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Original article

Physical and mental effects of 12 weeks of hot yoga: A controlled open-label study

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Abstract

The effects of a 60-minute hot yoga program twice a week for 12 weeks in healthy women who were aware of their lack of exercise were examined and compared with a control group. The analysis subjects were divided into two groups: a control group (19 subjects, age 43.6 ± 10.3 years, BMI 26.4 ± 0.8) and a hot yoga group (34 subjects, age 44.1 ± 9.5 years, BMI 25.7 ± 0.4), and the hot yoga practitioners were further divided into a wood stone floor group and a regular floor group (17 subjects each). Measurements after 12 weeks showed improvement in skin condition (water content, wrinkles, and texture), improvement in mental stress according to the SF-8 questionnaire, mild improvement in weight, body shape, and flexibility, decrease in HbA1c, and increase in expression of sirtuin gene *SIRT6*. No adverse events were noted. A comparison of regular and woodstone floors showed a greater improvement in SF-8 body score when using woodstone floors, and blood tests showed an avoidable decrease in RBC and Hb, a significant increase in total protein, and a mild increase in CPK, which was considered an advantage of the woodstone floors. In the present study, a 12-week hot yoga program conducted with potentially inactive women showed a wide variety of effects and increased *SIRT6* expression as a factor involved in the mechanism of action. The results suggest that hot yoga, a combination of thermotherapy and yoga, is a safe health promotion method that increases sirtuin activity and enhances the efficiency of health promotion effects both mentally and physically.

KEY WORDS: hot yoga, yoga, heat therapy, sirtuin, heat shock protein (HSP), SIRT1, SIRT6

Introduction

Yoga is an ancient Indian practice that trains the body and mind. Through a combination of breathing exercises, postures known as yoga postures, and meditation, the goal is to relieve tension in the body and mind and to achieve mental stability and ease. Yoga is usually practiced under natural conditions without temperature or humidity control (room temperature yoga). In Japan, the room temperature is around 28 °C, so the amount of sweating is not so great. Considering the Indian climate, it is expected that the practice developed in a much hotter and more humid environment and that the amount of sweating would have been higher. Hot yoga, in contrast, takes place in an environment with a room temperature of 38-40 °C and humidity of 55-65%. In a hot and humid environment, the same movements are physically demanding and can lead to dehydration if proper hydration is not maintained. However, the advantage is that even slow movements are suitable for beginners who are not good at exercising, as they can exert a moderate physical load and work up a pleasant sweat.

Thermal therapies such as "onsen" therapy and "ganban (bedrock)" baths are popular in Japan. These are known for their relaxing effects and reduction of mental and physical stress. Warming the body, *i.e.*, increasing deep body

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temperature, relaxes blood vessel walls, dilates blood vessels, and improves blood flow. Genes and proteins (*i.e.*, heat shock proteins) induced by elevated body temperature have also been reported.

Hot yoga is a treatment in which heat therapy is added to room-temperature yoga, however, not enough information is available on the effects of hot yoga on the body. Therefore, we conducted an open study on healthy female subjects to examine the effects of participation in a hot yoga program twice a week for 12 weeks on the body in an uncontrolled open study ¹). The results showed that the expression of the sirtuin gene *SIRT6* (silent information regulator-6) increased, reactive oxygen species (ROS) decreased, and skin conditions (water content, elasticity, and texture) improved. In this controlled open study, we examined the effects of a hot yoga program on skin condition, oxidative stress, and sirtuin gene expression, and compared the differences when the program was administered on a regular floor and a wood stone floor.

Method

Subjects

The subjects were 91 healthy women between the ages of 20 and 59 years old, who were aware of their lack of exercise and who were able to take hot yoga lessons of 60 minutes per session at least twice a week for 12 weeks without fail. From among the applicants, 60 participants who met the selection criteria and did not meet the exclusion criteria were selected according to the judgment of the study investigator.

The selection criteria are as follows:

- 1) Japanese women between the ages of 20 and 59.
- 2) Those aware of their lack of exercise.
- Must be able to attend at least two hot yoga classes a week for 12 weeks, 60 minutes per class.
- 4) Those who have received a full explanation of the purpose and content of the study, understand it well, volunteer to participate, and agree to participate in the study in writing.

Exclusion criteria are listed below;

- 1) Those who are currently in hospital or being treated with drugs or herbal remedies for any disease.
- Those on a diet and exercise regimen under medical supervision.
- 3) Current or previous history of serious illness.
- 4) Women around menopause who are experiencing significant changes in their physical condition.
- 5) Persons who may show skin allergic symptoms, *i.e.*, atopic dermatitis.
- 6) History of or current drug or alcohol dependence.
- 7) Those who have started taking medicines, healthy food, supplements that affect the study within the last month or will start during the study period.
- Pregnant, lactating, or planning to become pregnant or lactating.
- 9) Currently exercising at least three times/week for at least one hour.

- 10) Have never attended hot yoga on a regular basis (except for trial only).
- 11) Work for a yoga studio, gym, or other fitness-related company, or have a family member or close relative living with them who works for a yoga studio, gym, or other fitness-related company.
- 12) Who have participated or are currently participating in another clinical trial within one month prior to obtaining consent, or will participate during the study period.
- 13) Persons who have been determined by the investigator to be ineligible for participation in this study.

Fig. 1 shows the number of subjects tested.

Study design

The study was a controlled, open-label study.

The allocation manager, who was not involved in the study, first divided the 60 subjects into the following two groups, taking their age and weight into consideration to prevent bias;

A control group: 20 subjects who did not practice hot yoga and recorded their daily life.

Hot yoga group: 40 subjects who practice hot yoga at least twice a week.

Next, the 40 hot yoga group members were divided into the following two groups according to the difference in the flooring material of the studio where the hot yoga was performed;

The wood stone studio (WSS) group: 20 participants who participated in hot yoga on the wood stone floor.

Hot yoga on regular floor (RF) group: 20 participants.

Subjects in the WSS group and subjects in the RF group took a 60-minute hot yoga lesson under the guidance of an instructor in an indoor environment with room temperature around 35 °C and humidity around 60 % at a designated hot yoga studio LAVA store at least twice a week for 12 weeks (from May to August, 2021). Subjects were allowed to choose either the beginner program or the basic program depending on their individual physical condition on the first day of the program. The programs that were implemented are shown in *Table 1*.

Overall body structure measurements (weight, BMI, body fat percentage), site measurements (circumference of waist, hips, bust, arms, thighs, and calves), flexibility measurements (forward and backward bending), skin measurements (skin water content, VISIA image analysis), and aging-related marker measurements (*SIRT1, SIRT2, SIRT3*) before and 12 weeks after study initiation *SIRT6, hTERT, hTERC*, 8-OHdG, and ROS), stress assessment (SF-8), blood pressure measurement, blood biochemistry, and hematological tests were performed. Subjects recorded the presence and severity of adverse events and lifestyle habits during the study period in a daily logbook.



Fig. 1. Number of examinees.

WSS, woodstone studio, RS, regular floor; FAS, full analysis set; PPS, per protocol set.

	YOGA BEGINNER										
	Block of Yoga sequence	Yoga Poses Names (Asanas) in Sanskrit & English	Duration								
1	session preparation instructions	session preparation instructions	1.5 min								
2	warm-up	warm-up -Breath -Shoulder joint -Spine	6.5 min								
3	Asana-Prone	Dandayamna Bharmanasana / Balancing Table Pose sequence including a preparatory pose (hip strech) and follow-up pose (child pose)	7.0 min								
4		Anjaneyasana / Low lunge sequence including a preparatory pose (twist pose) and follow-up pose (half sprit).	6.0 min								
5		Phalakasana / plank pose	0.5 min								
6	Asana-Sitting	Bhujangasana 🖊 cobra pose	0.5 min								
7		Salabhasana / Locust pose	0.5 min								
8		Balasana / Child's pose	0.8 min								
9		Adho Mukha Shvanasana / Downward-facing dog	0.8 min								
10		Uttanasana / Standing Forward Bend	1.0 min								
11	Asana-Standing	Utthita Trikonasana / Extended triangle pose sequence including four preparatory Poses (high lunge, eagle pose, wild-legged forward bend, and Squat)	14.0 min								
12		Phalakasana / plank pose	0.8 min								
13		Ardha Bhujangasana / Half cobra pose	0.8 min								
14	Asana-Sitting	Dhanurasana / Bow pose	0.8 min								
15		Balasana / Child's pose	0.8 min								
16		Upavistha konasana / Wide-angle seated forward bend	6.0 min								
17		Ardha Setubandhasana / half bridge pose	0.8 min								
18	Cool-down	Eagle twist with hip strech	3.5 min								
19		Pawanmuktasana / Wind-relieving pose	0.8 min								
20		Shavasana / Corpse Pose	5.0 min								
21	Relaxation	Breath	1.0 min								
22		session closing instructions	1.0 min								
		total	60.0 min								

