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Original article

Hot yoga increases SIRT6 gene expression, inhibits ROS generation, and improves skin condition

Takahiro Ishikawa [1,2], Eri Ito [1], Takumi Okada [1], Takahiko Sumi [1]

1) LAVA International, Inc. Tokyo, Japan.
2) River City Clinic, Tokyo, Japan.

Abstract

Accumulating evidence suggests that yoga promotes a healthy life expectancy in humans; however, the molecular basis of this effect is not clear. Moderate exercise is known to activate sirtuin family genes by improving blood circulation and prevent aging. Hot yoga might have a particularly strong benefit to activate these sirtuin family genes, because prolonged exposure to high temperatures coupled with high humidity can increase blood flow. Here, we investigated the impact of hot yoga on characteristic features of aging by assessing body composition and blood biochemistry indexes in 48 healthy female volunteers (aged 20 to 59 years) before and after completion of a hot yoga program consisting of two 60 minutes lessons per week for 12 weeks. *SIRT6* expression was increased and ROS levels were decreased in volunteers after completing the hot yoga program compared with before starting. Furthermore, we demonstrated that completion of the hot yoga program increased the moisture content in the stratum corneum and elasticity and improved skin texture. These results suggest that hot yoga can have a positive impact on various features associated with aging, suggesting that it might be useful in delaying senescence.

KEY WORDS: hot yoga, anti-aging, reactive oxygen species (ROS), sirtuin, telomerase

Introduction

Recently, an increase in lifestyle diseases and medical problems related to aging, such as diabetes mellitus, arteriosclerosis, myocardial infarction, stroke, and cancer, has become a serious problem in many countries [1-4]. Thus, identifying a way to overcome the biological impacts of senescence would help reduce the onset of the aforementioned medical conditions; further, such an improvement could even potentially reduce the risk of metabolic conditions such as hyperglycemia, dyslipidemia, and hypertension [5].

Yoga is a form of exercise in which participants sustain varied poses and slow movements, and that has become particularly popular among young women. Regular yoga exercise has a positive impact not only in terms of well-being (including on depression and anxiety), but also on aging-related conditions such as diabetes mellitus, cardiovascular diseases, and Alzheimer's disease, as well as on senescence [6-9]. These beneficial effects of exercise, regardless of yoga, are thought to be mediated by the promotion of cutaneous blood flow [10-13]. In addition, exercise under appropriate heat stress and high humid conditions is expected to increase cutaneous blood flow with skin temperature [14-16]. However, it is not known whether yoga performed in a high-temperature, high-humidity environment, an activity called hot yoga, has age-associated diseases and conditions.

Increases in catecholamines and cortisol are thought to be one of the causes of anxiety and depression, which have been reported to be suppressed by regular yoga practice [17]. Several reports have suggested that adrenaline and noradrenaline generate substantial oxidative stress in the body through sustained activation of the β-adrenergic receptor signaling system [18, 19]. High levels of circulating catecholamines and cortisol accelerate cutaneous aging [20, 21]. Accumulation of damage to the vascular endothelium during aging leads to increased levels of reactive oxygen species (ROS) in cells, which has been linked to apoptosis and damage to lipids, proteins, and nucleic acids [22].

Exercise and caloric restriction have been proposed to prolong life by upregulating genes involved in longevity,

Correspondence to: Takahiro Ishikawa
LAVA International, Inc.
Aoyama Bld. 9F, 1-2-3, Kitaaoyama, Minato-ku, Tokyo, 107-0061, Japan.
TEL: +81-3-6362-7523 FAX: +81-3-3470-3418
e-mail: ishikawa@rivercity-clinic.jp

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such as those in the sirtuin family and telomerases, or by promoting the scavenging of ROS [22-26]. A previous study reported that regular yoga practice promotes telomerase activity and sirtuin expression [9].

The sirtuin family is one of the most well-studied gene families in antiaging research, and many studies have reported evidence that sirtuins are involved in the regulation of longevity [23].

One sirtuin family member, SIRT1, is an NAD-dependent histone deacetylase, and SIRT1-mediated deacetylation suppresses cellular senescence via deacetylation of various transcription factors, such as p53, FOXO1, NF-κB, and PGC-1α [27]. Previous studies have shown that exercise and caloric restriction protect against aging-associated diseases via the activation of SIRT1 [28]. In addition, resveratrol, the most abundant polyphenol in red wine, slows the effects of aging through inducing SIRT1 activation [29].

Another sirtuin family member, SIRT6 has attracted attention as a therapeutic target involved in diabetes, cardiac hypertrophy, carcinogenesis, and premature aging syndrome [29-33]. *SIRT6* knockout mice exhibit growth retardation, lymphopenia, loss of subcutaneous fat, kyphosis and lordosis, and severe metabolic defects during development, which eventually leads to death after 4 weeks [33]. A previous report showing that muscle specific *SIRT6* knockout mice exhibited remarkably reduced endurance exercise capacity compared with controls [34].

Finally, telomeres are of interest in aging research because they gradually shorten each time a cell divides, and suppressing telomere shortening is expected to prolong cell lifespan [35]. Telomerase is an enzymatic complex that can maintain telomere length; it is composed of an RNA component (TERC), a reverse transcriptase component (TERT), and other regulatory subunits [36, 37]. TERT is highly expressed in cancer cells, but it is expressed at exceedingly low levels or not at all in most normal cells [38]. However, several research groups have demonstrated that continuous exercise promotes TERT expression in normal tissue, an effect that is closely related to the suppression of senescence through the extension of telomeres [39-41].

Here, we focused on indicators that are associated with aging in 48 healthy female volunteers by analyzing adrenal gland hormones, oxidative stress markers, and anti-aging genes in the peripheral blood as well as the skin condition. Overtraining can induce catecholamines and interfere with the effects of the above exercises, so we set up a beginner course and a basic course and asked the participants to choose the best one.

Methods

Recruitment and management of volunteers

The volunteers were recruited for the hot yoga trials by Souken Co., Ltd. (Tokyo, Japan) and signed an informed consent form before joining the hot yoga trial. The volunteers participated in yoga lessons in an environment with a constant temperature of approximately 35°C and humidity of 60% (*i.e.*, hot yoga). Initially, 56 healthy Japanese women aged 20 to 59 years participated in 60-minute of hot yoga lessons twice weekly for 12 weeks (from April 2019 to July 2019). This research aimed to examine the effects of moderate, comfortable exercise. Therefore, to achieve a similar level of exertion amongst volunteers, they were asked to select either the beginner program or the basic program according to their individual physical condition on the first day of the program. The volunteers conducted the programs presented in ***Table 1*** at LAVA hot yoga studio under the direction of an instructor.

During the trial period, the volunteers were asked to maintain approximately the same sleep, diet, and other lifestyle patterns as before the trial and were not allowed to use additional skin care products, makeup, or health supplements. The trial started with 56 participants, but 8 chose to withdraw through convenience; thus, 48 participants (39.2 ± 8.7 years, means ± standard error) were included in the final analysis. A week before the hot yoga trial begins and a week after completion, blood samples were collected from the participants in the clinical laboratory of Souken. Blood collection tubes containing EDTA-2NA (Falco Biosystems, Kyoto, Japan) were used to obtain 12 mL volume of whole blood samples. Of the obtained blood, 1 mL was stored as a whole blood sample at −80°C as it was, and 3 mL was centrifuged at 4°C and 3,000 g for 20 minutes to extract the supernatant and stored it as a plasma sample. The remaining 8 mL of blood was stored frozen on dry ice and delivered to SRL Co., Ltd. (Tokyo).

The study was initiated after the approval of the Shiba Palace Clinic Ethics Review Committee (#142621-26161).

