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Association of traditional Chinese medicine body constitution and cold syndrome with leukocyte mitochondrial functions

An observational study

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Abstract

Body constitution in traditional Chinese medicine (TCM) refers to the holistic and relatively durable state of an individual, based on the qi and blood assessment, and TCM syndrome is defined as the theoretical abstraction of disease-symptom profiles. The biological basis as related to mitochondria, which produce most of the cellular energy, has not been well studied. This study aimed to elucidate the association of mitochondrial function with TCM body constitution and cold syndrome. Body constitution and cold syndrome in TCM were assessed using the Constitution in Chinese Medicine Questionnaire (CCMQ). The mitochondrial function of peripheral leukocytes was evaluated based on oxygen consumption rate (OCR) and enzyme activity; OCR reflects mitochondrial activity and the capacity to produce adenosine triphosphate (ATP). Cellular adenosine nucleotides and malondialdehyde levels were determined using high-performance liquid chromatography to assess the potential bioenergetic mechanisms. A total of 283 adults participated in this study. Leukocytes from subjects with a balanced constitution had higher OCRs than those with unbalanced constitutions. Yang deficiency and cold syndrome also demonstrated lower energy metabolism, as indicated by reduced basal metabolic rate and cellular levels of ATP and malondialdehyde. Decreased mitochondrial enzyme activity has been observed in individuals with the cold syndrome. Unbalanced body constitutions in TCM impair mitochondrial function in leukocytes, which may contribute to the high disease susceptibility. Cold syndrome is characterized by reduced mitochondrial mass, which may explain its symptoms of low-energy metabolism and cold intolerance.

Abbreviations: ATP = adenosine triphosphate, BMR = basal metabolic rate, CCMQ = constitution in Chinese medicine questionnaire, OCR = oxygen consumption rate, TCM = traditional Chinese medicine.

Keywords: body constitution, cold syndrome, mitochondria, traditional Chinese medicine, yang-deficiency

1. Introduction

Human body constitution refers to the holistic and relatively stable characteristics of the morphological structure, physiological functions, and psychological state of humans.[1–3] It also reflects the state of qi, blood, yin, and yang, as described in traditional Chinese medicine (TCM).[2–4] Accordingly, constitution types based on TCM have been classified and assessed using questionnaires.[5,6] Among these questionnaires, the Constitution in Chinese Medicine Questionnaire (CCMQ) developed by Wang et al has been widely used in healthcare and disease prevention in many countries, including China, Canada, Japan, Korea, and the USA.[1,3,5–7] Recently, it has been used to evaluate the treatment effects of Chinese medicine on COVID-19 rehabilitation.[8]

In the CCMQ, body constitution is categorized into 1 balanced type and 8 unbalanced types (yang-deficiency, yin-deficiency, qi-deficiency, phlegm dampness, heat dampness, blood stasis, qi-stagnation, and inherited special).[1] A balanced constitution is a non-symptomatic or healthy type, whereas unbalanced types are in a suboptimal health state and are more susceptible to certain pathogenic factors and related diseases.[1,4,6,9,10] For example, individuals with a yang-deficient constitution often have whitish skin and deep, thready, and weak pulses.[10] They are vulnerable to cold-damp invasion, which is independently associated with cerebral infarction.[11] Yin-deficiency and phlegm dampness are associated with the occurrence of diabetes[12] and hypertension.[1] A syndrome or pattern is an integral and essential part of TCM theory, and

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can be defined as the theoretical abstraction of the symptom profiles of diseases.[13,14] Cold syndrome is characterized by susceptibility and aversion to cold and is typically observed in individuals with a yang-deficient constitution.