 Table 1. Hot yoga implementation program (choice of three programs below)

	YOGA BASIC										
	Block of Yoga sequence	Yoga Poses Names (Asanas)	Duration								
1	session preparation instructions	session preparation instructions	1.0 min								
2		Breath	1.0 min								
3		Jathara Parivartanasana / Revolved belly pose	2.0 min								
4		Pawanmuktasana / Wind-relieving pose	1.0 min								
5		Kapalahbhati / Skull-shining breath	2.0 min								
6		Marjarasana / Cat cow pose	1.5 min								
7	warm-up	Parsva balasana \nearrow Thread the needle	1.5 min								
8		Uttana Shishosana 🗡 Extended Puppy Pose	1.0 min								
9		Balasana / Child's pose	1.0 min								
10		Anjaneyasana 🖊 Low lunge	2.0 min								
11		Parighasana / Gate pose	2.0 min								
12		Balasana / Child's pose	1.0 min								
13		Utkatasana / Chair pose	4.0 min								
14		Ardha chandrasana / Half moon pose	1.5 min								
15		Parivrtta utkatasana / chair twist pose	1.5 min								
16		Tadasana / Mountain pose	1.0 min								
17		Garudasana / Eagle pose									
18		Parsvottanasana / Intense side stretch pose									
19	Asana-Standing	Parivrtta Sanchalasana / Low lunge twist pose	1.0 min								
20		Virabhadrasana II/ Warrior II Pose	1.0 min								
21		Utthita Trikonasana / Extended triangle pose	1.0 min								
22		Prasarita Padottanasana / Wide Legged Forward Bend									
23		Natarajasana / Lord of the dance pose									
24		Vrksansana / Tree pose	1.5 min								
25		Tadasana 🖊 Mountain pose	2.0 min								
26		Phalakasana / plank pose	1.0 min								
27		Bhujangasana 🖊 cobra pose	1.0 min								
28		Dekasana / Airplane pose	1.0 min								
29	Asana-Prone	Dhanurasana / Bow Pose	1.0 min								
30		Balasana / Child's pose	1.0 min								
31		Ustrasana / Camel pose	1.0 min								
32		Sasangasana / Rabbit pose	1.0 min								
33		Upavistha konasana 🖊 Paschimottanasana	2.0 min								
34	Asana-Sitting	Krounchasana / Heron pose	1.0 min								
35	8	Ardha Matsyendrasana / Half lord of the fishes Pose	1.0 min								
36		Paschimottanasana / Seated forward bend	1.0 min								
37		Ardha Setubandhasana / half bridge pose	1.0 min								
38	Cool-down	Pawanmuktasana / Wind-relieving pose	1.0 min								
39		Pawanmuktasana / Wind-relieving pose	2.0 min								
40	Relaxation	Shavasana / Corpse Pose	5.0 min								
41		session closing instructions	1.0 min								
		total	60.0 min								

	PELVIC ALIGNMENT YOGA									
	Block of Yoga sequence	Yoga Poses Names in English	Dur	ation						
1	Session preparation instruction	Session preparation instruction	2.0	min						
2	Neck stretching	Rotate the neck while breathing	3.0	min						
3	Lesson the joints of the fast	Plantar flexion and dorsiflexion of the ankle joint								
4	plantar flavion and dersiflavion	Internal / external rotation of the hip joint	5.0	min						
5	plantal nexion and doisinexion	Ankle rotation								
6		Pull and stretch the hip joint								
7	Loosen the hip joint	Sole pose	8.0	min						
8		Hip joint internal rotation stretch								
9		Raising and lowering the ischium								
10	Loosen the pelvis	Round and bend back	8.0	min						
11		Waist rotation								
12		Psoas major muscle training	2.0							
13	Psoas major training Lunge pose		2.0	m1n						
14		Raise and lower legs with prone position								
15		Bow pose								
16	Back training	Child's pose	3.0	min						
17		Downward-facing dog								
18		Frog pose								
19		Cat pose								
20		Balance pose								
21		Child's pose								
22		Downward-facing dog	12.0							
23	Core training	Swan pose	13.0	min						
24		Downward-facing dog								
25		Twist pose								
26		Wind Relieving pose								
27		Half lotus pose								
28	Spine stretch	Side bending	5.0	min						
29		Bridge pose								
30	II'm inint studeb	Wind Relieving pose	4.0							
31	hip joint stretch	Adductor muscle stretch	4.0	min						
32	Pelvic / spine stretch	Spinal rotation	2.0	min						
33	Delevation	Corpse pose	5.0							
34	Relaxation	Session closing instuctions	5.0	0 min						
		total	60.0	min						

Assessment Items

Measurement of aging-related markers

As previously reported ¹, expression levels of *hTERT*, *hTERC* and sirtuin genes (*SIRT1*, *SIRT6*) were measured in blood samples by real-time PCR. 8-OHdG was detected in plasma samples using a highly sensitive ELISA kit (Nikken Zyle, Shizuoka, Japan) and ROS was detected in plasma samples using an OxiSelectTM In Vitro ROS/RNS Assay Kit (Cosmo Bio, Tokyo, Japan) was used to measure ROS and RNS, respectively. Measurements were performed at the Japan Clinical Trial Association (Tokyo).

Skin measurement

For skin properties and functional evaluation, stratum corneum water content was measured, and spots and wrinkles were measured by image analysis. For these measurements, after washing the face with a commercially available cleanser, the skin was allowed to acclimate by resting for 20 minutes in a room maintained at a temperature of 21 ± 2 °C and a humidity of 50 ± 10 RH%. Skin moisture content was measured using a Corneometer (CM825; Courage & Khazaka, Köln, Germany), and the measurement site was the cheek. Facial skin images were analyzed using VISIA Evolution (Canfield Imaging Systems, Fairfield, NJ, USA) to evaluate blemishes (light spots), wrinkles, uneven coloration (texture), pores, and porphyrins. The measurement area was the entire face.

Stress assessment

Stress assessment was evaluated with the Japanese version of the SF-8 health-related quality of life scale.

Body Structure

Body weight and body fat percentage were measured using a body composition analyzer (BC-313; Tanita, Itabashiku, Tokyo). The circumference of the waist, hips, bust, arms, thighs, and calves were measured by the examiner using a contact method with a tape measure. Flexibility was measured in forward bending and backward bending by the examiner using a contact method with a tape measure (measuring the distance between the fingertips and the floor surface).

Blood pressure

Systolic and diastolic blood pressures were measured.

Blood biochemistry

Blood samples were used to measure the following: total protein (TP), albumin, total cholesterol, triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and albumin. alkaline phosphatase (ALP), leucine dehydrogenase (LDH) [IFCC], γ -glutamyl trans peptidase (γ -GTP), creatine phosphokinase (CPK), total bilirubin, urea nitrogen creatinine, sodium (Na), potassium (K), chlor, calcium (Ca), inorganic phosphorus (P), serum blood

sugar, HbAlc [NGSP], low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), uric acid (UA), insulin-like growth factor-I (IGF-I).

Hematological tests

The following items were measured using blood samples: white blood cell count, red blood cell count, hemoglobin, hematocrit, platelet count, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), and MCHC (mean cell hemoglobin concentration). Blood biochemical tests and hematological tests were performed at the Japan Clinical Trial Institute (Shinjuku, Tokyo).

Statistical analysis

Statistical analysis was performed using Excel statistics (Social Information Service, Tokyo, Japan) and a paired-t test. Corresponding t-tests were used to compare each item to its pre-test, and Student's t-tests were used to compare the change in each group from pre-test to 12 weeks posttest. Data multiplicity was not considered and there were no missing values. Both tests were two-tailed, and a risk rate of less than 5% (p < 0.05) was considered a significant difference.

Subject of analysis

The study started with 60 participants, but 6 dropped out in the hot yoga group (3 in the WSS group and 3 in the RF group). In all cases, the reason for dropping out of the study was discontinuation of participation for personal reasons. Of the 54 patients in the analysis population (full analysis set: FAS), one non-intervention control group member had lost a large amount of weight (-7.3 kg) from 62.2 kg before the start of the study (week 0) to 54.9 kg after 12 weeks, and the case review committee concluded that it was appropriate to exclude this person from the analysis.

