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Article

Cardiovascular and Thermal Responses to Cold Exposure during Exercise in Iron Deficient Anemic Individuals

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Abstract

Background: Temperature regulation is impaired in iron deficient anemic humans and rats at rest during cold exposure. However, there is a paucity of data regarding the interplay of cold exposure, anemia and exercise on thermal and cardiovascular responses. Therefore, we aimed to explore thermal and cardiovascular responses of individuals with chronic mild iron deficient anemia during exercise in the cold compared to controls. **Methods:** Nine anemic (5 F, 4 M) and 9 control (5 F, 4 M) individuals, matched for body fat, size, mass but different by design in hematological parameters and physical fitness, participated in the study. The participants cycled in cold (11 °C, 40% RH) and neutral (22 °C, 40% RH) conditions at an intensity ~10% below the respiratory threshold until 1 °C increase in T_{re} or 1h exercise, whichever occurred first. **Results:** In the cold, the anemic individuals showed a lower rate of T_{re} rise compared to controls ($p=0.047$) and only controls increased heat production (VO_2) compared to the neutral condition ($p=0.035$). Moreover, in the cold, the anemic group exhibited lower T_{sk} than controls ($p=0.03$) and exaggerated blood pressure response compared to the neutral environment ($p<0.05$) due to heightened total peripheral resistance ($p<0.05$) and vasomotor response ($p<0.001$). **Conclusions:** In summary, chronic mild iron deficient anemia impaired temperature regulation as judged from the lower rate of T_{re} rise and an inability to activate further the metabolism at cold. Concomitantly, the anemic participants demonstrated increased cardiovascular strain. This is notable because anemia and these environmental conditions are encountered in the workplace, recreational activities and athletic endeavors.

Keywords: cardiovascular strain; cold intolerance; heat production; vasoconstriction; sympathetic response; temperature regulation; thermal sensation

1. Introduction

Iron deficiency anemia affects cardiovascular response and induces metabolic and hormonal disturbances both of which are essential for temperature regulation [1-3]. Early studies in resting humans and animals with iron deficiency anemia have shown impaired thermoregulation and altered metabolism during cold air or water exposure [4-7]. For instance, individuals with anemia experienced greater body heat losses due to a reduced ability to increase metabolism, as indicated by lower rectal temperature and oxygen uptake responses [5]. Additionally, due to enzymatic depletion

of iron, oxidative metabolism is impaired, and catecholamine levels are elevated [2,8-10]. These disturbances, at rest, are reversed when iron deficiency anemia is treated, highlighting the importance of adequate iron levels for maintaining proper thermoregulation and overall metabolic function [4].

However, there is a paucity of studies investigating the integrated responses of thermoregulatory and cardiovascular responses in anemic individuals at cold. Exercise and cold environment could independently tax cardiovascular responses [11] and, to the best of our knowledge, there are no investigations of whether the disadvantageous cardiovascular system of anemic individuals, in terms of oxygen delivery, would be able to serve both the thermoregulatory and metabolic demands of exercise simultaneously. It has been documented that during exercise in the cold the cardiovascular strain is amplified causing altered sympathetic function [12]. Cold exposure is characterized by a sympathetic nervous system excitation that causes cutaneous vasoconstriction [13] and elevated muscle sympathetic nerve activity [14] leading to increases in arterial blood pressure, and thus, heightening the risk of cardiovascular events more pronouncedly in individuals with underlying cardiovascular diseases [13,15-18].

Iron deficiency anemia is most prevalent in well trained pre-menopausal women, athletes, military personnel and pregnant women [19-21]. It also coexists in various diseases such as heart failure, hypertension, kidney failure and cancer [22-25]. Therefore, it is of great importance to examine the physiological and thermal behavioral responses of individuals with chronic iron deficiency during exercise in moderately cold environments as these conditions are common in work, sports and recreational activities.

This study aims to investigate how individuals with mild iron deficiency anemia regulate body temperature and respond to cardiovascular stress during exercise in moderate cold (11-12 °C) conditions. Understanding these responses is critical for improving the safety and performance of vulnerable populations under such environmental conditions.

We hypothesized that during exercise anemic participants compared to controls would demonstrate a slower rise in core temperature due to a compromised heat production which could not be counterbalanced by enhanced peripheral vasoconstriction and augmented blood pressure response.