Many studies have demonstrated the biological basis of TCM constitutions[3,5,6] and cold syndromes.[13,15,16] Unbalanced constitution is often associated with impaired immunity.[10,17] Individuals with yang-deficiency also exhibit alterations in energy metabolism.[18–20] The cold intolerance symptoms of cold syndrome are related to an imbalance of the nerve-endocrine-immune system[13,15,16] and impaired thermogenesis in the mitochondria.[21]

Mitochondria are cell powerhouses, producing the majority of adenosine triphosphate (ATP), a high-energy compound, which directly regulates the biological processes. Mitochondria and ATP not only participate in energy metabolism but also play pivotal roles in immune regulation.[22–24] Since leukocytes are the key players in immune responses and an unbalanced constitution is characterized by higher disease susceptibility, individuals with unbalanced constitutions may have fewer leukocyte mitochondrial functions than those with a balanced constitution. Therefore, we evaluated the mitochondrial function of leukocytes in individuals with different body constitutions. We observed that those with unbalanced constitutions had impaired mitochondrial functions. Cold syndrome also demonstrates a reduced energy state and enzyme activity. This study provides further evidence for the scientific basis of the TCM constitution and reveals the mechanisms underlying the low-energy metabolism of the cold syndrome.

2. Materials and methods

2.1. Study design and participants

The primary aim of this cross-sectional study was to investigate the association between mitochondrial function and the TCM body constitution. To exclude the influence of clinical disease, only the adults without detectable physical abnormalities were included in this study. The main outcome parameters were the constitution type and cellular oxygen consumption rate (OCR) that comprehensively represents the mitochondrial function. The constitution type was identified using the CCMQ. On the same day, peripheral blood was collected from a local medical institution and used for determining OCRs and mitochondrial enzyme activity. This study complied with the principles of the Declaration of Helsinki. The China Ethics Committee of Registering Clinical Trials approved the protocols (no. ChiECRCT20200169). Between April 2018 and October 2021, 283 eligible individuals from a university and local company were enrolled in this study. The participants were willing to complete the study procedures and provide informed consent.

2.2. Identification of body constitution and cold syndrome

The body constitution in TCM was determined using the CCMQ. The questionnaire consisted of 60 items scored on a 5-point Likert scale and had 9 subscales, each associated with a constitution type and the corresponding score.[1,5] A balanced constitution was defined by a balanced score of at least 60 and all other constitution types had scores of less than 40. An unbalanced constitution was defined as a score of at least 40 on its respective subscale. In addition, basal metabolic rates (BMR) were estimated using a predictive equation developed for Chinese adults: BMR = 13.88 × weight (kg) + 4.16 × height (cm) – 3.43 × age (years) – 112.40 × sex (men = 0; women = 1) + 54.34.[25]

There is no standard physical method to determine cold syndromes and questionnaires are frequently used for this purpose.[13,16] Four questions in the CCMQ were selected to evaluate the cold syndrome. They cover the essential elements of cold syndrome, including cold locations, cold sensations, and warmth-seeking behaviors.[13,26] The questions were as follows: Did you feel cold easily in your abdomen, back, lower back, or knees? Were you sensitive to cold and tended to wear more clothes than others? Did you feel more vulnerable to the cold than others? Did you feel uncomfortable when you drank or ate something cold, or did you avoid drinking or eating something cold? Each question had 5 options: "not at all" (1 point), "scarcely" (2 points), "sometimes" (3 points), "often" (4 points), and "always" (5 points). The sum of the points for each question (that is, the cold syndrome score) indicates the severity of the cold syndrome. A total score of 4 to 6 points indicated that none of the questions were answered as "often" or "always," whereas a total score of 14 to 20 indicated that at least 2 "often" or one "always" options were selected as answers; these 2 groups were categorized as "absent" and "confirmed" cold syndromes, respectively.

2.3. Leukocyte isolation

Approximately 7 mL of peripheral blood was collected for leukocyte isolation and the subsequent analysis of mitochondrial function. Blood was collected via venous puncture in vacuum tubes containing 10% acid-citrate-dextrose buffer. After centrifugation at 1300 × *g* for 15 min at room temperature (20–23°C), the leukocyte-rich layer was removed and washed with phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 5.6 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.4). The cells were then recollected after centrifugation at 550 × *g* for 5 min and the remaining erythrocytes were lysed using lysis buffer (155 mM NH_4Cl, 10 mM $NaHCO_3$, and 0.1 mM EDTA, pH 7.4) on ice for 3 min. Finally, the leukocytes were harvested and washed once with PBS. Mitochondrial function was immediately evaluated using OCRs and mitochondrial enzyme activity.

