Shivering during sleep: Relationship between muscle blood flow and fiber type composition

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Abstract. The present study considers in rabbit: i) the relationship between muscle blood flow (BF) increase and fiber-type composition during shivering; ii) the influence of the vigilance states (Quiet Wakefulness, QW; Synchronized Sleep, SS; Desynchronized Sleep, DS) on this relationship. The results show that muscle BF increase during shivering is proportional to the slow-twitch oxidative (SO) fiber component in QW and SS; in DS the proportionality is lost. This is in accordance with the disappearance of shivering, together with all thermoregulatory effector responses, in this sleep state. Another muscle circulation pattern occurring at low ambient temperature, the relationship between BF increase and muscle depth, also disappears in DS. This confirms that the integrative control of muscle circulation, like other integrative mechanisms, is impaired during DS.
Key words. Shivering; sleep; thermoregulation; muscle blood flow; muscle fiber composition; microspheres.

It has been shown that muscle blood flow (BF) increases during shivering thermogenesis (cold-induced metabolic heat production due to involuntary rhythmic muscular activity) in different mammalian species: rat[1], sheep[2,3] and ox[4]. In muscle, the metabolic profile of different fiber types is closely matched by their microvascular supply[5]. The resulting functional characteristics entail an ordered recruitment pattern during voluntary exercise in mammals[6]; since shivering can be viewed as a mild form of isometric exercise, it is reasonable to surmise that it may have an analogous progression of recruitment. Moreover, the threshold and gain of the thermoregulatory effector mechanisms, including shivering, are affected by the level of vigilance (arousal[7], sleep[8], anesthesia[9]). In particular a strong, mutual influence exists between sleep and thermoregulatory processes[10]. The present study therefore addresses these issues. Firstly, what is the relationship between muscle BF increase and fiber type composition during shivering in the rabbit? Secondly, how is this relationship affected by the behavioral condition: Quiet Wakefulness (QW), Synchronized Sleep (SS), Desynchronized Sleep (DS)?

Methods

The present study is based on a series of experiments in which the overall adjustments of peripheral circulation to a thermal load during sleep were also assessed[11].

Animal preparation. Male New Zealand white rabbits (3200–3800 g) were used. Under general anesthesia (pentobarbital sodium, 40 mg/kg s.c. 45 min after premedication with Flunitrazepam, 0.5 mg/kg i.m.[12]), electrodes were chronically implanted for standard electroencephalographic (EEG), electromyographic (EMG, neck muscles) and electrooculographic (EOG) recordings. Silastic catheters (0.63 mm i.d., 1.17 mm o.d.) were positioned in the aortic bulb via the common carotid artery for injection of microspheres, and in the abdominal aorta via the femoral artery for withdrawal of reference blood samples. Calcium heparin ($\approx$ 1500 IU/day) was continuously administered through an osmotic pump (ALZET, Palo Alto, CA) implanted s.c. in the interscapular region.

Experimental procedure. The animals were kept in the animal colony at an ambient temperature (Ta) in the thermoneutral zone (18 $\pm$ 1 °C). They were exposed to 'neutral' (18 °C, 5 animals) or low (0 °C, 5 animals) Ta during the experimental session; at low Ta the activation of shivering thermogenesis was apparent in EMG recordings. A relatively mild thermal stimulus was chosen in order to minimize interference with the sleep-waking cycle. On the day of the experiment, blood pressure and heart rate were recorded, blood gases measured, and blood flows evaluated with the radioactive microsphere technique[13]. Microspheres were labeled with ^{57}Co, ^{113}Sn or ^{85}Sr and injection order was randomly assigned to the different states of the sleep-wake cycle (QW, SS and DS). Each animal received three injections of approximately 350,000 microspheres; injection time was chosen according to polygraphic and behavioral criteria. Microsphere injections lasted $\approx$ 10 s. Given the short circulation time in the rabbit (10.5 s[14]), the duration of the DS episode at both ambient temperatures, under our experimental conditions (84 $\pm$ 26 s, 0 °; 73 $\pm$ 10 s, 18 °C; mean $\pm$ S.D.) allowed measurements to be completed in most cases. On the few occasions when an animal awoke before completion of the measurements, all data were discarded.

Animals were killed with a thiopental sodium overdose. The different tissues were dissected, weighed and stored in plastic vials. The radioactivity in the tissue samples was then measured with a well-type gamma counter (Nucleus, Oak Ridge, TN, modified from model 1000). Blood flow values were determined for the muscles whose fiber-type composition (slow-twitch oxidative, SO; fast-twitch oxidative glycolytic, FOG; fast-twitch glycolytic, FG) had been previously determined in our laboratory[15], and is summarized in figure 1 (NB: only fiber type composition is presented).