2. Materials and Methods

2.1. Participants

Eighteen young adult participants, nine (5 females, 4 males) with chronic mild iron deficiency anemia (inclusion hematological criteria were: $10 < \text{Hb} < 12 \text{ g/dL}$, $34 < \text{Hct} < 37\%$, ferritin $< 15 \mu\text{g/L}$) [26] and nine (5 females, 4 males) controls with normal [Hb], serum iron (Fe) and ferritin volunteered for the study (Table 1). An a priori power analysis (G Power 3.1.9.7. software) indicated that a minimum of sixteen participants (8 per group) was required to achieve a statistical power greater than 0.85, effect size $\eta_p^2 > 0.06$ (medium) and α value ≤ 0.05 , in a mixed 3-way ANOVA design (group \times condition \times time). Both groups had no previous history of cardiovascular and pulmonary diseases and were free of musculoskeletal injuries and thyroid diseases. Before visiting the lab, the participants were instructed to avoid heavy meals (4h), intense exercise (24h) and not consume alcohol and caffeinated beverages (12h). All female participants had regular menstrual cycle and were not using contraception pills. In addition, to avoid the confounding effects of female hormones on core temperature and physiological responses, all female participants exercised during the follicular phase of menstrual cycle [27], which was assessed with urine sticks. The experimental procedures had the approval of the local Ethical Committee (1157/11-12-2019). All possible risks and benefits of the procedures were thoroughly explained to the participants, and their written consent was obtained. The two groups were well matched for age, height, body mass, body surface area and %fat. However, they were different by design for fitness level using the prediction equation of differences in $\text{VO}_{2\text{max}}$

with corresponding differences in hemoglobin concentration as reported by Calbet and colleagues [28]. More specifically, initially, fitness and hematological parameters of the anemic participants were measured and thereafter they were matched with control participants based on [Hb]-corrected $\text{VO}_{2\text{max}}$ values.

2.2. Study Design

Prior to the main experiments, the participants visited our lab on two occasions, including a baseline blood status assessment, a seven-site body fat calculation [29] and a familiarization with experimental procedures and the equipment/instrumentation. Thereafter, on two different days, with at least 48-h interval, the participants performed $\text{VO}_{2\text{max}}$ tests on cycle ergometer (Lode, Groningen, The Netherlands), one in cold (11 °C and RH: 40%) and one in neutral (22 °C and RH: 40%) environment. The load of incremental exercise was initially set at 20 W for all participants and then increased by 20 W every min for females and by 30 W for males until volitional exhaustion. The highest 20-s values were averaged to determine $\text{VO}_{2\text{max}}$.

During experimental protocols, 1 h before the experiment, participants arrived at the lab and ingested 5 mL/BM of water. They then emptied their bladder, and their body mass was measured (Bilance Salus, Italy) while wearing only shorts (males) or shorts and a fitted top (females). Afterwards, their capillary blood Hct% and [Hb] were determined. Then, they remained seated for 5 min while baseline rectal and skin temperatures and blood pressure were recorded, serving as idle values. If rectal temperature differed more than 0.2 °C from the value recorded in the first visit, the experiment was postponed for another day. The participants remain seated for another 20 min either in neutral or cold environment in a randomized and counterbalanced order. Then, they performed the prolonged cycling exercise protocol in the corresponding environmental condition (neutral: 22 °C, 40% RH and cold: 11 °C, 40% RH), (ANN: anemia + neutral, ANC: anemia + cold & CONN: control + neutral, CONC: control + cold): at an intensity 10% below the second ventilatory threshold (VT2) until either their rectal temperature (T_{re}) increased by 1 °C or until 1 h had passed, whichever occurred first. Participants were not allowed to drink water throughout the experiment (including resting period and exercise) and wore the same clothing across all conditions: shorts, socks, T-shirt and shoes. Each exercise session was separated by an interval of at least 48 h between measurements. Also, the hydration status was assessed in every visit using Hct% values and body mass [30].

2.3. Measurements

Core body temperature was recorded with a probe (MSR 145, Henggart, Switzerland) at rectum, 10-12 cm past the anal sphincter, sampled and stored at 30-s intervals by MSR data logger (Modular Signal Recorder, MSR Electronics GmbH, Henggart, Switzerland). Skin temperature was measured at four sites (forearm, fingertip, calf, chest) using thermocouples (MSR 147WD, Henggart, Switzerland) attached to the skin with a single piece of adhesive tape, and a weighed mean skin temperature (T_{skin}) was calculated [31,32]. The forearm–fingertip temperature difference ($T_{\text{f-t}}$) was used as an index of skin vasomotor tone, which is reproducible during exercise [33].

Minute mass flow of local sweat rate secreted in the forearm was measured continuously during the experiment at a frequency of 200 Hz by means of a ventilated capsule, positioned on a 5.72-cm² area of skin at the right forearm and ventilated at a rate of 1 L/min with room air. The temperature and humidity of air entering and exiting the capsule were measured with thermocouples (TSD 202A, BIOPAC, Systems Inc.) and capacitance hygrometers (Delta), respectively. Sweating rate (SwR) was estimated from the difference between the temperature and the humidity of inflowing and outflowing air. Temperature and humidity sensors were appropriately calibrated before their use. Also, thermal sensation was assessed with a scaled questionnaire (1, cold; 3, cool; 5, slightly cool; 7, neutral; 9, slightly warm; 11, warm; 13, hot) (ASHRAE, 1966, modified). Gas exchange and ventilatory variables (VO_2 , VCO_2 RER, VE) were recorded continuously throughout experiments breath by breath via open-circuit spirometry (Ultima CPX, MedGraphics, USA). Before each test, the gas analyzers for O_2 and CO_2 and the pneumotachograph were calibrated with two different gas mixtures