2.4. Determination of cellular OCR

In mitochondria, most ATP is produced via oxidative phosphorylation, the process which consumes oxygen.[27] The OCRs reflect the general mitochondrial function through oxygen consumption and ATP production via oxidative phosphorylation and are increasingly used to evaluate mitochondrial functions under various conditions.[28–30] In this study, the mitochondrial function of leukocytes was primarily evaluated by measuring OCRs, including basal OCR, maximal OCR, and reserve respiratory capacity.[27] Basal OCR reflects oxygen consumption for basal metabolism and the maintenance of ATP homeostasis. Maximal oxygen consumption occurs when cellular ATP is in crisis or during the collapse of mitochondrial membrane potential induced by FCCP, an uncoupler of oxidative phosphorylation. Reserve respiratory capacity is calculated as the difference between the maximal and the basal OCRs, as a measure of the cellular ability to respond to an increase in energy demand.[29,31]

Basal and maximal OCRs were measured using a fluorescent oxygen probe.[32] Briefly, freshly isolated leukocytes were seeded at 5.0×10^5 cells/well in 14.5 µL of culture medium into a 384-well microplate. Next, 5 µL of the Mito Xpress-Xtra O_2 sensitive probe (Luxcel Biosciences, Cork, Ireland) and 0.5 µL of DMSO (solvent control, for basal OCR) or FCCP (5 µM, constituted with DMSO, for maximal OCR) were added. A well containing only the medium and DMSO was used as blank. All reagents and microplates were prewarmed to 37°C. After sealing the wells with 3 drops of mineral oil to prevent the diffusion of ambient O_2, time-resolved fluorescence was kinetically measured using a microplate reader. Fluorescence measurements and intensity calculations were performed in accordance with the manufacturer's instructions. OCR was expressed as the increase in the rate of fluorescence lifetime and was normalized to the

cell number in each well (μs/h/million cells). Measurements were conducted for 60 cycles over 1 h and each sample was measured in duplicate. The total time taken from blood sample collection to the completion of OCR measurements was approximately 5 to 6 h.

2.5. Assays of mitochondrial enzyme activities

Enzymes play important roles in mitochondrial function. Citrate synthase is a marker exclusive to the mitochondrial matrix that serves as a surrogate for the quantitative count of mitochondria.[33] The respiratory chain complexes I to V are directly responsible for ATP production. To determine their activity, leukocytes were lysed by freezing for 30 s in liquid nitrogen, followed by thawing at room temperature. The measurements were performed spectrophotometrically at 37°C in a 96-well microplate. Citrate synthase, respiratory chain complex I (NADH ubiquinone oxidoreductase), II (succinate ubiquinone reductase), and I + III (NADH cytochrome c oxidoreductase) were assayed as described by Spinalzzi et al[34] Complex V (ATP synthase) activity was determined according to the method described by Ma et al[35] Activities were expressed as the change in reactants in 1 min and were normalized to cell numbers (nmol/min/million cells).

2.6. Determination of ATP, ADP, AMP, and malondialdehyde

ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP) are adenosine nucleotides that are involved in energy production and immune regulation. Malondialdehyde is a product of lipid peroxidation and is used as a biomarker of oxidative stress.[36] Their values in leukocytes were simultaneously determined using high-performance liquid chromatography.[36] Briefly, 100 μL of leukocyte suspension was mixed with 400 μL of 6% perchloric acid solution. After cold incubation for 10 min, the mixture was centrifuged at 10,000 × *g* for 5 min. Next, 300 μL of the supernatant was neutralized with 40 μL of 2 mol/L potassium carbonate. After the mixture was filtered through a 0.22-μm filter, 10 μL of the filtrate was loaded for chromatographic analysis.

