Original Article

Mechanism of the Huangguoshu waterfall forest environment's promotive effect on human health in Guizhou, China

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Background: The aim of this study was to investigate the specific mechanisms underlying the human health-promoting effects of the forest environment at Huangguoshu Falls, Guizhou, China.

Methods: Ninety-five participants were recruited and an eye tracker was used to record fixation and sweep indices. A questionnaire was also used to evaluate the effects of different subject environments on human emotions, perceived recovery and preferences. Thereafter, 24 participants with chronic fatigue syndrome (CFS) were recruited and the participants' fatigue and stress-related scale indices and inflammatory factor levels were examined. Serum metabolites of the participants under different time waterfall forest interventions were detected by ultra performance liquid chromatography-quadrupole-time of flight mass spectrometry (UHPLC-Q/TOF-MS).

Results: Eye tracking paradigm analysis showed that the "waterfall" element was the most interesting element for participants and that the "charm" of the waterfall forest environment could be well perceived by participants. Scores on the Fatigue Scale, Anxiety Scale and Depression Scale decreased as the duration of treatment in the waterfall forest environment increased. Levels of inflammatory factors decreased after treatment in the waterfall forest environment. At the same time the level of antioxidants, represented by L-ascorbic acid, increased significantly.

Conclusions: The charm of the Huangguoshu waterfall scenery could be perceived by the participants and have a positive modulating effect on mood and cognitive function. In addition, the unique mixture of negative oxygen ions in this environment can increase the content of endogenous antioxidants and balance the metabolism of choline and amino acids.

Keywords: Huangguoshu waterfall; healing environment; natural therapy; chronic fatigue syndrome (CFS); waterfall forest environment

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Introduction

Environmental change is closely related to human health (1,2). Several studies have revealed that a natural environment can provide a more significant promotive effect on human health than can a green urban landscape environment or a virtual environment of natural pictures. Even very short contact with the natural environment or just looking out of the window at natural scenery can

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help individuals improve their emotional and mental state, increase their attention, and thereby significantly enhance their cognitive ability (3-5). Other research has indicated that the natural environment is not only suitable pretherapeutically for the adjuvant therapy of some patients with chronic diseases, but can also serve preventively in the emotional or physiological regulation of healthy populations (6-8). As early as the 1990s, Japanese scholars were positing the concept of the "forest bath", which is a kind of implicit experience in a natural environment, especially a forest one. It was suggested that in the forest, people can enjoy the fragrance emitted by the forest and "suck" some of the substances and natural aromatics of the forest via the mouth and skin to further stimulate their body and mind, while perceiving the shape, color, and sound of animals and plants. This would ease the mind, dispel fatigue, relieve stress, and cultivate a healthy complexion (9). Relevant studies in Germany have demonstrated that the natural environment can contribute to releasing stress, and Germany has taken the lead in establishing the first forest recreation sites in the world (10-13). At present, the concept of a healing environment is a more comprehensive generalization and summary of "forest bath" and "natural therapy", which makes the understanding of the medicalization of natural environmental resources more diverse and profound (12,13). Based on Germany's experience, Japan has developed various alternative therapies based on forest resources, including horticultural therapy, wilderness healing and climatic terrain therapy, and other "nature-based medicine" approaches. The Japanese government has developed a variety of alternative therapies based on forest resources, incorporating "nature-based medicine" such as horticultural therapy, wilderness healing and climatic terrain therapy. Existing research in forest medicine is mainly in the service of Japanese forest therapy, but forest medicine cannot support a wide range of complementary alternative therapies using forests. Therefore, there is an urgent need to reorganize the theory of forest and health from a practical point of view, and to provide systematic support for practice. For the first time, this study explored the promotive effect and underlying mechanism of the Huangguoshu waterfall forest environment in Guizhou Province, China (hereafter referred to the as waterfall forest environment) on human psychology (emotion and cognition) and physiology (chronic fatigue state).

Research site

Huangguoshu waterfall is the largest waterfall in Asia, with a height of 77.8 meters and a width of 101 meters. The geographical coordinates are 105°35′–105°42′ E and 25°52′–26°02′ N. The average altitude is about 900 meters and the annual average temperature is 16 ℃. Huangguoshu waterfall is rich in water resources and is located in a subtropical plateau monsoon humid climate with temperate weather and abundant rainfall. The scenic spot has a high forest vegetation coverage and is characterized by a surface environment of carbonate rocks with many algae, mosses, and vascular plants. Hence, Huangguoshu waterfall is an ideal site for researching a healing environment (14). We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3787/rc).

Methods

Part I

Recruitment of healthy participants

The recruitment of participants and the experimental processes were supervised by the ethics committee. In this study, participants were recruited through online poster advertising or by on-the-spot recruitment. Before the experiment, the participants signed written consent of voluntary participation and filled in a statistical table of personal information. The experiment recruited a total of 104 participants. Due to failures in data recording, incomplete questionnaires, and other factors, the data of 9 samples were eliminated, and 95 valid samples were finally obtained. The inclusion and exclusion criteria are shown in Appendix 1.

Experiment process of data recording by eye tracker

Each participant anonymously filled in a basic information form. Each participant was required to complete experiments at 3 research sites. The selected research sites were all in the Huangguoshu waterfall scenic area, and each site had unique landscape elements. The specific environments of the 3 research sites are shown in *Figure 1*. The photos were taken on a sunny day in the summer from 11 a.m. to 2 p.m., showing the natural and artificial landscapes under similar light.

The participants randomly visited each research site. The eye-tracking data collected in this part of the experiment involved binocular tracking using Tobii Pro Glasses 3 (Tobii, Danderyd, Sweden). The observation time of participants wearing the eye tracker at each

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Figure 1 Panoramic view of the 3 different research sites. (A) Waterfall forest environment, (B) park grass environment, (C) urban street environment.

interaction point was 1 minute.

Scale detection of mood changes

The profile of mood states (POMS) scale was used to determine the restoration of perceptual ability of participants under different environments.

Scale detection of psychological restoration

The Perceived Restorativeness Scale (PRS) was used to evaluate the psychological restoration of participants. The scale consists of 18 items, including perceptions of environmental fascination, being away, extension, and compatibility perception. Each item was accurately translated into Chinese and assessed on a 5-point Likert scale ranging from 1= "not agree at all" to 5= "very much agree". Each participant was required to perceive only 1 selected environment. The average duration of the test per participant is about 10 minutes.

Detection of individual environmental preference

The 5-point Likert scale was used to assess the participant's preference level for interaction points (ranging from 1= "extremely dislike" to 5= "extremely like").

Part II

Recruitment of patients with chronic fatigue syndrome (CFS)

Patients with CFS diagnosed in the psychology and psychiatric outpatient departments of the Affiliated Hospital of Guizhou Medical University and Guizhou Provincial People's Hospital from January 2010 to January 2019 were recruited. All patients met the 2003 CFS criteria of the US Centers for Disease Control and Prevention (CDC). Among the 32 patients with CFS aged 20 to 55 years, a total of 24 participants who met the inclusion criteria and voluntarily agreed to participate in this study were finally selected. Chronic fatigue is the intermediate state between physiological fatigue and pathological fatigue. Therefore, the determination of chronic fatigue not only needs to exclude physiological fatigue (the characteristics of easy recovery after rest; i.e., short-term fatigue), but also needs to exclude organic diseases, mental diseases (such as depression), and other related diseases. With the help of professional psychologists, the perception ability and psychological changes of participants were evaluated before enrollment. The inclusion criteria referred to the diagnostic criteria of CFS formulated by Centers for Disease Control and Prevention (CDC) in 1994. The specific inclusion, exclusion, and withdrawal criteria are shown in Appendix 2.

Experimental grouping

The eligible participants with the same demographic characteristics were divided into 2 groups. A random number generator was adopted to assign numbers between 1 and 24 for demographic analysis of participants. The study was fully explained to all participants in writing, with special emphasis on the study's purpose, the precise procedure to be used, and any possible adverse events.

Experimental procedures

The detailed daily schedule was as *Figure 2*.

Other variables and the means by which they were controlled are described below: (I) drinking and smoking were restricted 24 hours before and throughout the

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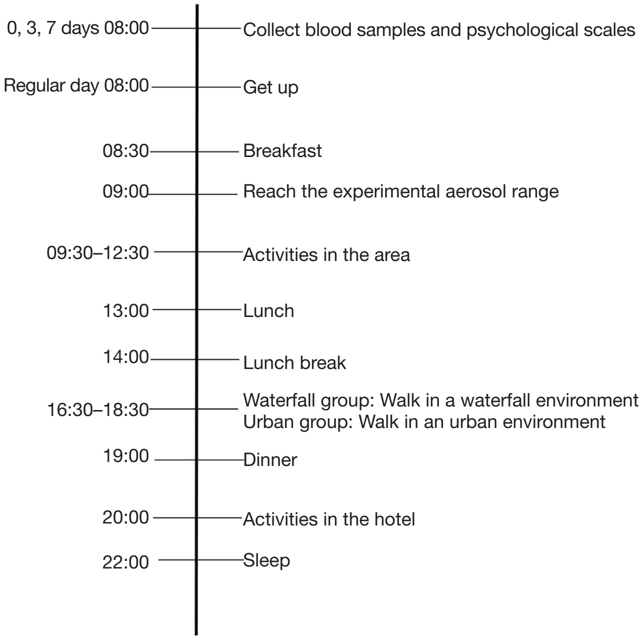

Figure 2 The detailed daily schedule.

experiment; (II) an 8-hour fast was required 8 hours before blood drawing in each experiment; (III) no other therapeutic regimens, including other types of natural environment therapy, were implemented; (IV) excessive activity outside the experimental area was limited; (V) excessive use of electronic mobile devices (more than 8 hours per day) or unhealthy daily habits, such as staying up late, were discouraged; and (VI) a regular diet was provided throughout the experimental period. On this basis, all participants in the waterfall group went to Huangguoshu waterfall scenic spot for natural environmental intervention. All the participants in the waterfall group stayed at a hotel (1,024 m above sea level) 500 m near the core scenic spot of Huangguoshu, and 3 meals a day were arranged by the hotel.

The participants in the urban group were required to work with a routine workload every day and to carry out activities in the urban landscape area within the same planning time. The diet was provided according to the scheme formulated by the research group to ensure daily balanced nutrition. All participants were required to wear the same type of fitness tracker during the experimental period that would detect the daily sleep time/quality and daily activity intensity to ensure that there was no statistical difference in the general value of these measures between participants.

Psychological scales

The fatigue scale (FS-14), Hamilton Anxiety Scale (HAMA), and Hamilton Depression Scale (HAMD) were scored before and after convalescence in the different environments.

Cognitive function

The paced auditory serial addition task (PASAT) was used to measure the changes in the cognitive level of the participants before and after convalescence in different environments.

Inflammatory and antioxidant indexes

One day and three days before the beginning of the experiment and the next day after the end of the experiment, 5.0 mL of elbow vein blood was taken from all participants and centrifuged at 3,000 ×g for 15 minutes to collect the supernatant. The levels of glucose, triglyceride, total cholesterol, and uric acid were detected using a biochemical analyzer within 24 hours. Cortisol (Elabscience, Wuhan, China), 5-hydroxytryptophan (5-HT)l (Elabscience, Wuhan, China), and antioxidant indexes were detected using enzyme-linked immunosorbent assay (ELISA) kitsl (Jiancheng, Nanjing, China).

