants

Sixteen healthy males participated in this study. They were all right handed and divided into two groups (experimental cold adaptation and control group) that had no statistical group difference in the following parameters; height, weight, body mass index, right forearm maximal girth, skin fold thickness and handgrip strength during maximal voluntary contraction (MVC). Skinfold thickness was measured at seven sites (forearm, upper arm, subscapular, abdomen, iliac spine, thigh and calf) by one experienced examiner using a skinfold caliper (Eiyoken-type; Meikosha Co. Ltd., Japan). Physical characteristics measured pre- and post-intervention are shown in Table 1. All experimental protocols in this study were designed according to the principle of the Helsinki Declaration and approved by the Institutional Review Board of Kyushu University. All participants were informed of the experimental procedures and gave their written informed consent before participation.

Procedures

The experimental cold adaptation group repeated forearm muscle cooling eight times on separate days in three weeks, while no cold exposure was conducted in the control group. Their right forearm was locally cooled by using cooling pads attached to the skin until their forearm muscle temperature fell to 25°C. Whole surface of the right forearm between the wrist and elbow was wrapped by several water perfusion cooling pads, in which 5°C water was circulated using a thermostatic bath system (LTB-400, ASONE, Japan). On average, about 70 minute was required to cool forearm muscle to 25°C. The forearm muscle temperature was measured by a deep body temperature monitor (CM-210, TERUMO, Japan) which detects the tissue temperature 5-10 mm below the skin surface using the zero heat flow method (Yamakage and Namiki 2003). This monitor measures skin surface temperature beneath a thermal insulating pad containing a heater, which equilibrates the skin temperature with the deep tissue temperature when heat flow from the skin is maintained to zero.

To evaluate adaptation in metabolism in the skeletal muscle, both groups undertook two experimental forearm cooling tests, three weeks apart (Pre and Post), until their right forearm muscle temperature fell to 25°C. Before the first local cooling test, all participants practiced the protocol including submaximal (10% of predetermined MVC) isometric handgrip exercise using a handgrip dynamometer (T.K.K.5710b, Takei Scientific Instruments, Japan). On the day of the forearm cooling test, participants came to the laboratory at least one hour prior to starting the protocol. After changing into shorts and T-shirts, they rested on a chair in a climate chamber controlled at 24°C and 50% relative humidity, and sensors were attached to them. During the forearm cooling test they sat on a chair and placed their right arm on a table holding the hand grip dynamometer fixed on the table. At the beginning of the test, normothermic baselines of measurement items described below at rest and during submaximal isometric contraction (10%MVC) were measured before starting forearm cooling. After the measurement of normothermic baseline, their right forearms were locally cooled by the water perfusion cooling pads attached to the skin, as the same manner of the repeated forearm cooling described above. During the cooling protocol, participants repeated 25-second isometric handgrip (10% MVC) for every 2°C reduction in the forearm muscle temperature (around 35, 33, 31, 29, 27 and 25°C).

Measurements

Changes in muscle hemodynamics (changes in oxy- and deoxy-hemoglobin content) were continuously measured by spatially resolved near-infrared spectroscopy (NIRS) (NIRO-200, Hamamatsu Photonics, Japan) every 0.5 seconds. The optodes of the NIRS, with a 3 cm distance between an illuminant and a detector, were housed in an optically dense black vinyl holder to ensure the position and were attached on the skin above the flexor digitorum (around 5-10 cm distal from the cubital fossa) using double-sided adhesive tape. A fixed path length (10.6 cm) was used in this study

as suggested by the manufacturer. The resting oxygen consumption in normothermic forearm muscle was evaluated by the slope of the concentration change of oxy-hemoglobin (O_2Hb) during 15-second arterial occlusion using an upper-arm cuff inflated to 220 mmHg with a rapid cuff inflator system (E20 and AG101, Hokanson, USA). The calculation of tissue oxygen consumption (tissue$\dot{V}O_2$) was based on previous studies (van Beekvelt et al. 2001; van Beekvelt et al. 2002). The rate of decrease in concentration of O_2Hb (-d(O_2Hb)/dt) was manually detected using a 5-second stable linear slope of O_2Hb around 5-10 seconds after occlusion. Then, the unit of -d(O_2Hb)/dt [μmol Hb/L/sec] was converted into milliliters O_2 per 100 gram tissue per minute [mLO_2/100g/min] taking into account that each Hb molecule binds four O_2 molecules, the molar gas volume in STPD is 22.4 L and muscle density is 1.04 kg/L, using the following equation:

$$\text{Tissue}\dot{V}O_2 = -d(O_2Hb)/dt \cdot 4 \cdot 22.4/1000 /1.04/10 \cdot 60 \ [mLO_2/100g/min]$$