a) 12% O₂ and 5% CO₂ balanced in N₂ and b) 21% O₂ and 0.01% CO₂ balanced in N₂ and a 3-L syringe (Ultima CPX, MedGraphics, USA), respectively. Metabolic energy expenditure was calculated as follows:

$$M (W) = \dot{V}O_2 \frac{\left(\frac{RER - 0.7}{0.3}\right) e_c + \left(\frac{1 - RER}{0.3}\right) e_f}{60}$$

where “RER” is respiratory exchange ratio (VCO₂/VO₂), “e_c” is the caloric equivalent of a liter of oxygen when carbohydrates are oxidized (21.1 kJ) and “e_f” is the caloric equivalent of a liter of oxygen when fat is oxidized (19.6 kJ).

Systolic blood pressure (SP) and diastolic blood pressure (DP) were continuously recorded beat by beat noninvasively via a photoplethysmograph with the cuff attached on the middle finger of the left hand (Finometer 2003, FMS, The Netherlands). Stroke volume (SV) was estimated via the Modelflow method [34], while mean arterial pressure (MAP), cardiac output (CO) and total peripheral resistance (TPR) were calculated by BeatScope software (version 1.a.). The method of photoplethysmography has been shown to have very high validity correlation (0.93-0.99) with invasive methods such as arterial line placement during dynamic exercise [35]. To minimize measurement noise, the left hand was carefully and comfortably immobilized in a stable structure so as not to constrain maximal effort during exercise. The heart rate (HR) was monitored with a standard lead II electrocardiogram (ECG 100C, BIOPAC Inc., USA). The Hct% values were determined using Hct scale (Haematocrit Reader, Hawksley Inc., UK) after a 5-min centrifugation of 75 µL capillary blood at 11,500 rpm (Micro Haematocrit Mk5 Centrifuge, Hawksley, UK) with a resolution of 1% units. The [Hb] values were determined by a portable photometer (Diaspect Tm) using 10 µL of capillary blood.

2.4. Statistical Analysis

Normality of data was assessed using Shapiro-Wilk test. A Student’s paired two-tailed *t*-test was employed for comparing participants’ characteristics. Resting values of rectal and skin temperatures were analyzed using ANOVA for two factors (group × condition). The same analysis was also performed for rate of rectal temperature rise, heat production values and initiation of sweating. Three-way ANOVA was conducted to analyze cardiovascular and thermoregulatory responses with [Hb] as between-subject factor and two within-subject factors (condition and time). The cardiovascular and thermal responses presented in the figures were analyzed for the common 20-min period of exercise since there was time variation to achieve the targeted T_{re} of 38 °C. A Tuckey post hoc test was used to assess the significance of parametric analysis. For significant time related interactions, separate two-way ANOVA was conducted at each time point of interest. Moreover, partial eta squared as measures of effect size was used, where $\eta^2 = 0.01$ is considered small, 0.06 moderate, and >0.14 as large. A violation of normality was found for thermal sensation; thus, initially a Friedman test was conducted to discriminate overall group effects (anemia vs. control). Thereafter, a Wilcoxon Signed-Rank Test was used to detect differences between conditions within the same group and a Mann-Whitney test for independent groups at each time point. Data is presented as mean±SD. The significance level was set at $p \leq 0.05$.

3. Results

3.1. Sample Characteristics

The two groups differed by design in blood variables ($p < 0.001$) and were matched for anthropometric characteristics ($p > 0.05$) (Table 1). [Hb] was by 21% lower in the anemic group ($p < 0.001$) and VO_{2max} by 12.5%, but overall group difference did not reach statistical significance ($p = 0.06$). Also, there was no effect of cold exposure in VO_{2max} for both groups (ANN: 40.6±4.7, ANC: 41.1±4.9 & CONN: 46.3±7.1, CONC: 46.7±6.6 mL·kg⁻¹·min⁻¹) ($p > 0.05$). No group differences for MAP were detected before the thermal provocation for resting data ($p = 0.98$), (ANN: 94±9; ANC: 96±7 vs.

CONN: 94±6, CONC: 95±6). Also, there were no differences between groups for baseline values regarding T_{sk} and T_{re} in Neutral and Cold conditions, respectively.

3.2. Cardiovascular Responses

There was a significant group x environment interaction for MAP ($p=0.031$, $\eta_p^2=0.26$) and for TPR ($p=0.043$, $\eta_p^2=0.23$) and post-hoc analysis indicated that they were significantly higher in the cold condition compared to neutral, but only in the anemic group the values reached significance, both at rest and during exercise ($p<0.001$) (Figure 1).