Untargeted metabolomics

The number of subjects enrolled in each group in this experiment was small, if comparing the changes in serum metabolites in other settings. The accuracy of the results is reduced due to individual differences in the situation. Therefore, we applied the metabolomic results of waterfall environment subjects for their own different intervention time control. Full spectrum analysis of the serum samples from participants exposed to the waterfall forest environment at baseline, 3 days, and 7 days was performed using the ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UHPLC-Q/TOF-MS). XCMS software was used to extract peaks and identify differential metabolites. Principal component analysis and discriminant analysis were conducted to screen differential metabolites. The Kyoto Encyclopedia of Genes and Genomes (KEGG) website was used for enrichment analysis of the 3 groups of differential metabolites to clarify the signal pathways that might be affected by the waterfall

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environment.

Statistical analysis

SPSS 24.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The counting data of the demographic characteristics of the participants are expressed in the number of cases (n) or percentage (%). The data of each group were subjected to normal distribution analysis and a test for homogeneity of variance before statistical analysis. For the data in normal distribution, comparisons of the mean of multiple samples were completed using 1-way analysis of variance (ANOVA), while the comparisons between groups were conducted using independent samples *t*-test.

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Medical Ethics Committee of Guizhou Provincial People's Hospital [No. (2019)60] and informed consent was taken from all the participants. The Affiliated Hospital of Guizhou Medical University was also informed and agreed with the study.

Results

General characteristics of healthy participants

The data sets from 95 valid questionnaires were imported into GraphPad software (GraphPad Software Inc., La Jolla, CA, USA) for statistical analysis (*Table 1*).

Fixation heat map and fixation trajectory map

A fixation heat map was used to display visual attention in color. The relationship between attention, attention time, and

Table 1 General characteristics profile of the participant group

Items	Range	Cases	Percentage (%)
Age (years)	<25	4	4.2
	25–30	26	27.37
	31–40	33	34.73
	41–50	20	21.05
	51–60	7	7.37
	>60	5	5.26
Education	Junior high school education or below	2	2.11
	Senior middle school (vocational and technical school)	16	16.84
	Bachelor's degree	61	64.21
	Master's degree or above	16	16.84
Occupation	Office or institution staff	71	74.74
	Technical or research personnel	11	11.58
	Freelancer	9	9.47
	Student	2	3.16
	Retiree	3	2.11
Daily sleep time	≤6 hours	15	15.79
	>6, ≤7 hours	38	40.00
	>7, <8 hours	37	38.95
	≥8 hours	5	5.26

Table 1 (*continued*)

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Table 1 (*continued*)

Items	Range	Cases	Percentage (%)
Frequency of recent tiredness (3 months)	Never	16	16.84
	Occasionally	42	44.21
	Sometimes	16	16.84
	Often	19	20.00
	Always	2	2.11
Exercise frequency	Occasionally	62	65.26
	Once a week	12	12.63
	2–3 times/week	13	13.68
	4–5 times/week	8	8.42
Exercise type	Mainly walking and running	–	
Exercise time	About 1 hour	–	
Smoking frequency	Never	74	77.89
	Occasionally (1–3 cigarettes/week)	6	6.32
	1–5 cigarettes/day	5	5.26
	6–10 cigarettes/day	4	4.21
	11–20 cigarettes/day	3	3.16
	More than 20 cigarettes/day	1	1.05
	Quit smoking	2	2.11
Recent feeling of anxiety, depression, or other psychological disorders	Yes	23	24.21
	No	72	75.79

color was red > yellow > green. The fixation trajectory was presented in the form of dots. The numbers in the trajectory map represented the trajectory order, and the larger the value, the later it was noticed. The more the numbers overlapped, the more times the participant looked back and the more attractive the element was to the participant. According to the fixation heat map of the waterfall forest environment, the participants paid the highest attention to the waterfall in the waterfall forest environment, and the fixation trajectory was relatively concentrated (*Figure 3*). The heat map of the park grassland environment showed that the fixation behavior of participants was jumping, indicating that the novelty of the environment was weak and the exploration desire of the participants was not strong (*Figure 4A,4B*). The participants' fixation in the urban street environment was messy and the trajectory dots were scattered, suggesting that the participants had difficulties in concentrating and failed to recover energy in this environment (*Figure 4C-4F*).

Eye-tracking analysis

Eye-tracking can tell the researchers exactly what subjects are looking at, so there's no need for self-reporting and guesswork. The eye movement of participants at 3 different research sites was analyzed. The participants showed the highest fixation count and frequency in the waterfall forest environment and the lowest fixation count and frequency in the urban street environment. The participants had the strongest information processing ability in the waterfall forest environment. Compared with the other 2 environments, the participants in the waterfall forest environment had the highest saccade count, as well as the fastest rate and the widest amplitude in unit time, suggesting that the participants were energetic in the waterfall forest environment and had a desire to explore the

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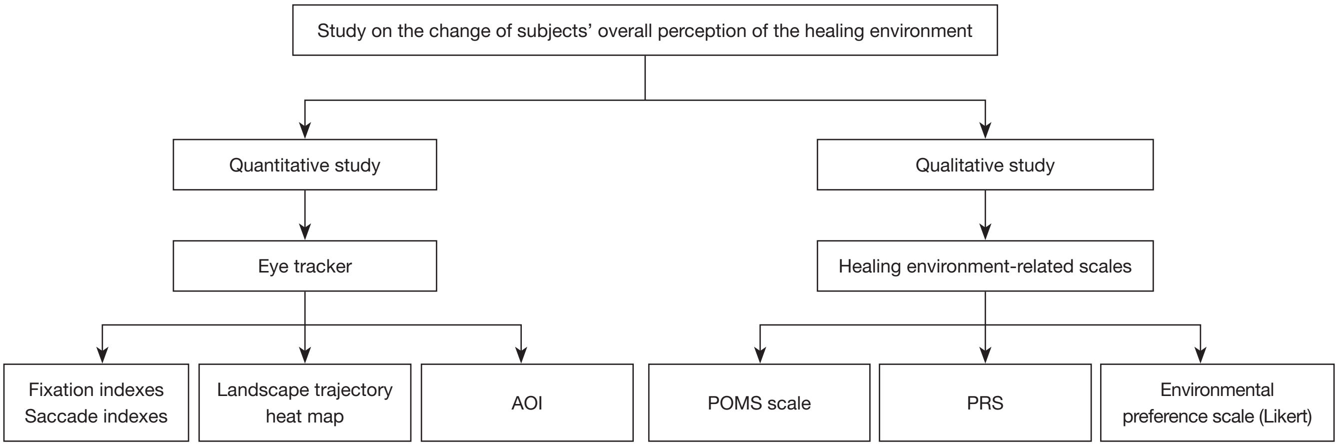

Figure 3 Environmental evaluation indicators for short-term visits to the waterfall forests environment. AOI, area of interest; POMS, profile of mood states; PRS, perceived restorative scale.

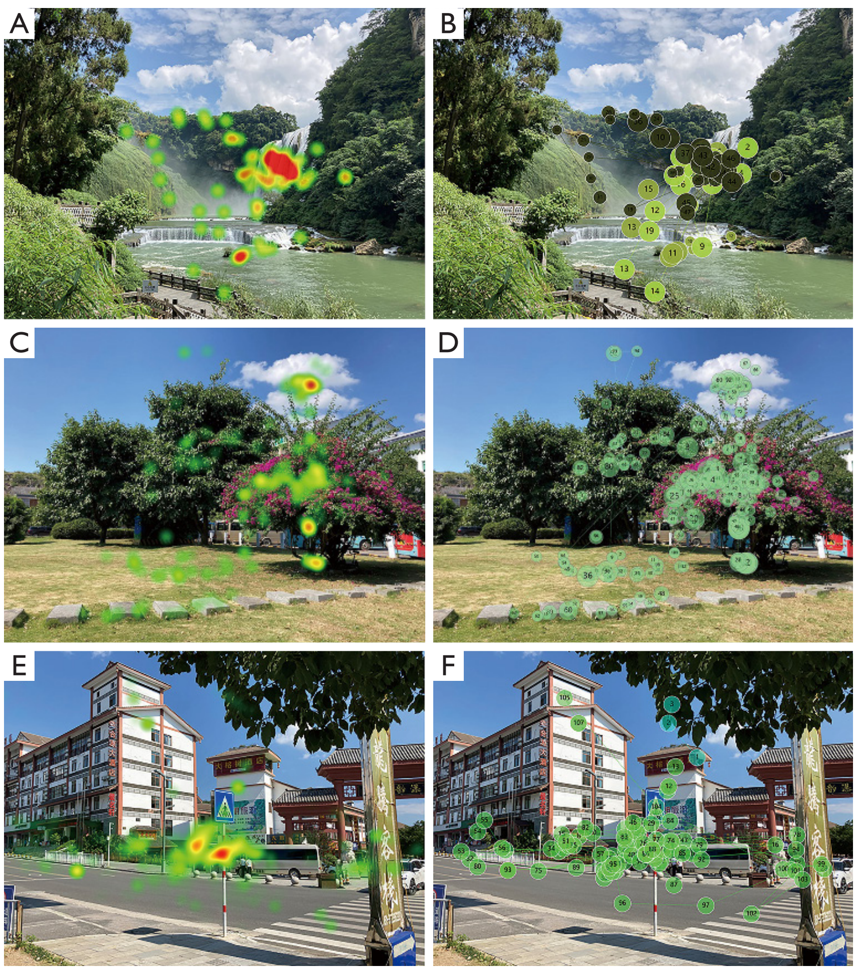

Figure 4 Eye movement diagram of the waterfall forest environment by using fixation heat map (A) and fixation trajectory map (B), and eye movement diagram of the park grass environment by using fixation heat map (C) and fixation trajectory map (D), and eye movement diagram of the urban street environment by using fixation heat map (E) and fixation trajectory map (F).