The efficacy analysis (PPS analysis) included the following; Control group: 19 subjects, 43.6 ± 10.3 years old.

Hot yoga group: 34 subjects (WSS group 17, RF group 17), 44.1 \pm 9.5 years old.

Safety analysis was performed on all subjects; all 60 subjects who received the intervention at least once were included in the safety analysis.

Ethical standard

In accordance with the ethical principles based on the Declaration of Helsinki (revised Fortaleza, October 2013) and the Ethical Guidelines for Medical Research Involving Human Subjects (partially revised in 2017), this study was approved by the Ethical Review Committee of the Expert Committee on Pharmaceutical Affairs Law (Committee Chairman: Hisao Hoga, Attorney at Law) and the consent explanation document was given to the subject. The purpose and methods of the study were fully explained in writing and orally, and free will consent was obtained in writing from the subjects. The study was registered and conducted in the UMIN Clinical Trials Registry (UMIN #000044157).

Results

Aging-related marker measurements (Table 2)

After 12 weeks of hot yoga, *SIRT6* was significantly elevated in the hot yoga group compared to the control group (p < 0.001); *SIRT1* was significantly elevated in the control group compared to the hot yoga group (p = 0.034). There were no significant changes in *hTERT*, *hTERC*, 8-OHdG, or ROS between the control and hot yoga groups. No significant changes were found between the WSS and RF groups in the measurement of aging-related markers.

Skin measurements (Table 3)

After 12 weeks of hot yoga, skin water content (+21.8%, p<0.001), wrinkles (-15.8%, p=0.002), texture (+18.3%, p = 0.002) and porphyrins (-31.1%, p = 0.003) were significantly improved in the hot yoga group compared to the control group. Skin water content significantly improved in the WSS group compared to the RF group (p = 0.019).

Mental and physical stress assessment (Table 4)

After 12 weeks of hot yoga, the Health-Related Quality

Table 2. Aging-related markers.

of Life Scale-SF-8 items "Vitality" (p = 0.019), "Social functioning" (p = 0.045), "Role emotional" (p = 0.003) and "Mental component summary" (p = 0.042) were significantly higher in the hot yoga group and significantly improved in the hot yoga group. The health-related quality of life scale-SF-8"Physical component summary" significantly improved in the WSS group compared to the RF group (p = 0.030).

Body structure (Table 5)

After 12 weeks of hot yoga, body weight (-1.9%, p = 0.012), BMI (-1.7%, p = 0.021) and body fat percentage (-3.4%, p = 0.002) were significantly lower in the hot yoga group than in the control group. In the regional measurements, circumferences of the waist (-5.4%, p < 0.001), hips (-2.0%, p < 0.001), bust (-2.4%, p < 0.001), arms (-3.5%, p < 0.001), thigh (-4.4%, p < 0.001), and calf (-1.7%, p = 0.046) were significantly lower in the hot yoga group than in the control group. With regard to flexibility, the distance between forward bending (p < 0.001) and back bending (p = 0.018) was significantly shorter in the hot yoga group than in the control group. No significant changes were noted between the WSS and RF groups in the body structure measurements.

T.	Group		Compariso	Group comparison	Group comparison		
Items		n	0 w	12 w	P value	(vs. Control)	(vs RF group)
		11	Mean SE	Mean SE		of change	of change
	Control	19	2.87 ± 0.21	5.23 ± 0.51	0.000 0.000	0.024	
CIDT1	Hot yoga	34	3.16 ± 0.20	4.23 ± 0.26	0.002 0.002	0.034	
51K11	WSS	17	3.12 ± 0.32	4.52 ± 0.41	0.016 0.016	0.187	0.200
	RF	17	3.20 ± 0.24	3.94 ± 0.31	0.048 0.048	0.012	0.299
	Control	19	1.59 ± 0.24	2.32 ± 0.18	0.000 0.000	0.000	
SIRT6	Hot yoga	34	1.48 ± 0.18	4.98 ± 0.31	0.000 0.000	0.000	
	WSS	17	1.50 ± 0.26	4.76 ± 0.47	0.000 0.000	0.000	0.406
	RF	17	1.46 ± 0.23	5.20 ± 0.38	0.000 0.000	0.000	0.490
	Control	19	3.98 ± 0.26	5.48 ± 0.32	0.000 0.000	0.520	
	Hot yoga	34	3.93 ± 0.20	5.22 ± 0.27	0.000 0.000	0.520	
HIEKI	WSS	17	3.95 ± 0.31	5.16 ± 0.41	0.003 0.003	0.490	0.606
	RF	17	3.91 ± 0.26	5.28 ± 0.35	0.000 0.000	0.705	0.090
	Control	19	2.76 ± 0.16	3.82 ± 0.22	0.003 0.003	0.079	
LTEDC	Hot yoga	34	3.80 ± 0.24	4.87 ± 0.25	0.003 0.003	0.978	
ITERC	WSS	17	3.82 ± 0.38	5.08 ± 0.35	0.024 0.024	0.729	0.575
	RF	17	3.78 ± 0.29	4.66 ± 0.34	0.076 0.076	0.746	0.575
	Control	19	0.16 ± 0.02	0.11 ± 0.01	0.051 0.051	0.227	
8-OHdG	Hot yoga	34	0.15 ± 0.01	0.12 ± 0.01	0.232 0.232	0.237	
(ng/mL)	WSS	17	0.13 ± 0.01	0.12 ± 0.02	0.709 0.709	0.237	0.467
	RF	17	0.16 ± 0.03	0.12 ± 0.02	0.232 0.232	0.750	0.407
	Control	19	78.56 ± 0.96	76.74 ± 1.51	0.340 0.340	0.065	
ROS	Hot yoga	34	77.73 ± 1.19	70.99 ± 1.03	0.001 0.001	0.065	
(nM)	WSS	17	77.54 ± 1.72	70.94 ± 1.36	0.019 0.019	0.139	0.041
	RF	17	77.92 ± 1.63	71.05 ± 1.54	0.020 0.020	0.131	0.941

T4	Group		Compariso	Group comparison	Group comparison			
Items		n	0 w	12 w	P value		(vs. Control)	(Vs KF group)
			Mean SE	Mean SE			of change	of change
	Control	19	36.54 ± 1.75	35.47 ± 1.60	0.239	0.239	0.000	
Skin water	Hot yoga	34	36.28 ± 1.45	44.18 ± 1.69	0.000	0.000	0.000	
content	WSS	17	35.04 ± 1.29	45.70 ± 2.11	0.000	0.000	0.000	0.010
	RF	17	37.51 ± 2.56	42.66 ± 2.60	0.001	0.001	0.000	0.017
	Control	19	116.29 ± 9.14	132.92 ± 9.37	0.150	0.150	0 105	
Spot	Hot yoga	34	121.38 ± 6.08	118.41 ± 5.34	0.399	0.399	0.105	
	WSS	17	113.38 ± 6.63	115.88 ± 6.98	0.694	0.694	0.275	0.121
	RF	17	129.38 ± 9.82	120.94 ± 8.04	0.006	0.006	0.039	0.121
	Control	19	118.00 ± 5.81	132.26 ± 10.19	0.098	0.098	0.002	
Wrinklag	Hot yoga	34	108.60 ± 4.72	91.41 ± 4.80	0.000	0.000	0.002	
WIIIKIES	WSS	17	102.59 ± 4.41	83.65 ± 5.86	0.001	0.001	0.001	0.578
	RF	17	114.62 ± 8.09	99.18 ± 7.12	0.002	0.002	0.003	0.578
	Control	19	8.33 ± 1.03	7.50 ± 0.94	0.066	0.066	0.002	
Taytura	Hot yoga	34	6.48 ± 0.61	7.67 ± 0.67	0.009	0.009	0.002	
Texture	WSS	17	6.11 ± 0.86	7.58 ± 0.93	0.076	0.076	0.015	0.517
	RF	17	6.86 ± 0.85	7.76 ± 0.96	0.027	0.027	0.004	0.517
	Control	19	865.63 ± 72.04	900.24 ± 65.39	0.721	0.721	0.626	
Dores	Hot yoga	34	839.63 ± 66.04	825.81 ± 57.51	0.537	0.537	0.020	
roles	WSS	17	809.85 ± 101.35	817.21 ± 91.88	0.828	0.828	0.790	0.247
	RF	17	869.41 ± 84.09	834.41 ± 69.12	0.250	0.250	0.493	0.347
	Control	19	1146.03 ± 113.24	1253.50 ± 125.58	0.426	0.426	0.002	
Dombruing	Hot yoga	34	1329.81 ± 124.98	915.84 ± 105.02	0.000	0.000	0.003	
Forphyrins	WSS	17	1484.47 ± 190.75	1016.32 ± 167.94	0.020	0.020	0.015	0.580
	RF	17	1175.15 ± 152.58	815.35 ± 121.36	0.000	0.000	0.005	0.309

Table 3. Skin measurements.