Table 1. Participants' baseline hematological and anthropometric characteristics.

Characteristics	AN	CON	p value
N (M/F)	9 (4/5)	9 (4/5)	
Height (cm)	169±8	171±8	.62
Body mass (kg)	62±8	63±11	.86
Body fat (%)	17±6	16±6	.79
BSA (m²)	1.72±0.15	1.74±0.18	.78
[Hb] (g/dL)	11.8±0.4	14.1±0.9	.0001
HCT (%)	37.1±1.8	42.3±2.7	.0001
Fe (µg/dL)	53±16	93±13	.0001
Ferritin (ng/mL)	15±9	56±12	.0001

Mean±SD. BSA: Body surface area, [Hb]: hemoglobin concentration, HCT: hematocrit, Fe: serum iron.

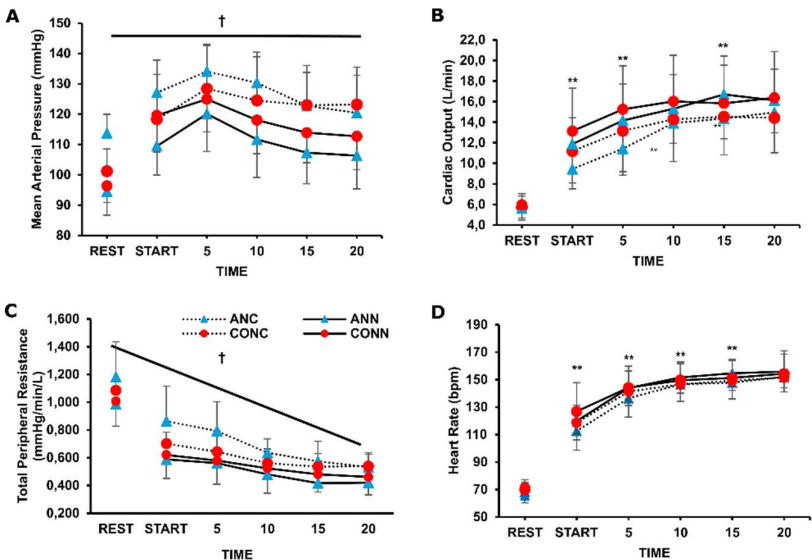


Figure 1. A) mean arterial pressure, B) cardiac output, C) total peripheral resistance and D) heart rate, during rest and 20 min of exercise, in anemic participants (n=9) at neutral (ANN) and cold (ANC) and for controls (n=9) in neutral (CONN) and cold (CONC) environmental conditions, respectively. †: significant difference between

ANC and ANN ($p<0.05$). ** significant difference between ANC and ANN ($p<0.01$). Data are presented as Mean \pm SD.

For CO responses an environment \times time interaction was revealed ($p=0.004$, $\eta_p^2=0.19$) and further analysis indicated that only in the anemic group the CO differed at specific time points between cold and neutral conditions during exercise (Figure 1B). Overall stroke volume increased from rest to exercise ($p=0.001$, $\eta_p^2=0.19$) and there was no difference in the cold (97 ± 26 mL) compared to the neutral (103 ± 26 mL) condition during exercise ($p=0.10$). Overall heart rate increased during exercise in both groups and conditions ($p<0.001$, $\eta_p^2=0.98$), and it was lower from the onset until the 15th min of exercise in ANC compared to ANN ($p=0.01$, $\eta_p^2=0.40$). Diastolic blood pressure was higher in ANC (93 ± 10 mmHg) compared to ANN (82 ± 10 mmHg) ($p=0.022$, $\eta_p^2=0.75$), while it was elevated in CONC compared to CONN only at the 15th and 20th min of exercise ($p=0.005$). Systolic blood pressure was elevated only in ANC (175 ± 21 mmHg) compared to ANN (155 ± 21) ($p=0.0004$, $\eta_p^2=0.37$) with no difference between CONC (166 ± 23 mmHg) and CONN (162 ± 23 mmHg) ($p=0.64$).

3.3. Thermoregulatory Responses

The rate of rectal temperature rise was lower in ANC compared to CONC ($p=0.047$, $\eta_p^2=0.22$) and ANN ($p=0.003$, $\eta_p^2=0.33$) (Table 2). Accordingly, exercise time to reach a specific temperature ($+1^\circ\text{C}$) was longer for ANC compared to ANN ($p=0.001$, $\eta_p^2=0.52$) and CONC ($p=0.021$, $\eta_p^2=0.26$) (Table 2).

Table 2. Participants’ temperature regulation parameters at rest and during exercise.