Evaluation of body composition

The body composition of subjects measured one week before the hot yoga trial started and one week after the trial was completed in the clinical laboratory of Souken. Before and after subjects completed the hot yoga program, the body weight, body mass index (BMI), muscle mass, fat volume, and body fat percentage of volunteers were evaluated using InBody 3.2 (InBody Japan Inc., Tokyo).

Measurement of catecholamine and cortisol concentrations

The analysis of adrenaline, noradrenaline, dopamine, and cortisol were commissioned by SRL Co., Ltd.

ROS assay

ROS levels were assessed with an OxiSelect™ In Vitro ROS/RNS Assay Kit (Cosmo Bio Co., Tokyo). Plasma samples (50 µL) were assessed for ROS levels with an OxiSelect™ In Vitro ROS/RNS Assay Kit (Cosmo Bio Co., Tokyo). Briefly, an unknown ROS or RNS sample, and standard was added to the wells with a catalyst that accelerates the oxidation reaction. After a short time of incubating, the oxidation reaction began with the addition of prepared DCFH probes prepared in all wells. Finally, the prepared samples were analyzed with a Fluoroskan microplate fluorometer (Thermo Fisher Scientific Co., Yokohama, Japan) at an excitation wavelength of 480 nm and an emission wavelength of 530 nm to detect 2',7'-dichlorofluorescein (DCF). The free radical content in the unknown sample was determined by comparing it with the DCF standard curve.

Table 1. Hot yoga programs. The volunteers were asked to participate in a beginner or basic yoga program according to their individual physical condition on the first day of the program.

■ 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 min
4	Asana-Sitting	Anjaneyasana／Low lunge sequence including a preparatory pose (twist pose) and follow-up pose (half sprit).	6 min
5		Phalakasana／plank pose	0.5 min
6		Bhujangasana／cobra pose	0.5 min
7		Salabhasana／Locust pose	0.5 min
8		Balasana／Child's pose	0.75 min
9		Adho Mukha Shvanasana／Downward-facing dog	0.75 min
10	Asana-Standing	Uttanasana／Standing Forward Bend	1 min
11		Utthita Trikonasana／Extended triangle pose sequence including four preparatory Poses (high lunge, eagle pose, wild-legged forward bend, and Squat)	14 min
12	Asana-Sitting	Phalakasana／plank pose	0.75 min
13		Ardha Bhujangasana／Half cobra pose	0.75 min
14		Dhanurasana／Bow pose	0.75 min
15		Balasana／Child's pose	0.75 min
16		Upavistha konasana／Wide-angle seated forward bend	6 min
17	Cool-down	Ardha Setubandhasana／half bridge pose	0.75 min
18		Eagle twist with hip strech	3.5 min
19		Pawanmuktasana／Wind-relieving pose	0.75 min
20	Relaxation	Shavasana／Corpse Pose	5 min
21		Breath	1 min
22		Session closing instructions	1 min
		total	60 min

■ Yoga basic			
Block of Yoga sequence		Yoga Poses Names (Asanas)	Duration
1	Session preparation instructions	Session preparation instructions	1 min
2	Warm-up	Breath	1 min
3		Jathara Parivartanasana／Revolved belly pose	2 min
4		Pawanmuktasana／Wind-relieving pose	1 min
5		Kapalahbhati ／Skull-shining breath	2 min
6		Marjarasana／Cat cow pose	1.5 min
7		Parsva balasana ／Thread the needle	1.5 min
8		Uttana Shishosana ／Extended Puppy Pose	1 min
9		Balasana／Child's pose	1 min
10		Anjaneyasana／Low lunge	2 min
11		Parighasana ／Gate pose	2 min
12		Balasana／Child's pose	1 min
13	Asana-Standing	Utkatasana／Chair pose	4 min
14		Ardha chandrasana／Half moon pose	1.5 min
15		Parivrtta utkatasana／chair twist pose	1.5 min
16		Tadasana／Mountain pose	1 min
17		Garudasana／Eagle pose	1.5 min
18		Parsvottanasana／Intense side stretch pose	1.5 min
19		Parivrtta Sanchalasana／Low lunge twist pose	1 min
20		Virabhadrasana Ⅱ／Warrior Ⅱ Pose	1 min
21		Utthita Trikonasana／Extended triangle pose	1 min
22		Prasarita Padottanasana／Wide Legged Forward Bend	1.5 min
23		Natarajasana／Lord of the dance pose	2 min
24		Vrksansana／Tree pose	1.5 min
25		Tadasana／Mountain pose	2 min
26	Asana-Prone	Phalakasana／plank pose	1 min
27		Bhujangasana／cobra pose	1 min
28		Dekasana／Airplane pose	1 min
29		Dhanurasana／Bow Pose	1 min
30		Balasana／Child's pose	1 min
31		Ustrasana／Camel pose	1 min
32		Sasangasana／Rabbit pose	1 min
33	Asana-Sitting	Upavistha konasana／Paschimottanasana	2 min
34		Krounchasana／Heron pose	1 min
35		Ardha Matsyendrasana／Half lord of the fishes Pose	1 min
36		Paschimottanasana／Seated forward bend	1 min
37	Cool-down	Ardha Setubandhasana／half bridge pose	1 min
38		Pawanmuktasana／Wind-relieving pose	1 min
39		Pawanmuktasana／Wind-relieving pose	2 min
40	Relaxation	Shavasana／Corpse Pose	5 min
41		Session closing instructions	1 min
		total	60 min

8-OHdG assay

Plasma samples (500 µL) were ultrafiltrated using a Microsep Advance Centrifugal Device 10K (Nihon Pall, Tokyo), and the 8-OHdG (8-oxo-2'-deoxyguanosine) level was assessed with a highly sensitive ELISA kit for 8-OHdG (Nikken Seil, Shizuoka, Japan). Briefly, 50 µL of 8-OHdG standard solution or ultrafiltered sample was dispensed into each well, and then 50 µL of the primary antibody solution was added to each well, and the mixture was reacted at 37 °C for 1 hour. After completion of the reaction, the reaction solution of the well was discarded, the well was washed with 250 µL of the lavage fluid and then 100 µL of the secondary antibody solution was applied into the well reacted with the primary antibody and reacted at 37 °C for 1 hour. After completion of the reaction, the reaction solution in the well was discarded, and 250 µL of the lavage fluid was dispensed for washing, followed by, 100 µL of the color coupler solution was dispensed into the wells where the reaction was carried out, and the reaction was carried out at room temperature for 15 minutes, finally 100 µL of the reaction quenching solution was added to the wells to stop the reaction. The prepared samples were measured using a Varioskan plate reader (Thermo Fisher Scientific Co., Yokohama) at an absorbance 450 nm.

qRT-PCR analysis of SIRT1, SIRT6, hTERC, and hTERT

Total RNA was extracted from whole blood (250 µL) with RNAiso (Takara, Otsu, Japan) according to the manufacturer's instructions. Reverse transcription polymerase chain reaction (RT-PCR) and qRT-PCR were carried out according to the instructions from the Thunderbird SYBR qPCR/RT Set (Toyobo, Osaka, Japan) in a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The reaction program was initiated at 95 °C for 20 seconds, followed by 40 cycles of denaturation at 95 °C for 5 seconds and annealing and extension at 60 °C for 20 seconds, and the melting curve was determined at 95 °C for 15 seconds, at 60 °C for 1 minute, at 95 °C for 15 seconds. The mRNA expression levels were normalized to those of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and analyzed standard curve method. The primer sequences used are listed in ***Table 2*** [42-45].