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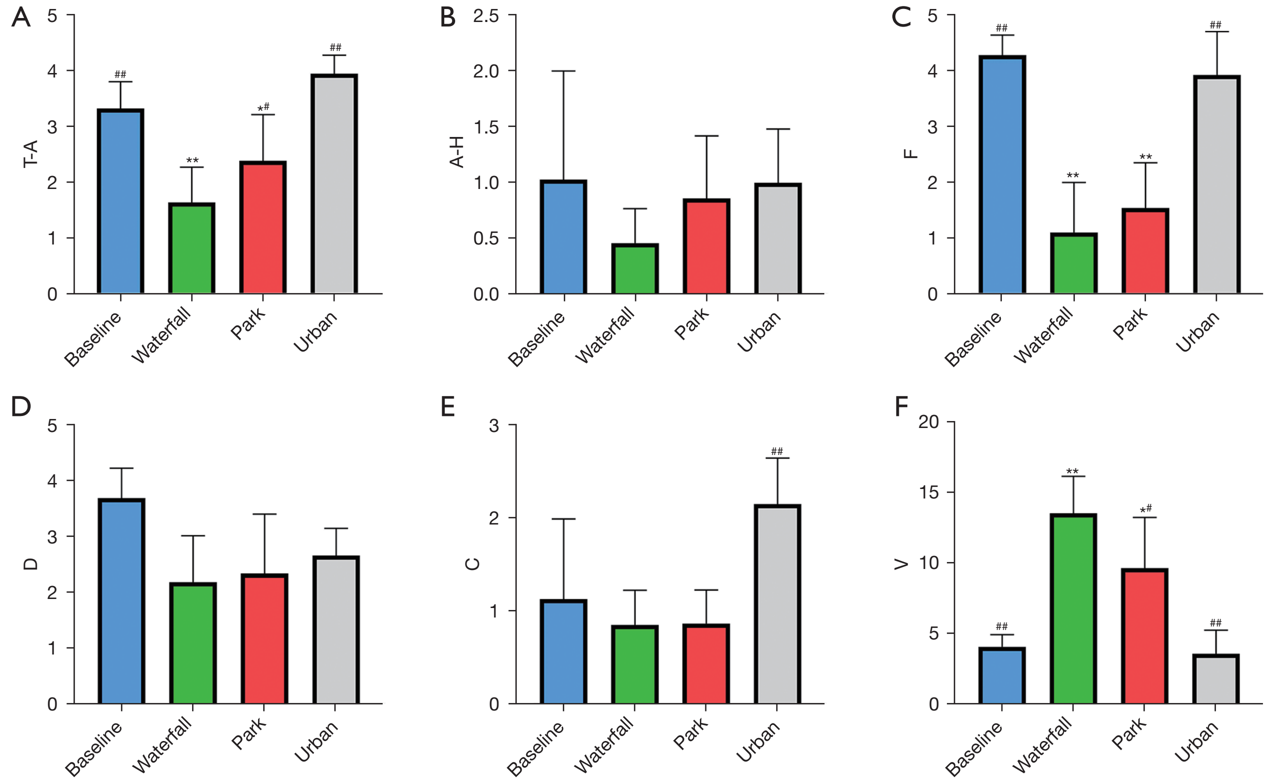

Figure 5 Three landscape environments with different AOI divisions. (A,D) Waterfall forest environment, (B,E) park grass environment, and (C,F) urban street environment. *, P<0.05 and **, P<0.01 indicate significant difference between other group and baseline; $^{\#}$, P<0.05 and $^{\#\#}$, P<0.01 indicated that there were significant differences between the Waterfall and other groups. AOI, area of interest; T-A, tension-anxiety; A-H, anger-hostility; F, fatigue; D, depression; C, confusion; V, vigor.

Table 2 Eye-tracking paradigm of the 3 research sites ($\bar{x}$ ± SD)

Testing sites	Fixation count/times	Fixation frequency (times/s)	Saccade count/times	Saccade rate/(°/s)	Saccade amplitude°
Waterfall forest	83.33±13.4$^{\#\#}$	1.35±0.28	55.66±8.73	205.33±9.07$^{\#\#}$	16.42.33±1.05$^{\#\#}$
Park grassland	64.53±6.35**	1.19±0.06	50.19±13.22	179.46±18.87**	12.34±1.04**
Urban street	47.24±11.54**	1.00±0.17	61.5±7.50	156.00±2.64**$^{\#\#}$	9.20±1.14**$^{\#\#}$

**, P<0.01 indicated a significant difference between each group and the waterfall forest group; $^{\#\#}$, P<0.01 indicate that there a significant difference between each group and the park grassland group. SD, standard deviation.

scene's elements.

Areas of interest analysis

A regression count was used to represent the degree of interest in a given element module, with the regression count being directly proportional to the degree of preference. The eye-tracking analysis revealed that each landscape scene had 5 areas of interest (AOIs) with the most concentrated data, as shown in *Figure 5*. After the eye-tracking data is collected, the device is turned off, the Secure Digital (SD) memory card is removed from the recording unit (RU), inserted into the card reader, and all the data on the SD card is copied or cut to the computer with the Tobii Pro Lab.Obtain the Tobii Lab software installation package from the official Tobii Pro website (https://www.tobiipro.com/zh/) The proportion of regression count in different AOIs is shown in *Tables 2,3*.

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Table 3 Regression count of different AOIs in percentages

Research sites	AOI	Regression count	Proportion
Waterfall forest	Waterfall	37	46.83
	Forest	16	20.25
	Sky	2	2.53
	Water	18	22.79
	Plank road	6	7.60
Park grassland	Tree	8	13.57
	Flower	16	27.12
	Sky	21	35.59
	Grassland	7	11.86
	Plank road	7	11.86
Urban street	Street	3	6.38
	Sign	10	21.28
	Vehicle	16	34.05
	Advertisement	7	14.89
	Tree	3	6.38
	Building	11	23.40

AOI, area of interest.

Analysis of participants' mood changes in different landscape environments

The POMS scale was used to evaluate the mood changes of participants in the waterfall forest environment, park grass environment, and urban street environment. This scale is specifically shown in the Appendix 3 of the paper and includes elements of tension-anxiety (T-A), depression (D), anger-hostility (A-H), vigor (V), fatigue (F), confusion (C), and friendliness.

The participants in the waterfall forest environment showed the lowest T-A score of 1.65±0.63, while the participants in the urban street environment had the highest T-A score of 3.95±0.33 ($P<0.01$), indicating that the participants were most relaxed in the waterfall forest environment and that this environment temporarily eliminated the experience of inner tension. In terms of A-H, the scores ranked as follows: baseline > urban street > park grassland > waterfall forest. The results were not statistically different among the groups, but the waterfall forest environment showed the most obvious effect on improving anger. In terms of F, both the waterfall forest environment and park grassland environment effectively improved the fatigue state of participants. However, the score of participants in the waterfall forest environment was relatively lower, suggesting that fatigue improvement was more effective. In terms of D, the natural environments had the tendency to reduce depression, but there was no significant difference. In terms of C, the scores ranked as follows: urban street > baseline > park grassland > waterfall forest. The urban street environment with more dynamic landscapes (vehicles and pedestrians) increased the participants' inner confusion, while the waterfall forest environment made the participants' inner experience more calm. In terms of V, compared with the baseline, the score of participants in the waterfall forest environment were significantly increased, suggesting that the waterfall forest landscape was conducive to the recovery of vigor in these participants. After a comprehensive comparison of the 3 environments, it was found that the mood state of participants had deteriorated after they visited the urban street environment and that this was accompanied by a downward trend of positive emotions and confused thoughts; compared with the park grassland environment, the waterfall forest environment significantly decreased the participants' negative emotion indexes and increased the participants' positive emotions, as shown in *Table 4* and *Figure 6*.

Analysis of restorative perception in different landscape environments

The participants' restorative perception in different landscape environments was statistically analyzed. The evaluation scale was composed of 4 dimensions comprising a total of 18 items. Each item had 5 options, and the score was between 1 and 5: the higher the score, the stronger the perception degree of the item. The scale involved perceptions of fascination, being away, extension, and compatibility of the landscape environment. The specific statistics are shown in *Table 5*. There were significant differences in the perception of the participants across the 3 landscape environments. The perception level of participants was the highest in the waterfall forest landscape environment and the lowest in the urban street environment. The participants' perception of the waterfall forest environment was generally good. In terms of fascination, the average perception of participants were greater than 4, suggesting that the waterfall forest environment had a strong attraction to the participants;

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Table 4 Changes in POMS scores in the different environments ($\bar{x}$±s)

Environments	T-A	A-H	F	D	C	V
Baseline	3.33±0.47	1.03±0.97	4.25±0.36	3.68±0.53	1.13±0.85	4.13±0.86
Waterfall	1.65±0.63**	0.46±0.31	1.10±0.89**	2.18±0.82	0.86±0.37	13.61±2.62**
Park	2.39±0.83*#	0.86±0.56	1.54±0.80**#	2.33±1.06	0.87±0.36#	9.71±3.59*#
Urban	3.95±0.33##	1.00±0.48	3.90±0.77**##	2.66±0.48	2.15±0.49#	3.64±1.67**##

Note: *, P<0.05 and **, P<0.01 indicates a significant differences between groups compared with baseline; #, P<0.05 and ##, P<0.01 indicates a significant difference between groups compared with the waterfall group. POMS, profile of mood states; T-A, tension-anxiety; A-H, anger-hostility; F, fatigue; D, depression; C, confusion; V, vigor.

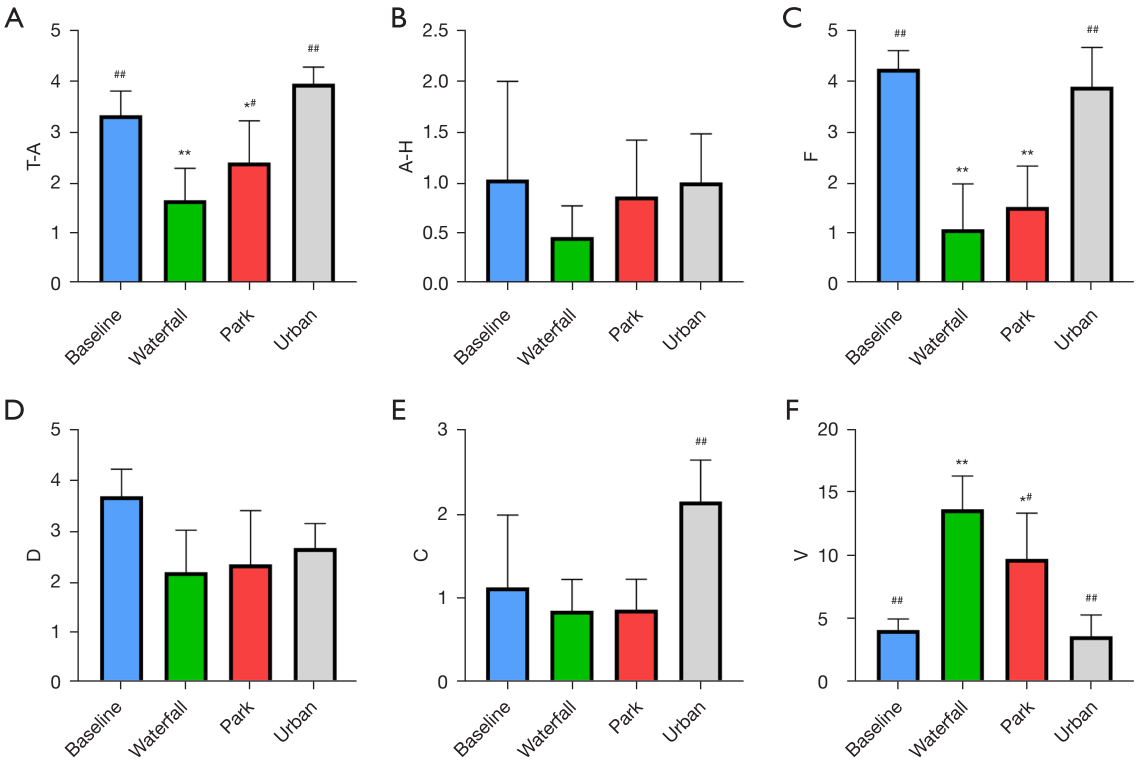

Figure 6 Changes in POMS scores in different environments. (A) T-A for tension scale, (B) A-H for anger, (C) F for fatigue, (D) D for depression, (E) C for confusion, and (F) V for vigor. *, P<0.05 and **, P<0.01 indicate significant differences between groups compared with baseline; #, P<0.05 and ##, P<0.01 indicate significant differences between groups compared with the waterfall group. POMS, profile of mood states; T-A, tension-anxiety; A-H, anger-hostility; F, fatigue; D, depression; C, confusion; V, vigor.