2.7. Statistical analysis

The data were analyzed using SPSS Statistics for Windows (version 22.0; IBM Corp., Armonk, NY). Analysis of variance (ANOVA) and independent *t* tests were used to evaluate differences among multiple groups and between 2 groups, respectively. Parametric (Pearson's coefficient) or nonparametric (Spearman's coefficient) correlation analyses were used to evaluate possible correlations between factors of interest. The *P* value was set at $P < .05$.

3. Results

In total, 283 participants were recruited, including 155 females and 128 males (Table 1). They were healthy, as indicated by normal liver and kidney function indicators (data not shown), fasting blood sugar levels, and blood pressure. All the participants completed a questionnaire to identify their body constitution. The mitochondrial function of peripheral leukocytes freshly isolated from 227 participants was evaluated based on basal OCR, maximal OCR, and reserve capacity. Leukocytes from the other participants, who mainly had balanced and yang-deficient constitutions, were used only for demining mitochondrial enzyme activity, cellular adenine nucleotides, and malondialdehyde.

3.1. Leukocyte OCRs correlate with body constitution and cold syndrome

Correlation analysis was initially used to identify the factors related to mitochondrial function. Bivariate correlation analysis showed that balanced constitution, yang-deficiency constitution, cold syndrome, and age were mostly correlated with OCR ($P < .05$, Table 2). Body constitution and syndrome also correlated with cellular malondialdehyde and ATP levels. Further analysis showed that male sex, body mass index (BMI), and BMR predict a high reserve OCR. Given that males had more balanced constitution (65/128 males vs 34/155 females), higher BMI (males 24.8 ± 3.5 vs females 22 ± 3.2 kg/m^2, $P < .001$), and BMR (males 1694 ± 176 vs females 1304 ± 108 kCal/m^2/day, $P < .001$), the correlation of sex, BMI, and BMR with the reverse OCR appears to be secondary to the correlation of body constitution with OCRs. When correlation analyses were performed within each constitution type, sex, BMI, and BMR were not significantly correlated with OCRs. These results indicate that OCRs vary with body constitution and age but are not related to gender. A comparison of the mean values of the OCRs was inconsistent with that of the correlation analysis. Compared with individuals with a balanced constitution, those with an unbalanced constitution had lower basal OCR, maximal OCR, and reserve capacity (Fig. 1). The difference was not related to sex, since it occurred both in the female (Fig. 1A; ANOVA, $P \leq .029$, $F \geq 4.9$ for all OCRs) and the male groups (Fig. 1B; ANOVA, $P < .001$, $F \geq 29.1$ for all OCRs).

Table 1

Details of subjects included in the study.

	Females	Males
Age (yrs)	33.2 ± 10.2 (17–69)	32.7 ± 7.9 (18–55)
Numbers	155	128
Body constitution		
Balanced	34	65
Unbalanced, yang-deficiency	65	19
Unbalanced, no yang-deficiency	56	44
Cold syndrome		
Absent	21	56
Intermediate state	96	62
Confirmed	38	10
Body mass index (kg/cm^2)	22 ± 3.2 (15.5–32.2)	24.8 ± 3.5 (17.8–39.4)
Basal Metabolic rate (kCal/d/m^2)	1304 ± 108 (1078–1790)	1695 ± 176 (1352–2394)
Fast blood sugar (mmol/L)	5 ± 0.4 (4.1–5.9)	5.2 ± 0.5 (4.3–6.8)
Systolic blood pressure (mm Hg)	109.9 ± 11.7 (84–139)	121.2 ± 10 (95–140)
Diastolic blood pressure (mm Hg)	71.4 ± 8.4 (54–88)	77.7 ± 7.9 (60–96)

Note: Data are shown as numbers or values (mean ± standard deviation [minimum–maximum]).