Evaluation of skin moisture content and elasticity

Skin assessment was performed one week before the hot yoga trial started and one week after completion in the clinical laboratory of Souken. The skin moisture content assessed at 3 points above the cheekbone and within the area 5 mm to the left and right of the points using a Corneometer CM825 (KOKO Kosmetikvertrieb, Köln, Germany). Three parameters of skin elasticity – the maximum skin amplitude, minimum skin amplitude, and efficiency of returning to the original state – were measured, as follows: a 4 cm sample area, which was located along the axis from beneath one ear lobe to the lip, was assessed three times with a Cutometer Dual MPA580 system (Courage + Khazaka electronic, Köln). All measurements were conducted in a room with a constant temperature and humidity (room temperature: 22 ± 2 °C, humidity: 50 ± 10 %).

Skin replica analysis

Skin replicas were obtained one week before the hot yoga began and one week after completion in the clinical laboratory of Souken. Skin replicas were assessed using previous reports as a reference [46]. Briefly, after stabilizing the head of each of the volunteers to minimize movement, 7-cm-long skin replicas of the area from the base of the ear lobe to the nose were obtained. Images were acquired with an ASA-03RXD system (Asahi Bio-Rad, Tokyo). All measurements were collected in a room with a constant temperature and humidity (room temperature 22 ± 2 °C, humidity 50 ± 10 %).

Statistical analysis

All results are expressed as the mean ± SE values. Mann-Whitney U tests were used to analyze statistical significance. The significance levels were set at $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. All the data necessary to evaluate the conclusion of a paper is present in the paper and/or in the supplementary information. Additional data is available from the author on request.

Table 2. qRT-PCR primer nucleotide sequence.

Gene	Forward	Reverse	Reference
GAPDH	5-ATGACATCAAGAAGGTGGTG-3′	5′-CATACCAGGAAATGAGCTTG-3′	57
SIRT1	5′-TCGCAACTATACCCAGAACATAGACA-3′	5′-CTGTTGCAAAGGAACCATGACA-3′	57
SIRT6	5′-CCACCAAGCACGACCGCCAT-3′	5′-CGCCCTCTCCAGCACACGG-3′	58
hTERT	5′-CGGAAGAGTGTCTGGAGCAA-3′	5′-TGACCTCCGTGAGCCTGTC-3′	59
hTERC	5′-GTGGTGGCCATTTTTTGTCTAAC-3′	5′-TGCTCTAGAATGAACGGTGGAA-3′	60

Results

Completion of a hot yoga program had not significantly change body composition other than body fat. First, the impacts of yoga on health were first investigated by analyzing body composition before and after participation in a 12-week hot yoga program. The body weight and BMI were slightly but non-significantly reduced after 12 weeks of hot yoga training (***Fig. 1-a, b***). The muscle mass did not change after completion of the hot yoga program (***Fig. 1-c***). However, the body fat mass levels and the total body fat percentage were significantly lower after hot yoga than they were before hot yoga ($p < 0.05$; ***Fig. 1-d, e***).

Plasma levels of catecholamines and cortisol after hot yoga

We measured the plasma levels of adrenaline, noradrenaline, dopamine, and cortisol in the volunteers before and after participation in the hot yoga program. Dopamine and cortisol levels did not differ significantly before and after hot yoga; however, the levels of adrenaline and noradrenaline were significantly lower after hot yoga than before training (***Fig. 2***).

Reduction in plasma levels of oxidative stress markers after hot yoga

Plasma ROS levels were measured before and after the hot yoga program, using DCF as an indicator. We found that ROS production was significantly reduced in the volunteers after hot yoga compared with before yoga (***Fig. 3-a***). We next compared the oxidative DNA damage present in individuals before and after completing the hot yoga program. The plasma levels of 8-OHdG, a measure of oxidative damage to DNA, were significantly lower in the post-yoga samples than in the pre-yoga samples (***Fig. 3-b***).

Decreased expression of aging-related markers in the blood after hot yoga

The upregulation of telomerase and sirtuins has been shown to have anti-aging effects [23, 35]. Thus, to continue our analysis of whether hot yoga had an impact on factors related to aging, we used quantitative real-time PCR (qRT-PCR) to measure the expression levels of *hTERT, hTERC, SIRT1* and *SIRT6* in blood samples collected from individuals before and after completion of the hot yoga program. The *SIRT6* mRNA level was significantly increased after hot yoga, but no significant differences were observed in the expression levels of *hTERT, hTERC* and *SIRT1* (***Fig. 4***).

Indicators of skin aging were improved after hot yoga

Senescence gradually causes progressive deterioration of skin function [47]. We thus examined the moisture content and elasticity of the skin, which are important measures of skin function. The moisture content in the stratum corneum in the study volunteers was significantly increased after completion of the hot yoga program relative to before beginning the program (***Fig. 5-a***). The maximum amplitude indicates how much the skin was pulled, and the minimum

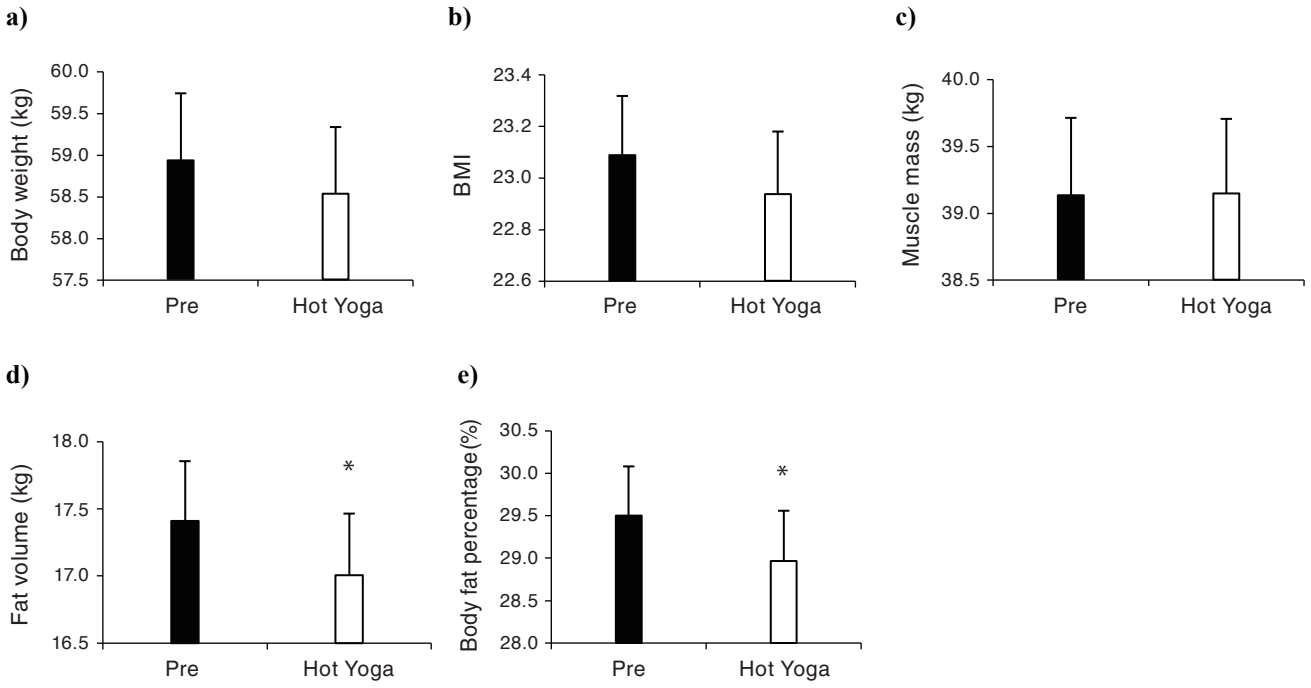

Fig. 1. **Evaluation of body composition in volunteers before and after yoga.**

Each participant individually participated in 60-minute hot yoga lessons twice weekly for 3 months. No significant changes in body weight **(a)**, BMI **(b)**, or muscle mass **(c)** were observed when comparing results from individuals before and after hot yoga, but the fat volume **(d)** and body fat percentage **(e)** differed significantly before and after hot yoga. The results were analyzed using the Mann-Whitney U test and are expressed as the mean ± SE, n = 48, * $p < 0.05$. BMI, body mass index; SE, standard error.