the participants were easily attracted by the waterfall forest and were willing to spend more time watching the waterfall, which was consistent with the Area of Interest (AOI) results in the analysis of eye-tracking data. Regarding the perception of being away, the perception degree of participants was high in the waterfall forest environment, but there were still differences in individual perception. The participants' perception scores of "very different from daily life" and "being able to alleviate the pressure in life and work" were high, indicating that the participants had a high perception that the waterfall forest environment contributed to relaxing the tension in daily life and releasing the pressure in life and work. Regarding extension perception, the score of "the constituent elements of the landscape are matched" of participants in the park grassland environment was higher than that in the waterfall forest

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Table 5 Perception of restorativeness in the urban forest environment ($\bar{x}$ ± SD)

Dimensions	Items	Waterfall	Park	Urban
Fascination	The landscape has its own characteristics	4.83±0.17	3.93±0.23	1.66±0.43
	Attention is drawn to many interesting things	3.67±0.33	2.01±1.43	1.19±0.86
	The urban forest environment is charming	3.33±0.79	2.38±0.28	0.5±0.04
	Desire to spend more time in the urban forest	3.27±1.07	2.50±0.55	1.50±1.03
Being away	A feeling of being free from worldly vision and not being bound	4.05±2.58	3.17±1.50	0.83±0.03
	Being able to temporarily stop thinking about the demands of life	3.58±1.30	3.76±1.36	0.67±0.62
	Being different from the environment of daily life	4.67±1.84	1.33±0.64	0.37±0.88
	Being able to relax a tense mood	3.83±1.84	2.13±0.57	0.22±0.05
	Being able to relieve stress in life and work	4.06±0.65	4.00±0.16	1.12±0.44
Extension	The constituent elements of the landscape are matched	3.27±0.47	3.87±0.58	4.05±0.37
	The landscape and surrounding facilities are coordinated	4.17±1.06	4.00±0.91	2.67±0.28
	Many places worth exploring and discovering	3.83±1.89	1.50±0.18	0.67±0.30
	Being able to extend many beautiful associations	3.83±1.73	1.67±0.98	0.89±0.90
	The overall environment and natural resources can meet the recreational needs	3.83±0.67	26.03±0.67	0.17±0.59
Compatibility	Being able to enjoy yourself	4.33±0.81	3.83±1.73	1.83±0.34
	Being able to quickly integrate into the urban forest environment	2.45±0.81	2.77±0.75	2.67±1.24
	The activities will not be inconsistent with the environment	3.19±1.01	3.40±0.69	0.33±0.04
	Having a sense of belonging to urban forests and integrating with nature	3.59±0.78	2.46±0.49	0.85±0.16

SD, standard deviation.

environment, indicating that the participants were generally satisfied with the constituent elements and landscape facilities of the park grassland. This might be due to the flat and open ground of the park grassland environment and well-kept man-made facilities, which is similar to other park environments participants typically engage with, resulting in a safe attachment. Meanwhile, there were various landscape elements in the waterfall forest environment that could stimulate the participants' desire to explore. There was no significant difference in the score of compatibility perception between the waterfall forest environment and park grassland environment, indicating that most of the participants could live in harmony with nature when they carried out various activities in the 2 environments. The average scores of the participants in the urban street environment in the items "the overall environment and natural resources can meet the recreational needs" and "a sense of belonging to the environment" were less than 0.5, with the standard deviation also being small, suggesting that the participants had difficulty meeting their recreational needs in the urban street setting and had no sense of belonging in this environment.

Participants' preferences for different environments

The participants had the highest preference for all aspects of the waterfall forest environment, which was followed by the park grassland environment and the urban street environment. The participants preferred landscapes with high naturalness, overlook, and sociality. The specific results are shown in *Table 6*.

Part II

General characteristics of CFS participants

This study recruited 24 participants with CFS including

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Table 6 Participants' preferences for the different environments ($\bar{x}$ ± SD)

Preference dimensions	Items	Waterfall	Park	Urban
Tranquility	No human noise or traffic noise	4.17±1.87	3.43±14.31	0.65±0.36
	Killing time without being in contact with too many people	2.28±0.78	3.02±0.78	0.37±0.50
Spatiality	A wide environment with space for human activities	4.16±1.41	3.96±0.94	0.26±0.19
Naturalness	A variety of natural elements, such as water and vegetation	4.44±1.37	3.35±1.19	0.34±0.13
	Perceived high green coverage	3.54±0.66	3.42±1.05	0.59±0.03
Diversity	Abundant animal and plant resources	4.14±1.05	3.07±0.07	0.29±0.02
Sheltered	Places where you can watch others play or yourself play	4.00±0.65	3.52±0.54	0.43±0.06
Overlook	A wide field of vision for people to look into the distance	4.65±1.46	2.68±0.56	0.17±0.03
Sociality	A place for social activities, such as dinner, dating, etc.	4.72±1.15	3.95±0.51	0.40±0.09
Culturality	Local decoration or ethnic minority style	2.28±0.48	1.73±0.67	4.13±1.63
	Detailed explanation, tips, and tour guidance	4.15±0.31	4.73±0.69	3.07±0.53

SD, standard deviation.

15 females (BMI 22.057±1.808) and 9 males (BMI 21.871±2.133). The average age of females was 42.333±8.731 years while that of males was 39.444±10.725 years. The demographic characteristics of the 2 groups of participants are shown in *Table 7*. There was no statistical difference in basic characteristics or lifestyle between the waterfall group and the urban group. The 2 groups of participants were well-matched.

Basic level of psychophysiological indicators

On the day before the experiment, the 2 groups of participants underwent venous blood sampling and filled in the relevant psychological scales to determine the baseline level of the participants. It could be seen from *Table 8* that the 2 groups of participants were well matched at the physiological and psychological levels (P>0.05). Due to the small number of samples, this study did not carry out a stratified analysis of gender or other characteristics.

Improvement of mood scales in different environments

Compared with the urban group, the HAMA and HAMD scores of the waterfall group were significantly decreased, suggesting that after 7 days of intervention in the different environments, the promotive effect of waterfall forest environment on mental health was more significant than that of the urban control group. Overall, the effect of environmental therapy after 7 days was better than that after 3 days (*Figure 7*).

Improvement of fatigue state in different environments

The results of the FS-14 fatigue scales are shown in *Tables 9,10* and *Figure 8*. The scores of somatic fatigue and mental fatigue in the waterfall forest group were lower than those in the urban group (P<0.05 or P<0.01).

Evaluation of cognitive function

The PASAT was conducted to evaluate the changes in information processing speed, executive ability, and cognitive level of participants. It could be seen from *Figure 9* that after the 7 days of waterfall forest environmental intervention, the number of attempts and correct attempts of participants in the PASAT were increased.

Determination of serum cortisol and 5-HT

The severity of mental illnesses like depression is positively correlated with serum cortisol level and negatively correlated with 5-HT level. *Figure 10* shows that the cortisol level of participants in the waterfall forest group was lower than that in the urban group, while the 5-HT level of participants in the waterfall forest group was higher than that in the urban group, suggesting that the long-term intervention of the waterfall forest landscape regulated the secretion of neurotransmitters and reduced the risk of mental illness.

Changes in physiological indexes after different environmental interventions

Figure 11 shows that the serum glucose, triglyceride, total

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Table 7 Demographic characteristics of participants with CFS ($\bar{x}$ ± SD)

Items	Urban landscape group	Waterfall forest group	P value
Number of participants	N=12 (male =5)	N=12 (male =4)	
Age (years)	39.58±7.95	42.58±12.15	0.48
BMI (kg/m^2)	22.578±1.832	22.411±2.054	0.84
Waistline (cm)	68.5±9.73	71.54±10.14	0.46
Systolic pressure (mmHg)	114.75±13.67	115.83±16.94	0.86
Diastolic pressure (mmHg)	78.83±7.76	74.58±8.60	0.22
Cholesterol (mmol/L)	5.07±0.89	4.99±0.62	0.78
Triglyceride (mmol/L)	2.24±1.30	2.19±1.43	0.92
Creatinine (μmol/L)	70.75±13.08	70.90±14.29	0.98
Average sleep time (h/day, 3 months before the experiment)	7.24±0.67	7.06±1.00	0.6
Coffee intake (%)	42	58	<0.001
≥360 mL/day	25	8.3	
<360 mL/day	17	50	
Tea intake (%)	41	41	
≥500 mL/day	25	8.3	<0.001
<500 mL/day	16.7	33.3	
Alcohol intake (%)	16.7	25	<0.001
≥20 g/day	8.3	8.3	
<20 g/day	8.3	16.7	
Drug abuse (marijuana, cocaine, and psychotropic substances)	0.92	0.67	<0.001
Exercise (%)	0.92	0.67	<0.001

CFS, chronic fatigue syndrome.

cholesterol, and creatinine in patients with CFS were decreased after 7 days of intervention in the different environments, but there was no significant statistical difference (P>0.05). After intervention in the waterfall forest environment, the level of serum uric acid was decreased, and the difference was statistically significant (P<0.05).

Changes in glutathione peroxidase, superoxide dismutase, and MDA levels after different environmental interventions

The serum levels of GSH-Px (glutathione peroxidase) and SOD (superoxide dismutase) in the participants in the waterfall forest group were increased significantly compared with those in the urban group (P<0.05; *Figure 12*); meanwhile, the level of MDA (3,4-Methylenedioxyamphetamine) was significantly decreased in the participants exposed to the waterfall forest environment, indicating that the waterfall forest environment was conducive to improvement in the endogenous antioxidant level.

Changes in inflammatory factor levels after different environmental interventions

Compared with the urban control group, the waterfall forest group showed decreased inflammatory indexes [interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α)] and infection indexes (IL-6 and IL-10) in the serum of participants (P<0.05, *Figure 13*).

Changes in serum metabolites after different environmental interventions

The above results demonstrated that waterfall forest environment improved the fatigue, depression, and immune

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Table 8 Comparison of the baseline level of psychophysiological indicators between the 2 groups ($\bar{x}$ ± SD)

Baseline	Urban landscape group	Waterfall forest group	P value
Hamilton Anxiety Scale (HAMA)	12.84±1.88	13.50±0.92	0.68
Hamilton Depression Scale (HMAD)	14.00±0.896	13.73±0.83	0.67
FS-14 somatic fatigue scale	6.67±0.89	6.50±1.09	0.69
FS-14 mental fatigue scale	4.17±0.94	4.75±0.97	0.15
PASAT (correct)	28.00±4.71	29.50±5.07	0.88
PASAT (attempt)	28.25±8.28	31.50±7.76	0.97
CORT (ng/mL)	16.79±1.98	17.91±1.80	0.45
5-HT (μg/L)	117.15±11.55	116.49±11.10	0.99
GLU (mmol/L)	5.18±0.83	5.68±0.36	0.31
uric acid (μmol/L)	351.50±10.47	350.75±9.43	0.56
GSH-Px (U/mL)	80.88±14.49	74.94±13.28	0.07
SOD (u/mL)	57.17±3.38	54.80±2.30	0.46
MDA (μmol/L)	7.79±0.76	8.32±1.07	0.17
IL-1β (pg/mL)	5.13±0.39	4.89±0.30	0.53
IL-6 (pg/mL)	8.41±0.69	8.90±1.45	0.79
IL-10 (pg/mL)	8.58±1.35	9.19±1.10	0.14
TNF-α (pg/mL)	10.25±2.01	12.33±1.24	0.15

PASAT, paced continuous addition task test; CORT, corticosterone; 5-HT, 5-hydroxytryptamine; GLU, glucos; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malonaldehyde; IL, immunoglobulin; TNF, tumor necrosis factor; SD, standard deviation.

