Original article

An observational study for the effects of a hot yoga program in a Wood Stone Studio on SIRT6 activation.

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Abstract

***Purpose*:** Hot yoga is a practice in which thermotherapy is added to ordinary yoga. A previous study reported a significant increase in SIRT6 mRNA expression following participation in a twice-weekly, 12-week program, but there was no significant difference between the regular floor (RF) and Wood Stone Studio (WSS). In the present study, female instructors who engaged in a hot yoga program for more than 6 months were evaluated and a comparative analysis between RF and WSS was conducted.

***Methods*:** Subjects were divided into two groups as instructors engaged in a hot yoga program for more than 6 months: 16 in the WSS group (29.7 ± 1.4 years, BMI: 35.7 ± 0.9) and 19 in the RF group (28.1 ± 0.8 years, BMI: 37.1 ± 0.9). The assessment items were the Anti-Aging Quality of Life Common Questionnaire (AAQOL), body composition, blood SIRT6 expression level, and natural killer (NK) cell activity, and multiple regression analysis was performed with SIRT6 expression level as the objective variable.

***Results*:** AAQOL showed that the WSS group tended to have less "muscle pain and stiffness," "anorexia," and "joint pain" than RF group ($p < 0.1$); SIRT6 expression and NK cell activity were not significantly different between two groups. Multiple regression analysis extracted age and BMI as factors lowering SIRT6 expression ($p < 0.05$) and WSS as a factor tending to activate ($p < 0.1$).

***Conclusion*:** The results suggest that the use of WSS may add some additional positive effects to the effect of hot yoga on SIRT6 expression. Further validation of the mechanism of action is needed.

KEY WORDS: hot yoga, sirtuin, SIRT6, natural killer (NK) cells, body composition

Introduction

Hot yoga is a combination of ordinary yoga and thermotherapy. Since there has been insufficient information on the effects of hot yoga on the body, two clinical trials [1,2)] were conducted. The studies focused on telomerase and sirtuin activity as aging-related markers.

In the first clinical trial, 48 healthy Japanese women (39.2 ± 8.7 years) were subjected to 60-minute hot yoga lessons twice a week for 12 weeks [1)]. The results showed an improvement in skin condition after 12 weeks. In addition, the expression levels of SIRT1, as well as SIRT6, hTERT, and hTERC in blood were measured by real-time PCR, and the results showed a significant increase in SIRT6 mRNA expression.

In the second clinical trial, the subjects for analysis were healthy women who were aware of their lack of exercise, and were divided into a control group (n = 19, age 43.6 ± 10.3 years, BMI 26.4 ± 0.8) and a hot yoga group (n = 34, age 44.1 ± 9.5 years, BMI 25.7 ± 0.4). 60-minute hot yoga lessons were given twice a week for 12 weeks [2)]. After 12 weeks, the results showed a significant increase in SIRT6 expression of HbA1c in the hot yoga group compared to the control group. Again, SIRT1, SIRT6, hTERT, and hTERC expression were measured, but only SIRT6 expression was found to be elevated. Furthermore, the hot yoga practitioners were divided into two groups, a wood stone studio (WSS) group and a regular floor (RF) group (n = 17 each), and two

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groups were compared. The results showed that there was no significant difference in SIRT6 activity between two groups, but in WSS group, the skin stratum corneum water content was significantly improved, and blood tests showed that a decrease in RBC and Hb was avoided, total protein was significantly increased, and CPK was slightly elevated.

In the current study, hot yoga instructors working for at least 6 months were divided into two groups, those working at WSS and those working at RF, and physical/mental symptoms, body composition, natural killer (NK) cell activity, and SIRT6 expression were measured, and multiple regression analysis was performed for factors affecting SIRT6 expression.

Method

Subjects

Subjects were female instructors affiliated with LAVA and working for at least 6 months at facilities with WSS or with RF, who met the selection criteria, did not meet the exclusion criteria, and were judged by the principal investigator to be eligible to participate in the study. From the subjects who met the selection criteria, did not violate the exclusion criteria, and were judged to be appropriate to participate in the study by the principal investigator, 16 subjects (29.7 ± 1.4 years) from WSS facilities and 19 subjects (28.1 ± 0.8 years) from RF facilities were selected, for a total of 35 subjects.