Considering the unbalanced constitution subgroups, each unbalanced constitution type had a lower OCR than that of the balanced constitution type (Table 3). The OCRs of yang-deficiency were not only significantly lower than the balanced constitution (Fig. 2A; $P < .001$ for all the OCRs) but also lower than the other unbalanced constitution types (unbalanced, no yang-deficiency; $P = .03$ for maximal OCR and $P = .016$ for reverse capacity). Each participant had a cold syndrome score based on 4 questions that were closely related to the cold syndrome. The scores were negatively correlated with OCRs (Table 2), indicating that OCRs decrease with an increase in the severity of cold syndrome. This was confirmed by the group analysis. Based on the cold score, the 3 cold syndrome groups were divided as follows: absent group with a score of 4 to 6

Table 2

Results of correlation analysis.

	Spearman correlation coefficient					
	Basal OCR	**Max OCR**	**Reserve OCR**	**Citrate synthase**	**Malon-dialdehyde**	**ATP**
Sex†	−0.026	0.087	**0.141***	−0.07	0.236	−0.057
Age	**0.352*****	**0.352*****	**0.285*****	−0.05	−0.227	0.173
Body mass index	0.039	**0.142a**	**0.186****	0.096	**−0.324***	0.039
Basal metabolic rate	−0.019	0.108	**0.162***	−0.026	0.15	−0.102
Balanced constitution†	**0.247*****	**0.314*****	**0.291*****	0.105	**0.300***	**0.284***
Yang-deficiency constitution†	**−0.145***	**−0.235*****	**−0.257*****	−0.161	**−0.284***	**−0.376****
Cold syndrome score	**−0.229*****	**−0.303*****	**−0.289*****	−0.097	**−0.379*****	**−0.389*****

Note:

†Nonparametric correlation was performed using ordered numbers for sex (female = 0, male = 1), balanced constitution (balanced constitution = 1, unbalanced constitution = 0), and yang-deficiency constitution (yang-deficiency = 1, no yang-deficiency = 0). Statistical significance of the correlation is indicated by:

*$P < .05$,

**$P < .01$, and

***$P \leq .002$. N = 227, 59, 49, and 65 for Oxygen consumption rates (OCRs), citrate synthase, malondialdehyde, and Adenosine triphosphate (ATP), respectively.

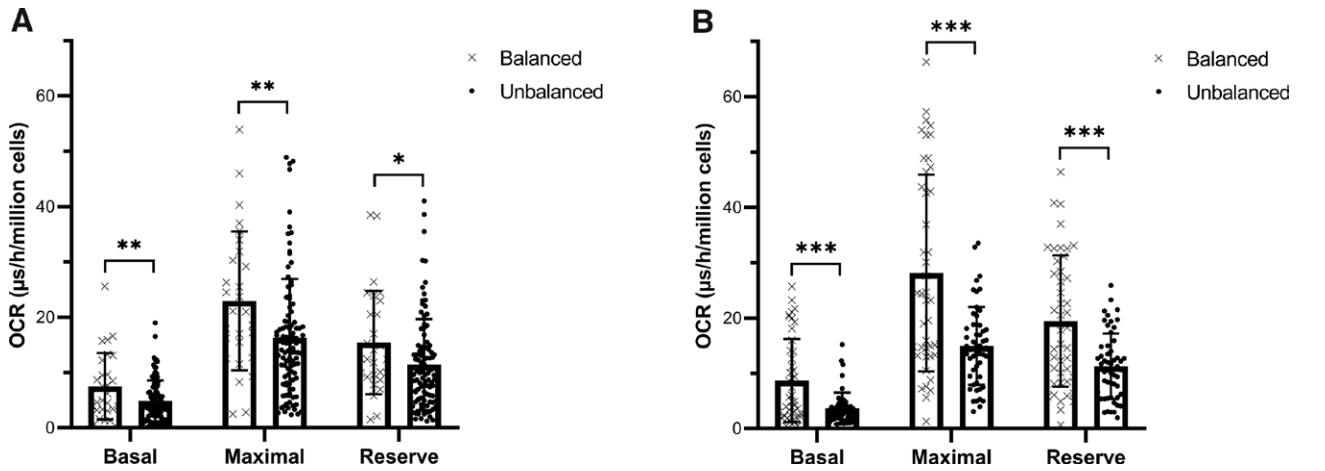

Figure 1. Oxygen consumption rates of leukocytes from adults with balanced and unbalanced constitutions. In both female (A) and male (B) groups, individuals with the balanced constitution (28 females and 45 males) had much higher Oxygen consumption rates (OCRs) than those with unbalanced constitutions (100 females and 54 males). ***$P < .001$, **$P < .01$, *$P < .05$.

