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Original article

Effects of exercise with ultra-soft rubber bands on glucose metabolism and endocrinology

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Abstract

***Purpose*:** Diet and exercise are important therapies for the treatment of glycative stress-related diseases (*e.g.*, type 2 diabetes and metabolic syndrome), which have been on the increase in recent years. In the present study, we investigated the effects of muscle loading exercise using an ultrasoft rubber band on glycative stress/advanced glycation endproduct (AGE) formation and endocrine indices in healthy subjects.

***Methods*:** Subjects were healthy men and women (2 males and 20 females, 44.9 ± 10.1 years) who performed the prescribed exercises using an ultrasoft rubber band for 6 minutes (6M group: n = 15) or 10 minutes (10M group: n = 7) once a day for 8 weeks. The primary endpoints were blood tests related to glucose metabolism (pentosidine, immune reactive insulin: IRI, HbA1c, fasting plasma glucose: FPG, skin AGE fluorescence: SAF), and endocrine-related blood tests (insulin-like growth factor-I: IGF-I, dehydroepiandrosterone-sulfate: DHEA-s, estradiol: E2, testosterone).

***Results*:** The percent change at 8 weeks (Total group) was significant in the primary endpoints pentosidine (−10.9 %, $p < 0.001$), IRI (−33.6 %, $p = 0.005$), HbA1c (−2.6 %, $p = 0.002$), DHEA-s (+16.1 %, $p = 0.010$), and in the secondary endpoint TG (−38.3 %, $p = 0.039$). FPG (−7.4 %, $p = 0.001$) was significantly reduced in the 10M group, and SAF (−5.3 %, $p = 0.021$) in the 6M group. Pre-intervention values for all items were within normal limits, and there were no significant differences in these items between men and women or in exercise duration; there were no significant changes in IGF-I, cortisol, E2, or total testosterone. No adverse events were noted in the safety endpoints and self-assessment findings.

***Conclusion*:** Exercise with this test product improved glucose tolerance, insulin resistance, AGEs (pentosidine and SAF), and DHEA-s, which is an important hormone for maintaining youth and health. Notedly, these parameters improved from the normal range. The results suggest that this treatment, which can be performed in a short period of time, is a safe and highly effective health promotion method.

***KEY WORDS*:** physical exercise, glucose metabolism, insulin resistance, sex hormones, glycation stress, ultrasoft rubber band

Introduction

Glycative stress is a condition in which the body is prone to the formation of short-chain aldehydes derived from carbohydrates and fatty acids. Short-chain aldehydes are highly reactive and induce carbonylative modifications of amino acids, *i.e.*, lysine, arginine, cycteine, residues that constitute proteins, resulting in the production of advanced glycation endproducts (AGEs). Eventually, AGEs increase the risk of developing various age-related diseases such as diabetic complications, Alzheimer's disease, cataracts, age-related macular degeneration, and skin aging [1-4)]. Notably, glycative stress induces a vicious cycle of decreased insulin production in pancreatic β-cells due to AGEs load and

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increased glycated insulin, which leads to impaired glucose tolerance and further contributes to glycative stress [5]. In order to interrupt this vicious cycle, it is important to take measures against glycative stress from an early stage.

The basis of glycative stress control is diet and exercise. Diabetes and postprandial hyperglycemia (blood glucose spike) cause carbohydrate-derived aldehyde production, while a high-fat diet causes fatty acid-derived aldehyde production [6]. Therefore, in addition to glycemic control, it is essential to correct dyslipidemia in parallel in order to control glycation. Therefore, a combination of diet and exercise therapy is recommended. We previously reported that muscle loading exercise using an ultrasoft rubber band (Kyowa Rubber) suppressed blood glucose spikes [7]. Expectedly, the controlling blood glucose spikes will suppress chain-response multispecies aldehyde production (aldehyde spark) and prevent subsequent vascular endothelial cell damage.

Subjects and methods

Subjects

Twenty-three healthy men and women, aged 20 to 65 years, who met the selection criteria, did not violate the exclusion criteria, and were judged by the principal investigator to be appropriate to participate in the study were included in the study as subjects.

The selection criteria are as follows:

1) Men and women between the ages of 20 and 65 at the time of obtaining consent to participate in the study.
2) Healthy individuals with no chronic physical diseases.
3) Persons who have been fully informed of the purpose and content of the study, are competent to consent, understand the study well, and voluntarily volunteer to participate in the study and are able to consent to participation in the study in writing.
4) Be able to come to the study site on the designated examination date and undergo the examination.
5) The study investigator has determined that the individual is appropriate to participate in the study.

