An Acad Bras Cienc (2020) 92(4): e20200012 DOI 10.1590/0001-3765202020200012
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

HEALTH SCIENCES

Dietary cucurbitacin E reduces high-strength altitude training induced oxidative stress, inflammation and immunosuppression

HEZHI XIE, XINLING TUO, FENG ZHANG, LAURA BOWEN, WEI ZHAO & YUYOU XU

Abstract: Professional athletes conduct high-intensitive hypoxic training often accompanied by the increase of many inflammatory-related cytokines and immunosuppression. Cucurbitacin E (CucE), as a triterpenoid isolated from Cucurbitaceae plants, exert potential anti-cancer and anti-inflammatory. However, it is unknown whether that the CucE could be used as dietary supplement for athletes to improve inflammatory response and immunosuppression. In this study, we established the simulative hypoxic training rat and monkey models and evaluated the effects of CucE on immune- and inflammation-related factors. Obvious improvement on pro-inflammatory factors and pro-lymphocyte proliferation activities were showed in CucE treated rats compared with the control. Further supplement of CucE in professional meals for cynomolgus monkeys with 4-weeks high-intensitive hypoxic training also exert effects on altitude-induced oxidative stress, inflammation and immunologic function. Furtherly, we explored the underlying mechanism of CucE in human Jurkat T cells and results showed that CucE may exhibit immunosuppressive effect by attenuating critical cytokine expression through down-regulating the NF-κB signaling pathway. In conclusion, CucE is expected to be a potential dietary supplement for athletes to ameliorate the inflammation and immunosuppression caused by high-intensitive exercise.

Key words: Athlete, cucurbitacin E, inflammation, immunosuppression, NF-κB.

INTRODUCTION

Athletes in several sports, such as basketball, need various modalities of hypoxic training to enhance their capacity of blood oxygen carrying for the better energy and stamina, and ultimately improve sports performance (Akpinar 2016, Bonci 2011, Ploszczyca et al. 2018). Up to now, many elite athletes and sports teams have often utilized high-strength hypoxic training for the preparation of different levels of competition, although, its efficacies remains controversial from a research perspective (Franchini et al. 2011, Hamlin et al. 2019). However, while the combined strict hypoxia and high-intensitive exercise brings the obvious improvement on athletic abilities for athletes, it also introduces the enormous risks on immune system, including immunological disturbances, infections and illness (Blume et al. 2018, Canis & Santos 2019, Doherty et al. 2019, Walsh 2018). Not only that, the obvious increase of systemic inflammatory cytokines following high-intensitive altitude training indicating a relatively unambiguous immune system response (Khanna et al. 2018, Shen et al. 2012). In addition, altitude training were also reportedly related to the subsequent increase in production of the free radical and oxidative stress (Quindry et al. 2016, Sinha et al. 2009).

According to several reported traditional ethno-biomedical researches (Oikawa et

al. 2012, Ozkan et al. 2016, Zhao et al. 2016), plants have been used to treat various human diseases including excessive oxidative stress and inflammation. Among numerous functional plants, cucurbits were demonstrated the abilities of immunoregulatory, anti-inflammatory and liver protection. Several bioactive compounds from cucurbits family were early recognized with main biological values in both *in vivo* and *in vitro* assays. Cucurbitacins are a group of tetracyclic triterpenes isolated from Cu cucurbitaceae plants possessing extensive pharmacological properties (Chen et al. 2012, Hussain et al. 2019). Cucurbitacin E (CucE), one of the abundant members of the cucurbitacin family, has similar pharmacological activities to other cucurbitacins. According to previous researches, the anti-cancer activity of CucE has been proposed to be mediated by inhibiting signal transducer and activator of transcription 3 phosphorylation in cancer cells (Jevtic et al. 2016, Murtaza et al. 2017). Beyond the reported anti-cancer activities, CucE has achieved more and more attention for its anti-inflammatory and immunoregulatory activities (Shi et al. 2015). However, whether the CucE could use as an effective dietary supplement to ameliorate the high-strength altitude training induced oxidative stress, inflammation and immunosuppression is largely unknown.

Thus, the aim of current study was conducted to determine whether the administration of CucE influences systemic oxidative stress and inflammation related biomarkers in response to the high-strength altitude training in rat and cynomolgus monkey models. We also aimed to explore the action mechanism of CucE on suppression of inflammatory response and immune abnormality in the levels of mRNA and protein expression in human Jurkat T cells.

MATERIALS AND METHODS

Materials

Cucurbitacin E, as a kind of Chinese herb extracts and dietary supplement, was obtained from Shanghai Aibo Biotechnology Co., Ltd (Shanghai, China). Mouse, rat and human lymphocytes were purchased from Shanghai Yan sheng Biotechnology Co., Ltd (Shanghai, China). Wistar rats and cynomolgus monkeys were obtained from Guang Dong Experimental Animal Center and were allowed with free access to diet and water, and acclimated to controlled ambient conditions of 12-h light/dark cycle before establishment of hypoxia training model. Jurkat T cells were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). Animal studies were approved by the Institutional Animal Care and Use Committee of the Soochow University, and performed according to the guidelines.

Establishment of rats and cynomolgus monkeys hypoxia training model

In the present study, the 4-6 weeks old SD rats, weighting 200-250 g, were subjected to weight-bearing swimming training by hanging a 10 g lead pellet in the lower abdomen and placing it in the Morris water maze simulating at 4000m. The rat's mouth and nose were submerged in water for 7 s, and then picked up and dried quickly. Training time interval: 1 time/d, 6 times/week, rest interval 1 d, total training 4 weeks. The experimental rats were randomly divided into 3 groups (n=6): (1) normal ones without hypoxia training. The rats with altitude hypoxia training orally treated (2) with CucE (1mg/kg) or (3) without CucE for 4 weeks.

High-strength altitude training of cynomolgus monkeys in the current study was performed utilizing altitude training model in primary way of previous reports (Shi et al. 2015).

Dietary supplementation with cucurbitacin E for cynomolgus monkey was carried out as follows: half amounts of cynomolgus monkeys were allocated to dietary supplementation with cucurbitacin E (50 μg/per day) whereas the other half ones were allocated to the control food treatment. Dietary supplementation with cucurbitacin E was provided twice a week.

Lymphocyte transforming assay

Rat serum and human serum were collected before and at week 2, 4, 5 (a week rest period) and incubated with mouse, human lymphocytes and mouse, rat lymphocytes, respectively. lymphocyte transformation indexes were measured with previous reported MTT method (Wei et al. 2003).