Tissue$\dot{V}O_2$ of the normothermic forearm during isometric handgrip (10% MVC) was also evaluated by a similar technique. Participants were asked to keep constant handgrip strength for 25 seconds with arterial occlusion for the latter 15 seconds. After starting forearm cooling, tissue$\dot{V}O_2$ of the right forearm at rest and during isometric handgrip (10% MVC) was evaluated for every 2°C reduction in the forearm muscle temperature using the same technique as for the normothermic baseline.

Skin temperature on the right forearm ($T_{forearm}$) was measured by thermistor sensors and a data logger (LT-8A, Gram Corporation, Japan) every two seconds. Skin blood flow (SkBF) in the forearm skin above the flexor digitorum was measured by laser Doppler flowmetry (FLO-C1, OMEGAWAVE, Japan). The right forearm skin thermistor and a laser Doppler probe were attached on the skin around 10 mm distal site from the NIRS probe. The SkBF data were sampled using an A/D converter (Powerlab/16SP, AD Instruments, Australia) and recorded at 0.5 second intervals using a personal computer. Since the laser Doppler flow signal does not provide an absolute measurement of blood flow,

the voltage output of the laser Doppler measurement was normalized (%SkBF) relative to maximal levels (100%), which were measured during reactive hyperemia after right arm arterial occlusion, and the minimum value (0%) during the occlusion conducted before starting cooling. To minimize the artifact due to the movement of the laser Doppler probe, participants were asked to keep their right arm as stable as possible on a fixed table. The 15-second averaged %SkBF and skin temperature just before the arterial occlusion for evaluating resting muscle oxygenation for every 2°C reduction in forearm muscle temperature was analyzed later.

Statistics

Data representative for baseline prior to cooling and each 2°C reduction in the forearm muscle temperature (tissue$\dot{V}O_2$, $T_{forearm}$ and %SkBF) were analyzed by a repeated measures two-way (muscle temperature × pre-post intervention) analysis of variance (ANOVA). This analysis was conducted separately for each control and experimental cold adaptation group. After determining the main effects, pair-wise post-hoc tests were conducted between pre- and post-intervention tests at indicated muscle temperatures, and among muscle temperatures within pre- and post-test groups. Additionally, pre-intervention group differences were tested by two-way (muscle temperature × group) ANOVA followed by Student's *t*-tests between groups at each muscle temperature, using the measured pre-test data. Significant differences were established at $P<0.05$. All data are presented as mean values and standard error (SE).

Results

Physical characteristics

No difference was observed in any physical characteristics between groups or in pre- and post-intervention forearm cooling within a group (Table 1).

Control group

Based on the data measured in the pre-intervention forearm cooling test, there was no statistical difference between the control and experimental groups in any variables (tissue $\dot{V}O_2$, $T_{forearm}$ and %SkBF) at each muscle temperature. The control group did not show significant differences in any variables at each muscle temperature between pre and post forearm cooling tests (Fig. 1a-4a).

Tissue oxygen consumption in the experimental group

The resting forearm tissue$\dot{V}O_2$ of the experimental group at each 2°C muscle temperature reduction pre- and post-cooling is shown in Figure 1b. There is a significant main effect of muscle temperature (P=0.014) in the two-way ANOVA (Fig 1b). However, post-hoc analysis indicated no statistical difference among tissue$\dot{V}O_2$ at any muscle temperatures between pre- and post-intervention tests.