Items	Group		Comparison o	Group comparison (vs. Control)	Group comparison (vs RF group)		
Items	Group	n	0 w	12 w	P value	_ Comparison	Comparison
	Control	10	$\frac{\text{Mean}}{51.00 \pm 1.42}$	$\frac{1}{52.60 \pm 0.67}$	0.564 0.564	of change	of change
Physical	Unit viana	- 19	51.90 ± 1.43	52.00 ± 0.07	0.304 0.304	0.353	
functioning	Hot yoga	17	51.10 ± 0.08	53.13 ± 0.38		0.289	
(PF)	w 33	17	51.93 ± 0.92	53.94 ± 0.32	0.050 0.050	0.388	0.948
		1/	50.39 ± 0.96	52.31 ± 0.64	0.039 0.039	0.423	
Dala abasiaal	Control	19	52.34 ± 1.00	52.79 ± 0.82	0.634 0.634	0.377	
(RP)	Hot yoga	34	$\frac{51.47 \pm 0.73}{52.20 \pm 1.00}$	53.00 ± 0.53	0.064 0.064		
(141)	w88	17	52.29 ± 1.00	54.45 ± 0.40	0.085 0.085	0.260	0.445
	RF	17	50.64 ± 1.01	51.55 ± 0.85	0.419 0.419	0.747	
	Control	19	52.65 ± 1.83	50.34 ± 1.89	0.197 0.197	0.101	
Bodily pain	Hot yoga	34	51.83 ± 0.92	53.03 ± 0.96	0.312 0.312		
(DP)	WSS	17	50.45 ± 1.48	53.86 ± 1.31	0.053 0.053	0.021	0.057
	RF	17	53.21 ± 0.99	52.20 ± 1.38	0.521 0.521	0.578	
-	Control	19	51.07 ± 1.29	52.41 ± 1.37	0.278 0.278	- 0.191	
General health	Hot yoga	34	51.47 ± 0.86	54.75 ± 0.60	0.000 0.000		
(GH)	WSS	17	50.95 ± 1.46	55.53 ± 0.96	0.003 0.003	0.079	0.119
	RF	17	51.98 ± 0.90	53.98 ± 0.67	0.044 0.044	0.668	
	Control	19	51.83 ± 1.26	51.51 ± 1.54	0.690 0.690	- 0.019	
Vitality (VT)	Hot yoga	34	51.91 ± 0.75	54.44 ± 0.64	0.007 0.007		
(10000)	WSS	17	51.17 ± 1.12	53.72 ± 1.03	0.067 0.067	0.069	0.983
	RF	17	52.65 ± 0.95	55.17 ± 0.74	0.055 0.055	0.060	01202
0 1	Control	19	51.84 ± 1.54	51.20 ± 1.87	0.582 0.582	- 0.045	
functioning	Hot yoga	34	49.94 ± 1.24	52.68 ± 1.08	0.026 0.026	0.045	
(SF)	WSS	17	50.06 ± 1.67	53.93 ± 0.89	0.023 0.023	0.025	0.344
(51)	RF	17	49.82 ± 1.82	51.42 ± 1.92	0.379 0.379	0.297	0.544
	Control	19	51.75 ± 1.08	50.60 ± 1.33	0.235 0.235	0.002	
Role emotional	Hot yoga	34	50.02 ± 0.96	52.71 ± 0.69	0.001 0.001	- 0.003	
(RE)	WSS	17	49.34 ± 1.36	52.35 ± 0.99	0.033 0.033	0.014	0.685
	RF	17	50.69 ± 1.32	53.07 ± 0.96	0.011 0.011	0.008	0.005
	Control	19	50.55 ± 1.46	50.30 ± 1.34	0.822 0.822	0.0(7	
Mental health	Hot yoga	34	50.45 ± 0.90	52.84 ± 0.83	0.010 0.010	0.067	
(MH)	WSS	17	50.63 ± 1.26	51.67 ± 1.20	0.438 0.438	0.455	0.124
	RF	17	50.27 ± 1.30	54.01 ± 1.08	0.004 0.004	0.015	0.124
	Control	19	51.31 ± 1.37	51.53 ± 1.19	0.890 0.890		
Physical	Hot yoga	34	50.81 ± 0.60	52.38 ± 0.57	0.048 0.048	- 0.455	
component	WSS	17	50.85 ± 1.01	54.06 ± 0.78	0.007 0.007	0.128	0.020
summary (PCS)	RF	17	50.77 ± 0.64	50.70 ± 0.59	0.949 0.949	0.879	0.030
	Control	19	49.99 ± 1.32	49.25 ± 1.67	0.621 0.621		
Mental	Hot yoga	34	49.10 ± 0.97	51.89 ± 0.97	0.001 0.001	- 0.042	
component	WSS	17	48.76 ± 1.48	50.73 ± 1.37	0.159 0.159	0.181	0.000
summary (MCS)	RF	17	49.43 ± 1.24	53.05 ± 1.30	0.000 0.000	0.014	0.298

Table 4. Mental and physical stress assessment.

Tuble C. Doug composition.	Table 5. Bod	y composition.
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Items	Group		Comparison o	Group comparison (vs. Control)	Group comparison (vs RF group)			
items	Group	n	0 w	12 w	P value		Comparison	Comparison
			Mean SE	Mean SE			of change	of change
Body weight	Control Hot yoga	<u> </u>	$\frac{66.09 \pm 2.02}{64.89 \pm 1.23}$	$\frac{66.13 \pm 2.03}{63.63 \pm 1.20}$	0.929	0.929	0.012	
(kg)	WSS	17	64.46 ± 1.50	63.03 ± 1.20 63.43 ± 1.62	0.004	0.004	0.036	
	RF	17	65.33 ± 1.94	63.83 ± 1.77	0.015	0.015	0.029	0.463
	Control	19	26.39 ± 0.84	26.41 ± 0.89	0.917	0.917		
5.44	Hot yoga	34	25.67 ± 0.39	25.23 ± 0.39	0.001	0.001	0.021	
BMI	WSS	17	25.81 ± 0.50	25.50 ± 0.52	0.015	0.015	0.090	0.290
	RF	17	25.53 ± 0.60	24.96 ± 0.57	0.013	0.013	0.027	0.280
	Control	19	37.54 ± 1.13	37.60 ± 1.20	0.831	0.831	0.002	
Body fat	Hot yoga	34	36.66 ± 0.74	35.40 ± 0.80	0.000	0.000	0.002	
percentage (%)	WSS	17	37.24 ± 1.00	36.45 ± 1.01	0.000	0.000	0.018	0.069
	RF	17	$36.08 \ \pm \ 1.08$	34.34 ± 1.19	0.002	0.002	0.003	0.007
	Control	19	89.42 ± 2.22	89.83 ± 1.88	0.585	0.585	0.000	
Waist (cm)	Hot yoga	34	89.56 ± 1.19	84.72 ± 1.14	0.000	0.000	0.000	
	WSS	17	89.35 ± 1.43	84.90 ± 1.51	0.000	0.000	0.000	0.607
	RF	17	89.77 ± 1.90	84.55 ± 1.72	0.001	0.001	0.000	
Hips (cm)	Control	19	100.66 ± 1.57	100.88 ± 1.44	0.585	0.585	0.000	
	Hot yoga	34	99.60 ± 0.94	97.64 ± 0.94	0.000	0.000		
	WSS	17	100.09 ± 1.15	98.61 ± 1.37	0.019	0.019	0.021	0.217
	RF	17	99.11 ± 1.47	96.66 ± 1.24	0.000	0.000	0.000	
	Control	19	97.12 ± 1.78	98.56 ± 1.62	0.003	0.003	0.000	
Bust (cm)	Hot yoga	34	95.02 ± 0.91	92.77 ± 0.80	0.000	0.000		
	WSS	17	95.10 ± 1.14	92.94 ± 1.14	0.000	0.000	0.000	0.778
	RF	17	94.94 ± 1.42	92.60 ± 1.14	0.000	0.000	0.000	
	Control	19	29.97 ± 0.94	30.65 ± 1.03	0.007	0.007	0.000	
Arms (cm)	Hot yoga	34	29.11 ± 0.37	28.11 ± 0.36	0.000	0.000		
	WSS	17	28.95 ± 0.48	28.15 ± 0.50	0.000	0.000	0.000	0.087
	RF	17	29.28 ± 0.56	28.06 ± 0.52	0.000	0.000	0.000	
	Control	19	51.83 ± 1.11	52.26 ± 1.07	0.165	0.165	0.000	
Thigh (cm)	Hot yoga	34	51.81 ± 0.68	49.53 ± 0.67	0.000	0.000		
	w 88	17	52.11 ± 0.96	50.15 ± 1.04	0.000	0.000	0.000	0.260
		20	$\frac{51.51 \pm 0.95}{27.06 \pm 0.70}$	48.92 ± 0.80	0.000	0.000	0.000	
	Hot yoga	20	$\frac{37.96 \pm 0.70}{27.07 \pm 0.45}$	38.09 ± 0.70	0.372	0.572	0.046	
Calf (cm)	wss	17	$\frac{37.97 \pm 0.43}{27.70 \pm 0.61}$	$\frac{37.32 \pm 0.38}{27.22 \pm 0.61}$	0.074	0.074	0.002	
		17	37.79 ± 0.01	$\frac{37.22 \pm 0.01}{37.42 \pm 0.98}$	0.311	0.311	0.245	0.827
	Control	10	$\frac{38.13 \pm 0.03}{1.50 \pm 0.81}$	$\frac{37.42 \pm 0.98}{1.95 \pm 0.91}$	0.225	0.225	0.245	
Forward	Hot yoga	3/	$\frac{1.30 \pm 0.81}{4.90 \pm 1.05}$	$\frac{1.33 \pm 0.31}{2.30 \pm 0.73}$	0.225	0.225	0.000	
bending (cm)	WSS	17	4.90 ± 1.05	2.30 ± 0.73	0.001	0.001	0.023	
	RE	17	4.94 ± 1.94	$\frac{2.75 \pm 0.97}{1.85 \pm 1.08}$	0.040	0.040	0.023	0.563
	Control	19	117.21 + 2.24	117.05 ± 1.00	0.754	0.754		
Back bending	Hot yoga	34	$\frac{1121}{121.84} \pm 1.21$	119.82 ± 1.34	0.001	0.001	0.018	
(cm)	WSS	17	121.74 ± 1.55	120.06 ± 1.69	0.020	0.020	0.074	
- *	RF	17	121.94 ± 1.85	119.58 ± 2.08	0.025	0.025	0.052	0.557
		- '	121.51 = 1.05					