Determination serum levels of cytokines and immunoglobulin

Quantitative determination of serum levels of IL-2, IL-6, IL-8, IL-10, IL-1β and TNF-α were conducted using enzyme-linked immunosorbent assay (ELISA) kit, which were purchased from R&D Systems (Minnesota, America). Serum immunoglobulin levels (including IgA, IgG, and IgM) were detected using Abbott Laboratories automatic biochemical analyzer (Chicago, America). All the operations were conducted according to the user protocol.

Cell culture and detection

Jurkat T cells were cultured in RPMI-1640 medium with 2mM, 10% FBS, 50 μg/mL streptomycin, and 50 U/mL penicillin at 37°C. Cytotoxicity assays were conducted without or with different concentration of CucE under the stimulation of PDB (0.5 μM) plus Ion (0.5 μg/mL). Proliferation of cells were quantified according to the measurement of the absorbance at 450 nm by using a Bio-Rad 680 microplate reader (Hercules, America).

Detection of PBMC activated surface molecule

The peripheral blood mononuclear cells (PBMCs) of volunteers was separated by density gradient centrifugation, and then were cultured without or with different concentration of CucE (1, 3, 10 μM) under or not under the stimulation of PDB (0.5 μM) plus Ion (0.5 μg/mL). The PBMCs were washed by using PBS (0.1% NaN_3 and 2% fetal calf serum) and fixed by 4% paraformaldehyde. The further detection of PBMC activated surface molecule (CD25 and CD69) were conducted using the FACSCalibur flow cytometer (BD, America) after 24h culture.

Western blot analysis and RT-PCR analysis

Western blot analysis were conducted to evaluate the Jurkat T cell lystate as described previously (Wang et al. 2015). All the antibodies used in these detection were obtained from Cell Signaling Technology (Boston, America). Each band of gel was quantified by Quantity One® 1-D software (Bio-Rad, America). Total RNA were obtained from Jurtkat T cells, and subsequently transcribed into cDNA by using PrimeScript RT kit (Thermo Fisher, America). The primers used in this PCR and relative quantification of gene expression were conducted as previously described (Wang et al. 2015).

Data analysis

All measured variables are presented as mean ± SD. Differences in all parameters were tested using one-way ANOVA. *P* values lower than 0.05 were considered significant.

RESULTS

Effects of CucE on antioxidant capacity, oxidative stress and inflammation biomarkers in rat model of stimulating altitude training

In this study, the rats in stimulating altitude training group were trained by means of swimming for 4 consecutive weeks at 4000 m followed by reoxygen training for 1 week. As a result, the rat model of altitude training were successfully established. Rats in the normal control group 1ived and trained in normal conditions, and those in the other groups all trained with swimming in the stimulating altitude environment for 4 weeks.

During the training and CucE administration period, body weight changes of rats in the stimulating acute hypoxia training groups (treated with CucE or not) and normal condition group were showed in Figure 1. The body masses in all the 3 groups were positively increasing, whereas the growth rate of weight in hypoxia training group (without CucE) was lowest indicating that the CucE treatment exert improvement on the normal weight gain.

Changes in serum pro-inflammatory factors were furtherly detected and showed in Figure 2. Except for the IL-10, plasma levels of IL-6, IL-8, IL-2, IL-1β and TNF-α in altitude training model rats were all significantly increased after 4-week experiment period, whereas the rats treated with CucE effectively reverse abnormality of these cytokines indicating the potential anti-inflammatory effects of CucE in high-intensitive training individuals.

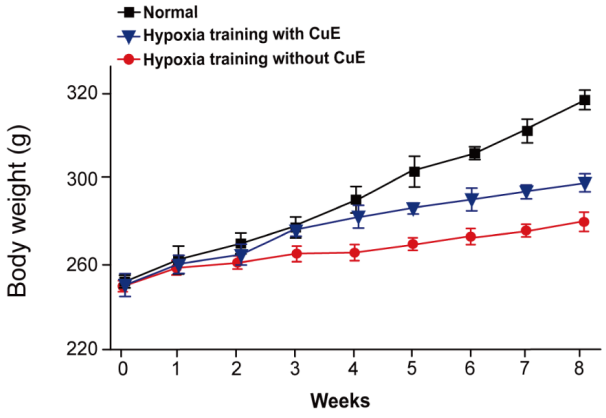

Figure 1. Changes of body weight during the 8-weeks experiment in the three groups. Results are presented as means ± SEM (n=6 each group).

Effects of serum from altitude training rats on mice lymphocyte transformation

Furthermore, we evaluated the effects of the serum collecting from cynomolgus monkeys before, at week 2, 4 (after hypoxia training) and week 5 (after a week rest period) on mouse or rat lymphocyte proliferation. As is showed in Figure 3a, serum from normal humans (without hypoxia training) exert stable activity to promote mouse or rat lymphocyte proliferation, whereas the serum in two hypoxia training group (without CucE) showed obvious inhibition effects on lymphocyte proliferation indicating that the high-intensity training may induced immunosuppression. In addition, administration of CucE in cynomolgus monkeys significantly improve the immunosuppression and help to rapidly return to a normal baseline after a week rest period.

Effects of CucE on altitude-induced oxidative stress, inflammation and immunologic function in cynomolgus monkeys

As is illustrated in Figure 4, plasma levels of IL-6, IL-8 and TNF-α of cynomolgus monkeys were significantly increased ($P<0.05$), and the plasma level of IL-10 was obviously decreased abnormality after four-week hypoxia training, whereas the administration of CucE effectively reduce the abnormality of these cytokines. Above results indicated that there are inflammation reaction exists in cynomolgus monkeys after high-intensitive hypoxia training,