Forearm tissue$\dot{V}O_2$ during submaximal isometric handgrip in the experimental group showed a significant main effect of muscle temperature (P<0.001), pre- and post-cooling (P=0.001), and a significant interaction between those factors (P=0.022) in the two-way ANOVA (Fig 2b). Tissue$\dot{V}O_2$ during submaximal handgrip was significantly higher after local cooling than pre-test for baseline, 35, 33 and 31°C muscle temperature (P<0.05). In the pre-test, tissue$\dot{V}O_2$ during submaximal handgrip at 27 and 25°C muscle temperature was significantly lower than that at baseline (P<0.05). In the post-test, tissue$\dot{V}O_2$ during handgrip at 31 to 25°C muscle temperature was significantly lower than that at baseline (P<0.05).

Forearm skin blood flow and skin temperature

The resting %SkBF in the right forearm just before occlusion at each 2°C muscle temperature reduction pre- and post-cooling is shown in Figure 3. In the experimental group, a significant main

effect of muscle temperature ($P<0.001$) was observed in the %SkBF in the two-way ANOVA (Fig 3b). %SkBF at 35°C (when cooling just was started) to 25°C muscle temperature was significantly lower than at baseline both pre- and post-intervention ($P<0.05$, Fig 3b). No statistical difference was observed between pre- and post-intervention at each muscle temperature level.

Right forearm skin temperature was gradually decreased with the reduction of muscle temperature; a significant main effect of muscle temperature ($P<0.001$), pre- and post-intervention ($P=0.039$), and a significant interaction between those factors ($P=0.008$), was shown in the two-way ANOVA in the experimental group (Fig 4b). Forearm skin temperature tended to be higher post-test at each muscle temperature level and a significant difference was observed at 33°C ($P<0.05$, Fig 4b).

Discussion

This study investigated the effect of repeated local forearm muscle cooling on the adaptation in the skeletal muscle metabolism. The major finding is that the forearm tissue oxygen consumption during submaximal isometric handgrip was significantly greater post-intervention local cooling test than pre-test in the range of thermoneutral baseline to 31°C muscle temperature level ($P<0.05$). This result indicated that repeated forearm muscle cooling may facilitate metabolism in skeletal muscle. On the other hand, resting oxygen consumption during forearm muscle cooling was relatively constant among muscle temperature levels and no change was found between pre and post intervention. This might be because the energy demand of resting muscle was too small to be affected by the suppression of metabolism in hypothermic muscle.

In this study, the change in skeletal muscle metabolism was examined after repeated local severe cold exposure which did not induce shivering. Thus, the present results were not affected by the muscle training effect due to repeated shivering during the adaptation process, as suggested by Cannon and Nedergaard (2004). Additionally, as there were no morphological change in the girth and skinfold

238 thickness on the forearm between pre- and post-intervention (Table 1), there would be no muscular
239 hypertrophy. Thus, the increased tissue$\dot{V}O_2$ after the repeated forearm cooling intervention would
240 mainly be due to some qualitative changes in the skeletal muscle tissue.

241 It is well known that a decline of muscle temperature slows enzymatic processes as a result of the
242 Q10 effect (Barany 1967; Bennett 1985). Additionally, suppression of O_2-uploading from hemoglobin
243 in lower temperature tissue (the Bohr Effect) would restrict the supply of O_2 to the muscle fibers.
244 Moreover, O_2 supply to the muscle tissue is restricted by the lower muscle blood flow in the
245 hypothermic muscle (Abramson et al. 1958; Thorsson et al. 1985). The suppressed muscle metabolism
246 would be a factor in the impairment of physical performance in the cold (Bennett 1985; Wakabayashi
247 et al. 2015). In this study, a significantly greater tissue$\dot{V}O_2$ during submaximal isometric handgrip was
248 observed after the repeated forearm muscle cooling in the experimental group, who repeatedly
249 experienced these metabolic suppressions in their skeletal muscle.

250 We propose a potential mechanism for this adaptation. Distribution of muscle fiber type was
251 assessed in Korean diving women who routinely exposed their body to cold water (Bae et al. 2003).
252 Divers had a greater percentage of type IIx and a lower proportion of type IIa fibers in the vastus
253 lateralis than control active women, whereas no group difference was observed in the percentage of
254 type I fibers. This suggested that repeated cold water immersion might induce the shift of type II
255 muscle fibers to the faster subgroup. In an animal study, a similar shift in fiber type from type I to type
256 IIa fibers was observed in rat soleus muscle (predominantly Type I) after intermittent cold exposure
257 (Walters and Constable 1993). Although, there is a discrepancy between the previous and the present
258 studies in the adaptation process with or without exercise, it is speculated that a greater number of
259 fast-twitch fibers might be recruited for the handgrip exercise following the intervention. This might
260 cause the increase of tissue$\dot{V}O_2$ after the cold acclimation period, as fast-twitch fibers are less
261 economical than slow-twitch fibers (Crow and Kushmerick 1982; Krustrup et al. 2008).