The selection criteria are as follows:
(1) Female instructors affiliated with LAVA and working at WSS facilities for at least 6 months or working at RF facilities for at least 6 months.
(2) Healthy and free from chronic physical diseases, including skin diseases.
(3) Be able to come to the office on the designated examination date and take the examination.
(4) Persons who are deemed appropriate by the investigator to participate in the study.

Exclusion criteria are as follows:
(1) Currently receiving drug treatment for any disease.
(2) Patients with a history or current medical history of mental illness, sleep disorder, diabetes, hypertension, dyslipidemia, or other serious illness.
(3) Those with a history or current history of serious disorders of the uterus, liver, kidney, heart, lungs, blood, etc.
(4) Persons with a history of taking medication for the purpose of disease treatment in the past month (excluding abortive medication for headache, common cold, *etc*.)
(5) Pregnant, lactating, or of child-bearing potential.
(6) Currently participating in another human clinical trial, or who have not yet completed 3 months of participation in another human clinical trial.
(7) Other subjects who are judged by the investigator to be inappropriate for this study.

Study design

This study was an observational study. Subjects were asked to comply with the following management items on the day before the examination:
(1) Visit before the examination. Avoid irregular lifestyle (lack of sleep, binge drinking, eating, *etc*.) on the day before the visit.
(2) Alcohol consumption is prohibited on the day before the visit.
(3) Excessive exercise other than that required by the instructor on the day before the visit was prohibited.
(4) Fasting from 12 hours prior to the scheduled blood examination until the end of the examination, and no intake of anything other than water.

On the day of the visit, subjects underwent physical measurements (height, weight, and body composition), blood pressure and pulse measurements, blood tests (SIRT6 mRNA expression level and NK cell activity), Anti-Aging Quality of Life Common Questionnaire (AAQOL), and an interview by the physician.

WSS

WSS is a studio in which ore stones are placed under cypress flooring, which is further heated by underfloor heating. It was designed with the expectation of a relaxing effect from the phytoncide released from the cypress and an efficient increase in core temperature from the ore containing "silica" with the potential to emit far infrared rays.

Examination item

SIRT6 expression

Blood samples were collected from subjects in PAXgene® RNA vacuum blood collection tubes (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and measured at Immunoanalytical Research Center, Inc. (Okayama, Japan).

NK cell activity

Blood was collected from subjects into NK cell activity lymphocyte storage containers and measured at LSI Medience Inc. (Tokyo, Japan). NK cytotoxicity against K-562 cells was evaluated by ^{51}Cr release assay.

Body composition measurement

InBody 770K (InBody; Koto-ku, Tokyo) to analyze body composition (body water content, protein, mineral content, body fat), muscle and fat (body weight, muscle mass, lean body mass), obesity index (BMI, body fat ratio), muscle balance (upper body, lower body, upper/lower body), muscle mass and development rate by site (right arm, left arm, trunk, right leg and left leg), body water content (extracellular water ratio), body fat by site (right arm, left arm, trunk, right leg and left leg), water content by site (right arm, left arm, trunk, right leg, left leg, intracellular/extracellular), basal metabolic rate, bone mineral amount, somatic cell mass, and skeletal muscle mass index (SMI).

Anti-Aging QOL Common Questionnaire (AAQOL)

Using AAQOL[3], subjects were asked to answer the physical condition (33 questions) and mental symptoms (21 questions) on a 5-point scale of "1. Not at all," "2. Almost none," "3. A little," "4. Moderately," and "5. Highly," as well as lifestyle (6 questions) in numerical form.

Statistical analysis

Appropriate statistical software such as SAS (SAS 9.4) or SPSS (Statistics 26) was used for statistical analysis, and comparisons between the WSS bed group and the RF group were performed by means of an unpaired t-test (F test for equal variance, Student's t test for equal variance, and Aspin-P values obtained from the Welch's t test were employed. Data from AAQOL were treated as nonparametric, and Mann-Whitney's U test was employed. Multiple regression analysis was performed using Excel statistics (Social Information Service, Tokyo, Japan). Both were two-tailed tests, with a risk rate of less than 5% (p < 0.05) judged as a significant difference and less than 10% (p < 0.1) as a significant trend.