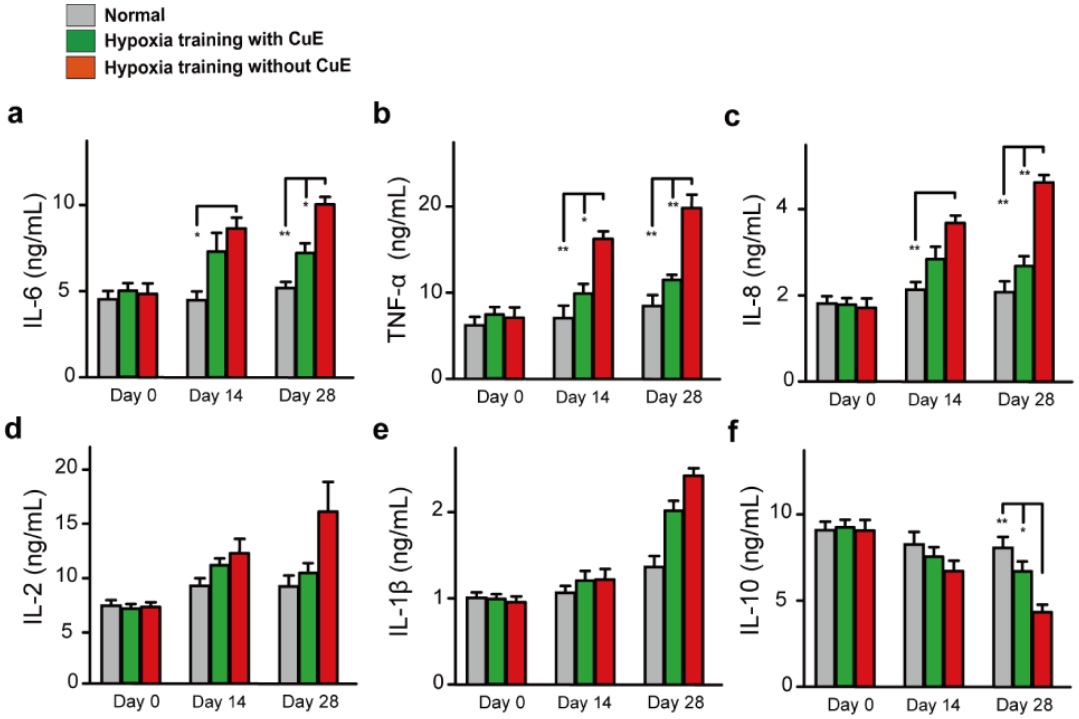

Figure 2. Changes in serum pro-inflammatory factors. (a) IL-6; (b) TNF-α; (c) IL-8; (d) IL-2; (e) IL-1β; (f) IL-10. Results are presented as means ± SEM (n=6 each group). *P< 0.05, **P< 0.02.

and CucE could improve the training induced inflammation.

As shown in Figure 5, serum level of IgG and IgA were gradually decreased in cynomolgus monkeys during the four-week altitude training period, and administration of CucE could obviously reverse these changes.

According to previous reports (Hui et al. 2019, Vajapey et al. 2014), many free radicals were caused by high-intensitive training and accumulated due to weaker scavenging capacity of anti-oxidation enzyme system in athletes. Then the extensive cellular damage comes with the inhibition of T and B lymphocyte cells. In this study, cell ratio and quantity of B cells, T cells and $CD3^+CD4^+$ T cells were all significantly decreased after 4-weeks altitude training, whereas the CucE treatment kept the lymphocyte subsets in a relatively stable state. In addition, $CD3^+CD4^+$ T cells were nor obviously changed in both cynomolgus monkey groups with or without CucE (Table I).

Effects of serum from altitude training cynomolgus monkeys on mice lymphocyte transformation

Furthermore, we evaluated the effects of the serum collecting from cynomolgus monkeys before, at week 2, 4 (after hypoxia training) and week 5 (after a week rest period) on mouse or rat lymphocyte proliferation. As is showed in Figure 6, serum from normal cynomolgus monkeys (without hypoxia training) exert stable activity to promote mouse or rat lymphocyte proliferation, whereas the serum in two hypoxia training group (without CucE) showed obvious inhibition effects on lymphocyte proliferation indicating that the high-intensity training may induced immunosuppression. In addition, administration of CucE in cynomolgus monkeys significantly improve the immunosuppression and help to rapidly return to a normal baseline after a week rest period.

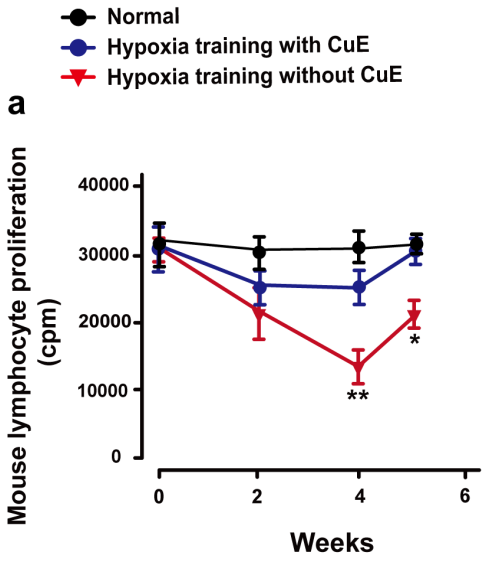

Figure 3. Response of the (a) mouse and (b) human lymphocyte proliferation to the rat serum from normal, Hypoxia training with or without CuE administration. Results are presented as means ± SEM (n=6 each group). ***P*< 0.02, **P*< 0.05 vs normal group; #*P*< 0.05 vs. hypoxia training ones with administration of CuE.