Another potential mechanism for the greater tissue $\dot{V}O_2$ after cold adaptation is an increment of O_2 delivery caused by the growth of capillary density. Bae et al. (2003) reported that Korean diving women had a significantly greater capillary number per muscle fiber of the vastus lateralis compared to that in the active control group. In animal studies, chronic cold exposure induced an increase in capillary density and/or the capillary to fiber ratio in rats (Suzuki et al. 1997; Deveci and Egginton 2002) and in guinea pigs (Sillau et al. 1980). The greater tissue $\dot{V}O_2$ after cold adaptation could be explained by the improvement of the oxygen supply to the muscle tissues by microvascular remodeling (angiogenesis). In this study, since the tissue $\dot{V}O_2$ was evaluated by a slope of the oxy-hemoglobin change during arterial occlusion, the blood circulation was limited in the local body part. However, better blood perfusion before occlusion might enable greater oxygen uptake in the occluded muscle tissue.

Additionally, a significant positive relationship between the increase of total daily energy expenditure during mild cold exposure and the increase of mitochondrial uncoupling of isolated human skeletal muscle biopsies taken after the cold exposure has been reported (Wijers et al. 2008; Wijers et al. 2011). On the other hand, no significant change in human skeletal muscle mitochondrial uncoupling was observed after repeated mild cold exposure (van der Lans et al. 2013). The timing of the muscle biopsy, when the sample was taken with (Wijers et al. 2008) or without (van der Lans et al. 2013) mild cold exposure, could influence the result of muscle respiration. The increased muscle metabolism observed in the present study might be due to enhanced muscle oxidative capacity via mitochondrial uncoupling and/or a greater number of mitochondria (Bruton et al. 2010). In this study, skin temperature of the cooled forearm tended to be higher after intervention, whereas there was no change in the skin blood flow. Thus, the higher skin temperature would be due to a greater thermogenesis in the subcutaneous skeletal muscle tissue, rather than to skin vasodilation.

In conclusion, after repeated local forearm muscle cooling, the forearm muscle oxygen

consumption during a submaximal isometric handgrip was facilitated at muscle temperatures higher than 31°C.

Acknowledgements

The authors wish to thank all those who participated in this study. We would also like to express our gratitude to Mr Mutsuhiro Fijiwara for his technical support. This study was supported by a Grant-in-Aid for Scientific Research (No. 09J03584, No. 26291099) from the Japan Society for the Promotion of Science, and the DESCENTE and ISHIMOTO Memorial Foundation for the Promotion of Sports Science. There is no conflict of interest in relation to this work.

295 **Table 1** Physical characteristic of the experimental and control groups pre- and post-intervention

		Experimental group		Control group	
		Pre	Post	Pre	Post
Age	yrs	21.9 (0.4)	-	22.1 (1.1)	-
Height	cm	171.3 (2.6)	171.3 (2.6)	168.5 (1.2)	168.5 (1.2)
Body weight	kg	59.8 (2.4)	60.2 (2.4)	55.3 (1.3)	55.4 (1.3)
Body mass index	$kg \cdot m^{-2}$	20.4 (0.5)	20.5 (0.5)	19.5 (0.4)	19.5 (0.5)
Mean skin fold thickness	mm	7.6 (0.8)	7.4 (0.8)	6.6 (1.0)	6.5 (1.0)
Forearm maximal girth	cm	24.3 (0.4)	24.3 (0.3)	24.0 (0.4)	24.1 (0.3)
Forearm skin fold thickness	mm	3.0 (0.2)	3.2 (0.3)	3.3 (0.2)	3.1 (0.2)
Handgrip MVC	kg	47.4 (2.5)	-	45.1 (3.3)	-

296

297 Values are means (standard error). Mean skin fold thickness was calculated from the measured values
298 at seven sites (forearm, upper arm, subscapular, abdomen, iliac spine, thigh and calf). Maximal
299 voluntary contraction (MVC) during isometric handgrip was measured. No statistical difference was
300 observed in any physical characteristics between groups or between pre- and post-intervention.