Ethical standards

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), the Ethics Committee for Clinical Studies was held at the "Glycative Stress Research Association" (Shinjuku-ku, Tokyo) to discuss and approve the ethics and validity of the study (GSE 2022-005). A clinical trial pre-registration was conducted for this study (UMIN #000049014).

Results

SIRT6 expression

Table 1 shows 0.957 ± 0.059 for WSS group and 0.895 ± 0.039 for RF group, with no significant difference between two groups by the t-test (p = 0.376).

NK cell activity

Table 1 shows 48.65 ± 3.04 for WSS group and 49.93 ± 3.81 for RF group; no significant difference was found between the groups by t-test (p = 0.804).

Body composition (*Table 2*)

Muscle mass by region (right arm) was significantly lower in WSS group than in the RF group (p = 0.023).

AAQOL (*Table 3*)

There were no significant differences between WSS group and RF group, but there were significant trends in "muscle pain and stiffness" (p = 0.096), "anorexia" (p = 0.072), and "joint pain" (p = 0.061), all of which tended to be lower in WSS group than in RF group, indicating that symptoms were mild.

Multiple regression analysis with SIRT6 expression as the objective variable (*Table 4*)

In this analysis, SIRT6 expression level was defined as the objective variable, and factors that increase SIRT6 expression level were predicted. For explanatory variables, factors that showed even mild correlation with SIRT6 expression level were selected as candidates, and the optimal combination was explored using a stepwise method. The explanatory variables for the first step were age, body composition, blood pressure, length of service, and presence of WSS. Finally, WSS, age, BMI, right arm development rate, and body water content (right arm) were defined as explanatory variables; for WSS, a dummy variable was created and transformed to = 2 with WSS and 1 without.

Factors decreasing SIRT6 expression were age ($\beta = -0.0207$, p = 0.0046) and BMI ($\beta = -0.0769$, p = 0.0039); WSS showed a tendency to activate SIRT6 expression ($\beta = 0.1076$, p = 0.0996).

Table 1. SIRT6 expression and NK cell activity.

		Mean		SE	p value
SIRT6 mRNA expression	WSS	0.957	±	0.059	0.376
	RF	0.895	±	0.039	
NK cell activity (%)	WSS	48.65	±	3.04	0.804
	RF	49.93	±	3.81	

WSS group: n = 16, RF group: n = 19. Analyses by Student's t test. WSS, Wood Stone Studio; RF, regular floor; NK, natural killer; SE, standard error.

Table 2. Body component.

			Mean	SE	p value
Total Body Water	L	WSS	27.85 ± 0.72		0.285
		RF	28.92 ± 0.67		
Protein	kg	WSS	7.41 ± 0.19		0.231
		RF	7.73 ± 0.18		
Minerals	kg	WSS	2.713 ± 0.075		0.383
		RF	2.805 ± 0.071		
Body fat mass	kg	WSS	11.08 ± 0.85		0.598
		RF	10.57 ± 0.41		
Weight	kg	WSS	49.06 ± 1.48		0.608
		RF	50.02 ± 1.15		
Muscle mass	kg	WSS	35.73 ± 0.92		0.281
		RF	37.11 ± 0.86		
Lean body mass	kg	WSS	37.98 ± 0.98		0.283
		RF	39.45 ± 0.92		
BMI	kg/m^2	WSS	19.09 ± 0.49		0.723
		RF	19.31 ± 0.37		
Percent body fat	%	WSS	22.31 ± 1.26		0.405
		RF	21.12 ± 0.61		
Balance (upper body)		WSS	1.1 ± 0.1		0.479
		RF	1.3 ± 0.1		
(lower body)		WSS	1.0 ± 0.0		1.000
		RF	1.0 ± 0.0		
(vertical)		WSS	1.9 ± 0.2		0.240
		RF	1.6 ± 0.2		
Right arm (muscle mass)		WSS	1.636 ± 0.064		0.112
		RF	1.781 ± 0.061		
(development rate)		WSS	91.60 ± 7.57		**0.028**
		RF	97.49 ± 1.72		
Left arm (muscle mass)		WSS	1.590 ± 0.065		0.199
		RF	1.712 ± 0.065		
(development rate)	kg	WSS	88.91 ± 1.85		0.093
		RF	93.64 ± 1.97		
Trunk (muscle mass)	%	WSS	16.01 ± 0.41		0.183
		RF	16.79 ± 0.39		
(development rate)	kg	WSS	99.13 ± 1.27		0.151
		RF	101.44 ± 0.97		
Right leg (muscle mass)	%	WSS	6.162 ± 0.189		0.787
		RF	6.231 ± 0.170		
(development rate)	kg	WSS	109.32 ± 1.91		0.593
		RF	108.01 ± 1.54		
Left leg (muscle mass)	%	WSS	6.122 ± 0.188		0.668
		RF	6.232 ± 0.172		
(development rate)	kg	WSS	108.61 ± 1.90		0.805
		RF	108.01 ± 1.54		
ECW/ TBW		WSS	0.3842 ± 0.0014		0.224
		RF	0.3821 ± 0.0011		