Blood pressure (Table 6)

No significant changes in blood pressure measurements were found between the control and hot yoga groups.

Blood biochemistry (Table 7)

After 12 weeks of hot yoga, HbA1c was significantly lower in the hot yoga group compared to the control group (-1.36%, p < 0.001). Calcium significantly increased in the hot yoga group compared to the control group (+1.39%, p = 0.005).

HbA1c was significantly lower in the RF group compared to the WSS group (p = 0.020). Total protein was significantly higher in the WSS group than in the RF group (p = 0.022); CK was significantly higher in the RF group than in the WSS group (p = 0.028).

Hematological tests (Table 8)

After 12 weeks of hot yoga, there were no significant changes in hematological tests between the control and hot yoga groups.

Red blood cell count (p = 0.008), hemoglobin (p = 0.018), and hematocrit (p = 0.034) were significantly higher in the WSS group than in the RF group.

Safety

There were no safety-related adverse events during or after the study period that were causally related to the use of hot yoga.

Table 6. Blood pressure.

T.	a a		Comparison	Group comparison	Group comparison				
Items	Gro	up		0 w	12 w	P value		(vs. Control)	(vs RF group)
				Mean SE	Mean SE			of change	of change
	Control		19	117.11 ± 2.35	118.89 ± 2.05	0.013	0.013	0.216	
Systolic blood	Hot yoga		34	119.56 ± 1.82	120.41 ± 1.66	0.202	0.202	0.510	
(mmHg)		WSS	17	118.29 ± 2.62	118.76 ± 2.53	0.605	0.605	0.242	0 568
× 0,		RF	17	120.82 ± 2.48	122.06 ± 2.07	0.225	0.225	0.641	0.508
	Control		19	80.32 ± 0.89	81.11 ± 0.87	0.279	0.279	0.068	
Diastolic blood	ic blood Hot yoga		34	79.26 ± 0.80	80.09 ± 0.59	0.076	0.076	0.908	
(mmHg)		WSS	17	78.53 ± 1.14	79.41 ± 0.84	0.114	0.114	0.917	0.808
~ 6/		RF	17	80.00 ± 1.09	80.76 ± 0.78	0.320	0.320	0.981	0.090

SE, standard error.

Table 7. Blood biochemistry.

-	-		Comparison of before and after							Group comparison	Group comparison
Items	Grou	ıp		0	W	1	12 w		alue	(vs. Control)	(vs RF group)
			n	Mean	SE	Mean	SE			of change	Comparison of change
	Control		19	7.40 =	± 0.08	7.44	± 0.08	0.520	0.520	0.440	
Total protein	Hot yoga		34	7.36	± 0.05	7.34	± 0.06	0.674	0.674	0.440	
(g/dL)		WSS	17	7.26	± 0.05	7.35	± 0.08	0.156	0.156	0.601	0.022
		RF	17	7.45	± 0.07	7.32	± 0.08	0.077	0.077	0.076	0.022
	Control		19	4.39	± 0.07	4.37	± 0.08	0.736	0.736	0.085	
Albumin	Hot yoga		34	4.45 =	± 0.04	4.44	± 0.04	0.694	0.694	0.985	
(g/dL)		WSS	17	4.37 :	± 0.06	4.42	± 0.06	0.375	0.375	0.370	0.006
		RF	17	4.54	± 0.04	4.46	± 0.06	0.149	0.149	0.380	0.090
T ()	Control		19	225.84	± 9.29	217.37	± 8.94	0.125	0.125	0.751	
1 otal Cholesterol	Hot yoga		34	219.24	± 7.61	212.85	± 6.00	0.111	0.111	0.731	
(mg/dL)		WSS	17	205.88	± 10.09	206.65	± 6.79	0.887	0.887	0.225	0.066
		RF	17	232.59	± 10.45	219.06	± 9.65	0.021	0.021	0.502	0.000