Figure captions

Fig. 1 Resting forearm tissue oxygen consumption at each 2°C muscle temperature reduction pre- and post-intervention. Values are mean ± SE of each control (a) and experimental group (b).

Fig. 2 Forearm tissue oxygen consumption during isometric handgrip at each 2°C muscle temperature reduction pre- and post-intervention. Values are mean ± SE of each control (a) and experimental group (b). *Significant difference between pre- and post-intervention tests.

Fig. 3 Normalized skin blood flow in the forearm just before occlusion at each 2°C muscle temperature reduction pre- and post-intervention. Values are mean ± SE of each control (a) and experimental group (b). The skin blood flow data are normalized relative to peak value.

Fig. 4 Forearm skin temperature just before occlusion at each 2°C muscle temperature reduction pre- and post-intervention. Values are mean ± SE of each control (a) and experimental group (b). *Significant difference between pre- and post-intervention tests.

References

Abramson DI, Kahn A, Tuck S, Jr., Turman GA, Rejal H, Fleischer CJ (1958) Relationship between a range of tissue temperature and local oxygen uptake in the human forearm. I. Changes observed under resting conditions. J Clin Invest 37 (7):1031-1038. doi:10.1172/JCI103684

Bae KA, An NY, Kwon YW, Kim C, Yoon CS, Park SC, Kim CK (2003) Muscle fibre size and capillarity in Korean diving women. Acta Physiol Scand 179 (2):167-172. doi:10.1046/j.1365-201X.2003.01185.x

Barany M (1967) ATPase activity of myosin correlated with speed of muscle shortening. J Gen Physiol 50 (6):Suppl:197-218

Bennett AF (1985) Temperature and muscle. J Exp Biol 115:333-344

Bruton JD, Aydin J, Yamada T, Shabalina IG, Ivarsson N, Zhang SJ, Wada M, Tavi P, Nedergaard J, Katz A, Westerblad H (2010) Increased fatigue resistance linked to Ca2+-stimulated mitochondrial biogenesis in muscle fibres of cold-acclimated mice. J Physiol 588 (Pt 21):4275-4288. doi:10.1113/jphysiol.2010.198598

Cannon B, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. Physiol Rev 84 (1):277-359. doi:10.1152/physrev.00015.2003

Crow MT, Kushmerick MJ (1982) Chemical energetics of slow- and fast-twitch muscles of the mouse. J Gen Physiol 79 (1):147-166

Deveci D, Egginton S (2002) Differing mechanisms of cold-induced changes in capillary supply in m. tibialis anterior of rats and hamsters. J Exp Biol 205 (Pt 6):829-840

Krustrup P, Secher NH, Relu MU, Hellsten Y, Soderlund K, Bangsbo J (2008) Neuromuscular blockade of slow twitch muscle fibres elevates muscle oxygen uptake and energy turnover during submaximal exercise in humans. J Physiol 586 (Pt 24):6037-6048. doi:10.1113/jphysiol.2008.158162