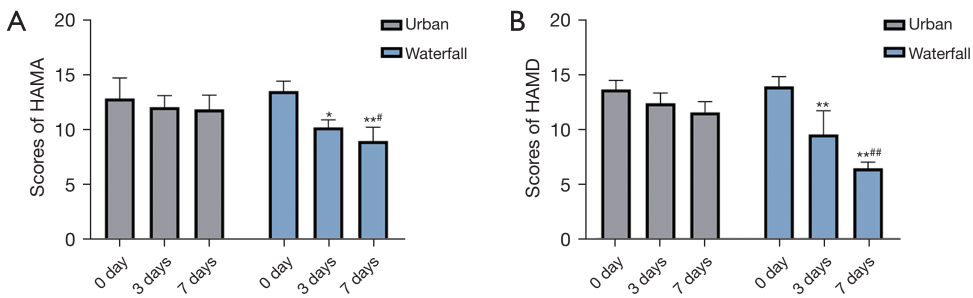

Figure 7 Changes in mood scales in the 2 groups of participants at different intervention times. (A) HAMA and (B) HAMD. *, P<0.05 and **, P<0.01 indicate a significant difference between day 0 and day 3; #, P<0.05 and ##, P<0.01 indicate a significant difference between day 0 and day 7. HAMA, Hamilton Anxiety Scale; HAMD, Hamilton Depression Scale.

indexes of CFS patients. We next explored the effect of the waterfall forest environment on serum metabolites in patients with CFS. We tried to avoid individual differences between samples, and performed mass spectrometry and chromatography analyses on the serum samples of participants in the waterfall forest group at baseline (A), day 3 (B), and day 7 (C).

For the structural identification of the 3 groups of metabolites, a self-built database was searched by means of accurate mass number matching (<25 ppm) and secondary spectrum matching. A total of 102 serum nontargeted metabolites were identified. In *Table 11*, the comparison of A *vs.* B shows 11 differential metabolites were upregulated and 28 were downregulated, the comparison of A *vs.* C

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Table 9 Comparison of FS-14 somatic fatigue scale scores in the different groups of participants ($\bar{x}$ ± SD)

Group	Baseline	Day 3	Day 7
Urban control	6.66±0.89	5.92±1.00	5.50±0.80
Waterfall forest	6.50±1.09	5.75±0.97	3.66±0.98$^{##}$

$^{##}$, P<0.01 indicates a significant difference between groups compared with the Waterfall forest group. FS, fatigue scale; SD, standard deviation.

Table 10 Comparison of FS-14 mental fatigue scale scores in different groups of participants ($\bar{x}$ ± SD)

Group	Baseline	Day 3	Day 7
Urban control	4.17±0.94	4.25±0.97	4.16±1.11
Waterfall forest	4.75±0.96	4.66±1.07	2.66±0.78$^{##}$

$^{##}$, P<0.01 indicates a significant difference between groups compared with the Waterfall forest group. FS, fatigue scale; SD, standard deviation.

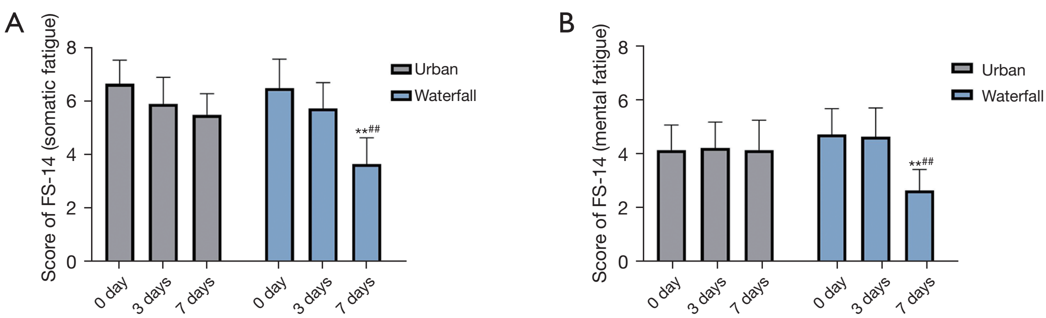

Figure 8 Changes in fatigue scale scores in the 2 groups of participants at different intervention times. (A) FS-14 somatic fatigue score and (B) FS-14 mental fatigue scores. FS, fatigue scale. **, P<0.01 indicate significant difference between other group and baseline; $^{##}$, P<0.01 indicated significant differences between the Waterfall and other groups.

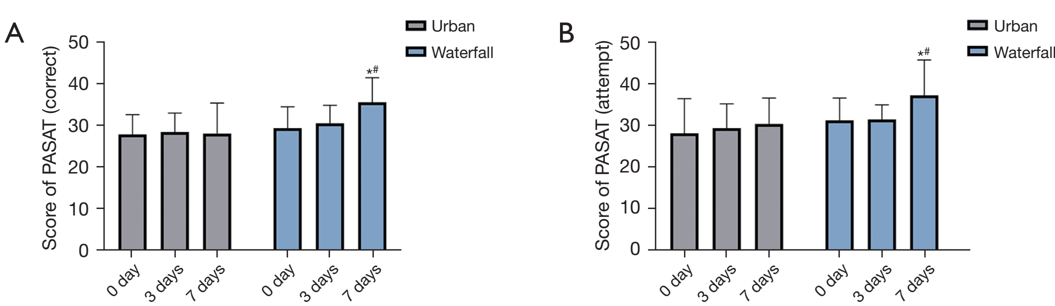

Figure 9 Evaluation of cognitive function in the 2 groups of participants at different intervention times. (A) PASAT correct and (B) PASAT attempts. *, P<0.05 indicate significant difference between other group and baseline; $^{#}$, P<0.05 indicated significant differences between the Waterfall and other groups. PASAT, paced auditory serial addition task.

shows 15 differential metabolites were upregulated and 25 were downregulated, and the comparison of B *vs.* C, shows 8 differential metabolites were upregulated and 15 were downregulated.

Analysis of differential metabolites

The differential metabolite results of groups A (day 0) *vs.* B (day 3), A (day 0) *vs.* C (day 7), and B (day 3) *vs.* C (day 7) are presented in volcano plots and clustering analysis plots (*Figures 14-16*).

The differential metabolites between groups

Compared with the baseline level, the levels of serum amino acids (ornithine, glutamate, valine, taurine, etc.) and antioxidants (L-ascorbic acid, L-cysteine, etc.) were increased, while those of creatinine and glucuronic acid were decreased on the third day of the waterfall forest environmental intervention. Compared with the baseline level, the level of branched chain amino acid Val-Ser related to antifatigue on the seventh day of the waterfall forest environmental intervention was significantly

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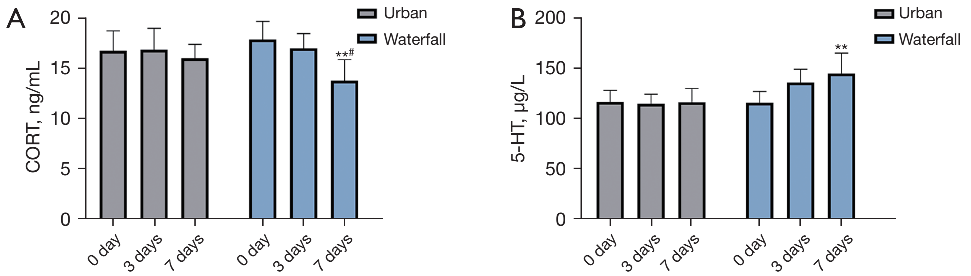

Figure 10 Changes in serum neurotransmitter levels in the 2 groups of participants at different intervention times. (A) Changes in cortisol and (B) 5-HT levels. **, P<0.01 indicate significant difference between other group and baseline; #, P<0.05 indicated significant differences between the Waterfall and other groups. CORT, cortisol; 5-HT, 5-hydroxytryptophan.

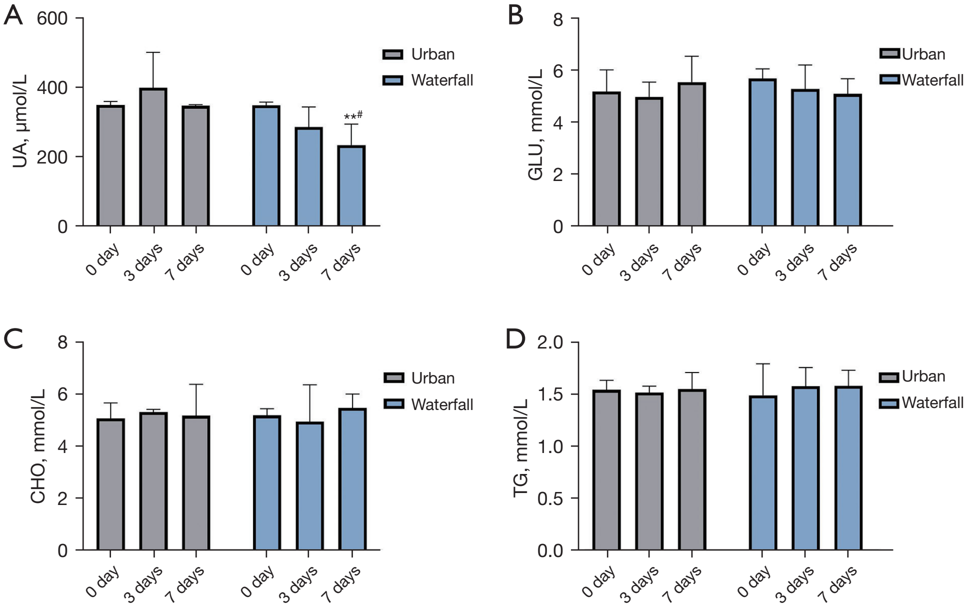

Figure 11 Changes in physiological indicators in two groups of participants at different intervention times. **, P<0.01 indicate significant difference between other group and baseline; #, P<0.05 indicated significant differences between the Waterfall and other groups. UA, uric acid; GLU, glucose; CHO, cholesterol; TG, triglyceride.

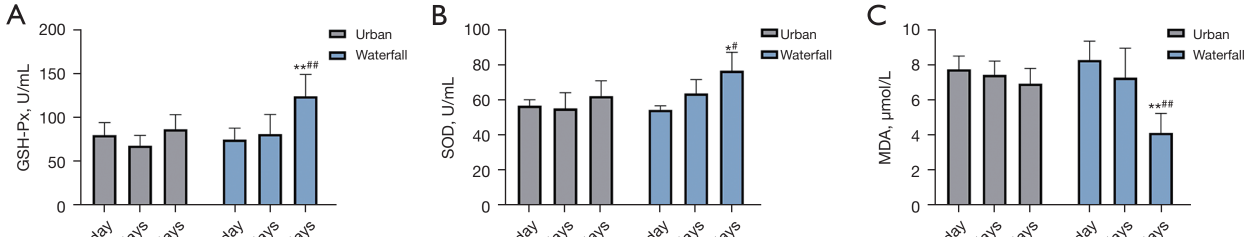

Figure 12 Changes in (anti) oxidants in the 2 groups of participants at different intervention times. *, P<0.05 and **, P<0.01 indicate significant difference between other group and baseline; #, P<0.05 and ##, P<0.01 indicated that there were significant differences between the Waterfall and other groups. GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malonaldehyde.