Data reduction. Given the dispersion of the measured BFs in each muscle, median values (ml/min per 100 g tissue) were considered for each behavioral state and

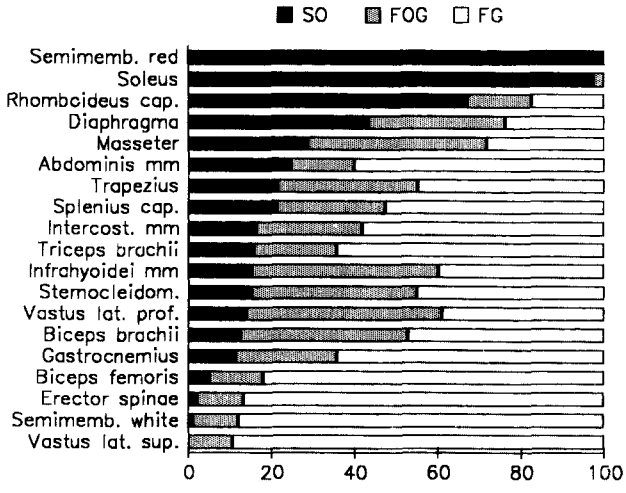

Figure 1. Percentage fiber-type composition in different muscles of rabbit. SO: slow-twitch oxidative; FOG: fast-twitch oxidative-glycolytic; FG: fast-twitch glycolytic. Data from Lenzi et al.[15].

ambient temperature. In order to assess the statistical significance of BF differences a three-way analysis of variance (ANOVA) was performed (dependent variable: muscle BF; factors: muscle denomination with 19 levels, behavioral state with 3 levels, Ta with 2 levels). Since statistical significance resulted for all three factors, but also for the interactions, three separate two-way ANOVA were then performed, one for each behavioral condition (dependent variable: muscle BF; factors: muscle denomination and Ta).

Within each behavioral state, Ta effects on muscle BF were then related to fiber-type composition. Since FOG percentage within the fast-twitch fiber group was positively correlated ($r = 0.854$, $p < 0.001$) with the percentage of SO fibers within the muscle, the contribution of FOG fibers could not be singled out and FG and FOG fibers were considered together. The resulting two groups (SO; FOG + FG) being complementary, BF changes were analyzed versus the SO component only.

Results

Median BF values (ml/min per 100 g tissue) in different muscles are presented in figure 2. It can be seen that, with respect to thermoneutrality, at low Ta most muscles show higher BF in QW and SS, but not in DS. Accordingly, the 2-way ANOVA performed within each behavioral state indicates that the main effect of muscle denomination is significant ($p < 0.01$) in all behavioral states (i.e. in all behavioral states BF differs in different muscles). On the contrary, the main effect of Ta is significant ($p < 0.01$) in QW and SS only (i.e. Ta significantly affects muscle BF in QW and SS, not in DS).

Moreover, significant interaction ($p < 0.001$) between Ta and muscle denomination (see 'Methods', 3-way ANOVA) indicates that the influence of Ta on BF varies in different muscles. Various conditions may underlie this diversified effect of Ta on muscle BF; the present study