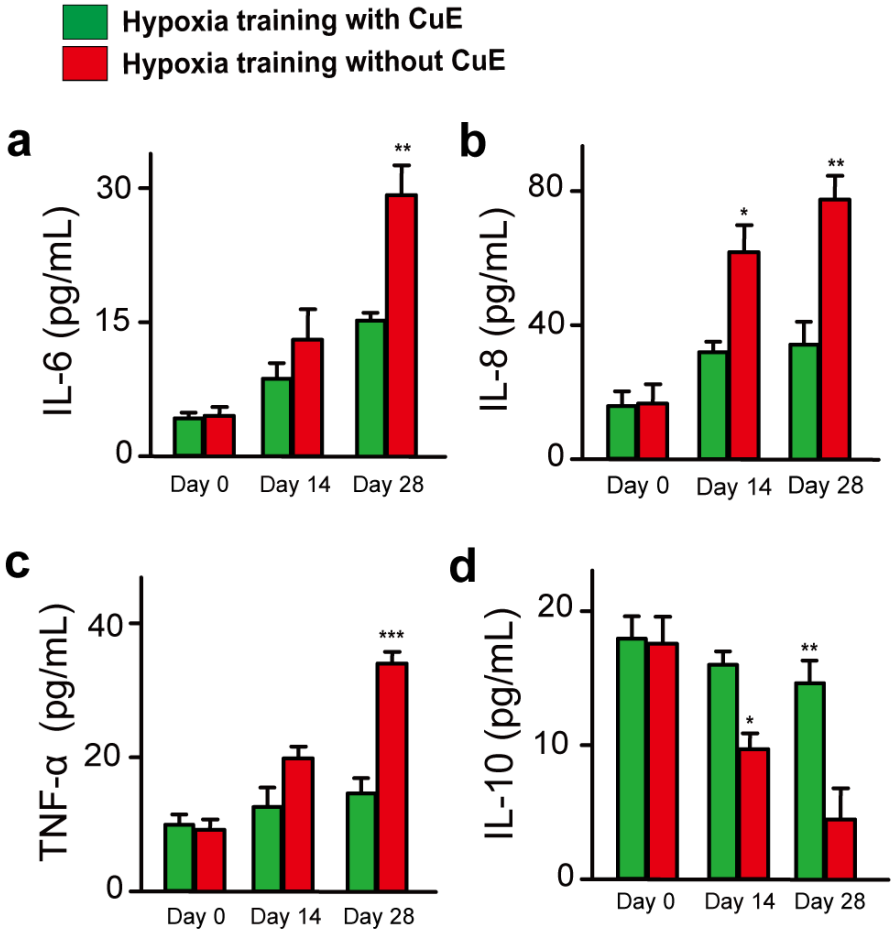

Figure 4. Effects of the administration of CuE on the serum pro-inflammatory factors in hypoxia training basketball athletes. (a) IL-6; (b) IL-8; (c) TNF-α; (d) IL-10. Results are presented as means ± SEM (n=6 each group). **P*< 0.05, ***P*< 0.02, ****P*< 0.001.

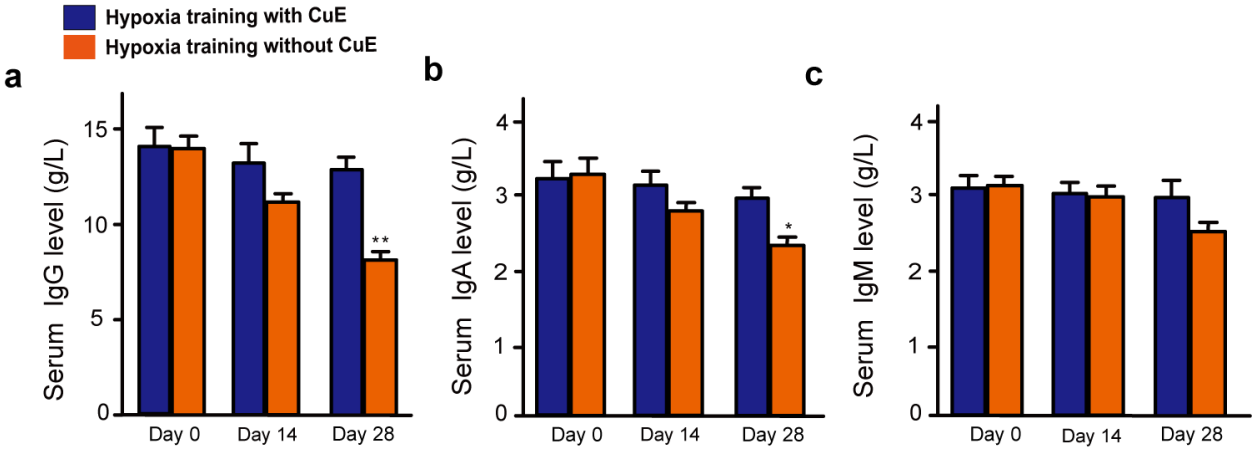

Figure 5. Effects of 4-week altitude training on serum hemoglobin of basketball athletes. Results are presented as means ± SEM (n=6 each group). ***P*< 0.02, **P*< 0.05 versus hypoxia training ones treated with CuE.

Table I. Effects of 4-week altitude training on the lymphocyte subsets of basketball athletes.

Lymphocyte subsets		0 week		4 weeks	
		+CuE	-CuE	+CuE	-CuE
Cell ratio (%)	B cells	9.5 ± 2.1	9.5 ± 2.1	8.2 ± 2.8	6.0 ± 2.4*
	NK cells	25.4 ± 8.2	26.1 ± 6.2	25.2 ± 7.1	31.0 ± 9.9
	T cells	53.1 ± 11.3	58.2 ± 9.5	55.2 ± 11.5	51.2 ± 8.2
	$CD3^+CD4^+$ T cells	29.2 ± 5.1	31.9 ± 3.2	28.1 ± 6.8	21.5 ± 3.1**
	$CD3^+CD8^+$ T cells	21.8 ± 5.6	20.1 ± 6.2	25.2 ± 7.1	31.0 ± 9.9
Cell quantity (cells/μL)	B cells	258.4 ± 51.3	236.1 ± 72.2	225.2 ±63.4	131.5 ± 59.4**
	NK cells	688.4 ± 132.5	712.2 ± 122.3	722.6 ±283.9	915.6± 211.2*
	T cells	1221.4 ± 322.1	1301.4 ± 292.9	1343.2 ± 514.7	1137.4 ± 313.2*
	$CD3^+CD4^+$ T cells	848.2 ± 218.1	828.1 ± 251.2	798.1 ±129.2	521.2± 91.3**
	$CD3^+CD8^+$ T cells	669.5 ± 188.1	700.2 ± 111.9	698 ±223.1	683.2± 211.2
	$CD3^+CD4^+$/ $CD3^+CD8^+$	1.26 ± 0.21	1.18 ± 0.12	1.14 ± 0.15	0.76 ± 0.14*

***P*< 0.02, **P*< 0.05 vs hypoxia training ones with administration of CuE.

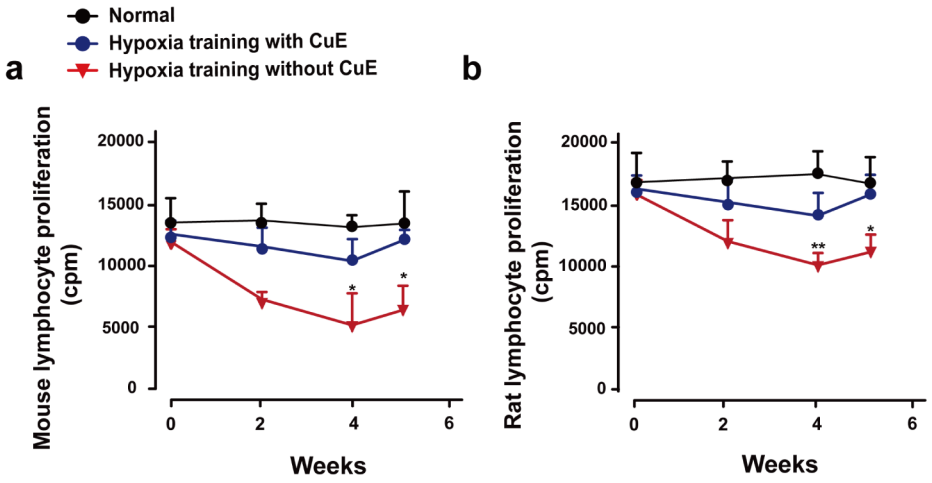

Figure 6. Response of the (a) mouse and (b) rat lymphocyte proliferation to the rat serum from the human and the ones under hypoxia training with or without CuE administration. Results are presented as means ± SEM (n= 6 each group). *P< 0.05, **P< 0.02 versus normal group.