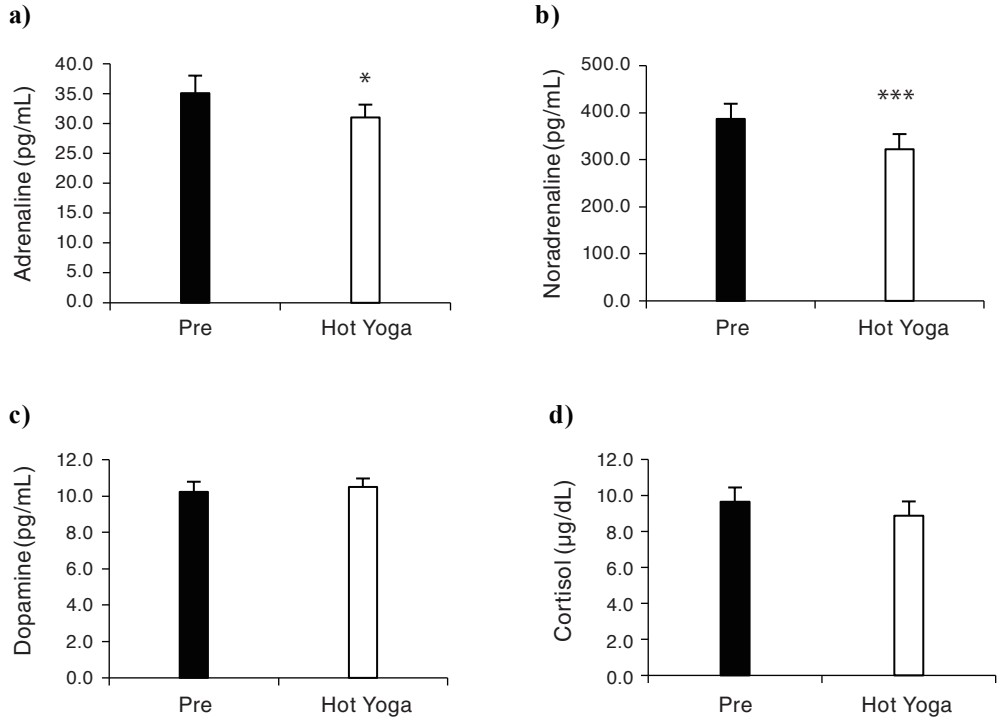

Fig. 2. **Changes in plasma catecholamine and cortisol concentrations before and after hot yoga lessons.**

Changes in plasma catecholamine and cortisol concentrations before and after hot yoga lessons. Plasma was obtained from subjects before initiation of the hot yoga program and after. The concentrations of adrenaline **(a)** and noradrenaline **(b)** were significantly higher after hot yoga than before individuals began the hot yoga program, but the concentrations of dopamine **(c)** and cortisol **(d)** did not change significantly. The results were examined by Mann-Whitney U test and are expressed as the mean ± SE values, n = 48, * $p < 0.05$, *** $p < 0.001$. SE, standard error.

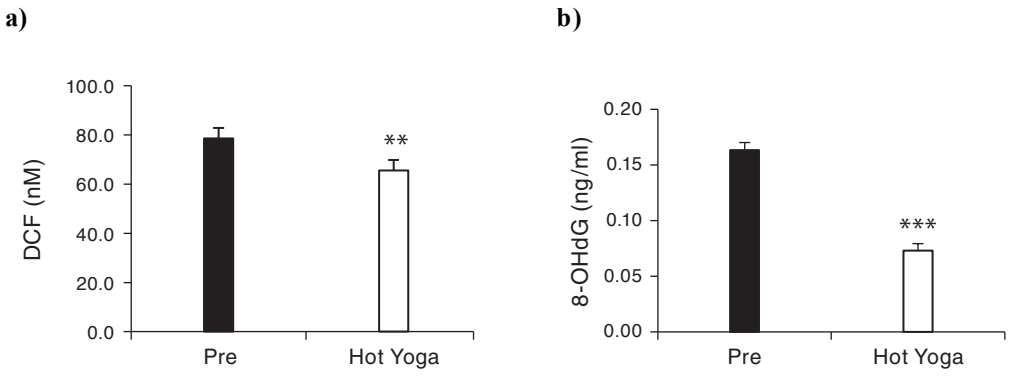

Fig. 3. **Effect of hot yoga on oxidative stress.**

(a) DCF. After completion of the hot yoga program, plasma ROS levels were significantly decreased compared to the levels observed before the program started. **(b)** 8-OHdG. The percentage of oxidized DNA damage in plasma from the different groups was evaluated by quantifying 8-OHdG levels. Hot yoga significantly decreased the level of 8-OHdG compared to the levels observed before the program. The results were analyzed using the Mann-Whitney U test and are expressed as the mean ± SE, n = 48, ** $p < 0.01$, *** $p < 0.001$. ROS, reactive oxygen species; DCF, 2',7'-dichlorofluorescein; 8-OHdG, 8-oxo-2'-deoxyguanosine; SE, standard error.

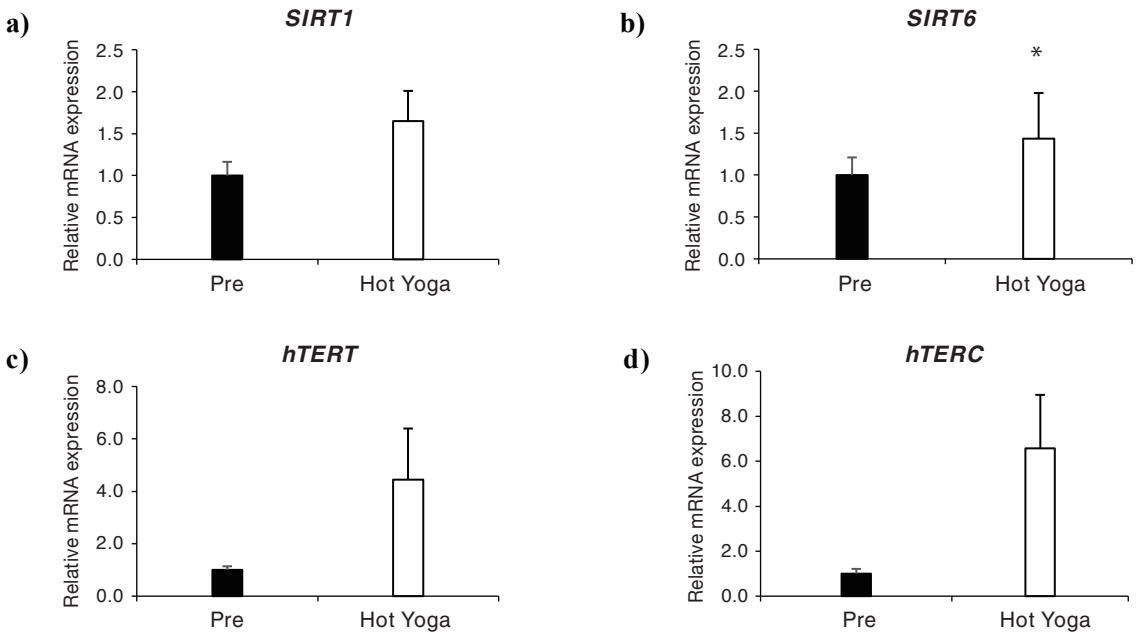

Fig. 4. **SIRT6 expression is changed by hot yoga.**

Blood was collected from subjects before beginning and after completing the hot yoga program. The mRNA expression levels of *SIRT6* **(b)** in individual samples were significantly higher after completing the hot yoga program than they were prior to its initiation; however, no differences were seen in the *SIRT1* **(a)**, *hTERT* **(c)** and *hTERC* **(d)** mRNA expression levels between the two groups of samples. The expression levels of the analyzed genes were normalized to that of *GAPDH*. The mRNA expression levels in the hot yoga group (white bars) are presented relative to the levels before beginning hot yoga (represented as 1.00; black bars). The results were analyzed using the Mann-Whitney U test and are expressed as the mean ± SE, n = 48, * $p < 0.05$. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SE, standard error.