Table 3

Oxygen consumption rates of leukocytes from adults with various body constitution types.

	OCR (μs/h/million cells, mean ± SD)			
Body constitution	**Basal**	**Maximal**	**Reserve**	**n**
Balanced	8.4 ± 7.1	27 ± 17.9	18.6 ± 12.6	73
Unbalanced	4.4 ± 3.5	15.8 ± 9.5	11.4 ± 7.5	154
Yang-deficiency	4.2 ± 3.1	13.9 ± 8	9.7 ± 6.3	66
Yin-deficiency	4 ± 3.3	15.5 ± 8.5	11.5 ± 6.7	57
Qi-deficiency	4.3 ± 3.5	14.9 ± 8.2	10.6 ± 6.5	76
Phlegm-dampness	4.3 ± 2.9	16.1 ± 8.6	11.8 ± 7.1	61
Heat-dampness	4.3 ± 3	14.9 ± 7.8	10.7 ± 6.5	62
Blood-stasis	4.7 ± 3.1	14.9 ± 7	10.2 ± 5.4	43
Qi-stagnation	4.4 ± 3	14.7 ± 6.8	10.2 ± 5.1	58
Inherited-special	4 ± 2.4	14.5 ± 7.6	10.5 ± 5.9	22

Note: Comparisons between the balanced and unbalanced types were performed using the independent sample *t* test. All or each of the unbalanced types had lower OCRs than the balanced type ($P < .001$).

(n = 59), intermediate group with a score of 7 to 13 (n = 131), and the confirmed group with a score of 14 to 20 (n = 37). The ANOVA results showed that the 3 groups had significant differences in all OCRs (Fig. 2B; $P < .001$, $F \geq 8.3$). The confirmed group had lower values than all other groups ($P \leq .008$, independent sample t test).

3.2. Yang-deficiency and cold syndrome are associated with low energy metabolism and mitochondrial enzyme activity

To further characterize the mitochondrial function, the activity of mitochondrial enzymes and the amount of adenosine nucleotides in the leukocytes of some participants were determined (Table 4). Cellular ADP and AMP were positively correlated with the activity of mitochondrial enzymes (Pearson's coefficients were between 0.43 and 0.69, $P \leq .002$, n = 49). Individuals with a yang-deficient constitution or cold syndrome had low values of BMR, cellular ATP, and malondialdehyde, suggesting a decreased energy state along with reduced oxidative stress in both conditions.

In addition, individuals with confirmed cold syndrome had lower mitochondrial enzyme activity than those with an absent cold syndrome (Table 4). Among these enzymes, citrate synthase is a mitochondrial marker.[33] When the activities of complex I, II, I + III, and V were normalized with that of citrate synthase, the difference could not be observed (complex I: 2.02 ± 0.48 vs 1.7 ± 0.73, $P = .213$; complex II: 1.17 ± 0.28 vs 1.27 ± 0.46, $P = .521$; complex I + II: 0.88 ± 0.16 vs 0.86 ± 0.14, $P = .79$; complex V: 12.77 ± 2.52 vs 11.84 ± 2, $P = .599$).

4. Discussion

Mitochondria have been associated with qi in TCM, and their roles in the development of yang and qi-related diseases have been postulated.[24] In this study, we experimentally demonstrated the association between mitochondrial functions and TCM body constitution and cold syndrome, which reflects an individual's qi state. The decreased mitochondrial function of leukocytes may account for the high disease susceptibility of individuals with unbalanced constitutions and decreased energy metabolism in those with the cold syndrome.