Body fat mass			Mean		SE	p value
Right arm	kg	WSS	0.70	±	0.06	0.400
		RF	0.65	±	0.03	
	%	WSS	78.37	±	6.04	0.273
		RF	70.90	±	2.76	
Left arm	kg	WSS	0.73	±	0.06	0.496
		RF	0.69	±	0.02	
	%	WSS	80.95	±	5.94	0.346
		RF	74.61	±	2.83	
Trunk	kg	WSS	4.80	±	0.47	0.760
		RF	4.64	±	0.24	
	%	WSS	94.51	±	9.02	0.721
		RF	91.01	±	4.69	
Right leg	kg	WSS	2.00	±	0.13	0.349
		RF	1.86	±	0.06	
	%	WSS	87.10	±	5.58	0.302
		RF	80.80	±	2.90	
Left leg	kg	WSS	1.99	±	0.13	0.390
		RF	1.87	±	0.06	
	%	WSS	86.64	±	5.52	0.313
		RF	80.55	±	2.86	
Body water content						
Right arm	L	WSS	1.271	±	0.050	0.109
		RF	1.384	±	0.048	
Left arm	L	WSS	1.235	±	0.051	0.189
		RF	1.332	±	0.050	
Trunk	L	WSS	12.49	±	0.32	0.190
		RF	13.08	±	0.31	
Right leg	L	WSS	4.806	±	0.148	0.798
		RF	4.857	±	0.132	
Left leg	L	WSS	4.780	±	0.148	0.691
		RF	4.860	±	0.135	
Intracellular	L	WSS	17.16	±	0.44	0.242
		RF	17.88	±	0.42	
Extracellular	L	WSS	10.69	±	0.29	0.372
		RF	11.04	±	0.26	
Skeletal muscle mass	kg	WSS	20.37	±	0.57	0.244
		RF	21.31	±	0.54	
Basal metabolic rate	kcal	WSS	1190.1	±	21.1	0.278
		RF	1222.1	±	19.8	
Bone mineral amount	kg	WSS	2.248	±	0.061	0.297
		RF	2.338	±	0.058	
Somatic cell mass	kg	WSS	24.56	±	0.62	0.240
		RF	25.59	±	0.60	
SMI	kg/m^2	WSS	6.01	±	0.14	0.460
		RF	6.14	±	0.11	

WSS group: n = 16, RF group: n = 19. Body component measured by "In Body". Analyses by Student's t test. WSS, Wood Stone Studio; RF, regular floor; BMI, body mass index; ECW, extracellular water; TBW, total body water; SMI, skeletal muscle mass index; SE, standard error.

Table 3. AAQOL.