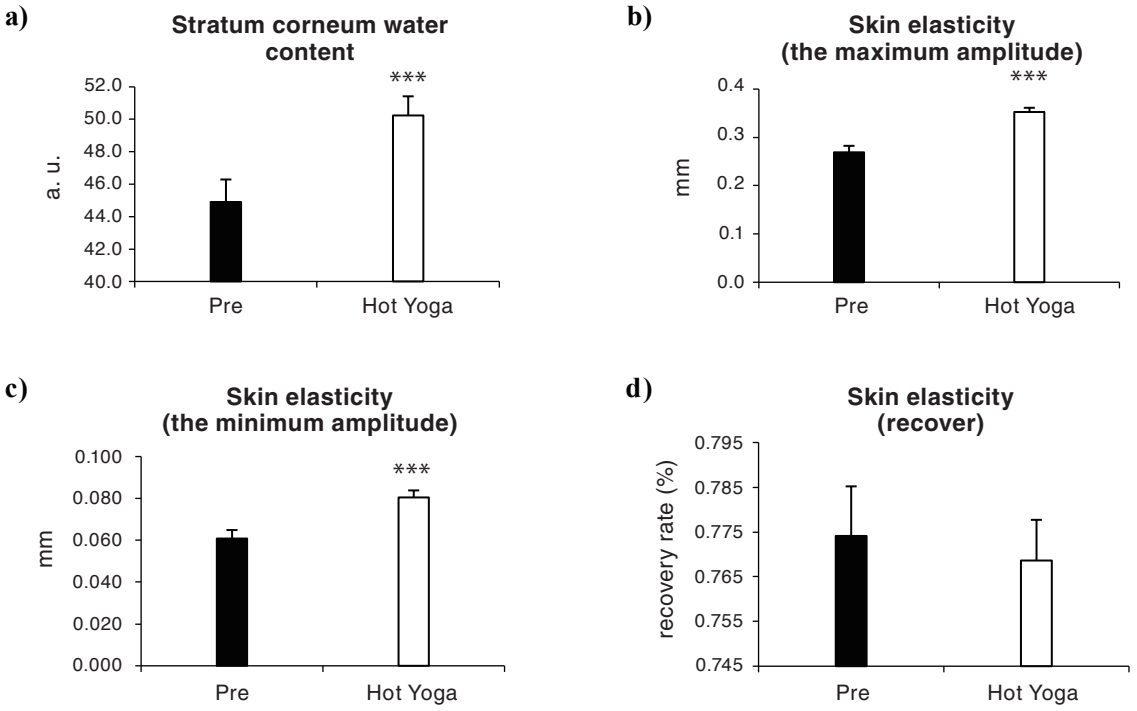

Fig. 5. **Protective effect of hot yoga against skin aging.**

(a) Skin corneum water content: The amount of water in the corneum. **(b)** Skin elasticity (the maximum amplitude): Height of the skin drawn into the opening when sucked and released at a negative pressure of 300 mbar (mm). **(c)** Skin elasticity (the minimum amplitude): Height of the skin drawn into the opening after 2 seconds of suction and release with a negative pressure of 300 mbar (mm). **(d)** Skin elasticity (recover): Ratio of the maximum amplitude to the minimum amplitude value (%). The moisture content of the stratum corneum was measured before and after completion of the hot yoga program. The moisture content in individuals who completed the hot yoga program was significantly higher than it was for them prior to beginning hot yoga. The skin elasticity was estimated in individuals before beginning and after completing the hot yoga program. The maximum and minimum amplitude of skin elasticity was significantly higher in individuals after completion of the hot yoga program than it was before starting it. The results were analyzed using the Mann-Whitney U test and are expressed as the mean ± SE, n = 48, *** $p < 0.001$. SE, standard error.

amplitude indicates how much the pulled skin returned, and the larger both values, the higher the elasticity of the skin. The return rate is the ratio of the maximum amplitude to the minimum amplitude, which a high value indicates a resilient skin. As shown in the ***Fig. 5-b, c,*** cutaneous skin elasticity, in terms of both the maximum and minimum amplitude values, was significantly increased after completion of the hot yoga program, however, the ability of the skin to return to its original position did not differ (***Fig. 5-d***).

The texture of the skin gradually deteriorates with aging [48]. To evaluate the extent of skin texture repair, we conducted a skin replica analysis.

A replica whose skin surface shape was transferred with a silicone-based impression agent was irradiated with parallel light at an angle of 30°C, and the obtained shaded was imaged with a CCD (charge coupled device) camera, and a skin texture was analyzed by data processing. Green indicates areas recognized as high and brown indicates areas recognized as deep (***Fig. 6-a***). The skin texture volume ratio is the number of the perceived texture volume divided by 100 per millimeter square meter, the number of skin texture is the number of pieces per millimeter straight line, and the depth of skin texture is the average depth of the texture within the unit area. The higher the value, the better the result of these skin textures.

The volume and number of skin texture points were significantly increased in the volunteers after completion of the yoga program, and the mean depth of the skin texture points was significantly reduced (***Fig. 6-b, c, d***). These findings suggest that a hot yoga regimen can improve the overall smoothness of skin. Taken together, the findings described here reveal the impact of hot yoga on various characteristics associated with aging, suggesting that it may have a role in suppressing aspects of senescence.

Discussion

Although yoga has been reported to inhibit aging-related diseases, the anti-aging effects of hot yoga performed in a high-temperature, high-humidity environment remains poorly understood. Since hot yoga is performed under high temperature and high humidity conditions, we expected that it would be effective in changing body composition, reducing adrenal gland hormone and oxidative stress, and increasing the expression of anti-aging genes.