	AN		CON	
	N	C	N	C
Tre Rest ($^\circ\text{C}$)	37.02 \pm 0.1 3	37.08 \pm 0.1 5	37.05 \pm 0.1 3	37.06 \pm 0.2 0
Tre End exercise ($^\circ\text{C}$)	38.01 \pm 0.1 0	37.93 \pm 0.1 5	38.02 \pm 0.1 6	38.03 \pm 0.1
Tre increase ($^\circ\text{C}\cdot\text{min}^{-1}$), value $\times 100$	2.69 \pm 0.9	1.84 \pm 0.9*	2.74 \pm 0.4	2.88 \pm 0.5
Exercise time to increase $+1^\circ\text{C}$ or 1h duration (min)	41.3 \pm 12.1	52.8 \pm 9.3*	35.8 \pm 5.4	38.2 \pm 8.8
Hprod (W)	610 \pm 105	606 \pm 107	645 \pm 204	710 \pm 236*
Hprod ($\text{W}\cdot\text{kg}^{-1}$)	9.8 \pm 1.4	9.7 \pm 1.0	10.1 \pm 1.7	11.1 \pm 2.2*
Hprod ($\text{W}\cdot\text{m}^{-2}$)	355 \pm 48	352 \pm 42	365 \pm 78	402 \pm 94*
Threshold ($^\circ\text{C}$) for SwR increase ($\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$)	0.15 \pm 0.1	0.33 \pm 0.2**	0.14 \pm 0.0	0.21 \pm 0.1**

Mean \pm SD. AN: $n=9$ and CON: $n=9$. Tre: rectal temperature. Hprod: heat production in absolute (W), relative to body mass ($\text{W}\cdot\text{kg}^{-1}$) and relative to body surface area ($\text{W}\cdot\text{m}^{-2}$). SwR: sweat rate. *: Significant difference between environments ($p<0.05$). **: Significant difference between environments ($p<0.01$). #: significant difference between groups for the same environment.

A significant group \times condition interaction was noted for oxygen uptake ($p=0.016$, $\eta_p^2=0.31$) and post hoc analysis revealed that VO_2 was higher only in the control group during exercise in the cold (CONC: 1852 ± 626 mL/min) compared to thermoneutral (CONN: 2031 ± 727 mL/min) ($p=0.026$) (Figure 2), whereas in the anemic group the VO_2 remained unchanged in the two conditions (ANN: 1759 ± 314 mL/min vs. ANC: 1725 ± 321 mL/min) ($p=0.92$). Metabolic heat production (Hprod) in absolute values during exercise (Table 2) was higher in CONC (710 ± 236 W) than in CONN (645 ± 204 W) ($p=0.035$, $\eta_p^2=0.25$), but similar between ANC (610 ± 106 W) and ANN (606 ± 107 W). Also, Hprod was not different between ANN and CONN in relative values as well (Table 2).

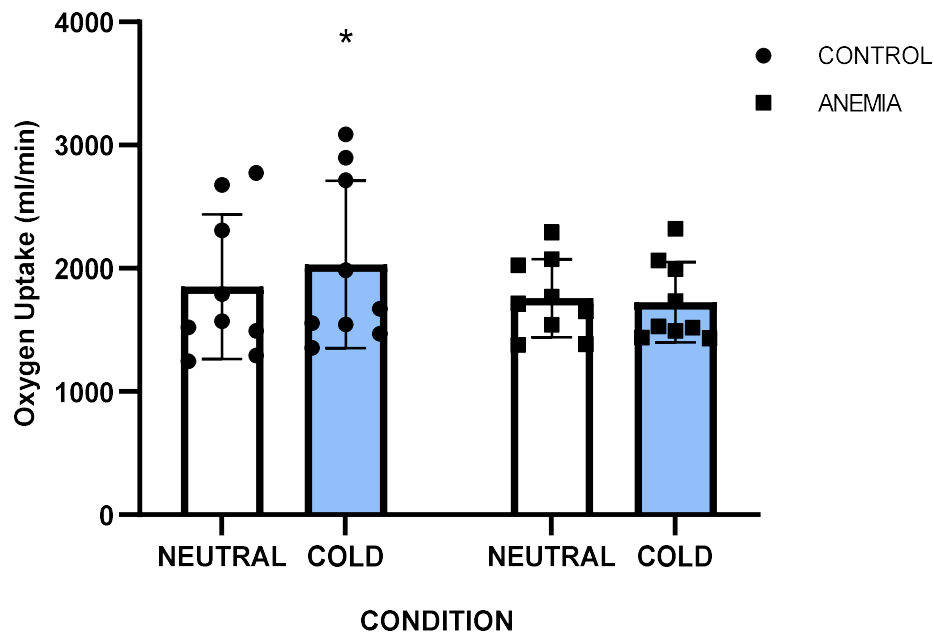


Figure 2. Oxygen uptake responses during exercise for anemic (n=9) and control (n=9) participants, in neutral and cold environmental conditions. A 2-way ANOVA (group x condition) was conducted with a Tuckey post hoc analysis. *: Significant difference between environments ($p=0.036$). Data are presented as Mean \pm SD.