Mechanism of potential immunomodulatory action of cucurbitacin E

In this study, we chose the PDB+Ion activated Jurkat T cell as the model to initially evaluate the potential immunomodulatory action and mechanism of CucE. As is showed in Figure 7a, there is no significant inhibition of CucE within 12 hours incubation on cell proliferation when its concentration was 10 μM or less in WST-1 test. When the incubation time was extended to 24 hours, the cell viability was more than 90% (10μM) indicating that the cytotoxicity of CucE incubation was limited at the relatively low concentration (10μM), and the doses of CucE used in the subsequent in vitro assays were determined to 1, 3, and 10 μM. In addition, human PBMCs, which were activated by incubation of PDB (0.5 μmol/L) and Ion (0.5 μg/ml) were stimulated in the presence or absence of CucE for consecutive 48 h. Expression of the CD69 and CD25 within $CD3^+$ T cells were assessed using FACS method, and results showed that CueE treatment exerts both inhibiting effect on CD25 and CD69. Activated T cells secret some cytokines, such as IL-2 and TNF-α, play very important roles in immune system response. We furtherly investigated effects of CucE treatment on the transcript levels of IL-2, TNF-α and IFN-γ. Quantitative RT-PCR results proved that the treatment of PDB+Ion significantly increased the mRNA levels of above cytokines, and the CucE incubation exert obvious down-regulatory effect in a dose-dependent manner (Figure 7c). Similar inhibition effects on protein levels of above cytokines were also showed in Figure 7d and suggested that incubation of CucE attenuate the immune response of T cells by inhibiting the cytokines both in mRNA and protein levels.

As CucE significantly decreased both mRNA and protein levels of IFN-γ, IL-2 and TNF-α, and NF-κB pathways are crucial in modulating the transcription, expression of several immune system related cytokines according to the previous reports (Hsu et al. 2016, Yao et al. 2014). Therefore, we conducted subsequent experiment to evaluate the effects of CucE on the NF-κB signaling in PDB+Ion activated Jurkat T cells. As shown in Figure 8, analysis of western blot revealed that treatment of CucE obviously reduced the phosphorylation levels of p65 and IκB which were significantly stimulated by incubation of PDB+Ion. We furtherly examined whether CucE treatment could affect the NF-κB/p65 nuclear accumulation. Analysis of western blot showed that the stimulation of PDB+Ion induced a significant nuclear accumulation in pre-treated Jurkat T cells, whereas the treatment

of CucE decreased the NF-κB/p65 level in the nucleus.

Above results indicate that the CucE down-regulates the NF-κB signal pathway via inhibiting phosphorylation of NF-κB and the nuclear translocation of NF-κB/p65.

DISCUSSION

In order to improve the blood oxygen capacity and endurance for the better sports performance, athletes of basketball and other similar sports often need hypoxic training under different intensity. However, the often utilized high-strength hypoxic training brings not only the significant improvement on athletic abilities, but also induces the enormous risks on immune system, such as hypoimmunity and immunological disturbances.

Cucurbitacin are kinds of cytotoxic triterpenoid substances which are originally isolated from Cucurbitaceae plants. Several cognates of cucurbitacin were previous identified (Hunsakunachai et al. 2019, Lui et al. 2009), and their pharmacological efficacies had also been reported, such as anti-fertility, purgative and anti-tumor activities. Previous researches revealed that CucE, as a promising compound with wide spectrum of pharmacological properties, possesses enormous anti-tumor and

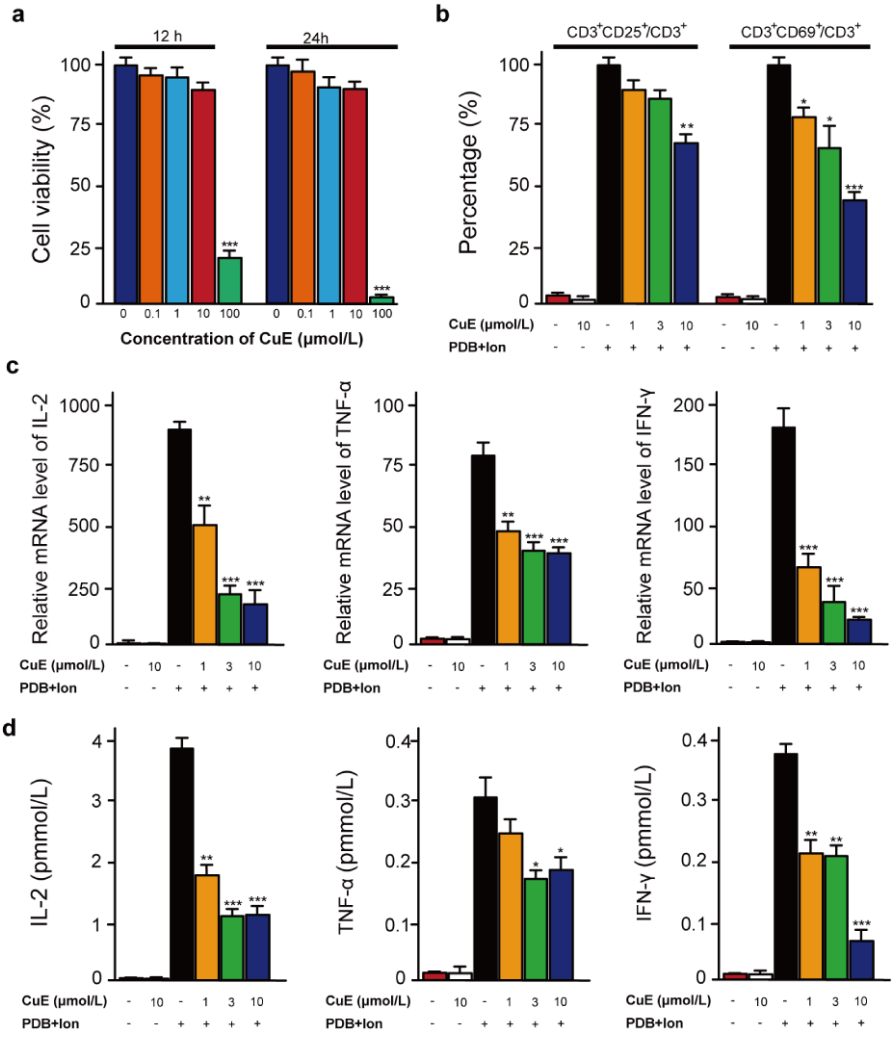

Figure 7. Effects of the incubation of CucE on PBMC and Jurkat T cells. (a) Cytotoxicity of CucE on human Jurkat T cells. (b) Inhibition of CD69 and CD25 expression on T cells by CucE treatment. Effects of the incubation of CucE on the (c) mRNA and (d) protein levels of cytokine in Jurkat T cells. Results are presented as means ± SD (n= 3 each group). *P< 0.05, **P< 0.02, ***P< 0.001 versus PDB+Ion group.

anti-Inflammation potential (Hussain et al. 2019, Jevtic et al. 2016, Murtaza et al. 2017).