In the current study, we assessed how a 12-week hot yoga regimen impacted factors related to aging, and we found that this regimen reduced both the mass

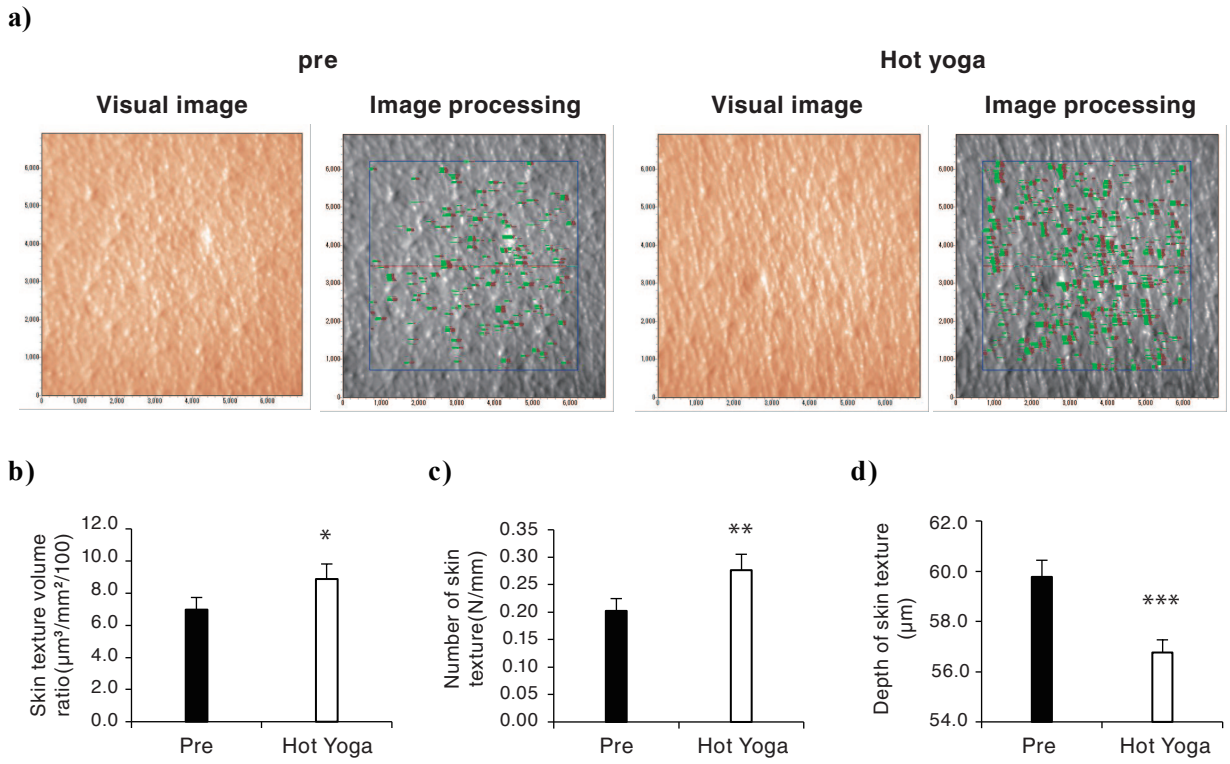

Fig. 6. **The skin texture improving effect of hot yoga.**

a) Skin texture visual image. Visual images of skin replicas and image analysis of the skin texture before and after hot yoga. **b)** Skin texture volume ratio: The number of perceived skin textures divided by 100 per millisquare meter. **c)** Number of skin texture: The average depth of skin texture within the unit area. **d)** Depth of skin texture: The number of skin texture per millimeter straight line. Green indicates areas that are recognized as high, and brown indicates areas that are recognized as deep. The volume, number, and depth of skin texture points in three-dimensional skin replicas was evaluated by image analysis. Quantification of the volume, number, and apparent depth of skin texture points. The volume and number of skin texture points after hot yoga were significantly larger than they were before the lesson program began. The apparent depth of the skin was smaller in the skin replicas after the hot yoga trial period than it was before the trial period. The results were analyzed using the Mann-Whitney U test and are expressed as the mean ± SE, n = 48, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

and percentage of body fat. The results of this research are consistent with previous studies measuring body composition changes after completion of the hot yoga with Dual energy X-ray absorptiometry (DXA)[49]. An increase in the amount of body fat has been associated with a variety of major aging-related diseases, such as obesity, arteriosclerosis, diabetes, and cancer[30, 50, 51]. Exercising in a warm, high-humidity environment promotes blood flow[52], which helps to burn body fat. Therefore, these results of this study suggest that hot yoga is effective for preventing diseases caused by fat accumulation[53]. Catecholamines and cortisol have a vasoconstrictive effect, resulting in a reduction in blood flow[20, 21]; therefore, we studied whether the concentrations of adrenaline, noradrenaline, dopamine, and cortisol changed in individuals who participated in the hot yoga program. Our findings suggest that hot yoga might enhance vasodilation via decreases in adrenaline and noradrenaline but that dopamine may not modulate blood flow. Vasoconstriction due to adrenaline or noradrenaline causes hypertension, which can lead to dangerous diseases such as cardiac hypertrophy, coronary atherosclerosis, angina, myocardial infarction, and psychological disorders[54]. In addition, since adrenaline and noradrenaline produce ROS through β-adrenergic receptor[18, 19], we assumed that Hot yoga might be effective in protecting against oxidative stress.

As humans age, cellular ROS levels gradually accumulate, which has been linked to lifestyle diseases through damage to lipids, proteins, and nucleic acids[22]. The increase in blood flow that occurs during exercise plays an important role in the antioxidant response by mediating the shear stress cells are exposed to[55]. A previous study showed that shear stress activates the longevity regulator sirtuin and protects blood vessels via suppression of mitochondrial ROS production[54]. Shear stress has also been shown to promote the nuclear translocation of the antioxidant response transcription factor Nrf2[56], and sirtuin has been revealed to act as a coactivator of Nrf2, indicating a possible role for increased blood flow as an initiator of these processes[57]. Therefore, we examined induction of SIRT1 and SIRT6 mediated by hot yoga, and found that the mRNA expression of *SIRT6* was increased in blood samples after hot yoga. Based on the findings stated above and the results we presented here, we hypothesize that hot yoga may enhance the activation of SIRT6 via a mechanism mediated by shear stress. SIRT6 is also a central regulator of longevity and aging[58]; thus, in hot yoga participants, upregulated SIRT6 may inhibit cellular senescence through its histone deacetylation function, but further studies are necessary to explore these suggestions.

Like the activation of sirtuin family members, the activation of telomerase has also been associated with promotion of blood flow, and telomerase activation has been demonstrated by an increase longevity in humans and other mammals[35]. Interestingly, the lifespan of SIRT6-overexpressing transgenic mice was shown to be prolonged in male but not in female mice[58], However, while these laboratory animals are grown in a stress-free and comfortable environment, humans, both men and women are exposed to various environmental stresses such as exhaust gas, particulate matter, and ultraviolet (UV). When exposed to ultraviolet rays, ROS is produced, but *Sirt6* increased expression of may alleviate skin aging due to oxidative stress.

This study did not reveal significant differences in the telomerase components hTERT and hTERC when comparing data collected before and after completion of the hot yoga program, although their expression did exhibit an increasing trend after hot yoga. Thus, reevaluation with an increased number of samples would help to determine if this is a meaningful phenomenon.

Changes in the skin surface, such as dry skin, rough skin, itchiness, and wrinkles, are among the most visible signs of aging, and they occur due to lose of moisture of and elasticity in the skin, respectively[59]. In the present study, we found that skin moisture content and elasticity were significantly increased in individuals after completing the hot yoga regimen compared to their baseline levels; these changes might prevent the formation of wrinkles with aging.

We also found that the skin texture of the subjects was improved after hot yoga. Younger people have better, shallower skin texture than older people[60]. Therefore, we hypothesize that in addition to suppressing mechanisms involved in senescence in the skin, hot yoga could rejuvenate the appearance of the skin, but more work is needed to validate this hypothesis. Although this study demonstrated the effects of short-term exercise long-term results must be observed in the future to unambiguously demonstrate the anti-aging effects of Hot yoga.

Conclusion

Our results raise the possibility that continuous hot yoga exercise suppresses catecholamines and thus promotes blood flow, which is expected to protect against ROS-induced senescence by modulating *SIRT6* expression, as modeled in ***Fig. 7***. However, the specific mechanism by which SIRT6 is activated by hot yoga to facilitate anti-aging signaling is unclear. Further studies are necessary to define the exact link between SIRT6 and hot yoga training.