	~ .					0.0.40		
	Control	19	122.74 ± 17.31	106.21 ± 14.31	0.068	0.068	0.993	
TG (mg/dL)	Hot yoga	17	$\frac{111.59 \pm 14.35}{102.52 \pm 16.27}$	95.21 ± 10.31	0.223	0.223	0.901	
		17	$\frac{103.33 \pm 10.27}{110.65 \pm 22.48}$	$\frac{83.39 \pm 10.80}{106.82 \pm 17.07}$	0.073	0.073	0.801	0.793
	Control	1/	119.03 ± 23.46	100.82 ± 17.07 10.16 ± 1.32	0.010	0.010	0.000	
AST (GOT)	Hot yogo	24	$\frac{17.47 \pm 0.70}{10.06 \pm 0.50}$	19.10 ± 1.32	0.030	0.030	0.316	
(U/L)	WSS	17	19.00 ± 0.33	25.09 ± 2.19	0.103	0.105	0.370	
(• • =)		17	$\frac{19.00 \pm 0.74}{10.12 \pm 0.01}$	23.37 ± 3.30 21.71 + 1.38	0.237	0.054	0.579	0.477
	Control	10	10.12 ± 0.01 16.16 ± 2.04	18.63 + 2.86	0.092	0.092	0.550	
ALT (GPT)	Hot yoga	34	10.10 = 2.01 17.94 + 0.99	26.59 ± 5.25	0.103	0.103	0.255	
(U/L)	WSS	17	17.47 ± 1.21	29.65 ± 9.54	0.223	0.223	0.332	
	RF	17	18.41 ± 1.56	23.53 ± 4.27	0.217	0.217	0.538	0.505
	Control	19	71.58 ± 3.96	69.32 ± 3.86	0.141	0.141	0.000	
	Hot voga	34	65.82 ± 2.40	67.62 ± 2.69	0.311	0.311	0.081	
ALP(U/L)	WSS	17	63.18 ± 3.20	68.18 ± 4.18	0.077	0.077	0.024	
	RF	17	68.47 ± 3.45	67.06 ± 3.38	0.506	0.506	0.740	0.066
	Control	19	185.21 ± 5.29	196.37 ± 8.81	0.116	0.116		
LDH [IFCC]	Hot yoga	34	182.12 ± 3.99	187.91 ± 5.19	0.169	0.169	0.503	
(U/L)	WSS	17	181.35 ± 4.88	188.88 ± 8.31	0.317	0.317	0.717	0.604
	RF	17	182.88 ± 6.31	186.94 ± 6.22	0.336	0.336	0.377	0.681
γ-GTP (U/L)	Control	19	32.63 ± 9.06	30.37 ± 9.09	0.503	0.503		
	Hot yoga	34	24.00 ± 2.93	27.97 ± 3.94	0.136	0.136	0.146	
	WSS	17	20.71 ± 3.40	29.24 ± 6.41	0.088	0.088	0.070	0.004
	RF	17	27.29 ± 4.64	26.71 ± 4.58	0.747	0.747	0.660	0.084
	Control	19	96.47 ± 11.05	121.42 ± 34.20	0.493	0.493	0.707	
CPK (U/L)	Hot yoga	34	82.32 ± 5.39	97.82 ± 8.36	0.014	0.014	0.797	
	WSS	17	76.35 ± 9.06	78.88 ± 8.69	0.673	0.673	0.542	0.028
	RF	17	88.29 ± 5.47	116.76 ± 12.73	0.008	0.008	0.925	0.020
	Control	19	0.62 ± 0.06	0.68 ± 0.07	0.055	0.055	0 192	
Total Bilirubin	Hot yoga	34	0.52 ± 0.02	0.66 ± 0.05	0.003	0.003	0.172	
(mg/dL)	WSS	17	0.54 ± 0.04	0.65 ± 0.07	0.089	0.089	0.543	0 509
	RF	17	0.51 ± 0.03	0.68 ± 0.07	0.015	0.015	0.164	0.505
	Control	19	12.81 ± 0.67	11.88 ± 0.79	0.153	0.153	0.325	
BUN (mg/dL)	Hot yoga	34	15.64 ± 2.73	11.79 ± 0.52	0.189	0.189	0.525	
	WSS	17	11.85 ± 0.69	10.66 ± 0.70	0.117	0.117	0.780	0.367
	RF	17	19.43 ± 5.25	12.91 ± 0.65	0.270	0.270	0.344	
	Control	19	0.67 ± 0.02	0.67 ± 0.02	0.885	0.885	0.224	
Creatinine	Hot yoga	34	0.71 ± 0.02	0.73 ± 0.01	0.077	0.077		
(mg/dL)	WSS	17	0.72 ± 0.02	0.72 ± 0.02	0.777	0.777	0.753	0.183
	RF	17	0.70 ± 0.02	0.74 ± 0.02	0.009	0.009	0.053	
	Control	19	140.84 ± 0.34	140.84 ± 0.50	1.000	1.000	0.423	
Na (mmol/L)	Hot yoga	34	141.00 ± 0.28	141.38 ± 0.28	0.222	0.222		
	WSS	17	141.12 ± 0.46	141.18 ± 0.36	0.904	0.904	0.923	0.300
	RF	10	140.88 ± 0.31	141.59 ± 0.42	0.083	0.083	0.187	
		19	$\frac{4.48 \pm 0.07}{4.28 \pm 0.05}$	4.19 ± 0.03	0.001	0.001	0.762	
K (mmol/L)	HOL YOGA	17	$\frac{4.38 \pm 0.03}{4.22 \pm 0.07}$	$\frac{4.11 \pm 0.03}{4.11 \pm 0.02}$	0.000	0.000	0.404	
	W 8 8	17	$\frac{4.32 \pm 0.07}{4.42 \pm 0.07}$	4.11 ± 0.03	0.001	0.001	0.494	0.399
	KF	1/	4.43 ± 0.07	4.12 ± 0.03	0.001	0.001	0.8//	

	Control	19	103.74 ± 0.49	103.53 ± 0.49	0.561	0.561	0.065	
C1(mmol/L)	Hot yoga	34	104.06 ± 0.25	103.00 ± 0.23	0.000	0.000	0.005	
CI (IIIII0I/L)	WSS	17	104.12 ± 0.36	102.71 ± 0.33	0.001	0.001	0.019	0 106
	RF	17	104.00 ± 0.33	103.29 ± 0.31	0.111	0.111	0.373	0.190
	Control	19	9.21 ± 0.08	9.14 ± 0.10	0.172	0.172	0.005	
C_{a} (mg/dL)	Hot yoga	34	9.31 ± 0.04	9.44 ± 0.04	0.007	0.007	0.005	
Ca (Ing/uL)	WSS	17	9.34 ± 0.05	9.46 ± 0.05	0.022	0.022	0.009	0 808
	RF	17	9.28 ± 0.06	9.42 ± 0.07	0.099	0.099	0.032	0.070
	Control	19	3.53 ± 0.09	3.56 ± 0.11	0.765	0.765	0.760	
P(mg/dL)	Hot yoga	34	3.39 ± 0.08	3.46 ± 0.08	0.393	0.393	0.709	
r (llig/uL)	WSS	17	3.31 ± 0.12	3.41 ± 0.11	0.387	0.387	0.658	0.724
	RF	17	3.48 ± 0.11	3.52 ± 0.12	0.738	0.738	0.952	0.724
	Control	19	94.11 ± 3.34	90.21 ± 3.56	0.243	0.243	0.044	
BS (Serum)	Hot yoga	34	91.21 ± 1.48	87.56 ± 0.91	0.014	0.014	0.944	
(mg/dL)	WSS	17	89.41 ± 2.05	87.06 ± 1.29	0.199	0.199	0.678	0.264
	RF	17	93.00 ± 2.05	88.06 ± 1.26	0.038	0.038	0.790	0.304
HbAlc [NGSP]	Control	19	5.44 ± 0.12	5.50 ± 0.12	0.030	0.030	0.000	
	Hot yoga	34	5.40 ± 0.05	5.33 ± 0.04	0.005	0.005	0.000	
(%)	WSS	17	5.35 ± 0.07	5.33 ± 0.07	0.548	0.548	0.054	0.020
	RF	17	5.46 ± 0.07	5.33 ± 0.05	0.002	0.002	0.000	0.020
	Control	19	131.26 ± 7.45	128.47 ± 7.38	0.538	0.538	0.017	
LDL-C	Hot yoga	34	128.09 ± 6.59	126.59 ± 5.88	0.652	0.652	0.817	
(mg/dL)	WSS	17	117.94 ± 8.13	120.76 ± 6.20	0.619	0.619	0.437	0.105
	RF	17	138.24 ± 9.77	132.41 ± 9.78	0.102	0.102	0.589	0.195
	Control	19	67.63 ± 4.94	65.74 ± 4.23	0.264	0.264	0.001	
HDL-C	Hot yoga	34	66.15 ± 2.47	66.94 ± 2.49	0.583	0.583	0.224	
(mg/dL)	WSS	17	65.82 ± 3.19	67.59 ± 3.27	0.444	0.444	0.199	0.507
	RF	17	66.47 ± 3.78	66.29 ± 3.74	0.924	0.924	0.488	0.507
	Control	19	4.77 ± 0.25	4.75 ± 0.26	0.900	0.900	0.057	
T TA ((1T)	Hot yoga	34	4.77 ± 0.20	4.93 ± 0.21	0.152	0.152	0.357	
UA (mg/dL)	WSS	17	4.75 ± 0.33	4.98 ± 0.37	0.094	0.094	0.233	0.520
	RF	17	4.79 ± 0.24	4.88 ± 0.20	0.616	0.616	0.644	0.538
	Control	19	152.11 ± 9.49	150.95 ± 9.79	0.812	0.812	0.000	
IGF-I	Hot yoga	34	159.12 ± 6.79	150.03 ± 7.80	0.128	0.128	0.298	
(ng/mL)	WSS	17	161.06 ± 10.08	156.00 ± 12.03	0.648	0.648	0.746	0.500
	RF	17	157.18 ± 9.07	144.06 ± 9.71	0.009	0.009	0.076	0.500