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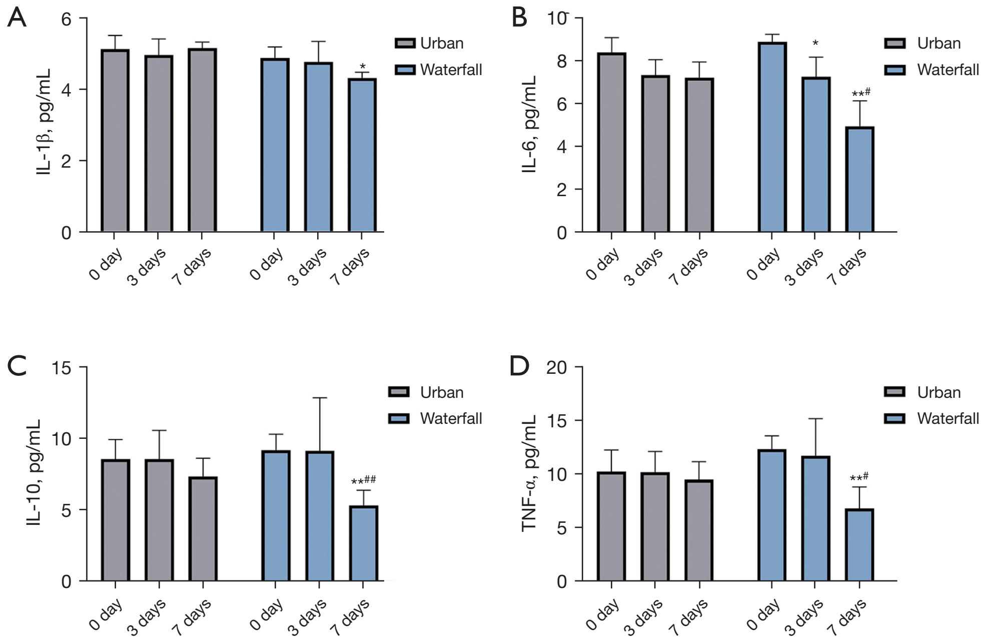

Figure 13 Changes in inflammatory factors in the 2 groups of participants at different intervention times. *, P<0.05 and **, P<0.01 indicate significant difference between other group and baseline; #, P<0.05 and ##, P<0.01 indicated that there were significant differences between the Waterfall and other groups. IL, immunoglobulin; TNF, tumor necrosis factor.

Table 11 Quantitative statistics for serum metabolites of participants in the waterfall forest group (N=12)

Comparisons	Up-	Down-	All-
A *vs.* B	11	28	39
A *vs.* C	15	25	40
B *vs.* C	8	15	23

Note: Comparisons, the comparison groups; Up-, upregulated differential metabolites; Down-, downregulated differential metabolites; All-, all differentially expressed metabolites; A, the baseline level; B, waterfall forest environment intervention day 3; C, waterfall forest environment intervention day 7.

increased. Compared with the third day of the waterfall forest environmental intervention, the level of endogenous chlorpromazine was increased on the seventh day of the intervention, suggesting that with the extension of time, the mood of patients with CFS tended to be calmer, with reduced anxiety. The levels of serum bilirubin and palmitic acid were decreased. The results are detailed in Appendix 3.

Analysis of the KEGG metabolic pathway

After comparing the results at 3 different time points, we obtained the differential metabolite data and submitted them to the KEGG website for pathway analysis. The results revealed that all serum metabolites were involved in 125 metabolic pathways. The most relevant 20 signal pathways were selected and a bubble diagram was drawn in R language (*Figure 17*). The larger the abscissa rich factor value is, the higher the enrichment degree; the redder the bubble color is, the smaller the P value; and the larger the bubble shape is, the more differential metabolites enriched in this pathway. *Figure 17* shows that the metabolites were more related to signal pathways such as choline metabolism in cancer, Parkinson disease, and amino acid transport.

Discussion

With the heightened emphasis on environmental and health issues, medical geography has been developing vigorously. Meteorology and environmental science are continuously advancing, gradually intersecting with medicine science, and society is becoming increasingly aware that an excellent natural environment is a valuable health resource (15). The environment has a great impact on human health. Compared with the simple

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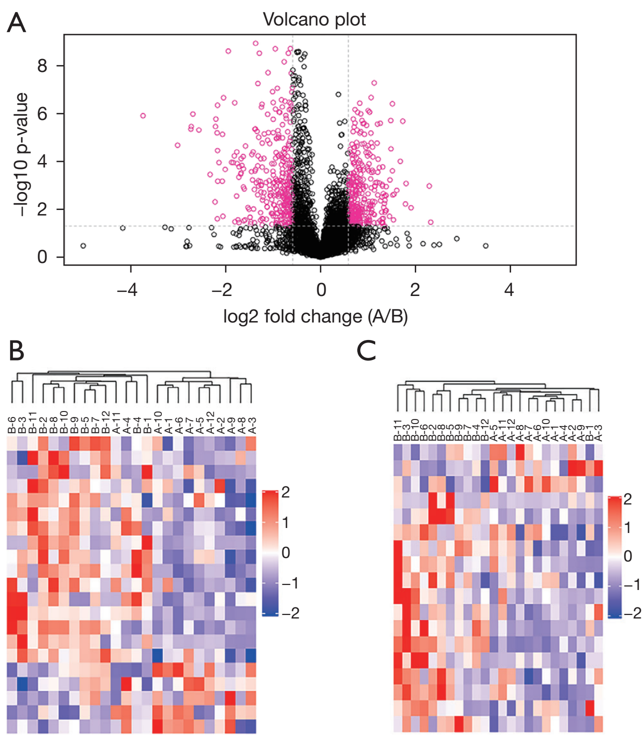

Figure 14 Differential metabolite detection results of group A *vs.* B. (A) Volcano plot, (B) positive ion pattern clustering analysis plot, and (C) negative ion pattern clustering analysis plot. Black: the overlap of the metabolites of the two groups of samples; Red: the metabolites of the two groups of samples.

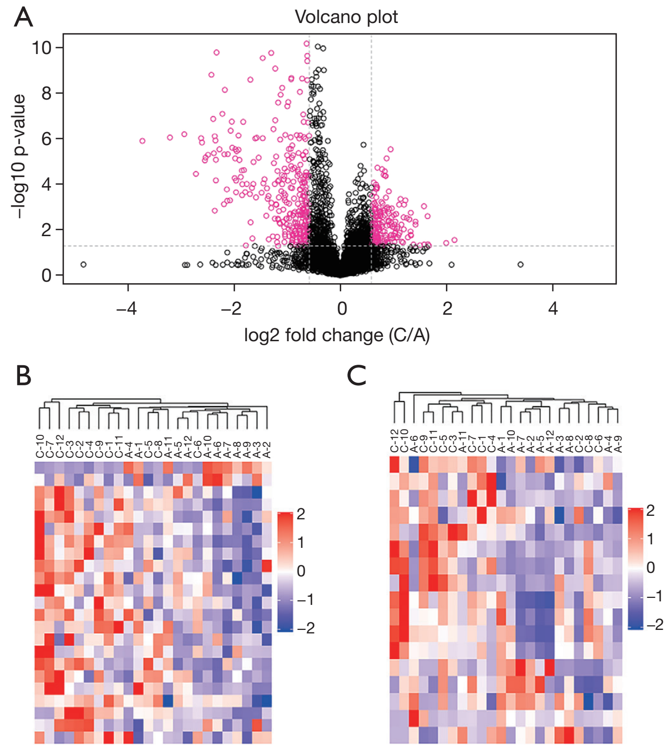

Figure 15 Differential metabolite detection results of group A *vs.* C. (A) Volcano plot, (B) positive ion pattern clustering analysis plot, and (C) negative ion pattern clustering analysis plot. Black: the overlap of the metabolites of the two groups of samples; Red: the metabolites of the two groups of samples.

forest environment, the Huangguoshu waterfall forest environment has more advantages for human health (1). The Huangguoshu waterfall is the largest waterfall in Asia with great potential energy. Due to the massive drop of a body of water, water molecules are split to form rich negative oxygen ions (16). The forest coverage rate in the scenic area is as high as 85%, and the forest vegetation consists mainly of shrub forest and theropencedrymion formed by natural growth, presenting a complete ecological system environment (2). The ecosystem in this area has strong anti-interference ability, and the vigorous photosynthesis of the vegetation also produces an abundance negative oxygen ions (17). Mixed negative oxygen ions from different sources hover stably in the environment, and finally, positively charged debris, bacteria, and other substances sink into the water or ground. Hence, the overall air quality of the scenic spot is excellent (18). Meanwhile, the pedestrian plank road in the scenic spot meanders through the natural vegetation and dense forest, providing convenient conditions for people to fully immerse in the atmosphere of mixed negative oxygen ions.

In this study, the participants experienced a short-term residence in different environments. The combination of eye tracking technology and scale evaluation was then used to clarify the role of waterfall forest environment in improving the participants' positive emotions. The fixation heat map and trajectory map of the waterfall forest environment suggested that the waterfall itself was the landscape element most focused on by the participants. The same results were obtained in the eye-tracking paradigm analysis and AOI analysis. Additionally, the average fixation count of participants in the waterfall forest environment was significantly higher than that in the urban street and park grassland environments, indicating that the participants required a longer time for cognitive processing of the waterfall forest environment and possessed a stronger desire for active cognitive exploration. The

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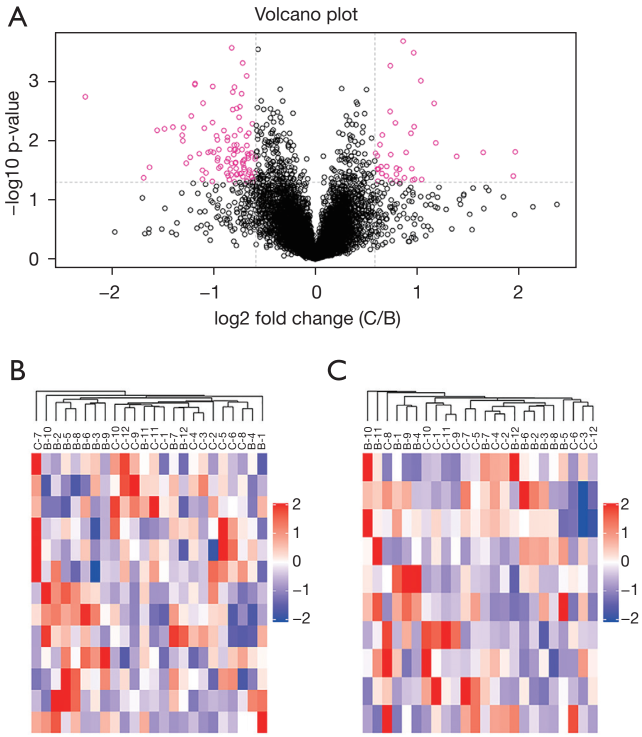