Conflict of interest declaration

This research was conducted with a research fund from LAVA International, Inc, which the author belongs to.

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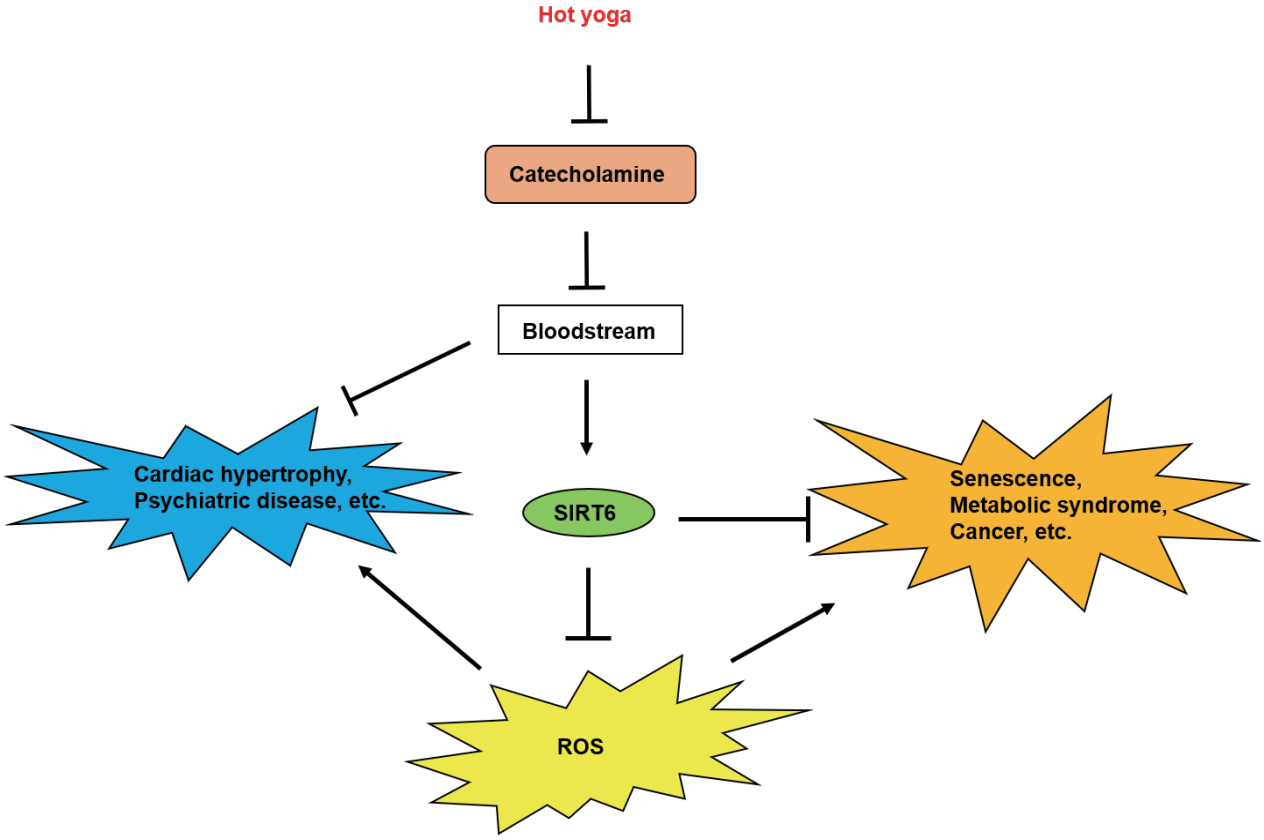

Fig. 7. **Schematic diagram showing the mechanism of hot yoga action.**
ROS, reactive oxygen species.

References

1) Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature*. 2001; 414: 782-787.
2) Wang JC, Bennett M. Aging and atherosclerosis: Mechanisms, functional consequences, and potential therapeutics for cellular senescence. *Circ Res*. 2012; 111: 245-259.
3) Soriano-Tárraga C, Giralt-Steinhauer E, Mola-Caminal M, et al. Biological age is a predictor of mortality in ischemic stroke. *Sci Rep*. 2018; 8: 4148.
4) Childs BG, Durik M, Baker DJ, et al. Cellular senescence in aging and age-related disease: From mechanisms to therapy. *Nat Med*. 2015; 21: 1424-1435.
5) Higashi Y, Kihara Y, Noma K. Endothelial dysfunction and hypertension in aging. *Hypertens Res*. 2012; 35: 1039-1047.
6) Guddeti RR, Dang G, Williams MA, et al. Role of yoga in cardiac disease and rehabilitation. *J Cardiopulm Rehabil Prev*. 2019; 39: 146-152.
7) Danucalov MA, Kozasa EH, Afonso RF, et al. Yoga and compassion meditation program improve quality of life and self-compassion in family caregivers of Alzheimer's disease patients: A randomized controlled trial. *Geriatr Gerontol Int*. 2017; 17: 85-91.
8) Gallagher A, Kring D, Whitley T. Effects of yoga on anxiety and depression for high risk mothers on hospital bedrest. *Complement Ther Clin Pract*. 2020; 38: 101079.
9) Tolahunase M, Sagar R, Dada R. Impact of yoga and meditation on cellular aging in apparently healthy individuals: A prospective, open-label single-arm exploratory study. *Oxid Med Cell Longev*. 2017; 2017: 7928981.
10) Tabara Y, Yuasa T, Oshiumi A, et al. Effect of acute and long-term aerobic exercise on arterial stiffness in the elderly. *Hypertens Res*. 2007; 30: 895-902.
11) Brown BM, Peiffer JJ, Martins RN. Multiple effects of physical activity on molecular and cognitive signs of brain aging: Can exercise slow neurodegeneration and delay Alzheimer's disease? *Mol Psychiatry*. 2013; 18: 864-874.
12) Santos-Parker JR, LaRocca TJ, Seals DR. Aerobic exercise and other healthy lifestyle factors that influence vascular aging. *Adv Physiol Educ*. 2014; 38: 296-307.
13) Tanaka H. Antiaging effects of aerobic exercise on systemic arteries. *Hypertension*. 2019; 74: 237-243.
14) Gonzalez-Alonso J. Human thermoregulation and the cardiovascular system. *Exp Physiol*. 2012; 97, 340-346.
15) Sawka MN, Cheuvront SN, Kenefick RW. High skin temperature and hypohydration impair aerobic performance. *Exp Physiol*. 2012; 97: 327-332.