Items	Group	Comparison of before and after					Group comparison	Group comparison
		n	0 w	w 12 w P value		alue	(vs. Control)	(Vs RF group)
			Mean SE	Mean SE			of change	of change
WBC (/µL)	Control	19	7184.21 ± 360.84	6526.32 ± 379.30	0.019	0.019	0.546	
	Hot yoga	34	6508.82 ± 242.91	5661.76 ± 254.58	0.000	0.000		
	WSS	17	6270.59 ± 375.34	5258.82 ± 394.96	0.000	0.000	0.298	0.354
	RF	17	6747.06 ± 297.41	6064.71 ± 290.08	0.025	0.025	0.949	
RBC (×10 ⁴ /µL)	Control	19	464.95 ± 7.71	465.74 ± 7.39	0.853	0.853	0.546	
	Hot yoga	34	446.38 ± 4.26	450.65 ± 4.91	0.277	0.277		
	WSS	17	440.82 ± 7.35	454.94 ± 7.99	0.012	0.012	0.049 0.335	0.008
	RF	17	451.94 ± 3.86	446.35 ± 5.52	0.278	0.278		
Hb (g/dL)	Control	19	12.87 ± 0.37	12.83 ± 0.39	0.729	0.729	0.647	
	Hot yoga	34	13.30 ± 0.16	13.34 ± 0.18	0.767	0.767		
	WSS	17	13.10 ± 0.22	13.44 ± 0.26	0.038	0.038	0.065	0.018
	RF	17	13.49 ± 0.21	13.24 ± 0.24	0.184	0.184		
Ht (%)	Control	19	39.15 ± 0.85	40.05 ± 0.87	0.040	0.040	0.870	
	Hot yoga	34	39.65 ± 0.36	40.64 ± 0.44	0.013	0.013		
	WSS	17	39.22 ± 0.49	41.01 ± 0.61	0.001	0.001	0.148	- 0.034
	RF	17	40.07 ± 0.52	40.26 ± 0.61	0.736	0.736	0.318	
PLT (×10 ⁴ /μL)	Control	19	35.43 ± 1.63	50.09 ± 15.87	0.367	0.367	0.314	
	Hot yoga	34	28.78 ± 0.97	27.03 ± 0.93	0.002	0.002		
	WSS	17	26.79 ± 1.13	25.19 ± 1.18	0.022	0.022	0.318	0.790
	RF	17	30.76 ± 1.43	28.88 ± 1.30	0.045	0.045		
MCV (fL)	Control	19	84.60 ± 2.17	86.36 ± 2.17	0.000	0.000	0.476	
	Hot yoga	34	88.97 ± 0.97	90.36 ± 0.98	0.000	0.000		
	WSS	17	89.28 ± 1.73	90.51 ± 1.71	0.005	0.005	0.333 0.762	0.608
	RF	17	88.66 ± 0.85	90.22 ± 0.94	0.010	0.010		
MCH (pg)	Control	19	27.85 ± 0.93	27.71 ± 0.98	0.365	0.365	0.830	
	Hot yoga	34	29.86 ± 0.41	29.67 ± 0.40	0.139	0.139		
	WSS	17	29.86 ± 0.71	29.67 ± 0.70	0.209	0.209	0.850	0.082
	RF	17	29.86 ± 0.39	29.66 ± 0.40	0.374	0.374	0.861	0.982
MCHC (g/dL)	Control	19	48.62 ± 15.44	31.91 ± 0.42	0.306	0.306	0 2 2 7	
	Hot yoga	34	33.54 ± 0.19	32.79 ± 0.18	0.000	0.000	0.327	
	WSS	17	33.40 ± 0.28	32.75 ± 0.31	0.001	0.001	0.324	0.523
	RF	17	33.67 ± 0.25	32.84 ± 0.17	0.002	0.002	0.331	0.323

Table 8. Hematological tests.

Discussion

Summary of results

The effects of a 60-minute hot yoga program twice a week for 12 weeks on the body of healthy women, who were aware of their lack of exercise, were examined and compared to a control group.

The results showed improvement in skin condition (water content, wrinkles, and texture), improvement in physical and mental stress, improvement in body weight, shape, and flexibility, decrease in HBA1c, and increase in sirtuin gene (*SIRT6*) expression. No adverse events were noted. These results indicate that hot yoga has a wide range of effects and that *SIRT6* may be involved in some of the mechanisms of action.

Effects of yoga and bedrock on the body

The effects of yoga practice vary depending on the target age group, work environment, and presence or absence of disease. Among the elderly, mental health benefits such as improved sleep quality $^{2,3)}$ and increased social activity $^{4)}$ were observed.

In middle-aged adults, the following effects have been reported: alleviation of physical and mental stress⁵⁻¹², improvement of sleep quality^{10, 12-14}, and improvement of depressive symptoms⁸).

In young people, there have been reports of stress reduction $^{15,16)}$ and improvement of depressive symptoms $^{16)}$.

For high school students, yoga was not found to improve physical and mental stress, however, psychological effects such as recovery from fatigue, improvement of apathy¹⁷, elevation of mood, and alleviation of negative emotions¹⁸) have been reported. Many of the students who underwent the yoga program reported that they found it effective.

On the other hand, yoga did not reduce psychosomatic stress in elementary school students^{19,20}. It is possible that there was originally little room for reduction due to the low psychosomatic stress load. In recent years, due to the coronavirus outbreak, the response to yoga may be different from that of schoolchildren, who are more stressed, and further investigation is needed.

Tolerance and sensitivity to psychosomatic stress vary greatly among people and their living environment. Therefore, it is extremely important to conduct research and accumulate data on the Japanese population.

A psychological survey was conducted on 74 new yoga practitioners, and the results showed an increase in selfesteem, satisfaction with life, and motivation to live positively, and a decrease in interpersonal anxiety and perfectionism²¹.

The bedrock baths popular in Japan incorporate thermotherapy, a component of hot yoga.

In a study of 13 women (mean age 47.3 years) lying down three times for about 10 minutes each time on a hot stone as a bedrock bath, there were no significant changes in pulse wave velocity²²⁾. In a heart rate variability analysis of 21 healthy adult male and female subjects aged 21-36 years, bedrock bathing significantly increased blood flow rate²³⁾. In addition, bedrock bathing is associated with a transient increase in blood glucose as body temperature rises, which has been shown to be alleviated by drinking water²⁴⁾. This

is important information indicating the need for adequate hydration when administering thermal therapies, including hot yoga.

In this study, hot yoga improved various SF-8 stress indicators. Despite the fact that the yoga program was designed for beginners, it has demonstrated psycho-psychologybenefits.

Role of HSPs

The role of heat shock proteins (HSPs) is important in the mechanism of action of the thermic effect, one of the components of hot yoga. HSPs are produced in the body when cells are damaged and are involved in the synthesis of proteins necessary for the repair of damaged cells²⁵ and also have immunostimulatory effects such as activation of NK cells²⁶. It is assumed to be an important mechanism of action for alleviating mental and physical stress, relieving fatigue, promoting wound healing, and demonstrating immunostimulating power.

Hsp70 members include cytoplasmic stress-induced Hsp70 (Hsp72), homeostatic cytoplasmic Hsc70 (Hsp73), and ER-localized BiP(Grp78)²⁵⁾. Hsp70 has ATPase activity, promotes ATP hydrolysis, enhances peptide binding, and stimulates new protein synthesis. Concurrently, it restores the structure of denatured proteins and prevents abnormal aggregation^{27,28)}. Unrepairable proteins undergo ubiquitination and are transported to the proteasome for degradation. As a result, endoplasmic reticulum stress in the cell is reduced and apoptosis is inhibited.

HSPs also have a positive effect on sleep quality. In animal studies, administration of HSP70 close to the hypothalamus, the control center of the sleep-wake rhythm, prolongs "slow wave sleep" via GABA receptors and improves sleep quality ²⁹⁻³⁰).

No measurements were made on HSP in this study. Blood test results showed increased total protein and Hb, suggesting that HSP may increase protein synthesis as a result of reduced accumulation of abnormal intracellular proteins and reduced endoplasmic reticulum stress, which in turn stimulates cellular function. Whether hot yoga increases HSPs or not remains to be verified in the future.

Role of SIRT1

In this study, we measured mRNA for *SIRT1* and *SIRT6*, two sirtuins derived mainly from leukocytes. SIRT1 functions as an NAD⁺-dependent protein deacetylase and plays an important role in supplying NAD⁺, which is essential for the mitochondrial TCA cycle to function. Progressive cellular senescence³¹) and increased oxidative stress³²) decrease *SIRT1* expression. mRNA-binding protein HuR normally binds to *SIRT1* mRNA 3'UTR to form a complex, but oxidative stress causes the *SIRT1*-HuR complex to diverge, resulting in decreased *SIRT1* expression.³². Whereas, hypoxia³³ and exercise ³⁴ increase *SIRT1* expression.