In this study, we investigate the immunomodulatory effects of CucE on hypoxia training rat model, and carefully explored the potential related mechanism in human Jurkat T cells. In addition, the potential application of CucE for basketball athletes, who needs to often conduct hypoxic training, as a promising agent in modulating the adaptive immune response and alleviate athletes' elevated inflammatory cytokines were also evaluated in cynomolgus monkeys, the closest animal model to humans.

The weight change in this study mean a reflection of physical status of animals. As shown in Figure 1, the high-strength altitude training reduced the body weight without any supplement while the dietary CucE effectively reversed this trend indicating the protective effects of CucE on the integral physical status of rats.

Recent studies found cytokines play important roles in multiple physiological behaviors, including the cells proliferation, immune function and repairing effects of damaged tissues. The effects of high intensity

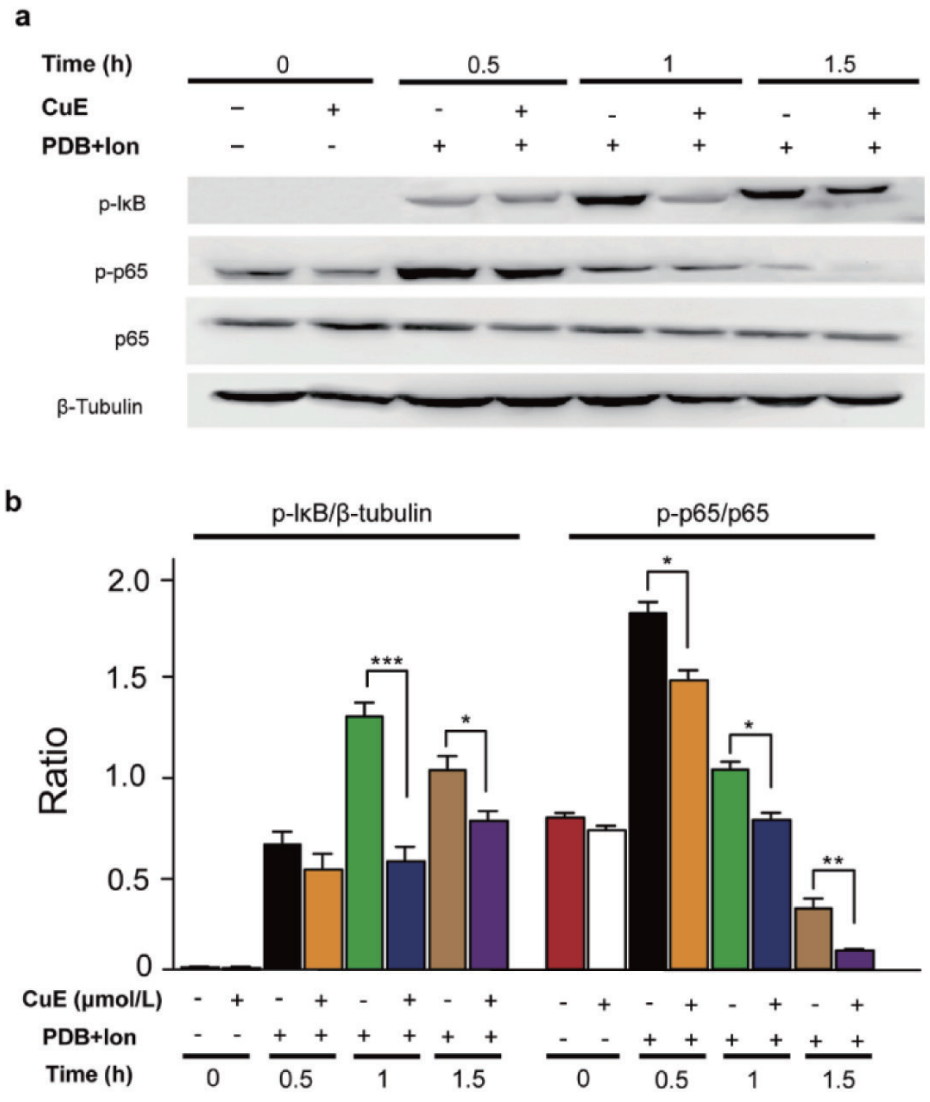

Figure 8. Inhibitory effect of CucE on the NF-κB signaling in Jurkat T cells. (a) Protein levels were determined by Western blotting. β-Tubulin was used as a loading control. (b) The relative densitometric ratios of p-IκB to β-tubulin or p-p65/p65. *P< 0.05, **P< 0.02, ***P< 0.001.

exercises on cytokines have been mentioned in many literatures, such as one-time strenuous exercise leads to a more than 100-fold increase in IL-6 level. However, there are few reports on the effects of long-term (over 2 weeks) intensive training on serum cytokines of animal models, especially for primates. Therefore, in this study, we investigated the effects of high-intensity and low-oxygen training on the changes of cytokines in both rat and primate models, as well as the biological effects of CucE. Results in Figures 2 and 4 showed that IL-6, IL-8 and TNF-alpha increased and IL-10 decreased indicating an abnormal state immune system of rat and cynomolgus monkey models after 4-weeks intensive training, whereas the administration of CucE efficiently improved these changes in both two species.

Previous reports have shown that certain immunosuppressive substances can be produced in the serum of athletes after high-intensitive exercise (Pedersen et al. 2011, Sato et al. 2016). In this study, we also found that incubating human and mouse lymphocytes with stress serum of rats (after 4-weeks altitude training) can significantly reduce transformation efficiencies, and serum from cynomolgus monkeys also has similar effects on the transformation of rat and mouse lymphocytes. Another similar biological phenomenon were also showed in the serum from CueE treated groups which exert a significantly reduced inhibition of lymphocyte transformation, suggesting that CucE, as a food or some other forms of supplement, has great potential to improve the immune suppression caused by excessive exercise.