Figure 16 Differential metabolite detection results of group B *vs.* C. (A) Volcano plot, (B) positive ion pattern clustering analysis plot, and (C) negative ion pattern clustering analysis plot. Black: the overlap of the metabolites of the two groups of samples; Red: the metabolites of the two groups of samples.

saccade count of participants in the urban street landscape was also higher than that in the waterfall forest and park grassland landscapes. The landscape elements of urban streets are more diverse, and the elements such as vehicles and pedestrians often appear dynamically and are relatively scattered, which leads to a high saccade count but a low saccade rate when the fixation of participants is switched between the 2 furthest elements of interest, suggesting that the urban street environment can cause distraction and reduce energy and cognitive ability (19). Individuals blink at a high frequency when they are in a state of pleasure (20). The increase of saccade rate and amplitude of participants in the water forest environment suggested that the participants' concentration, desire for exploration, and pleasant experience of the waterfall forest environment were increased, which was consistent with the trend in score of environmental "fascination" in the environmental preference scale. The POMS results showed that the participants' stress was released after exposure to the waterfall environment as evidenced by the decreased scores of tension, fatigue, depression, and anger. The PRS results also demonstrated that the fascination was the most perceptible dimension in the participants experiencing the waterfall environment, which might have been due to the salient difference between the scene and the participant's original daily living environment. Visually novel stimuli can make individuals attracted to special landscape elements, which also increases the fixation frequency and regression count, and then, the attention convergence effect can dramatically enhance the fascination perception (20). Peris *et al.* reported that the perception of tranquility is the most favored among those experiencing a natural environment (21). In this study, there was no significant difference between the waterfall forest environment and the park grassland environment in terms of tranquility, naturalness, or diversity, but the waterfall forest environment performed best in the 2 preference dimensions of overlook and sociality, which might have been related to the cultural background of the participants (22). This preference result also became the preference feature of the waterfall forest environment that differed from that of the other natural environments. This study selected Huangguoshu waterfall scenic spot as the research site, and the participants were mostly driven by autonomous motivation. However, there might have been some differences in the environmental cognition and preference of the group due to passive location tendency (23). It is necessary to increase the subgroup analysis of samples to explore the curative effect of motivation on "natural prescription".

In the second part of experiments, the results indicated that compared with those of the urban street–exposed participants, the scores of HAMA and HAMD scales of the waterfall forest-exposed participants showed a downward trend with the extension of intervention time. In the FS-14 scale, the score of psychological burnout was decreased significantly, suggesting that the waterfall forest environment had a ameliorative effect on the negative emotions and promoted energy recovery of patients with CFS. Individuals with severe CFS also often have cognitive impairment, and this study used the PASAT scale to assess cognitive function (24). The results showed that the waterfall environment notably improved the PASAT score and restored the cognitive function of participants with CFS, while the urban street environment had no improving effect.

Abnormal feedback of the hypothalamic-pituitary-adrenal (HPA) axis is an important mechanism of chronic

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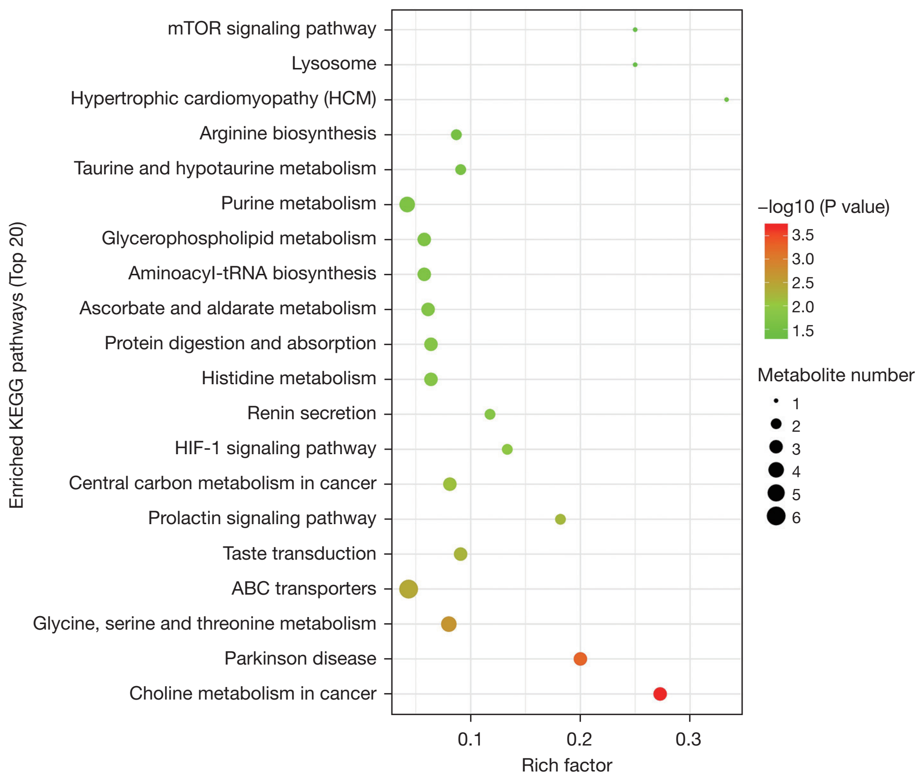

Figure 17 Analysis of KEGG pathway enrichment at different intervention times. KEGG, Kyoto Encyclopedia of Genes and Genomes.

fatigue (25,26). After recuperating in the waterfall forest environment, the participants exhibited an increased level of serum cortisol. It was thus speculated that the antifatigue mechanism of the waterfall forest environment may be related to maintaining the functional stability of the HPA axis and reducing the level of serum cortisol (27). Regarding other physiological indexes (glucose, triglyceride, total cholesterol, and creatinine), there was no significant difference among participants exposed to the different environments, which might be related to the short exposure time. Moreover, the CFS population often shows a tendency of increased uric acid (28,29). This study found that the blood uric acid level of participants was decreased after treatment in the waterfall forest environment, which was consistent with the results of metabolomics. A previous study has shown that fatigue produces a large number of oxygen-free radicals and lipid peroxides (30) to weaken the body's antioxidant effect. MDA, an indicator of lipid damage, is increased in those with CFS, while the activities of antioxidant SOD and GSH-Px are decreased (31). SOD protects cells from damage by removing superoxide anions in the microenvironment (32). GSH-Px specifically catalyzes the reduction of hydrogen peroxide and protects the cell membrane (33). According to the serological indexes, the waterfall forest environment notably improved the levels of endogenous GSH-Px and SOD in participants but reduced the level of MDA. Meanwhile, the levels of serum IL-1β, IL-6, and IL-10 were decreased after exposure to the waterfall forest environment, suggesting that the waterfall forest environment can reduce the level of inflammatory factors in the body (34). Serum TNF-α level has been shown to be elevated in patients with aging-related diseases such as Alzheimer disease (35). Our results showed that the TNF-α level decreased with the increase of exposure time in the waterfall forest environment. Based on the results of psychological scales and serological indexes, it could be surmised that the waterfall forest environment can enhance the anti-inflammatory effect of the body, stabilize the regulation of the neuroendocrine system, and reduce the occurrence of anxiety and depression in those with CFS.

There are two possible mechanisms of Huangguoshu waterfall environment regulating anxiety. First, the waterfall environment can stimulate the vision of the subjects living in the city for a long time, and can regulate the human

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internal environment such as oxytocin and other hormones. Second, the natural high concentration of negative oxygen ions in the waterfall environment can increase the content of endogenous antioxidants. Anxiety patients with oxidative stress is common. However, the present study only has revealed the preliminary phenomenon. Further research is needed to elaborate the relevant mechanism of this condition.

To further explore the effect of the waterfall forest environment on body metabolism, a combined analysis of chromatography and mass spectrometry was performed to clarify the effect of different exposure times to the waterfall forest environment on serum metabolites. The glutamate content of the participants was increased significantly, indicating that the disorders of glutamate and glutamine metabolism leading to fatigue were corrected after the treatment in the waterfall forest environment (36). The generation of endogenous L-ascorbic acid and other antioxidants was enhanced, which synergistically reduced the fatigue effect of excessive aerobic metabolism. Nitric oxide and superoxide in the body combine to form active nitrogen-free radicals [reactive nitrogen species (RNS)]. The increase of RNS level leads to DNA damage, and the decrease of choline level in serum results in DNA strand breakage and induces apoptosis (37). The level of choline was increased after the treatment in the waterfall forest environment, which could inhibit the microdamage of DNA or mitochondria. It was worth noting that after 7 days of the waterfall forest environmental intervention, the level of oleic acid was increased in a small range. We speculated that this might be related to the completion of liver glycogen reserve, the stability of body oxidation level, the repair of hepatocyte injury, and the repair of liver glycogen reserve under the waterfall forest environment. The mammalian target of rapamycin (mTOR) signal transduction pathway involves many critical processes, such as cell proliferation, differentiation, and protein composition. mTOR is regulated by upstream PI3K that participates in the synthesis of serine/threonine protein kinases and phosphatidylinositol kinase. Therefore, PI3K-regulated mTOR activation can reduce the symptoms of inflammatory response and metabolic resistance. Relevant studies have shown that negative emotions including anxiety and depression, as psychological stressors, can produce nonspecific stress responses and affect a variety of immune cell activities, immune recognition, and immune monitoring via the HPA axis and sympathetic nervous system, resulting in homeostasis imbalance and eventually the occurrence and development of tumors (38,39). Therefore, the environmental healing of waterfall forest may be related to choline metabolism in KEGG enrichment, but the specific mechanism needs to be further studied.

Conclusions

Short-term exposure to the waterfall forest environment can enhance inner peace and improve negative emotions. Mixed negative oxygen ions in the Huangguoshu waterfall forest environment can increase the content of endogenous antioxidants and balance amino acid metabolism.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Medical Ethics Committee of Guizhou Provincial People's Hospital [No. (2019)60] and informed consent was taken from all the participants. The Affiliated Hospital of

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Guizhou Medical University was also informed and agreed with the study.

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Supplementary

Appendix 1:

Inclusion criteria:

1. The Regular workplace is an urban environment.
2. I have not entered the tourist area for sightseeing recently (within 6 months).
3. Naked eye vision or corrected vision is above 1.0, no color blindness, color weakness and other conditions.

Exclusion criteria:

1. Visitors with mental illness requiring long-term medication and regular follow-up.
2. Tourists with cognitive dysfunction-related disorders.
3. Received anesthesia surgery recently (within 6 months).
4. Tourists dependent on drugs, alcohol, cocaine, marijuana, or amphetamines.

The demographic characteristics of tourists were subjected to a descriptive statistical analysis, as shown in Table 2. Experimental researchers are in the experiment

Visitors were advised to avoid excessive drinking the day before the experiment and avoid high intensity.

Dress guidance during nursery training and experiments.

Appendix 2

In the presence of the primary symptoms, four or more of the secondary symptoms were required to diagnose CFS.

CFS is diagnosed by the presence of 4 or more of the secondary symptoms.

1. Primary symptom: the presence of sleep fatigue that is difficult to eliminate if other organic diseases are excluded.

Duration: lasting for 6 months or more.