Physical symptoms		Mean		SE	p value
Tired eye	WSS	2.3	±	0.3	0.1661
	RF	2.9	±	0.3	
Blurry eyes	WSS	2.0	±	0.2	0.6737
	RF	1.9	±	0.3	
Eye pain	WSS	1.4	±	0.2	0.4533
	RF	1.7	±	0.2	
Stiff shoulders	WSS	3.1	±	0.3	0.6109
	RF	3.2	±	0.2	
Mascular pain / stiffness	WSS	2.4	±	0.2	**0.0956**
	RF	2.9	±	0.2	
Palpitations	WSS	1.3	±	0.2	0.8036
	RF	1.3	±	0.2	
Shortness of breath	WSS	1.3	±	0.1	0.5714
	RF	1.3	±	0.1	
Tendency to gain weight	WSS	2.4	±	0.3	0.2990
	RF	2.1	±	0.2	
Weight los/; thin	WSS	1.7	±	0.2	0.4075
	RF	1.5	±	0.2	
Lethargy	WSS	1.9	±	0.2	0.1337
	RF	2.4	±	0.2	
No feeling of good health	WSS	1.7	±	0.2	0.3020
	RF	2.0	±	0.2	
Thirst	WSS	1.8	±	0.2	0.6663
	RF	1.7	±	0.2	
Skin problems	WSS	2.3	±	0.3	0.7295
	RF	2.5	±	0.2	
Anorexia	WSS	1.2	±	0.1	**0.0719**
	RF	1.5	±	0.1	
Early satiety	WSS	1.6	±	0.2	0.8204
	RF	1.6	±	0.2	
Epigastralgia	WSS	1.6	±	0.2	0.8136
	RF	1.6	±	0.2	
Liable to catch cold	WSS	1.4	±	0.2	0.6881
	RF	1.5	±	0.1	
Coughing and sputum	WSS	1.6	±	0.2	0.8487
	RF	1.4	±	0.1	
Diarrhea	WSS	1.9	±	0.2	0.7321
	RF	1.9	±	0.2	
Constipation	WSS	2.0	±	0.4	0.1826
	RF	2.5	±	0.3	
Hair loss	WSS	1.4	±	0.2	0.4850
	RF	1.7	±	0.3	
Gray hair	WSS	1.4	±	0.3	0.9811
	RF	1.3	±	0.1	
Headache	WSS	2.1	±	0.3	0.3122
	RF	2.3	±	0.2	
Dizziness	WSS	1.4	±	0.2	0.1816
	RF	1.9	±	0.2	
Tinnitus	WSS	1.3	±	0.1	0.2265
	RF	1.6	±	0.2	
Difficulty hearing conversation	WSS	1.5	±	0.2	0.9655
	RF	1.4	±	0.2	
Lumbago	WSS	2.4	±	0.3	0.5325
	RF	2.2	±	0.3	

		Mean		SE	p value
Arthralgia	WSS	1.3	±	0.1	**0.0612**
	RF	1.6	±	0.1	
Edematous	WSS	2.4	±	0.2	0.5087
	RF	2.6	±	0.2	
Easily breaking into a sweat	WSS	3.3	±	0.3	0.2579
	RF	2.8	±	0.3	
Frequent urination	WSS	1.6	±	0.2	0.6122
	RF	1.8	±	0.2	
Hot flush	WSS	1.4	±	0.2	0.9839
	RF	1.5	±	0.2	
Cold skin	WSS	2.4	±	0.2	0.9179
	RF	2.5	±	0.3	