To further explore our hypothesis, we investigated the effects of CucE administration on serum concentration of immunoglobulin after high-intensitive exercises. Immunoglobulin, as the glycoprotein secretory by the mature plasma cells differentiated from B cells in response to stimulation of antigen, can directly against the corresponding pathogenic microorganism and toxins, and induce complement activation, phagocytosis, and so on. Immunoglobulin is main composition of the humoral immune and certainly related to health and fatigue status. Our results in Figure 5 showed that 4-weeks high-intensity training in cynomolgus monkeys led to a significant decrease in serum level of IgG, which was more sensitive than IgA and IgM, suggesting that humoral immune function of cynomolgus monkeys decreased after excessive exercise. Administration of CucE also could effectively improve the sharp decrease of IgG.

Percentage and count of NK cell, B cells, T cells and $CD3^+CD4^+$ T cells were all decreased significantly in the peripheral blood from cynomolgus monkeys after 4-weeks high-intensitive training. Under normal circumstances, the ratio of $CD3^+CD4^+$/ $CD3^+CD8^+$ is in a relatively balanced state which keeping the immune function also is stable. The decrease of $CD4^+/CD8^+$ ratio is a manifestation of cellular immunosuppression, which may cause immune dysfunction or immune diseases. According to previous reports (Campbell et al. 2012, McLeod and He 2010, Pinto et al. 2014), we suspect that the to many high-intensitive exercises cause too much free radicals accumulation leading extensive damage to immune cells, thus inhibiting the function of T lymphocytes and B lymphocytes. Meanwhile, the levels of B cells and Th cells were significantly decreased. Thus, we speculated that the inhibition of B cells and Th cells might be the main reason for the significant decrease of IgG in cynomolgus monkeys after high intensity exercises.

In the above studies, we found that intake of CucE can effectively reduce the occurrence of immunosuppression and inflammatory response in cynomolgus monkeys after

high-intensity exercise. In order to explain and explore the regulatory effect of CucE on adaptive immune function and its possible mechanism, as well as the potential of using CucE as an anti-inflammatory drug or a dietary supplement to alleviate the increase of pro-inflammatory cytokines in athletes.

As shown in Figure 7, CucE significantly suppressed the expression of CD69 and CD25 on the surfaces of $CD3^+$ T cells in PBMC stimulated with PDB plus Ion. PDB+Ion induced expression of IL-2, TNF-α and IFN-γ were dose-dependently inhibited by CucE. The mRNA levels of these cytokines in activated Jurkat T cells were also decreased upon CucE treatment. CucE decreased the phosphorylation levels of inhibitor of κB (IκB) and NF-κB/p65 in PDB+Ion stimulated cells. The nuclear translocation of NF-κB/p65 was also significantly suppressed in the presence of CucE. The phosphorylation of p38 MAPK, JNK and Erk1/2 was not decreased by CucE treatment.

The effects of CucE as a dietary supplement on oxidative stress, inflammation, and immunosuppression induced by excessive exercise were validated in rat and cynomolgus monkey models. The validation results of different species models are basically consistent, especially in non-human primates, indicating the application potential of this CucE.

CONCLUSION

Our study found that administration of CucE could efficiently inhibited the expression of some cytokines with negative effects, such as TNF-α, and down-regulated the NF-κB signaling pathway, suggesting that CucE has certain potential in regulating adaptive immune function and suppressing inflammatory response. In the follow-up study, we will investigate the long-term safety as well as acute toxicity of CucE administration under large dose. We will explore the combined administration of CueE with other dietary fiber and anti-inflammatory foods or as one of the additives in sports drinks for the better application prospect for alleviating athletic inflammation and immunosuppression in caused by regular high intensity training.

REFERENCES

AKPINAR S. 2016. Decreased interlimb differences in female basketball players. J Sports Med Phys Fitness 56: 1448-1454.

BLUME K, KORBER N, HOFFMANN D & WOLFARTH B. 2018. Training load, immune status, and clinical outcomes in young athletes: a controlled, prospective, longitudinal study. Front Physiol 9: Article 120.

BONCI LJ. 2011. Eating for performance: bringing science to the training table. Clin Sports Med 30: 661-670.

CAMPBELL KA, LIPINSKI MJ, DORAN AC, SKAFLEN MD, FUSTER V & MCNAMARA CA. 2012. Lymphocytes and the adventitial immune response in atherosclerosis. Circ Res 110: 889-900.

CANIS AV & SANTOS RVT. 2019. Performance and altitude: ways that nutrition can help. Nutrition 60: 35-40.

CHEN XP, BAO JL, GUO JJ, DING Q, LU JJ, HUANG MQ & WANG YT. 2012. Biological activities and potential molecular targets of cucurbitacins: a focus on cancer. Anti-Cancer Drug 23: 777-787.

DOHERTY R, MADIGAN S, WARRINGTON G & ELLIS J. 2019. Sleep and nutrition interactions: implications for athletes. Nutrients 11: 822-834.

FRANCHINI E, DEL VECCHIO FB, MATSUSHIGUE KA & ARTIOLI GG. 2011. Physiological profiles of plite judo athletes. Sports Med 41: 147-166.

HAMLIN MJ, WILKES D, ELLIOT CA, LIZAMORE CA & KATHIRAVEL Y. 2019. Monitoring training loads and perceived stress in young elite university athletes. Front Physiol 10: Article 34.

HSU CW, LI SC, CHANG NY, CHEN ZW, LIAO JW, CHEN TH, WANG JP, LIN JH & HSUAN SL. 2016. Involvement of NF-κB in regulation of Actinobacillus pleuropneumoniae exotoxin ApxI-induced proinflammatory cytokine production in porcine alveolar macrophages. Vet Microbiol 195: 128-135.

HUI Y, HUA JL & WANG C. 2019. Anti-oxidation and anti-aging activity of polysaccharide from Malus micromalus Makino fruit wine. Int J Biol Macromol 121: 1203-1212.

HUNSAKUNACHAI N, NUENGCHAMNONG N, JIRATCHARIYAKUL W, KUMMALUE T & KHEMAWOOT P. 2019. Pharmacokinetics of cucurbitacin B from Trichosanthes cucumerina L. in rats. BMC Complement Altern Med 19: 157168.

HUSSAIN H, GREEN IR, SALEEM M, KHATTAK KF, IRSHAD M & ALI M. 2019. Cucurbitacins as anticancer agents: a aatent review. Recent Pat Anti-Canc 14: 133-143.