2. Secondary symptoms: 1) Significant short-term memory loss or difficulty concentrating.

2) pain in the throat, similar to cold symptoms; 3) swelling or pain in the lymph nodes of the body; 4) localized fatigue without excessive exercise or muscle strain.

4) persistent local muscle soreness without excessive exercise or muscle strain, with or without affecting daily work life; 5) redness, swelling and pain at unrelated

(5) redness, swelling, and pain at the nodes; (6) the presence of unprovoked headache, which can be relieved by itself after rest; (7) persistent sleep at night

(7) poor recovery of energy after sleep; (8) physical or mental discomfort lasting 24 hours or more after physical or mental work.

Also (9) lack of sleep or reduced quality of sleep (10) reduced cognitive function and/or intolerance of standing position. etc. may also be

were considered as secondary symptoms.

In addition to meeting the diagnosis of CFS, the requirements of this study must also be met.

1 20-50 years of age Daily moderate-to-vigorous work in an urban environment, residing in an urban area

Male or female

2

CFS diagnosis certified by a professional neurologist, psychiatrist or psychologist.

3 BMI index: between 19 and 25

4

Recent (6 months and above) mental lethargy, depression, insomnia and forgetfulness, decreased sleep quality, decreased

Decreased attention span, etc.

5

With or without mild swelling and pain in the neck and axillary lymph nodes, but no significant abnormalities on physical examination and routine laboratory tests.

The physical examination and routine laboratory tests do not reveal any significant abnormalities.

2) Exclusion criteria.

1

Pregnant, pregnant or lactating women

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2

Exclude hypothyroidism, diabetes mellitus and other related cardiovascular, digestive, endocrine and neurological diseases.

Patients who need to take medication for a long time.

3

Patients who have recently (within 6 months) undergone anesthetic surgery.

4

No history of psychiatric disorders (post-traumatic stress disorder; bipolar disorder; any subtype of psychotic disorder; any subtype of psychotic disorder).

disorder; any subtype of delusional disorder; any subtype of dementia; anorexia nervosa; or bulimia nervosa; anxiety disorders.

bulimia nervosa; anxiety disorders; depression, etc.), and no history of psychiatric disorders in the immediate family.

5

Alcohol or other substance abuse for 2 years prior to diagnosis of chronic fatigue syndrome and at any time thereafter.

Substance abuse.

6

Infection with EBV, coxsackievirus, hepatitis virus, hepatitis B virus, or other drug abuse within 2 years prior to diagnosis of chronic fatigue syndrome and any time thereafter.

Coxsackie virus, hepatitis virus, HIV, HPV, etc. within 2 years and any time after the diagnosis of chronic fatigue syndrome.

7

Infection with parasitic diseases, etc. within 2 years prior to and any time after the diagnosis of chronic fatigue syndrome.

8

Infection with novel coronavirus, pneumonia, avian influenza, etc. within 2 years prior to and any time after the diagnosis of chronic fatigue syndrome.

Pneumonia, avian influenza and other infectious diseases.

9

Patients with less than secondary school education level

A thorough physical examination has been performed prior to subject enrollment and is required to provide within 3 months prior to the start of the trial

Physical examination report including (blood biochemistry, blood routine) and reduction of non-essential out-of-town travel residence. Avoid

Avoid bad habits such as staying up late, overeating, alcohol and drug abuse.

3) Withdrawal criteria

① Subjects cannot adapt to the environmental changes, have a significant decrease in sleep quality, poor appetite, and are eager to improve by withdrawing from the experiment.

The subject is unable to adapt to the environmental changes, has a significant decrease in sleep quality, poor appetite, and is eager to improve by withdrawing from the experiment.

② Subjects with serious acute pathology (respiratory system infection, digestive system inflammation) in the course of the experiment need

medication, or trauma requiring hospitalization, etc.

(③ Subjects had an accidental injury (car accident, fracture, cold, etc.), or a sudden important family event

(death of a relative, marriage, divorce, etc.)

1. The Regular workplace is an urban environment.

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Appendix 3

Table S1 Waterfall environmental intervention without time period out of serum differential metabolites (day 0 *vs.* day 3)

Name	VIP	Fold change	P value
L-Ascorbic acid	1.749688639	3.757247275	1.47825E-06
Glycerophosphocholine	3.576435214	1.833180498	1.84961E-05
D-Proline	1.701126648	1.635516268	3.70499E-05
Ornithine	1.460825829	1.562503015	0.000316483
Val-Ser	2.922387612	0.474781492	0.000967026
L-Pyroglutamic acid	2.290903109	1.667417385	0.0017219
L-Lysine	2.025500637	2.599338117	0.002681574
alpha-Linolenic acid	2.083028662	1.382391238	0.005368979
Betaine	2.381674296	1.182064834	0.008900451
Sarcosine	1.929126775	1.601244539	0.009053272
Imidazoleacetic acid	1.546243642	1.165388307	0.009208013
Choline	3.584788067	1.191994469	0.012319974
Eicosapentaenoic acid	1.309258668	2.107749321	0.013811266
Hypoxanthine	4.207576481	2.168396526	0.015308255
Taurine	1.059473259	0.652216715	0.022401585
1-Palmitoyl-sn-glycero-3-phosphocholine	14.57953293	1.190315404	0.022959218
L-Pyroglutamic acid	7.167804138	1.990140758	0.000100609
L-Cysteinesulfinic acid	1.073214663	1.20426907	0.005539491
Hypoxanthine	1.139014423	1.846467069	0.006345731
Taurine	1.712505107	0.584633099	0.007106044
Glyceric acid	1.10604714	1.429224562	0.011774181
Xanthine	1.897599503	1.43873385	0.038297941
Citrate	6.626677084	0.532716675	0.042072156
L-Lysine	1.751705502	1.312787659	0.043318066
Citramalic acid	1.690336408	1.72178441	0.044626331
Linoleic acid	5.296924558	1.357038041	0.045739806
L-Phenylalanine	2.141943154	1.216001992	0.048674149
L-Histidine	2.200263058	1.202201376	0.053785157
L-Pipecolic acid	1.791980336	1.135455009	0.063273996
Creatinine	4.548611321	1.729527172	0.063650339
1-Oleoyl-sn-glycero-3-phosphocholine	3.01434481	0.864768744	0.06817662
EDTA	2.431106629	0.660124429	0.071134598
DL-Indole-3-lactic acid	1.586797094	0.802751994	0.07824297
Hippuric acid	1.462270744	3.914723958	0.060578676
L-Malic acid	1.221469404	1.505481168	0.06530941
L-Histidine	1.691981377	1.975646644	0.076806111
L-Threonate	1.110118232	1.265652805	0.079433296
D-Glucuronate	1.088531366	1.221955013	0.081851471

Note: VIP is the variable weight value Fold change is the difference multiple, yellow background is the upregulation difference, and blue is the downregulation difference, the same below.

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Table S2 Waterfall environmental intervention without time period out of serum differential metabolites (day 0 *vs.* day 7)

Name	VIP	Fold change	P value
Val-Ser	3.996286549	0.254442827	2.85092E-06
L-Ascorbic acid	1.900943652	4.453823335	2.43817E-05
D-Proline	1.428322443	1.462687937	0.001254507
L-Pyroglutamic acid	2.149690657	1.571941352	0.001267235
L-Lysine	1.763759766	2.015373842	0.002243812
Glycerophosphocholine	2.142732477	1.369207093	0.006238053
L-Pipecolic acid	1.600367442	1.181846604	0.009240571
Sarcosine	1.786489535	1.48391883	0.013315921
trans-2-Hydroxycinnamic acid	1.026570607	1.392640174	0.013532596
Imidazoleacetic acid	1.45246483	1.140047862	0.013704703
DL-2,4-Diaminobutyric acid	1.489933632	1.297537316	0.014954471
1-Palmitoyl-sn-glycero-3-phosphocholine	13.88426449	1.167680569	0.01791303
L-Tyrosine	1.368957869	1.278483745	0.032417492
Tyramine	1.340769159	1.310268412	0.037642356
Ornithine	1.076612655	1.374749992	0.044008649
1-Stearoyl-sn-glycerol 3-phosphocholine	1.692083972	1.197412638	0.044632426
L-Pyroglutamic acid	6.562656846	1.882114888	0.000149735
Sphingosine-1-phosphate	1.14616173	2.461684135	0.004958508
L-Threonate	1.862095532	1.726142543	0.006794002
Myristic acid	1.148858946	1.375519774	0.011563791
Taurine	1.360630458	0.626637053	0.025981868
Citramalic acid	2.078685535	2.034240132	0.035893223
Linoleic acid	4.533349465	1.303102729	0.037327781
L-Phenylalanine	1.785767226	1.177883273	0.038565264
Betaine	4.794259534	1.123538502	0.051700011
1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine	1.995034903	1.236830041	0.057930908
L-Histidine	1.917047061	1.190343144	0.073179795
1-Myristoyl-sn-glycero-3-phosphocholine	1.590860163	1.260756746	0.079395464
Choline	2.819014071	1.161425912	0.081033281
Hypoxanthine	2.979211612	1.553241866	0.090376175
alpha-Linolenic acid	1.592180652	1.210867882	0.094232883
Dihydrothymine	1.06318009	0.562994373	0.05235444
alpha-Linolenic acid	1.907158665	1.354094953	0.05331124
cis-9-Palmitoleic acid	1.689056976	1.556403692	0.055705028
L-Malic acid	1.116111457	1.363942656	0.059996398
EDTA	6.954267601	0.360202642	0.074311805
Citrate	5.027840076	0.69414383	0.077858108
L-Lysine	1.677387096	1.22626004	0.078169042
Uric acid	1.571479619	0.821642449	0.08479962

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Table S3 Waterfall environmental intervention without time period out of serum differential metabolites (day 3 *vs.* day 7)

Name	VIP	Fold change	P value
L-Ascorbic acid	1.07727158	1.398233452	0.003454783
Val-Ser	3.42686793	0.53591564	0.00668246
Chlorpromazine	1.730424826	0.831216628	0.011520773
Glycerophosphocholine	5.27219663	0.746902498	0.013726953
Phosphorylcholine	1.08064895	1.218588011	0.017155457
Urocanic acid	1.953842113	1.524383687	0.017919473
D-Proline	1.25594993	0.609494016	0.022680521
Phe-Tyr	4.704838907	0.670167802	0.043746859
1-Oleoyl-sn-glycero-3-phosphocholine	12.37272528	1.214965597	0.04544287
Eicosapentaenoic acid	2.026470287	0.576290399	0.049085297
Glyceric acid	6.723166164	1.43104333	0.013766716
L-Isoleucine	4.853735592	1.351130959	0.022100339
L-Histidine	3.082299214	0.417375373	0.027454762
Embelin	1.407007531	1.411339981	0.029172403
cis-Aconitate	4.1316406	0.792818322	0.046189338
L-Proline	2.958241703	0.760718853	0.068354648
L-Leucine	4.96444089	1.326967997	0.070395273
Sarcosine	1.224370711	1.214901748	0.090168914
Citrate	7.673862736	1.545277749	0.053781966
Bilirubin	1.232775171	0.663733015	0.07604346
Palmitic acid	1.581496282	0.814959637	0.086667564
D-Glucuronate	1.086643933	0.869157594	0.095426076
Dihydrothymine	2.47926027	0.589382765	0.098690304

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