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Heme oxygenase activity increases after exercise in healthy volunteers

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Abstract

Heme oxygenase (HO) is an essential, rate-limiting protein which catalyzes the breakdown of heme to iron, carbon monoxide (CO), and biliverdin. The alpha methene bridge of the heme is eliminated as CO which can be measured as blood carboxyhemoglobin (COHb). Using blood concentrations of COHb as a measure reflecting HO activity, we tested the postulate that the activity of HO changes with exercise. Ten healthy, nonsmoking volunteers (5 females and 5 males with a mean age $\pm$ standard deviation of 25.7 ± 3.2 years), lifetime non-smokers with no history of respiratory diseases and not taking any medication, were included in the study. Subjects were exposed to filtered air for 2 hr while alternating exercise for 15 minutes on a cycle ergometer with rest for 15 minutes. Workload was adjusted so that subjects breathed at a ventilatory rate, normalized for body surface area, of 25 L/meter²/minute. Immediately before, immediately after, and the day following exercise, blood was drawn by standard venipuncture technique. COHb was determined using the IL 682 Co-Oximeter (Instrumentation Laboratory, Bedford, MA). COHb increased in each participant during the exercise session with the mean value ($\pm$ standard deviation) almost doubling (1.1 ± 1.6 to $2.1 \pm 1.6\%$) and returned to baseline by the following day ($1.3 \pm 1.3\%$). We conclude that exercise increases HO activity.

Keywords

Heme oxygenase; carboxyhemoglobin; carbon monoxide; hemoglobin; atmosphere exposure chambers

Introduction

Heme oxygenase (HO) is an essential, rate-limiting protein which catalyzes the breakdown of heme to iron, carbon monoxide (CO), and biliverdin. Most of the substrate originates from hemoglobin but 10 to 20% can be from other heme-proteins. The alpha methene bridge

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Disclosure statement

The authors declare no conflicts of interest.

of the heme is eliminated as CO. The CO produced through HO activity diffuses out of the cell and binds to hemoglobin (with 200 to 250 times the affinity of oxygen) in circulating erythrocytes; this vascular transport can be measured as blood carboxyhemoglobin (COHb). Subsequently, after controlling for variation in ambient concentrations, COHb in circulating blood can be employed as an index of HO activity [1,2]. In the lungs, COHb dissociates and CO is excreted in the exhaled breath. Accordingly, CO is present in measurable quantities in the exhaled air of normal subjects and concentrations correlate with blood COHb [3,4]. The measurement of expired CO can also be employed as an index of heme catabolism provided that the effect of variability in the ambient CO exposure is considered [5,6].

The relationship between human exercise and HO activity has been analyzed utilizing RNA quantification and protein expression in circulating blood cells [7–13]. An alternative approach to defining an association between exercise and HO activity includes measurement of the blood CO level. Using venous concentrations of COHb as a measure reflecting HO activity, we tested the postulate that the activity of HO changes with exercise.

Methods

Study population

Ten healthy volunteers were solicited by advertisements and included in this study. The study population included 5 females and 5 males (8 Caucasians and 2 Asian-Americans) with a mean age ($\pm$ SD) of 25.7 ± 3.2 years. Subjects were lifetime non-smokers with no history of respiratory diseases and not taking any medication during participation in the study. Subjects were informed of the procedures and potential risks and each signed a statement of informed consent. The protocol and consent form were approved by the University of North Carolina School of Medicine Committee on the Protection of the Rights of Human Subjects.

Study design

Prior to the exercise, peripheral blood was collected. Subjects then entered the chamber (approximately 1.83 meter $\times$ 1.83 meter $\times$ 2.44 meter in size), sat on a recumbent bicycle ergometer, and were exposed to filtered air for 2 hr. The subject alternated between 15 minutes of exercise on the bicycle ergometer and 15 minutes of rest. Exercise intensity, i.e. cycle ergometer workload, was adjusted so that subjects breathed at a ventilatory rate, normalized for body surface area, of 25 L/meter²/minute. In most subjects this approximated 50 L/minute (i.e. a VO_2 of approximately 1.0 L/min). A cycle ergometer work setting of about 100 watts achieved such a physiological response. Heart rate, electrocardiogram, and SpO_2 were monitored continuously.