Exclusion criteria are as follows;

1) Currently receiving medical treatment for any disease.
2) Patients with a history or current history of dyslipidemia, hypertension, diabetes, psychiatric disorders, sleep disorders, or serious illnesses.
3) Those who have been taking medication for the purpose of treatment of the disease in the past month (excluding those who have been taking medication for headache, menstrual cramps, common cold, *etc*.).
4) Those with a history or current history of serious disorders of the liver, kidney, heart, lungs, blood, etc.
5) Persons who have donated more than 200 mL of blood in the past month or 400 mL within the past 3 months.
6) Persons with severe anemia.
7) Persons who have difficulty in exercising for 10 minutes.
8) Persons whose daily alcohol consumption exceeds an average of 60 g/day of pure alcohol equivalent.
9) Persons who are likely to change their lifestyle during the test period (*e.g*., long trips, etc.)
10) Those who are currently, or within the past 3 months have been, or will be consuming functional foods or health foods that claim to be related to glucose metabolism (consumption for the purpose of health maintenance is acceptable).
11) Pregnant, lactating, or potentially pregnant women.
12) Currently participating in another clinical trial, or have not yet completed 3 months of participation in another human clinical trial.
13) Other subjects who are judged by the principal investigator to be inappropriate for this study.

Study Design

This was an open-label study in which subjects were divided into two groups according to the exercises they performed. Subjects performed the following exercises with an ultrasoft rubber band for 6 or 10 minutes once a day for 8 weeks. The study period was from August to October 2021.

- 6M group: Half set of exercises with ultra-soft rubber band (6 minutes)

1. Sidebend: topple over in 3 seconds, return in 3 seconds (2 minutes.)
2. Hip abduction: open in 3 seconds, return in 3 seconds. Left and right side. (2 minutes)
3. Marching: Raise and hold for 3 seconds, then lower. Hold for 3 seconds and bring down. (2 minutes).

- 10M group: Full set of exercises with ultra-soft rubber band (10 minutes)

1. Shoulder flexion: Raise for 3 seconds, lower for 3 seconds (2 minutes)
2. Side bends: 3 sec. to fall, 3 sec. to return (2 minutes)
3. Abduction: 3 seconds to open, 3 seconds to return. Left and right side. (2 minutes)
4. Knee Extension: Raise, hold for 3 seconds and lower. Left and right side. (2 minutes)
5. Marching: Raise, hold for 3 seconds, and lower. (2 minutes)

The ultra-soft rubber band used was manufactured by Kyowa Rubber Co. The study began with 23 subjects, and was completed with 22 subjects in total, with one subject in the 10M group discontinuing the study for personal reasons.

Endpoints

The primary endpoints were blood tests related to glucose metabolism/glycative stress, *i.e*., pentosidine, immune reactive insulin (IRI), HbA1c, fasting plasma glucose (FPG), skin AGE fluorescence (SAF) intensity, and endocrine-related blood tests, *i.e*., insulin-like growth factor-I (IGF-I), dehydroepiandrosterone-sulfate (DHEA-s). Fluorescent AGE levels were measured by AGE Reader mu (DiagnOptics, Groningen, The Netherlands), before, 2 weeks after, 4 weeks after, 6 weeks after, and 8 weeks after exercise, while blood tests were performed before, 4 weeks after, and 8 weeks after exercise.

Secondary endpoints were cortisol, cortisol/DHEA-s ratio, estradiol, total testosterone, and triglyceride (TG). Safety was evaluated by hematology, blood biochemistry, physician interview/adverse event assessment, and subject diary.

Statistical analysis

The test result values were tabulated into a cumulative table using Microsoft Office Excel 2016 (Microsoft Corp.). Statistical analysis was performed using appropriate statistical analysis software such as Excel Statistics (Shakai Joho Service, Tokyo, Japan), and the significance level for all tests was 5 % two-sided.

Analysis subjects

A total of 22 subjects who completed the study at the post-test case conference were included in the analysis. The analysis was conducted in the following three groups; 6M group (15 subjects), 10M group (7 subjects) and Total group (22 subjects), The age of the 15 patients in the 6M group (male: 1, female: 14) was 44.07 ± 11.26 years; the age of the 7 patients in the 10M group (male: 1, female: 6) was 46.57 ± 6.84 years; the age of the 22 patients in the Total group (male: 2, female: 20) was 44.86 ± 10.14 years (***Table 1***).

Table 1. Subject profile.

	Group	n	Mean		SD
	Total	22	44.86	±	10.14
Age	6 min	15	44.07	±	11.26
	10 min	7	46.57	±	6.84

SD, standard deviation, SD, standard deviation.

Ethical Review

This study was conducted in compliance with the Declaration of Helsinki (as amended at the 2013 WMA Fortaleza Meeting) and the Ethical Guidelines for Medical Research Involving Human Subjects (notified by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor and Welfare). The ethics committee for human trials was held at the "Society of Glycative Stress Research" (Shinjuku-ku, Tokyo), where the ethics and appropriateness of the study were approved (GSE 2021-007). A clinical trial pre-registration was conducted for this study (UMIN #000045177).