AMPK (AMP-activated protein kinase), which is important for cellular energy homeostasis, senses changes in the intracellular AMP/ATP ratio and changes its activity. When the AMP/ATP ratio increases due to exercise, AMPK is activated, resulting in phosphorylation of the transcriptional coactivator PGC-1 α . An increase in the intracellular NAD⁺/ NADH ratio is also triggered, leading to an increase in SIRT1 activity. SIRT1 further increases its activity by directly deacetylating phosphorylated PGC-1 α^{34} . AMPK activation leads to increased exercise endurance. Thus, exercise increases *SIRT1* expression, creating a virtuous cycle toward homeostasis of cellular and bodily functions and restoration of diminished function. Attempts to substitute drugs for this function are not recommended.

In the present study, hot yoga resulted in a decrease in body weight and BMI, and changes in body shape (decreases in waist, bust, hips, and thigh and lower leg circumference) due to a decrease in body fat. These effects should be attributed to the subjects' increased efficiency of energy production during daily activities and physical activity, homeostasis of glycolipid metabolism (*e.g.*, improved insulin resistance), and increased efficiency of fatty acid β -oxidation, in addition to the exercise associated with postural maintenance (posing) during hot yoga. In parallel with hot yoga, increasing the amount of physical activity in daily life can provide additional benefits.

As for thermal stimulation, it is not known whether *SIRT1* expression is increased, but it promotes the production of the heat shock protein HSP70. hsf1 (heat shock factor 1) is a transcription factor for the *HSP70* gene. Upon thermal stimulation, SIRT1 binds to HSF-1 and increases its transcriptional activity, leading to increased production of HSP70³⁵.

Although there was no significant increase in *SIRT1* expression in the present study compared to the control group, it is likely that the thermal stimulation of hot yoga may have promoted the induction of HSP70 production with an increase in transcriptional activity via the formation of the SIRT1/HSF-1 complex.

Many people in today's society face a potential lack of exercise and excessive fat intake. Under these circumstances, it is extremely important to maintain healthy *SIRT1* expression. In the liver, SIRT1 prevents a decrease in PPAR α signaling and fatty acid β -oxidation, leading to the prevention of fatty liver and steatohepatitis³⁶. In adipose tissue, SIRT1 prevents increased production of inflammatory adipocytokines, suppresses inflammatory responses, and leads to the prevention of steatosis³⁷.

Role of SIRT6

SIRT6 has NAD⁺-dependent histone deacetylase (HDAC) activity, which is characterized by weaker deacetylase activity and stronger demyristoylation activity than SIRT1, and should be regarded as a lysine deacylase³⁸). *SIRT6* knockout mice had shorter life spans, decreased white blood cell counts, and decreased bone density, and *SIRT6*-deficient cells were more susceptible to DNA damage³⁹). Male *SIRT6* transgenic mice had longer life spans, decreased serum IGF-I and increased IGFBP1 levels⁴⁰).

SIRT6 has been postulated to stabilize telomeres⁴¹, regulate the transcriptional activity of NF-kB⁴², and maintain DNA repair capacity³⁹. *SIRT6*-deficient cells are more susceptible to DNA damage.

There are few reports on the dynamics of SIRT6 in humans.

In the area of reproductive medicine, women with reduced fertility due to impaired ovarian function had lower levels of *SIRT1* expression with no difference in *SIRT6* expression compared to those with other causes⁴³. In the results of cyclic fasting in women who wanted to conceive, *SIRT1* expression was elevated, but *SIRT6* was not affected⁴⁴.

Daily consumption of a vitamin D-enriched yogurt drink for 12 weeks increased blood levels of SIRT1 and SIRT6 in type 2 diabetic subjects, and the vitamin D plus Ca-enriched yogurt drink enhanced the increase in SIRT6⁴⁵.

And in the present study, the hot yoga practice was found to increase SIRT6. Since the results are compatible to those of a previous report 1 , the findings are highly reproducible.

SIRT6 also affects the GH/IGF-I system; many *SIRT6* knockout mice die early in life due to hypoglycemia. *SIRT6* knockout mice exhibit phenotypes seen with aging, including growth retardation, smaller size, low insulin, decreased lymphocytes, decreased subcutaneous adipose tissue, decreased bone density, and kyphosis³⁸⁾. GH/IGF-1 levels are reduced, contributing to the smaller size of the individuals.

SIRT6 overexpressing mice are significantly longer-lived males, but not females, than wild-type mice, and have been reported to have lower IGF-I levels than males of wild-type mice⁴⁶. This can be interpreted as a compensatory increase in IGF-I in captive wild-type mice as a result of increased obesity tendency and insulin resistance due to stress and lack of exercise, while this compensatory increase is restored in SIRT6-overexpressing mice. SIRT6 overexpressing mice also have higher levels of IGF binding protein 1 (IGFBP-1), suggesting that SIRT6 is protective against high-fat dietary load ³⁹.

Inferring insulin resistance among the three groups in the present study, the mean waist diameter of the RFr group was the largest, and insulin resistance may be high. We interpret this as a significant decrease in serum IGF-I, which was compensatory elevated in the RF group, due to hot yoga.

Improvement of skin function

The improvement of skin function by hot yoga may be the result of the combined effects of several factors. The mechanisms of action include reduction of oxidative stress, involvement of HSPs, and involvement of *SIRT* gene expression.

Photoaging accounts for a large portion of skin aging and involves oxidative stress from UV exposure. In the present study, hot yoga significantly reduced ROS (p =0.065 in group comparison with the control group). UV exposure and oxidative stress are highly involved in wrinkle formation⁴⁷⁾. It is possible that hot yoga increased tolerance to oxidative stress and decreased ROS production, resulting in wrinkle suppression.

It is not known whether *SIRT1* expression is increased for thermal stimuli. However, thermal stimulation encourages SIRT1 to produce HSP70; HSF1 (heat shock factor 1) is a transcription factor for the *HSP70* gene. Upon thermal stimulation, SIRT1 binds to HSF1 and increases its transcriptional activity, leading to increased production of HSP70⁴⁸.

Although there was no significant increase in *SIRT1* expression in this study compared to the control group, it is quite possible that the thermal stimulation of hot yoga may have induced the production of HSP70 through SIRT1.

HSP70 affects skin function. Increased HSP70 in skin

keratinocytes reduces cellular damage (cell death, inflammatory response, and DNA damage) caused by UV exposure⁴⁹, and increased HSP70 in pigment cells suppresses melanin production and reduces the formation of dark spots⁵⁰.

Furthermore, it has been shown that hyperthermia increases HSP70 and suppresses wrinkle formation. Since the ability of cells to induce HSP70 is decreased in the elderly⁵¹, it may be advisable to actively incorporate thermal therapy such as hot yoga in the elderly.

Bipolar fractional radiofrequency (FRF) therapy is used in dermatology for the treatment of acne scars, acne vulgaris, and wrinkle removal. The expression levels of *FoxO3* and p53 were reported to be decreased ⁵². SIRT6 activity may be involved in the improvement of skin texture.

In the current study, hot yoga significantly improved skin water content, wrinkles (p = 0.002), and texture. The effects of hot yoga on skin were reproducible, as skin water content and texture were also improved in the previous study¹). We expect that these effects are the result of the synergistic effects of alleviation of oxidative stress, induction of HSP70, and increased *SIRT6* expression.

Comparison of RF group and WSS group

The comparison between the RF group and the WSS group showed that the WSS group had significantly better "Physical component summary", a comprehensive index of subjective physical symptoms related to quality of life on the SF-8, and blood tests showed that the WSS group avoided decreases in RBC, Hb, and Hct, significantly increased total protein, and had less elevated CPK. These are the advantages of the Woodstone floors. The only item that showed an advantage for the RF group was the HbA1c blood test, which was significantly lower for the RF group (-2.4%) but unchanged for the WSS group (-0.3%). Considering the above, it can be inferred that there are more advantages to using woodstone floors in hot yoga.

Safety

There were no adverse events attributable to hot yoga performed under proper supervision and guidance. The safety of this therapy was confirmed.

Conclusions

Aging, lack of exercise, and excessive fat intake result in the decline of various cellular and tissue functions, including the decline of HSPs and sirtuins. As a result, cellular homeostasis is compromised, resulting in reduced mitochondrial function, accumulation of senescent proteins, and increased endoplasmic reticulum stress. In this study, a 12-week hot yoga program was administered to women with potential physical inactivity and compared to a control group. The results showed improvement in physical and mental stress, weight loss, body shape, skin condition (wrinkles, texture, moisture retention), and an increase in sirtuin SIRT6. In a comparison between RF and woodstone floors, the latter were estimated to have more advantages. It was suggested that hot yoga, a combination of moderate heat therapy and yoga physical activity, is a safe treatment that increases sirtuin activity, acts synergistically with each other, and enhances the efficiency of health promotion effects both physically and mentally.

Conflict of interest declaration

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