Mitochondria are central regulators of ATP generation and immune function.[22,23] Their functions can be evaluated using OCRs.[28–30] Leukocyte OCR reflects immune functions[31,37] and has been suggested as a marker for assessing human health.[38,39] Our finding that the individuals with an unbalanced constitution

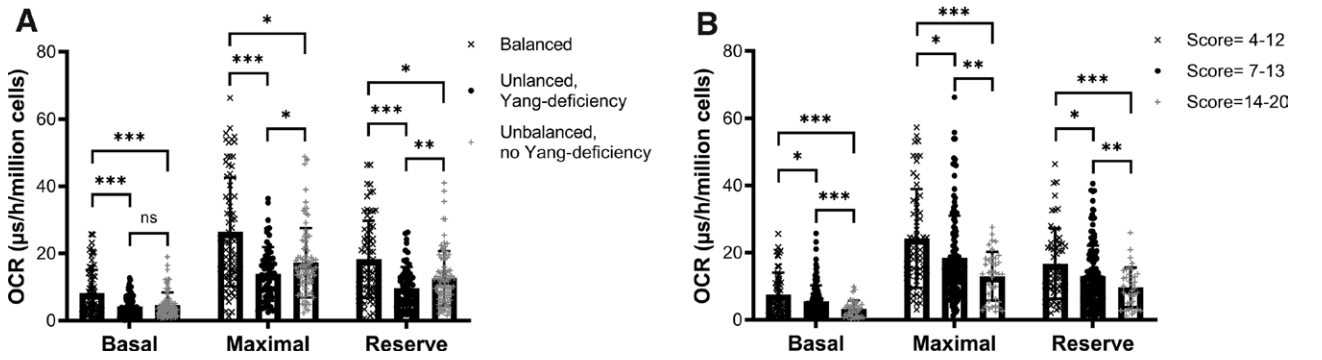

Figure 2. Oxygen consumption rates of leukocytes are lower in individuals with yang-deficiency constitution (A) and confirmed cold syndrome (B). A shows that yang-deficiency constitution (n = 66) had lower values compared to the balanced (n = 73) and no yang-deficiency unbalanced (n = 88) constitution types. B shows that the OCRs decreased with an increase in the seriousness of cold syndrome, indicated as the cold syndrome scores ($P < .001$ for all groups, ANOVA). ***$P \leq .001$, **$P < .01$, *$P < .05$, independent samples t test.

Table 4

Comparison of basal metabolic rate, adenosine nucleosides, malondialdehyde, and the activity of mitochondrial enzymes.

	Body constitution			Cold syndrome		
	Balanced	Yang-deficiency	P	Absent	Confirmed	P
Basal metabolic rate (kcal/m2/day)	1567 ± 266 (92)	**1370 ± 201 (78)**	<.001	1600 ± 230 (68)	1350 ± 204 (45)	<.001
Cellular contents (nmol/million cells)						
ATP	15.91 ± 4.02 (33)	**12.08 ± 4.34 (22)**	.001	16.52 ± 3.97 (24)	**11.28 ± 4.51 (11)**	.001
ADP	13.11 ± 7.27 (33)	10.54 ± 6.41 (22)	.185	13.59 ± 6.15 (24)	**8.07 ± 5.71 (11)**	.017
AMP	4.87 ± 1.73 (33)	5 ± 1.62 (22)	.774	4.88 ± 1.81 (24)	4.37 ± 1.59 (11)	.432
Malondialdehyde	4.28 ± 1.17 (25)	**3.46 ± 1.2 (14)**	.043	4.37 ± 1.05 (24)	**3.02 ± 1.85 (11)**	.039
Enzyme activity (nmol/min/million cells)						
Complex I	1.66 ± 0.67 (30)	1.39 ± 0.6 (15)	.192	1.58 ± 0.54 (22)	**0.97 ± 0.44 (6)**	.017
Complex II	0.88 ± 0.3 (30)	0.79 ± 0.29 (15)	.374	0.9 ± 0.25 (22)	0.71 ± 0.21 (6)	.118
Complex I + III	0.66 ± 0.25 (30)	0.68 ± 0.26 (15)	.809	0.68 ± 0.18 (22)	**0.5 ± 0.1 (6)**	.028
Complex V	9.98 ± 2.74 (30)	8.88 ± 2.83 (14)	.228	9.77 ± 2.55 (22)	**6.72 ± 0.89 (6)**	<.001
Citrate synthase	0.81 ± 0.24 (30)	0.73 ± 0.22 (15)	.311	0.79 ± 0.23 (22)	**0.58 ± 0.1 (6)**	.038