Exposure to filtered air

Exposure to filtered air was carried out at the Environmental Protection Agency's Human Studies Facility on the University of North Carolina campus. Subjects were monitored continuously and a duty physician was available at all times. Total exposure time was 2 hours. CO was scrubbed from the ambient air by passing it through a bed of HOPCALITE which oxidizes this gas to carbon dioxide. During the exposure to filtered air, the following

measurements were monitored: fine particulate matter (by tapered element oscillating microbalance, DataRam, and filter weight), ozone, NO₂, NO, total hydrocarbon, SO₂, and carbon monoxide. All are either zero or negligible except for total hydrocarbon which is between 1 and 3 ppm (all of which is methane). The exposure atmosphere was 40–50% relative humidity and 20° C.

Blood samples

Immediately before, immediately after, and the day following exercise, blood was drawn by standard venipuncture technique. COHb in an anti-coagulated (ethylenediaminetetraacetic acid or EDTA) sample of venous blood was determined in triplicate using the IL 682 Co-Oximeter (Instrumentation Laboratory, Bedford, MA).

Pulmonary function tests

Spirometry was measured immediately before, immediately after, and 24 hours after exercise. The subject inhaled completely and then exhaled rapidly and completely via a tube into a spirometer. From this maneuver, forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁) were derived.

Blood cytokine concentrations

Interleukin (IL)-1 β , IL-6, IL-8, and tumor necrosis factor (TNF)- α were measured in plasma using immunoassays (MesoScale Discovery, Rockville, MD).

Statistics

Data are expressed as mean values $\pm$ standard deviation. Differences between values of an endpoint at pre-exercise, post-exercise, and the following day were compared using one-way analysis of variance. The post-hoc test employed was Duncan's Multiple Range test. Two-tailed tests of significance were employed. Significance was assumed at $p < .05$.

Results

Pre-exercise hemoglobin, hematocrit, white blood cell count, serum sodium and potassium, pulmonary function tests (FVC and FEV₁), and COHb levels were all within the range of values considered normal (Table 1 and Figures 1 and 2).

Hemoglobin and hematocrit values were not significantly changed with exercise in filtered air (Table 1). Accordingly, there was no obvious evidence that the major substrate for HO (i.e. heme from hemoglobin) changed with bicycling. Similarly, exercise in filtered air was not associated with evidence of intravascular volume depletion. Serum sodium and potassium concentrations were not significantly altered with exercise in the filtered air (Table 1). In addition, bicycling in filtered air was not associated with changes in white blood cell count either immediately or 24 hours after suggesting lack of significant inflammatory response to this level of exercise (Table 1). Venous concentrations of IL-1 β , IL-6, IL-8, and TNF- α demonstrated no increases with exercise further supporting a lack of a significant impact of exercise on indices of systemic inflammation (Table 1). There was no impact of exercise on spirometry values. FVC and FEV₁ demonstrated no significant

decrement immediately following the exercise (Figure 1). Finally, COHb increased in each participant during the exercise session (Figure 2A). Mean COHb almost doubled during exercise (Figures 2B and 2C). There were no significant differences in increased COHb levels when expressed as fold-change (post-exercise/pre-exercise) (Figure 2C). Values of COHb returned to baseline by the following day (Figure 2A and 2B).

Discussion

Employing venous COHb concentration as an index of protein activity, HO catalysis of heme was demonstrated to increase with exercise. Neither the level nor the duration of exercise utilized in our study was excessive yet venous levels of COHb, reflecting HO activity, almost doubled. In other human investigation into a relationship between exercise and HO activity, elevated HO-1 RNA in human blood leucocytes and mononuclear cells was reported after a half-marathon and ultra-marathon [7, 8, 9]. HO-1 RNA in human lymphocytes also increased following exercise [10]. Protein expression of HO in human monocytes and lymphocytes were similarly elevated after exercise (11–13). Animal investigation has similarly demonstrated that exercise can increase HO-1 protein levels in tissues, enhance expression of HO-1 mRNA in the aorta, cardiac muscle, and kidney, and elevate HO and CO content in the blood (14–17). A prior study using measurement of exhaled CO to quantify HO activity observed an immediate decrease with exercise [18]. These results suggest that methodology using exhaled CO to measure HO activity may require more precise detection systems for analysis of breath than is currently available [19]. A specific mechanistic pathway for elevated HO activity after exercise is not provided. Prior studies have suggested a destruction of erythrocytes by mechanical trauma during exercising with a subsequent stimulation of HO expression [20,21]. However, non-traumatic exercise was used in our study and previous investigation does not support an association of hemolysis with this activity [22]. Accordingly, there were no decrements in either hemoglobin concentration or hematocrit among participants in our study of healthy subjects using a recumbent cycle and exercise-associated hemolysis is not proposed as underlying elevated HO activity. Exercise does provoke a transient inflammatory response [9,23]. Exercise-induced inflammation is associated with both elevated cytokine levels and increased oxidant generation [24–27]. The expression of HO can be directly stimulated by cytokines elevated following exercise [28,29]. Expression and activity of HO can be increased in inflammatory disease [30–32]. Subsequently, cytokines associated with exercise-induced inflammation could potentially affect elevations in HO expression and its subsequent activity. In our study, inflammation was evaluated using the white blood cell count and plasma levels of pro-inflammatory cytokines and these did not demonstrate any significant change with 2 hours of exercise in filtered air. It is concluded that inflammation did not confound the relationship between COHb and iron indices observed in this study. However, HO expression can be controlled at the level of transcription and both its promoter and ensuing activity are impacted by oxidative stress [33,34]. Exercise has been repeatedly observed to produce an oxidative stress [35,36]. It is reasonable to assume that the HO activity in the ten healthy volunteers could increase after exercise via oxidant-related pathways.