Mental symptoms		Mean		SE	p value
Irritability	WSS	2.1	±	0.2	1.0000
	RF	2.1	±	0.2	
Easily angered	WSS	1.9	±	0.2	0.5712
	RF	1.7	±	0.1	
Loss of motivation	WSS	2.0	±	0.2	0.6933
	RF	1.9	±	0.2	
No feeling of happiness	WSS	1.3	±	0.1	0.9165
	RF	1.4	±	0.2	
Nothing to look forward to in life	WSS	1.1	±	0.1	0.3157
	RF	1.3	±	0.1	
Daily life is not enjoyable	WSS	1.1	±	0.1	0.5098
	RF	1.2	±	0.1	
Loss of confidence	WSS	1.3	±	0.1	0.6723
	RF	1.3	±	0.1	
Reductance to talk with others	WSS	1.3	±	0.1	0.8399
	RF	1.3	±	0.2	
Depressed	WSS	1.3	±	0.2	0.9281
	RF	1.4	±	0.2	
Feeling of usefulness	WSS	1.2	±	0.1	0.3939
	RF	1.3	±	0.1	
Sallow sleep	WSS	2.0	±	0.3	0.5050
	RF	2.2	±	0.2	
Difficulty in falling asleep	WSS	2.1	±	0.3	0.7423
	RF	2.2	±	0.2	
Pessimism	WSS	1.7	±	0.2	0.6257
	RF	1.8	±	0.2	
Lapse of memory	WSS	1.9	±	0.2	0.6241
	RF	2.1	±	0.2	
Inablity to concentrate	WSS	1.9	±	0.2	0.3396
	RF	1.7	±	0.2	
Inability to solve problems	WSS	1.6	±	0.2	0.9703
	RF	1.6	±	0.2	
Inability to make judgments readily	WSS	1.5	±	0.2	0.5558
	RF	1.8	±	0.3	
Inability to sleep because of worries	WSS	1.8	±	0.2	0.7073
	RF	1.9	±	0.2	
A sense of tension	WSS	1.9	±	0.2	0.5417
	RF	1.7	±	0.2	
Feeling of anxiety for no special reason	WSS	1.9	±	0.2	0.6817
	RF	1.7	±	0.2	
Vague feeling of fear	WSS	1.4	±	0.2	0.9838
	RF	1.4	±	0.2	

WSS group: n = 16, RF group: n = 19. Analyses by Mann-Whitney's U test. WSS, Wood Stone Studio; RF, regular floor; AAQOL, Anti-Aging Quality of Life Common Questionnaire; SE, standard error.

Table 4. Multivariate analysis with SIRT6 mRNA expression as the objective variable.

Variable	Partial regression coefficient (β)	SE	t value	p value
Age	– 0.0207	0.0067	– 3.0727	**0.0046**
BMI	– 0.0769	0.0245	– 3.1383	**0.0039**
WSS (+)	0.1076	0.0633	1.7013	**0.0996**
Right arm development rate	– 0.0049	0.0075	– 0.6538	0.5184
Body water content (R)	0.4977	0.3415	1.4575	0.1557

WSS (+); n = 16 using WSS, WSS (–): n = 19 using RF. WSS, Wood Stone Studio; RF, regular floor; BMI, body mass index; (R), right side, SE, standard error.

Discussion

In our previous studies, we found that SIRT6 expression increased as well as skin condition and subjective symptoms improved after 12 weeks of participation in a hot yoga program[1,2]. Multiple regression analysis of factors affecting SIRT6 expression showed that age and BMI were factors that decreased SIRT6 expression. This analysis also showed that WSS tended to activate SIRT6 expression.

The sirtuin gene family plays an important role in the homeostasis of body functions. Among them, SIRT6 has NAD+-dependent histone deacetylase (HDAC) activity, which is characterized by its weaker deacetylase activity and stronger demyristoylation activity than SIRT1, and should be regarded as a lysine deacylase[4].

SIRT6 is known to stabilize telomeres[5], regulate the transcriptional activity of NF-kB[6], and maintain DNA repair capacity[7]. SIRT6-deficient cells are highly susceptible to DNA damage, and SIRT6-knockout mice are highly susceptible to DNA damage.

SIRT6 knockout mice are short-lived, have decreased leukocyte counts and bone density, and SIRT6-deficient cells are highly susceptible to DNA damage[7]. Proximal tubule-specific SIRT6 knockout mice have a type 4 collagen deposition and elicit a phenotype similar to that of diabetic tubular damage[8]. SIRT6 has attracted attention as a therapeutic target for diabetes[9], non-alcoholic steatohepatitis (NASH)[10] and other hepatic metabolic diseases[11], skin wound healing[12], cardiac diseases[13], cancer[14], and early aging syndrome[15].

Physical exercise[16] has been known as a lifestyle habit that activates SIRT6 in humans. As a result of this study and previous studies[1,2], we can say that hot yoga has newly been added to the list of SIRT6 activators.