Note: Data are presented as the mean ± standard deviation (number). P values, based on an independent samples t test, indicate the significance of the difference between balanced and yang-deficient constitution or between absent and confirmed cold syndrome. Absent and confirmed cold syndromes were defined as cold syndrome scores of 4 to 6 and 14 to 20, respectively.

have reduced leukocyte OCRs is consistent with impaired mitochondrial function and the resulting high disease susceptibility.[4,9,40]

Although cold syndrome is a typical feature of individuals with yang-deficiency, lower mitochondrial enzyme activity was observed only in those with cold syndrome. As a biomarker for mitochondria,[33] the decrease in the total activity of citrate synthase suggests a reduced mitochondrial number or mass. In addition, when the mitochondrial enzyme activities were normalized to citrate synthase, no significant difference was observed between individuals with and without cold syndrome. These results clearly indicate that the cold syndrome is accompanied by a lower number of mitochondria. This reduction in mitochondrial mass in cold syndrome may differentiate itself from the larger subset of yang-deficient individuals who have reduced mitochondrial function, but not necessarily reduced mitochondrial mass. This hypothesis will need to be confirmed in future studies. The impairment of enzymes may be one, but not the only, reason for decreased OCRs and low-energy states. Yao et al reported that the expression of genes regulating lymphocyte differentiation is associated with yang-deficiency.[20] Whether and how the genes are expressed differently in the leukocytes of other unbalanced constitution types also requires further investigation.

The association between mitochondrial function and body constitution can be interpreted within the context of networked bioenergetic physiology.[15] Here, we report a low-energy state of yang-deficiency and cold syndrome, as evidenced by the decreased levels of cellular adenosine nucleotides and a lower BMR. Cold syndrome is associated with an imbalance in hormone regulation.[15,16,21,41] Low levels of thyroid hormone expression in individuals with yang-deficiency may result in impaired thermogenesis in the mitochondria and subsequent cold syndrome.[21] We also observed that cold syndrome and yang-deficiency were accompanied by low levels of malondialdehyde in leukocytes, indicative of low oxidative stress, which is consistent with the increase in nitric oxide synthase 1 and glutathione peroxidase 1 in patients with cold syndrome.[15] Decreased oxidative stress is a consequence of low-energy metabolism because most oxidative substances originate from proton leakage from the mitochondrial respiratory chain reaction for ATP generation.

The present study has some limitations. Although we evaluated the energy-related functions of whole leukocytes, we did not assess the contribution of specific subgroups, such as peripheral blood mononuclear cells, which play vital roles in immune responses. Changes in immune function and the role of mitochondrial mass and function require further investigation. Finally, mitochondrial function, specifically OCR, was reduced in all unbalanced subtypes, and did not offer a distinguishing feature for differentiating subtypes.

5. Conclusions

Cold syndrome demonstrates reduced mitochondrial numbers in leukocytes, which may contribute to their low-energy metabolism and cold intolerance symptoms. Unbalanced constitutions present a significant reduction in leukocyte OCR, indicative of an impairment of immune function, which may contribute to the disease-prone features. Our findings further promote the scientific basis of body constitution classification in TCM and provide explanatory mechanisms for the low-energy metabolism of the cold syndrome. Given that the body constitution is the result of a qi-blood state with strong energetic connotations, a connection between the qi state and the mitochondria can be expected.

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Writing – review & editing: Yulong Wei, Tongju Li.

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