A group x condition interaction ($p=0.010$, $\eta_p^2=0.36$) was found for T_{skin} accounted for by the lower T_{sk} values at rest and throughout exercise observed in ANC compared to CONC ($p=0.028$) and ANN ($p=0.001$) (Figure 3A). Cold exposure delayed the onset of sweating in both groups ($p=0.001$). There was a strong tendency for difference between ANC (0.33 ± 0.2) and CONC (0.21 ± 0.1) ($p=0.056$). The sweat rate increased as time progressed ($p=0.0001$) and it was lower in cold environment irrespective of group ($p=0.004$, $\eta_p^2=0.40$). Also, SwR in ANC was lower than CONC during exercise ($p=0.024$) (Figure 3B).

There was a violation of normality for thermal sensation. Therefore, a Friedman test was conducted indicating that there is a condition effect for both the anemic and control groups ($p=0.001$). Thus, Nonparametric Wilcoxon was used as a post-hoc test and showed a difference between cold and neutral conditions for both groups ($p=0.001$). Lower thermal sensation values were noticed in ANC compared to CONC ($p=0.01$) according to Mann-Whitney test for independent groups. $T_{\text{forearm-finger}}$ was lower in the anemic participants than in controls ($p=0.004$, $\eta_p^2=0.42$) and higher in the cold than in the thermoneutral condition ($p<0.001$, $\eta_p^2=0.72$) (Figure 4B).

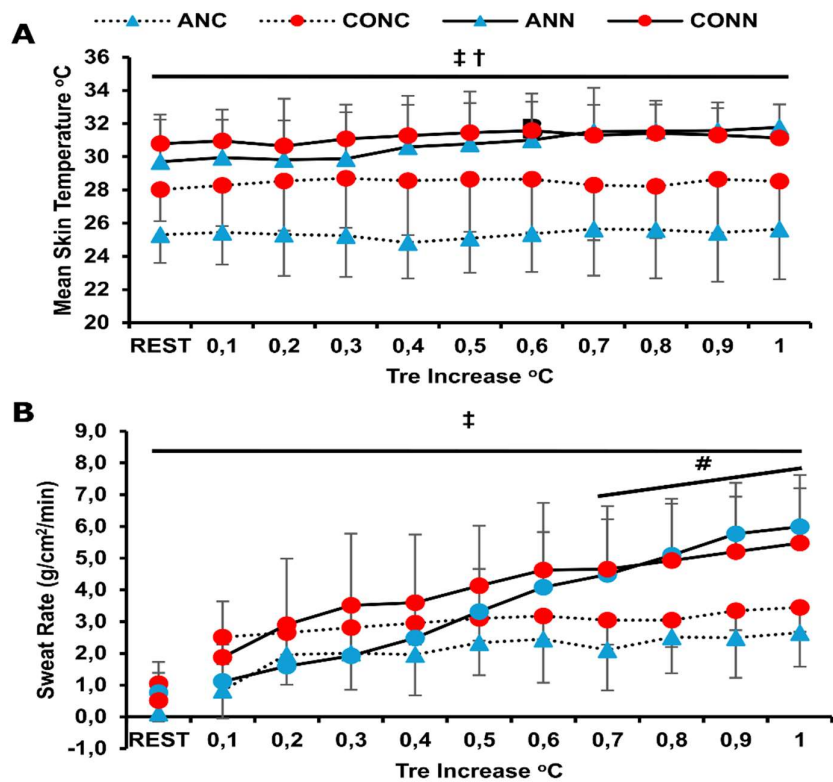


Figure 3. A) mean skin temperature and B) sweat rate plotted against change in rectal temperature during exercise in anemic participants (n=9) at neutral (ANN) and cold (ANC) and for controls (n=9) in neutral (CONN) and cold (CONC) environmental conditions, respectively. ‡: Significant difference between ANC and CONC (p<0.05). †: Significant difference only between ANC and ANN (p<0.05). #: significant difference between environments for both groups (p<0.02). Data are presented as Mean±SD.