341 Meyer CW, Willershauser M, Jastroch M, Rourke BC, Fromme T, Oelkrug R, Heldmaier G,
342 Klingenspor M (2010) Adaptive thermogenesis and thermal conductance in wild-type and
343 UCP1-KO mice. Am J Physiol Regul Integr Comp Physiol 299 (5):R1396-1406.
344 doi:10.1152/ajpregu.00021.2009
345 Mineo PM, Cassell EA, Roberts ME, Schaeffer PJ (2012) Chronic cold acclimation increases
346 thermogenic capacity, non-shivering thermogenesis and muscle citrate synthase activity in
347 both wild-type and brown adipose tissue deficient mice. Comp Biochem Physiol A Mol Integr
348 Physiol 161 (4):395-400. doi:10.1016/j.cbpa.2011.12.012
349 Nishimura T, Motoi M, Egashira Y, Choi D, Aoyagi K, Watanuki S (2015) Seasonal variation of
350 non-shivering thermogenesis (NST) during mild cold exposure. J Physiol Anthropol 34 (1):11.
351 doi:10.1186/s40101-015-0051-9
352 Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T,
353 Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M (2009) High incidence of
354 metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure
355 and adiposity. Diabetes 58 (7):1526-1531. doi:10.2337/db09-0530
356 Sillau AH, Aquin L, Lechner AJ, Bui MV, Banchero N (1980) Increased capillary supply in skeletal
357 muscle of guinea pigs acclimated to cold. Respir Physiol 42 (3):233-245
358 Suzuki J, Gao M, Ohinata H, Kuroshima A, Koyama T (1997) Chronic cold exposure stimulates
359 microvascular remodeling preferentially in oxidative muscles in rats. Jpn J Physiol 47
360 (6):513-520. doi:10.2170/jjphysiol.47.513
361 Thorsson O, Lilja B, Ahlgren L, Hemdal B, Westlin N (1985) The effect of local cold application on
362 intramuscular blood flow at rest and after running. Med Sci Sports Exerc 17 (6):710-713
363 van Beekvelt MCP, Colier WNJM, Wevers RA, van Engelen BGM (2001) Performance of
364 near-infrared spectroscopy in measuring local O2 consumption and blood flow in skeletal

muscle. J Appl Physiol (1985) 90:515-519

van Beekvelt MCP, van Engelen BGM, Wevers RA, Colier WNJM (2002) In vivo quantitative near-infrared spectroscopy in skeletal muscle during incremental isometric handgrip exercise. Clin Physiol Funct Imaging 22:210-217. doi:10.1046/j.1475-097X.2002.00420

van der Lans AA, Hoeks J, Brans B, Vijgen GH, Visser MG, Vosselman MJ, Hansen J, Jorgensen JA, Wu J, Mottaghy FM, Schrauwen P, van Marken Lichtenbelt WD (2013) Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. J Clin Invest 123 (8):3395-3403. doi:10.1172/JCI68993

van Marken Lichtenbelt WD, Daanen HA (2003) Cold-induced metabolism. Curr Opin Clin Nutr Metab Care 6 (4):469-475. doi:10.1097/01.mco.0000078992.96795.5f

van Marken Lichtenbelt WD, Schrauwen P (2011) Implications of nonshivering thermogenesis for energy balance regulation in humans. Am J Physiol Regul Integr Comp Physiol 301 (2):R285-296. doi:10.1152/ajpregu.00652.2010

van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ (2009) Cold-activated brown adipose tissue in healthy men. N Engl J Med 360 (15):1500-1508. doi:10.1056/NEJMoa0808718

Vybiral S, Lesna I, Jansky L, Zeman V (2000) Thermoregulation in winter swimmers and physiological significance of human catecholamine thermogenesis. Exp Physiol 85 (3):321-326

Wakabayashi H, Oksa J, Tipton MJ (2015) Exercise performance in acute and chronic cold exposure. J Phys Fitness Sports Med 4 (2):177-185. doi:10.7600/jpfsm.4.177

Walters TJ, Constable SH (1993) Intermittent cold exposure causes a muscle-specific shift in the fiber type composition in rats. J Appl Physiol (1985) 75 (1):264-267

Wijers SL, Schrauwen P, Saris WH, van Marken Lichtenbelt WD (2008) Human skeletal muscle

389 mitochondrial uncoupling is associated with cold induced adaptive thermogenesis. PLoS One
390 3 (3):e1777. doi:10.1371/journal.pone.0001777
391 Wijers SL, Schrauwen P, van Baak MA, Saris WH, van Marken Lichtenbelt WD (2011)
392 Beta-adrenergic receptor blockade does not inhibit cold-induced thermogenesis in humans:
393 possible involvement of brown adipose tissue. J Clin Endocrinol Metab 96 (4):E598-605.
394 doi:10.1210/jc.2010-1957
395 Yamakage M, Namiki A (2003) Deep temperature monitoring using a zero-heat-flow method. J Anesth
396 17 (2):108-115. doi:10.1007/s005400300026
397 Yoneshiro T, Aita S, Matsushita M, Kayahara T, Kameya T, Kawai Y, Iwanaga T, Saito M (2013)
398 Recruited brown adipose tissue as an antiobesity agent in humans. J Clin Invest 123
399 (8):3404-3408. doi:10.1172/JCI67803

All Figures were created using MS Excel 2010.