16) Rowell LB, Brengelmann GL, Murray JA, et al. Human metabolic responses to hyperthermia during mild to maximal exercise. *J Appl Physiol*. 1969; 26: 395-402.
17) Gallagher A, Kring D, Whitley T. Effects of yoga on anxiety and depression for high risk mothers on hospital bedrest. *Complement Ther Clin Pract*. 2020; 38: 101079.
18) Sun F, Ding XP, An SM, et al. Adrenergic DNA damage of embryonic pluripotent cells via β2 receptor signalling. *Sci Rep*. 2015; 5: 15950.
19) Corbi G, Conti V, Russomanno G, et al. Adrenergic signaling and oxidative stress: A role for sirtuins? *Front Physiol*. 2013; 4: 324.
20) Romana-Souza B, Santos Lima-Cezar G, Monte-Alto-Costa A. Psychological stress-induced catecholamines accelerates cutaneous aging in mice. *Mech Ageing Dev*. 2015; 152, 63-73.
21) Terao M, Katayama I. Local cortisol/corticosterone activation in skin physiology and pathology. *J Dermatol Sci*. 2016; 84: 11-16.
22) Redman LM, Smith SR, Burton JH, et al. Metabolic slowing and reduced oxidative damage with sustained caloric restriction support the rate of living and oxidative damage theories of aging. *Cell Metab*. 2018; 3: 805-815.
23) Imai S, Guarente L. It takes two to tango: NAD^+ and sirtuins in aging/longevity control. *NPJ Aging Mech Dis*. 2016; 2: 16017.
24) Balan E, Decottignies A, Deldicque L. Physical activity and nutrition: Two promising strategies for telomere maintenance? *Nutrients*. 2018; 10: 1942.
25) Done AJ, Traustadóttir T. Nrf2 mediates redox adaptations to exercise. *Redox Biol*. 2016; 10: 191-199.
26) Gordon LA, Morrison EY, McGrowder DA, et al. Effect of exercise therapy on lipid profile and oxidative stress indicators in patients with type 2 diabetes. *BMC Complement Altern Med*. 2008; 8: 21.
27) Lavu S, Boss O, Elliott PJ, et al. Sirtuins: Novel therapeutic targets to treat age-associated diseases. *Nat Rev Drug Discov*. 2008; 7: 841-853.
28) Boily G, Seifert EL, Bevilacqua L, et al. SirT1 regulates energy metabolism and response to caloric restriction in mice. *PLoS One*. 2008; 3: e1759.
29) Baur JA, Pearson KJ, Price NL, et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*. 2006; 444: 337-342.
30) Kuang J, Chen L, Tang Q, et al. The role of Sirt6 in obesity and diabetes. *Front Physiol*. 2018; 9: 135.
31) Sundaresan NR, Vasudevan P, Zhong L, et al. The sirtuin SIRT6 blocks IGF-Akt signaling and development of cardiac hypertrophy by targeting c-Jun. *Nat Med*. 2012; 18: 1643-1650.
32) Lerrer B, Gertler AA, Cohen HY. The complex role of SIRT6 in carcinogenesis. *Carcinogenesis*. 2016; 37: 108-118.
33) Mostoslavsky R, Chua KF, Lombard DB, et al. Genomic instability and aging-like phenotype in the absence of mammalian *SIRT6*. *Cell*. 2006; 124: 315-329.
34) Cui X, Yao L, Yang X, et al. SIRT6 regulates metabolic homeostasis in skeletal muscle through activation AMPK. *Am J Physiol Endocrinol Metab*. 2017; 313: E493-E505.
35) Tzanetakou IP, Nzietchueng R, Perrea DN, et al. Telomeres and their role in aging and longevity. *Curr Vasc Pharmacol*. 2014; 12: 726-734.
36) Cong Y, Shay JW. Actions of human telomerase beyond telomeres. *Cell Res*. 2008; 18: 725-732.
37) Savage SA, Bertuch AA. The genetics and clinical manifestations of telomere biology disorders. *Genet Med*. 2010; 12: 753-764.
38) Leão R, Apolónio JD, Lee D, et al. Mechanisms of human telomerase reverse transcriptase (hTERT) regulation: Clinical impacts in cancer. *J Biomed Sci*. 2018; 25: 22.
39) Werner C, Hanhoun M, Widmann T, et al. Effects of physical exercise on myocardial telomere-regulating proteins, survival pathways, and apoptosis. *J Am Coll Cardiol*. 2008; 52: 470-482.
40) Ludlow AT, Zimmerman JB, Witkowski S, et al. Relationship between physical activity level, telomere length, and telomerase activity. *Med Sci Sports Exerc*. 2008; 40: 1764-1771.
41) Laye MJ, Solomon TP, Karstoft K, et al. Increased shelterin mRNA expression in peripheral blood mononuclear cells and skeletal muscle following an ultra-long-distance running event. *J Appl Physiol*. 2012; 112: 773-781.
42) Yamamori T, DeRicco J, Naqvi A, et al. SIRT1 deacetylates APE1 and regulates cellular base excision repair. *Nucleic Acids Res*. 2010; 38: 832-845.
43) Chilton WL, Marques FZ, West J, et al. Acute exercise leads to regulation of telomere-associated genes and microRNA expression in immune cells. *PLoS One*. 2014; 9: e92088.
44) Patel PL, Suram A, Mirani N, et al. Derepression of hTERT gene expression promotes escape from oncogene-induced cellular senescence. *Proc Natl Acad Sci USA*. 2016; 113: E5024-5033.
45) Rubtsova M, Naraykina Y, Vasilkova D, et al. Protein encoded in human telomerase RNA is involved in cell protective pathways. *Nucleic Acids Res*. 2018; 46: 8966-8977.
46) Shiratsuchi E, Nakaba M, Yamada M. Elastin hydrolysate derived from fish enhances proliferation of human skin fibroblasts and elastin synthesis in human skin fibroblasts and improves the skin conditions. *J Sci Food Agric*. 2016; 96: 1672-1677.
47) Wang AS, Dreesen O. Biomarkers of cellular senescence and skin aging. *Front Genet*. 2018; 9: 247.
48) Czajka A, Kania EM, Genovese L, et al. Daily oral supplementation with collagen peptides combined with vitamins and other bioactive compounds improves skin elasticity and has a beneficial effect on joint and general wellbeing. *Nutr Res*. 2018; 57: 97-108.
49) Hunter SD, Laosiripisan J, Elmenshawy A, et al. Effects of yoga interventions practised in heated and thermoneutral conditions on endothelium-dependent vasodilatation: The Bikram yoga heart study. *Exp Physiol*. 2018; 103: 391-396.
50) Lee J, Jung S, Kim N, et al. Myocardial metabolic alterations in mice with diet-induced atherosclerosis: Linking sulfur amino acid and lipid metabolism. *Sci Rep*. 2017; 7: 13597.
51) Khandekar MJ, Cohen P, Spiegelman BM. Molecular mechanisms of cancer development in obesity. *Nat Rev Cancer*. 2011; 11: 886-895.
52) Petrofsky J, Berk L, Alshammari F, et al. The effect of moist air on skin blood flow and temperature in subjects with and without diabetes. *Diabetes Technol Ther*. 2012; 14: 105-116.
53) Petrofsky JS, McLellan K, Prowse M, et al. The effect of body fat, aging, and diabetes on vertical and shear pressure in and under a waist belt and its effect on skin blood flow. *Diabetes Technol Ther*. 2010; 12: 153-160.

54) Ferrari R, Camici PG, Crea F, et al. Expert consensus document: A 'diamond' approach to personalized treatment of angina. *Nat Rev Cardiol*. 2018; 15: 120-132.
55) Cunningham KS, Gotlieb AI. The role of shear stress in the pathogenesis of atherosclerosis. *Lab Invest*. 2005; 85: 9-23.
56) Satta S, Mahmoud AM, Wilkinson F, et al. The role Nrf2 in cardiovascular function and disease. *Oxid Med Cell Longev*. 2017; 2017: 9237263.
57) Pan H, Guan D, Liu X, et al. SIRT6 safeguards human mesenchymal stem cells from oxidative stress by coactivating NRF2. *Cell Res*. 2016; 26: 190-205.
58) Kanfi Y, Naiman S, Amir G, et al. The sirtuin SIRT6 regulates lifespan in male mice. *Nature*. 2012; 483: 218-221.
59) Choi JW, Kwon SH, Huh CH, et al. The influences of skin visco-elasticity, hydration level and aging on the formation of wrinkles: A comprehensive and objective approach. *Skin Res Technol*. 2013; 19: e349-355.
60) Lagarde JM, Rouvrais C, Black D. Topography and anisotropy of the skin surface with ageing. *Skin Res Technol*. 2005; 11: 110-119.