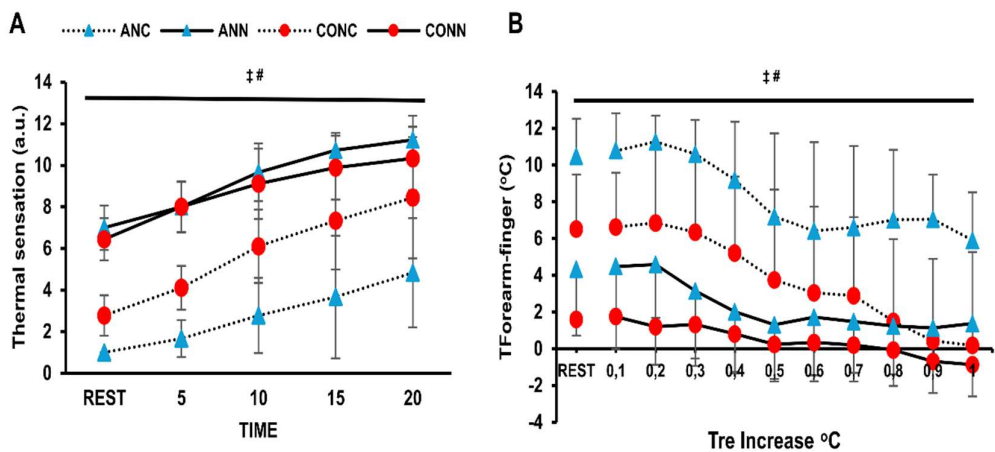


Figure 4. A) Thermal sensation at rest and specific time points during exercise and B) vasomotor activity (gradient of forearm-finger skin temperatures) plotted against change in rectal temperature during exercise in anemic participants (n=9) at neutral (ANN) and cold (ANC) and for controls (n=9) in neutral (CONN) and cold (CONC) environmental conditions, respectively. ‡: Significant difference between ANC and CONC (p<0.01). #: Significant difference between environments for both groups (p<0.05). Data are presented as Mean±SD.

4. Discussion

Previous studies in animals and humans with iron deficient anemia have focused primarily on temperature regulation responses during cold exposure at rest [5-7]. This is the first study that examined the interactive temperature, cardiovascular and thermal behavioral responses both at rest and during exercise in anemic individuals. The main findings of this study are that chronic mild iron deficiency anemia affects cardiovascular and thermoregulatory responses in individuals exposed to cold air conditions at rest and during exercise. Specifically, anemic individuals showed lower rate of rectal temperature rise and mean skin temperature, augmented peripheral vasoconstriction and exaggerated blood pressure response in the cold condition. Also, anemic individuals exhibited suppressed sweating rate responses. These findings suggest enhanced cardiovascular strain in anemic individuals, especially during exercise, probably inducing high cold vulnerability in this group of people.

4.1. Cardiovascular Responses

In young anemic individuals, elevated mean arterial pressure was recorded both at rest and during early exercise, more than in controls, in cold conditions. Even though anemia is generally characterized by increased vasodilatory capacity [36,37], this is the first study indicating a high blood pressure response in anemic individuals during cold exposure. This accentuated cardiovascular strain, likely due to increased sympathetic activation and peripheral vasoconstriction, could be of clinical importance given the frequent coexistence of anemia and cardiovascular disease, which may raise the risk of adverse events in cold environments. In the present study, the healthy participants exhibited elevated blood pressure response at rest (~5mmHg) and exercise (~10mmHg) in the cold compared to the neutral condition but this difference did not reach statistical significance likely due to a great individual variability [16,38]. The lower skin temperature observed in our anemic individuals in the cold is associated with increased total peripheral resistance, which is attributable to changes in cutaneous vasculature based on $T_{\text{finger-forearm}}$, although current data in young adults remain inconclusive as to whether sympathetic response also involves muscle vasculature [14,39,40]. Moreover, the hypertensive response may be driven by higher catecholamine levels in anemia, as supported by earlier animal and human research [4,8,9]. In our study, the cardiac output of anemic individuals was lower in the cold compared to the neutral condition due to lower heart rate response which is common during cold exposure, especially under extreme cold conditions where body temperature is reduced [11]. Also, intriguingly, the reduced heart rate of anemic individuals during exercise in the cold could be indicative of an attempt to attenuate cardiac output and, therefore, to blunt the exaggerated arterial blood pressure response, thus altering the operating point of HR baroreflex [41]. In our study, stroke volume remained relatively stable in both groups, in alignment with previous research showing that stroke volume is typically preserved or even elevated [42-45] and, thus, the central venous pressure (preload) was not different among conditions and groups. As arises from above, the hypertensive response displayed by the anemic individuals is mainly attributed to enhanced peripheral resistance. However, further research is warranted in anemic individuals regarding the purpose served and the precise autonomic responses recruited in cardiovascular control under cold stress.

4.2. Temperature Regulation Responses

Cutaneous vasoconstriction serves as a principal defending mechanism against body temperature reduction during cold exposure [11]. In our study, the anemic group showed higher vasoconstrictor activity in cold conditions compared to controls, as assessed by the finger-forearm skin temperature gradient, that resulted in lower mean skin temperature. Despite this, anemic individuals displayed a lower rate of rectal temperature rise in cold conditions compared to controls suggesting impaired heat generating mechanisms.