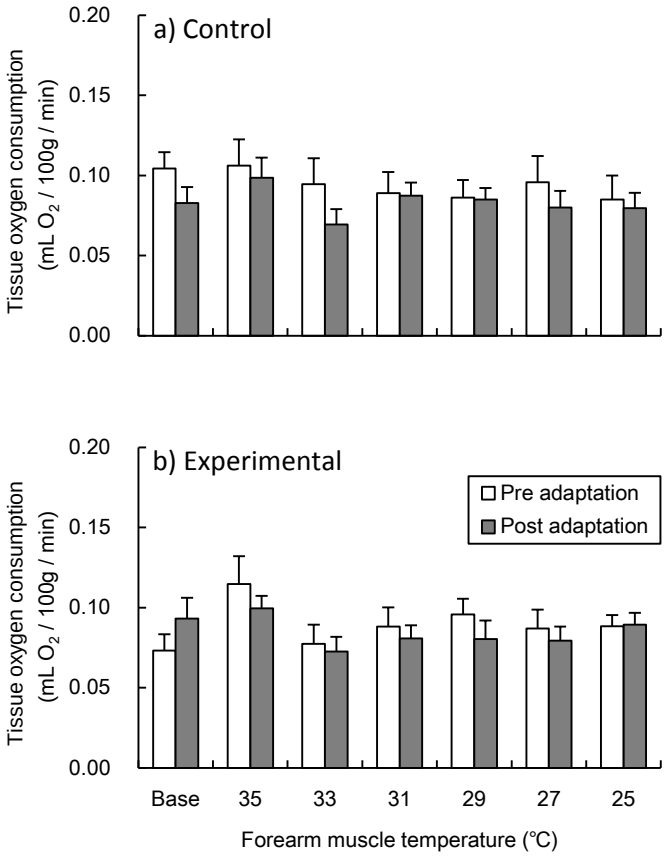

Fig. 1

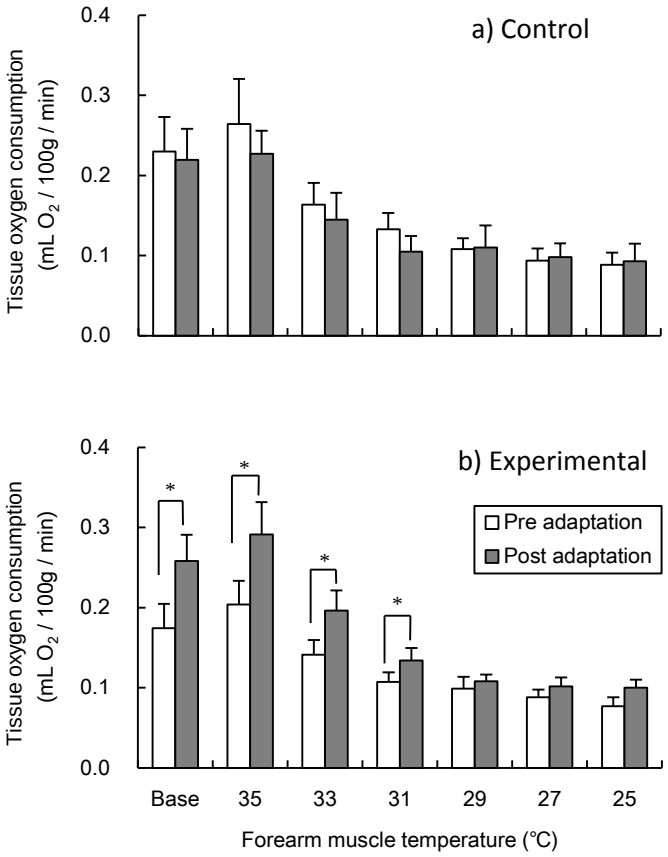
a) Control
b) Experimental
Tissue oxygen consumption (mL O2 / 100g / min)
0.4
0.3
0.2
0.1
0.0
Pre adaptation
Post adaptation
*
Base
35
33
31
29
27
25
Forearm muscle temperature (℃)