It is possible that some other source of CO was altered with exercise in this investigation accounting for the elevation in COHb observed in the volunteers after exercise. Such a source would contest any relationship between HO activity and exercise. However, endogenous CO results almost exclusively from the breakdown of heme by HO [2,37]. Enzymatic and non-enzymatic forms of auto-oxidation, photo-oxidation, and lipid peroxidation and *in vivo* oxidation of halomethanes can account for substantial CO generation but the exercise was in filtered air and no oxidative challenge was recognized during the two hours [2,37–39]. Regarding exogenous sources of CO, there are many in the normal environment. The most important is tobacco smoking which can elevate COHb concentration to high levels and environmental tobacco smoke (ETS) which can double the concentration [1,40–42]. Environmental CO can also result from automobile exhaust, industrial combustion, solid waste, gas heaters and stoves, and other home appliances [43]. Finally, increases in COHb concentrations can follow exposure to ambient air pollution [40,44]. The utilization of COHb as an index of HO activity requires absolute control for variation in the ambient concentration [1,2]. In this investigation, such control was exerted through the exercise being conducted in filtered air which had no measurable CO level. Accordingly, the observed changes in COHb can be attributed to exercise alone.

We conclude that HO activity increases with exercise in healthy volunteers. The biological significance of the enhanced expression of HO-1 remains unclear. HO has been demonstrated to protect cells against oxidative stress [45,46]. Furthermore, the oxidant-responsive property of HO-1 has led to its adoption as a specific and sensitive marker of oxidative stress at the cellular level *in vitro* [47]. Based on this, HO activity may potentially be upregulated by exercise to protect against an exercise-induced oxidative stress [48].

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Figure 1. Measures of forced vital capacity (A) and forced expiratory volume in one second (B) pre- and post-exercise and the following day. There were no changes in these indices of pulmonary function.

Figure 2. Carboxyhemoglobin (COHb) concentrations pre- and post-exercise and the following day (A). Mean COHb was significantly increased post-exposure relative to pre-exposure and the next day (B). No significant difference was evident between genders in increased COHb levels when expressed as fold-change (post-/pre-exercise) (C).

Table 1

Blood indices pre- and post-exercise in filtered air and the day following

	Pre-exercise	Post-exercise	Following day
Hemoglobin (g/dL)	13.6 ± 2.8	13.6 ± 3.5	13.1 ± 3.2
Hematocrit (%)	39.9 ± 9.8	39.8 ± 8.8	38.7 ± 9.2
White blood cell count (total/mm ³)	6.0 ± 4.7	6.3 ± 6.3	5.4 ± 2.8
Interleukin-1β (pg/mL)	1.9 ± 2.2	1.2 ± 1.3	1.6 ± 1.3
Interleukin -6 (pg/mL)	2.6 ± 1.6	2.9 ± 1.6	2.8 ± 1.4
Interleukin -8 (pg/mL)	4.5 ± 1.6	5.0 ± 1.9	4.7 ± 1.6
Tumor necrosis factor -α (pg/mL)	6.8 ± 1.9	6.7 ± 1.6	6.8 ± 1.9
Sodium (meq/L)	139 ± 3.2	139 ± 3.2	139 ± 3.2
Potassium (meq/L)	3.9 ± 0.9	4.2 ± 0.9	4.0 ± 0.6