VO₂ response has major role in temperature regulation in the cold [11]. In our study, the anemic participants had comparable metabolic heat production to controls in the neutral condition, but they could not increase their VO₂ responses in the cold as the healthy participants did and this is evident in their higher metabolic heat production both in absolute (W) and relative (W·kg⁻¹ & W·m⁻²) values. Our findings align with earlier findings that iron deficiency and iron deficiency anemia affected the metabolic responses during cold exposure in resting humans and animals. Particularly, Beard and colleagues (1990) in their study, after controlling for body fat and menstrual cycle, reported that anemic women had lower VO₂ values in the cold and their core temperature declined faster due to insufficient non-shivering thermogenesis mechanisms. The authors suggested that these responses are linked to lower thyroid hormones levels, since iron deficiency affects the conversion of T₄ to more drastic T₃, leading to diminished circulating levels of thyroxine and triiodo- thyronine in rats and humans [5]. This conversion to the more drastic T₃ requires iron [1] and is crucial for stimulating brown adipose tissue thermogenesis, essential for energy homeostasis in cold conditions [46,47].

Thermal sensation plays a critical role in initiating behavioral thermoregulation, but there is a lack of information regarding individuals with chronic iron-deficiency anemia. In our study, cold exposure reduced subjective thermal sensation in both groups, with anemic participants reporting even lower values. During exercise in the cold, their thermal sensation mirrored lower mean skin temperature, slower rectal temperature rise, and greater vasoconstriction, as indicated by finger-forearm gradient. These findings agree with previous studies showing that thermal sensation is influenced by skin and rectal temperatures [48]. Anemic individuals' higher cold sensitivity may reflect a compensatory behavioral mechanism for impaired heat production. On the other hand, it could be a greater intolerance which possibly arises from their diminished inability to maintain core temperature. Crucially, from a clinical perspective, cold intolerance is connected to earlier onset of ischemia or angina during exercise in coronary artery disease patients [49,50]. Further research should elucidate the thermal perception of anemic humans and the interactive effect of additional thermal factors, such as ageing, hypoxia and sleep deprivation on thermal sensation.

To our knowledge, the present study is the first one to examine the temperature regulation responses of iron deficient anemic humans during exercise in the cold with previous research focusing only on resting conditions [7,9,10]. In the present study the anemic participants also displayed lower local sweat rate in the cold in the same manner as T_{sk}. In fact, skin temperature modulates sweat rate responses [51] especially during cold exposure where a drop in T_{sk} reduces the sweat production without any observed change in body temperature [52].

The findings of this study on how iron-deficiency anemia alters cardiovascular and thermal responses during cold exposure could be applied in a wide range of areas, such as clinical practice, exercise performance, workers and military personnel. From a clinical perspective, these findings can help identify individuals at higher risk for adverse outcomes in cold environments, particularly in populations with complex cardiovascular conditions.

4.3. Methodological Considerations

This study is not without limitations. During cold exposure, the sympathetic nervous system is stimulated and, therefore, the autonomic cardiovascular responses play a pivotal role in maintaining the homeostasis of cardiovascular system both at rest and during exercise. Given that the anemic individuals showed a reduced heart rate with a concomitant increased mean arterial pressure it is suggested that the arterial baroreflex may be altered in this group; however, the study design did not allow for a thorough investigation of the role of autonomic regulation, especially the arterial baroreflex function. Therefore, more in-depth investigation of autonomic regulation is required in anemic populations. Moreover, the study was limited by the absence of skin blood flow measurements, since sympathetic noradrenergic vasoconstrictor nerves induce a rapid decrease in skin blood flow, in cold conditions [13]. However, we attempted to address this limitation by measuring forearm-finger skin temperature gradient which is considered as a valid qualitative index of cutaneous vasomotor tone during steady-state exercise [33]. Also, ecological validity was

compromised, as the participants’ light clothing did not accurately represent typical real-world settings; besides, a combination of cold temperatures with other environmental stressors (i.e. altitude, wet environment) could pose an additional challenge to metabolic responses [53].

5. Conclusions

In conclusion, our study highlights the significant impact of chronic mild iron-deficiency anemia on cardiovascular and thermoregulatory responses during cold exposure, particularly during exercise. Anemic individuals displayed a slower rise in rectal temperature. They also exhibited a lower skin temperature, as well as elevated vasoconstriction and total peripheral resistance, as a compensatory mechanism. The latter responses resulted in exaggerated blood pressure, indicating exaggerated cardiovascular strain compared to controls. Given the prevalence of anemia in individuals with cardiovascular diseases and in a significant portion of athletic population, more research is needed to adequately address the autonomic cardiovascular responses under various adverse environments.

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Abbreviations

The following abbreviations are used in this manuscript:

T _{sk}	Mean skin temperature
T _{re}	Rectal temperature
VO ₂	Oxygen uptake
TPR	Total peripheral resistance
MAP	Mean arterial pressure
CO	Cardiac output
HR	Heart rate
SwR	Sweat rate
H _{prod}	Heat production

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