Fig. 2

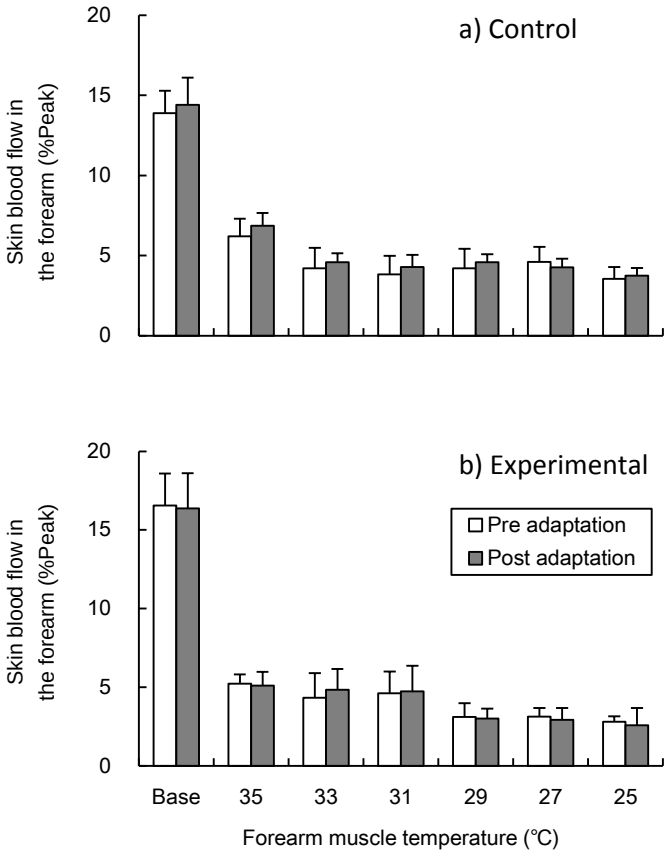
a) Control
b) Experimental
Pre adaptation
Post adaptation
Skin blood flow in the forearm (%Peak)
Skin blood flow in the forearm (%Peak)
20
15
10
5
0
Base
35
33
31
29
27
25
Forearm muscle temperature (℃)

Fig. 3

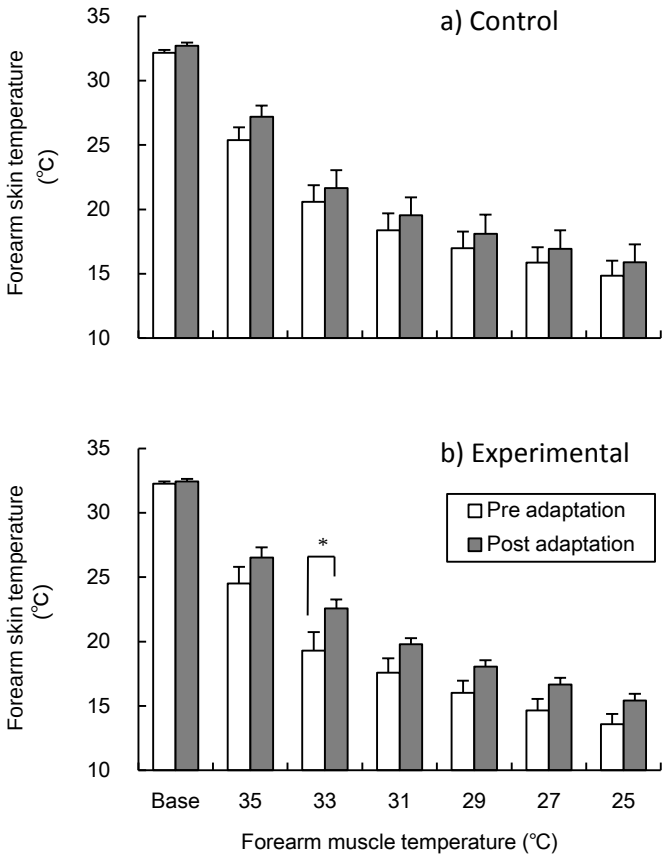
a) Control
b) Experimental
Forearm skin temperature (℃)
35
30
25
20
15
10
Pre adaptation
Post adaptation
*
Base
35
33
31
29
27
25
Forearm muscle temperature (℃)

Fig. 4