JEVTIC B, DJEDOVIC N, STANISAVLJEVIC S, DESPOTOVIC J, MIJKOVIC D & TIMOTIJEVIC G. 2016. Cucurbitacin E potently modulates the activity of encephalitogenic cells. J Agr Food Chem 64: 4900-4907.

KHANNA K, MISHRA KP, GANJU L, KUMAR B & SINGH SB. 2018. High-altitude-induced alterations in gut-immune axis: a review. Int Rev Immunol 37: 119-126.

LUI VW ET AL. 2009. Cucurbitacin I elicits anoikis sensitization, inhibits cellular invasion and in vivo tumor formation ability of nasopharyngeal carcinoma cells. Carcinogenesis 30: 2085-2094.

MCLEOD IX & HE Y. 2010. Roles of autophagy in lymphocytes: reflections and directions. Cell Mol Immunol 7: 104-107.

MURTAZA M, KHAN G, AFTAB MF, AFRIDI SK, GHAFFAR S, AHMED A, HAFIZUR RM & WARAICH RS. 2017. Cucurbitacin E reduces obesity and related metabolic dysfunction in mice by targeting JAK-STAT5 signaling pathway. PLoS ONE 12: e0178910.

OIKAWA D, AKAI R, TOKUDA M & IWAWAKI T. 2012. A transgenic mouse model for monitoring oxidative stress. Sci Rep-Uk 2: 229-236.

OZKAN G, KAMILOGLU S, OZDAL T, BOYACIOGLU D & CAPANOGLU E. 2016. Potential use of turkish medicinal plants in the treatment of various diseases. Molecules 21: 257-288.

PEDERSEN L, ELERS J & BACKER V. 2011. Asthma in elite athletes: pathogenesis, diagnosis, differential diagnoses, and treatment. Phys Sportsmed 39: 163-171.

PINTO JP ET AL. 2014. Physiological implications of NTBI uptake by T lymphocytes. Front Pharmacol 5: Article 24.

PLOSZCZYCA K, LANGFORT J & CZUBA M. 2018. The effects of altitude training on erythropoietic response and hematological variables in adult Athletes: a aarrative review. Front Physiol 9: Article 375.

QUINDRY J, DUMKE C, SLIVKA D & RUBY B. 2016. Impact of extreme exercise at high altitude on oxidative stress in humans. J Physiol-London 594: 5093-5104.

SATO K, IEMITSU M, KATAYAMA K, ISHIDA K, KANAO Y & SAITO M. 2016. Responses of sex steroid hormones to different intensities of exercise in endurance athletes. Exp Physiol 101: 168-175.

SHEN HT, DE ALMEIDA PE, KANG KH, YAO P & CHAN CW. 2012. Burn injury triggered dysfunction in dendritic cell response to TLR9 activation and resulted in skewed T cell functions. PLoS ONE 7: e50238.

SHI CC, LI HP, YANG YF & HOU LF. 2015. Anti-Inflammatory and Immunoregulatory Functions of Artemisinin and Its Derivatives. Mediat Inflamm 2015: 1-7.

SINHA S, RAY US, TOMAR OS & SINGH SN. 2009. Different adaptation patterns of antioxidant system in natives and sojourners at high altitude. Resp Physiol Neurobi 167: 255-260.

VAJAPEY R, RINI D, WALSTON J & ABADIR P. 2014. The impact of age-related dysregulation of the angiotensin system on mitochondrial redox balance. Front Physiol 5: Article 439.

WALSH NP. 2018. Recommendations to maintain immune health in athletes. Eur J Sport Sci 18: 820-831.

WANG L, LI C, LIN Q, ZHANG X & HE X. 2015. Cucurbitacin E suppresses cytokine expression in human Jurkat T cells through down-regulating the NF- B signaling. Acta Bioch Bioph Sin 47: 459-465.

WEI XT, SONG AM & XUE B. 2003. Effect of cadmium chloride on the immunocyte proliferation and cell apoptosis. J Environ Occupat Med 20: 411-414.

YAO W, YI L, XIAO-YU Z, LI-HUI X, DONG-YUN O, KUN-PENG L, HAO P, JIAN H & XIAN-HUI H. 2014. Ginsenoside Rg1 regulates innate immune responses in macrophages through differentially modulating the NF-κB and PI3K/Akt/mTOR pathways. Int Immunopharmacol 23: 77-84.

ZHAO XZ, LI FS, SUN WQ, GAO L, KIM KS, KIM KT, CAI L, ZHANG ZG & ZHENG Y. 2016. Extracts of magnolia species-induced prevention of diabetic complications: a brief review. Int J Mol Sci 17: 1629-1639.

How to cite

XIE H , TUO X, ZHANG F, BOWEN L, ZHAO W & XU Y. 2020. Dietary cucurbitacin E reduces high-strength altitude training induced oxidative stress, inflammation and immunosuppression. An Acad Bras Cienc 92: e20200012. DOI 10.1590/0001-3765202020200012.

Manuscript received on January 6, 2020;
accepted for publication on February 19, 2020

HEZHI XIE [1 #]
https://orcid.org/0000-0002-3871-5381

XINLING TUO [2 #]
https://orcid.org/0000-0002-6851-2948

FENG ZHANG[3]
https://orcid.org/0000-0002-3533-5420

LAURA BOWEN[3,4]
https://orcid.org/0000-0002-1784-7430

WEI ZHAO [5, 6]
https://orcid.org/0000-0001-8781-7192

YUYOU XU [5]
https://orcid.org/0000-0002-9379-4529

[1]Department of sports training, Guangzhou Sport University, Guangdong 510000, China

[2]Department of sports, Guangdong Mechanical and Electrical Polytechnic, Guangdong 510000, China

[3]Guangdong Provincial Youth Basketball Training Base, Guangdong 510000, China

[4]School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Edgbaston B15 2TT, UK

[5]Physical Training Research Center, Guangdong Justice Police Vocational College, Guangdong 510000, China

[6] Sports Medicine Center, Soochow University, Jiangsu 2150000, P.R. China

Correspondence to: **Yuyou Xu**
E-mail: yyx@ncstmc.cn
[#]These authors are regarded as co-first authors.

Author contributions

H.X; Designed the project, analyzed the data and wrote the manuscript. X.T and L.B; Performed the experiments and collected the relevant materials. F. ZH and W. ZH; Performed the biochemical analysis and interpreted the data. Y.X; Designed the project and supervised the research. All authors discussed the results and approved the final version of the manuscript.