Results

Primary endpoints

Blood tests related to glucose metabolism (pentosidine, insulin, HbA1c, FPG)

Pentosidine

In the male-female total analysis (***Table 2***), pentosidine went from 26.68 ± 7.96 pmol/mL to 24.11 ± 6.73 pmol/mL in the 6M group (−9.6%, p = 0.012), from 26.49 ± 5.23 pmol/mL to 22.91 ± 4.47 pmol/mL (−13.5%, p < 0.001) and from 26.62 ± 7.20 pmol/mL to 23.73 ± 6.13 pmol/mL (−10.9%, p < 0.001) in the Total group, with a significant decrease after 8 weeks. In the female analysis (***Table 3, Fig. 1-a***), there was a significant decrease from 26.24 ± 8.06 pmol/mL to 23.67 ± 6.75 pmol/mL (−9.8%, p = 0.011) in the 6M group, 26.72 ± 5.61 pmol/mL to 22.98 ± 4.83 pmol/mL (−14.0%, p < 0.001) and from 26.38 ± 7.41 pmol/mL to 23.47 ± 6.24 pmol/mL (−11.0%, p < 0.001) in the Total group, respectively, after 8 weeks.

Immuno reactive Insulin (IRI)

In the total analysis (***Table 2***), IRI decreased significantly after 8 weeks from 5.90 ± 3.04 μU/mL to 4.37 ± 2.05 μU/mL in the 6M group (−25.9%, p = 0.009) and from 6.84 ± 4.23 μU/mL to 4.54 ± 2.46 μU/mL in the Total group (−33.6%, p = 0.005), with a significant decrease after 8 weeks, but no significant change in the 10M group. In the female analysis (***Table 3, Fig. 1-b***), there was a significant decrease from 6.07 ± 3.07 μU/mL to 4.36 ± 2.12 μU/mL in the 6M group (−28.2%, p = 0.009) and from 6.38 ± 3.32 μU/mL to 4.56 ± 2.58 μU/mL in the Total group (−28.5%, p = 0.001), while there was no significant change in the 10M group.

HbA1c

In the total analysis (***Table 2***), HbA1c decreased significantly over 8 weeks from 5.97 ± 1.63% to 5.78 ± 1.48% (−3.2%, p = 0.004) in the 6M group and from 5.79 ± 1.37% to 5.64 ± 1.25% (−2.6%, p = 0.002) at 8 weeks, while there was no significant change in the 10M group. In the female analysis (***Table3, Fig. 1-c***), there was a significant decrease from 5.99 ± 1.69% to 5.81 ± 1.53% (−3.0%, p = 0.004) in the 6M group and from 5.80 ± 1.44% to 5.65 ± 1.31% (−2.6%, p = 0.002) in the Total group in the Total group and from 5.80 ± 1.44% to 5.65 ± 1.31% (2.6%, p = 0.002) in the Total group, but no significant change in the 10M group.

Fasting plasma glucose (FPG)

In the total analysis (***Table 2***), FPG showed a significant decrease after 8 weeks from 90.14 ± 8.51 mg/dL to 83.43 ± 7.69 mg/dL (−7.4%, p = 0.001) in the 10M group, but no significant change in the 6M and Total groups. In the female analysis (***Table 3, Fig. 1- d***), there was a significant decrease in the 10M group from 88.00 ± 7.23 mg/dL to 81.67 ± 6.87 mg/dL (−7.2%, p = 0.001), but he 6M and Total groups did not show any significant changes.

Intensity of SAF

In the combined male/female analysis (***Table 2***), SAF decreased significantly after 8 weeks from 2.08 ± 0.35 to 1.97 ± 0.33 (−5.3%, p = 0.021) in the 6M group, while there was no significant change in the 10M and Total groups. In the female analysis (***Table 3, Fig. 1-e***), there was a significant decrease in the 6M group from 2.09 ± 0.36 to 1.96 ± 0.34 (−6.2%, p = 0.021), but no significant change in the 10M and Total groups.

Endocrine-related blood tests

IGF-I

IGF-I did not vary significantly in either the male/female total or female groups (***Table 2, 3, Fig. 1-f***).

Table 2. Test results (Endpoints): Total male-female analysis.