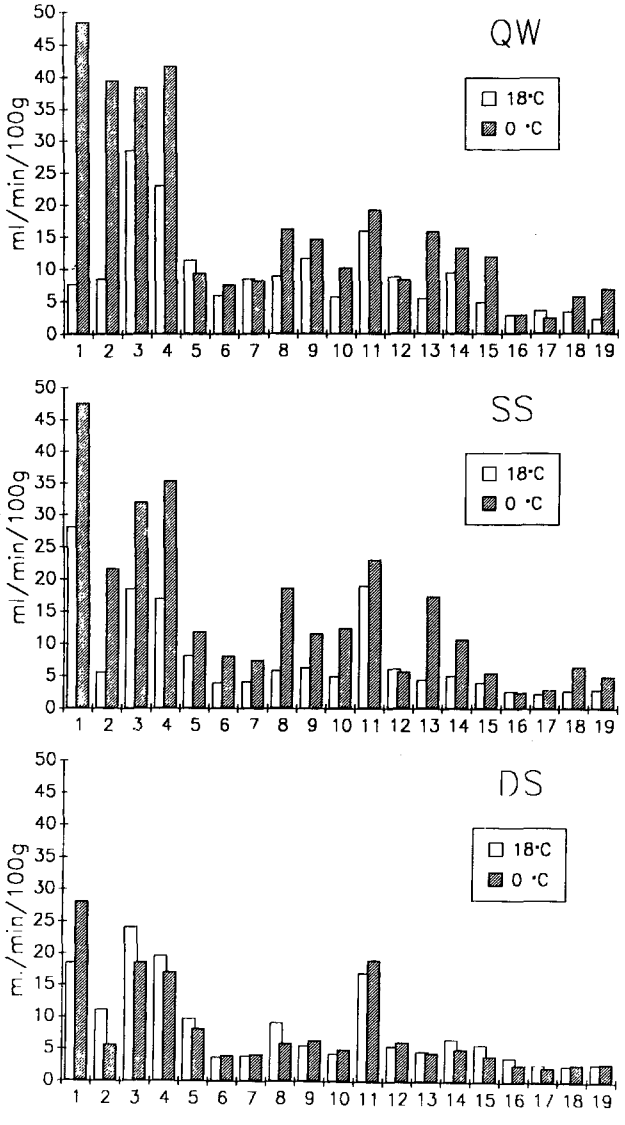

Figure 2. Blood flow values in different muscles of rabbit at neutral and low ambient temperatures. QW, quiet wakefulness; SS, synchronized sleep; DS, desynchronized sleep. Muscles: 1, semimembranosus red; 2, soleus; 3, rhomboideus capitis; 4, diaphragma; 5, masseter; 6, abdominis mm; 7, trapezius; 8, splenius capitis; 9, intercostales; 10, triceps brachii; 11, infrahyoidei; 12, sternocleidomastoideus; 13, vastus lat. prof.; 14, biceps brachii; 15, gastrocnemius; 16, biceps femoris; 17, erector spinae; 18, semimembranosus white; 19, vastus lat. superf.

considered the relationship between muscle BF increment at low Ta, and fiber type composition. In figure 3 the differences between median BF values at neutral and low Ta are plotted vs SO fiber composition, together with their regression lines. It can be seen that muscle BF increment at low Ta is positively correlated ($p < 0.001$) with SO fiber percentage in QW and SS, but not in DS. Beyond fiber type composition, other factors influence Ta effects on muscle BF (e.g., topological disposition: superficial vs deep location). In fact, the partial regression coefficient of muscle BF vs topological disposition is positive and significant ($p < 0.05$) in QW and SS. This confirms that the increase in muscle BF at low TA is

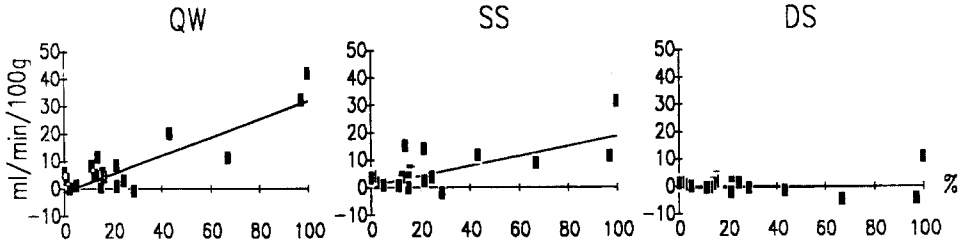

Figure 3. Differences between median blood flow values at neutral and low ambient temperatures are plotted vs the percent of SO (slow-twitch oxidative) fibers in the 19 muscles considered. Regression lines are also indicated.

higher in deeper layers; again, this relationship is lost in DS.

Discussion

Shivering, muscle blood flow and fiber type composition. The results of the present study show that the increase in muscle BF at low Ta when shivering is present is proportional to the SO fiber component of the muscle. Given the tight flow-metabolism coupling of the tissue, this indicates that shivering induced by a relatively mild thermal stimulus primarily involves the SO fiber population. This may correspond to the ordered recruitment pattern present in another form of muscle activation: locomotor activity. Laughlin and Armstrong[6] found an increase in SO fiber BF at a low speed of treadmill running and an increase in FOG fiber BF at a higher speed. Under our experimental conditions only one level of thermal load was applied, in order not to disrupt the sleep-wake cycle. It is reasonable to surmise that a stronger thermal load would also have activated the fast fiber component.

Whereas, to our knowledge, no data exist on this subject in mammals, shivering thermogenesis has been extensively studied in birds, revealing an ordered pattern of fiber recruitment with increasing intensity of thermal stimulation. In bantam cocks (*Gallus domesticus*) shivering thermogenesis close to threshold temperature involves aerobic muscles, whereas anaerobic muscles are recruited at lower Ta[16]. In pigeons (*Columba livia*) glycogen depletion in FG fibers occurs at extreme (– 25 °C) Ta only[17].

Shivering, muscle blood flow and the sleep-waking cycle. The increased muscle BF at low Ta in QW and SS but not in DS (fig. 2) is related to the presence and absence of shivering, respectively. It has been shown, in fact, that shivering, together with other thermoregulatory responses, disappears in DS[18]; as a consequence, the relationship between muscle BF increase and SO fiber component is also lost (fig. 3). The disappearance of thermoregulatory effector responses depends on the impairment of hypothalamic integrative activity in DS[19]. The same impairment underlies the disruption of another muscle circulation pattern, related to the contraction of the body 'core' at low Ta. According to this pattern, which corresponds to a topological (superficial vs inner layers) gradient, BF is higher in deeper muscles, muscle fiber composition being equal. This gradient abates in DS, confirming the loss of integrative control over peripheral circulation in this sleep stage[11].

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