In order to verify the quality improvement of hot yoga, this study compared the results of WSS and RF. The premise is that SIRT6 activation is observed with hot yoga in both WSS and RF. In a previous study[2], the WSS group was superior to the RF group in a comparison between the groups in terms of improvement in skin moisture content. However, the 12-week hot yoga program showed no significant difference in changes in SIRT6 activity between two groups.

In the present study, instructors who had been engaged in a hot yoga program for at least 6 months were compared between two groups. The results showed that multiple regression analysis identified age and BMI as negative factors (p<0.05, significant difference) that decreased SIRT6 activity, and WSS as a positive factor (p < 0.1, significant trend) that tended to increase SIRT6 activity, suggesting that WSS may be more beneficial for both program participants and instructors.

SIRT6 and NK cells

NK cells account for 10-30 % of lymphocytes in the blood and are immune response cells that play an important role in innate immunity. *In vivo*, NK cell activity varies with circumstances, and it is known that aging, smoking, and physical and mental stress reduce NK cell activity. When NK cells detect changes in antigens presented on the surface of cancer cells or virus-infected cells (loss of MHC class I antigen indicating "self" or presence of antigen indicating "not-self"), the active type receptors on NK cells are activated and attack the cells by cytokine secretion. When NK cells encounter normal cells, signals from inhibitory receptors predominate and they do not attack the normal cells.

When the regulatory mechanisms of NK activity are out of balance, they are involved in pathogenesis. In patients with rheumatoid arthritis (RA), an excess of CD38+ NK cells in the blood is involved in the development and progression of joint lesions[17]. NK/T-cell lymphoma (NKTCL), a rare tumor among non-Hodgkin's lymphomas, has increased SIRT6 expression[18]. This increased SIRT6 expression may be responsible for the intense aggressiveness of NKCTL cells.

CD38 is a single-transmembrane type II glycoprotein and is widely used as a cell differentiation marker for lymphocyte[19]. CD38 hydrolyzes NAD to ADP-ribose and cyclic ADP-ribose. As a result, NAD is reduced, resulting in decreased responsiveness (down-regulation) of SIRT6[20]. Decreased responsiveness of SIRT6 increases the expression of NKG2D ligand, which in turn increases the expression of cytokines[21]. CD38 is a key component of the NAD-regulated SIRT6 receptor, which is a key component of the NKG2D receptor.

Cyanidin-3-O-glucoside (C3G), a natural inhibitor of CD38, decreases CD38 expression and increases SIRT6 expression[20]. C3G decreases cytokine levels such as IL-1β,

IL-6, IL-17A, and TNF-α and restores NAD+ and NK cell levels NAD+ and NK cell levels.

On the other hand, in the case of age-related decline in NK cell function, it is accompanied by decreased SIRT6 expression [20, 21]. Therefore, it is speculated that restoring SIRT6 activity will lead to the recovery of NK cell function.

In this study, NK cell activity was measured, but there was no significant difference between WSS group and RF group. The reason for this is that there was no significant difference in SIRT6 activity between two groups. The target instructors were a healthy population, with an age of around 30 years and no lifestyle habits that would reduce NAD reduction due to excess aldehydes (impaired glucose tolerance, high-fat diet, and excessive alcohol consumption). Therefore, there was little age-related decline in SIRT6 and little room for improvement in SIRT6. If a clinical trial is planned in the future to extract differences in NK cell activity between WSS group and RF group, it could be necessary to recruit subjects with suspected NAD deficiency, where sufficient room for improvement of SIRT6 can be expected.

Conclusions

Two clinical trials have been conducted on hot yoga and confirmed that participation in the program twice a week for 12 weeks significantly increases SIRT6 mRNA expression. There was no difference in SIRT6 activity when compared to WSS and RF. In this study, multiple regression analysis was performed on female instructors who had been engaged in a hot yoga program for more than 6 months, and the presence of WSS was extracted as a factor that tended to increase SIRT6 activity, and the subjective symptoms of "muscle pain and stiffness," "loss of appetite," and "joint pain" were less severe in WSS group, suggesting that hot yoga using WSS has some positive effects. This suggests that hot yoga with WSS may have some positive effects. The mechanism of this effect may need to be verified in the future.

Conflict of interest declaration

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