	Group	n	Comparison of before and after						Group comparison (p value)
			0 w	4w	P value	8w	P value		8 w
			Mean SE	Mean SE	rate of change	Mean SE	rate of change		rate of change
Pentosidine (pmol/mL)	Total	22	26.62 ± 7.20	24.10 ± 6.32	**0.000**	23.73 ± 6.13	**0.000**		
	6min	15	26.68 ± 7.96	24.06 ± 6.69	**0.000**	24.11 ± 6.73	**0.007**		0.140
	10min	7	26.49 ± 5.23	24.19 ± 5.46	**0.031**	22.91 ± 4.47	**0.000**		
IRI (μU/mL)	Total	22	6.84 ± 4.23	4.85 ± 2.97	**0.026**	4.54 ± 2.46	**0.001**		
	6min	15	5.90 ± 3.04	3.86 ± 1.56	**0.026**	4.37 ± 2.05	**0.022**		0.308
	10min	7	8.84 ± 5.54	6.96 ± 4.00	0.514	4.91 ± 3.13	**0.028**		
HbA1c (%)	Total	22	5.79 ± 1.37	5.76 ± 1.28	0.650	5.64 ± 1.25	**0.001**		
	6min	15	5.97 ± 1.63	5.92 ± 1.52	0.462	5.78 ± 1.48	**0.002**		0.250
	10min	7	5.40 ± 0.17	5.41 ± 0.16	0.743	5.33 ± 0.19	0.252		
FPG (mg/dL)	Total	22	96.82 ± 45.57	97.95 ± 51.59	0.778	90.95 ± 29.62	0.140		
	6min	15	99.93 ± 54.61	102.80 ± 61.77	0.326	94.47 ± 34.93	0.611		0.116
	10min	7	90.14 ± 8.51	87.57 ± 5.60	0.356	83.43 ± 7.69	**0.001**		
Intensity of SAF	Total	22	2.08 ± 0.39	2.05 ± 0.34	0.585	2.00 ± 0.34	0.221		
	6min	15	2.08 ± 0.35	2.03 ± 0.29	0.114	1.97 ± 0.33	**0.016**		0.272
	10min	7	2.09 ± 0.47	2.09 ± 0.43	0.834	2.09 ± 0.35	0.735		
IGF-I (ng/mL)	Total	22	147.14 ± 47.72	150.86 ± 57.35	0.586	142.95 ± 47.21	0.409		
	6min	15	154.13 ± 53.34	155.27 ± 64.30	0.804	148.07 ± 50.82	0.266		0.661
	10min	7	132.14 ± 27.00	141.43 ± 36.71	0.333	132.00 ± 35.98	0.983		
DHEA-s (μg/dL)	Total	22	141.86 ± 79.76	160.41 ± 88.72	**0.006**	164.68 ± 99.24	**0.001**		
	6min	15	142.20 ± 80.26	169.40 ± 88.50	**0.001**	171.47 ± 109.11	**0.002**		0.145
	10min	7	141.14 ± 78.67	141.14 ± 86.09	0.695	150.14 ± 71.66	0.290		
Cortisol (μg/dL)	Total	22	7.61 ± 2.49	8.27 ± 3.17	0.197	7.75 ± 2.16	0.223		
	6min	15	7.92 ± 2.50	8.75 ± 2.83	0.100	7.93 ± 2.43	0.423		0.808
	10min	7	6.94 ± 2.31	7.23 ± 3.56	0.796	7.34 ± 1.37	0.310		
Cortisol/ DHEA-s	Total	22	0.07 ± 0.04	0.06 ± 0.04	0.936	0.06 ± 0.03	0.424		
	6min	15	0.07 ± 0.04	0.06 ± 0.03	0.303	0.06 ± 0.03	0.191		0.297
	10min	7	0.06 ± 0.04	0.07 ± 0.06	0.574	0.06 ± 0.03	0.670		
Estradiol (pg/mL)	Total	22	55.24 ± 86.50	49.97 ± 60.57	0.224	61.97 ± 76.29	0.138		
	6min	15	71.04 ± 99.37	49.58 ± 58.60	0.610	47.03 ± 60.57	0.623		0.170
	10min	7	21.37 ± 26.00	50.81 ± 64.57	0.274	93.97 ± 94.48	0.162		
Total testosterone (ng/mL)	Total	22	0.54 ± 0.82	0.58 ± 1.03	0.249	0.60 ± 0.99	0.355		
	6min	15	0.52 ± 0.82	0.60 ± 1.12	0.558	0.51 ± 0.85	0.668		**0.040**
	10min	7	0.60 ± 0.81	0.54 ± 0.79	**0.016**	0.80 ± 1.22	0.047		
TG (mg/dL)	Total	22	107.50 ± 82.92	126.09 ± 187.31	0.517	79.59 ± 46.48	**0.009**		
	6min	15	97.80 ± 48.98	83.27 ± 36.87	0.216	75.00 ± 42.87	**0.025**		0.770
	10min	7	128.29 ± 125.82	217.86 ± 308.22	0.119	89.43 ± 52.07	0.220		

See text for abbreviations.

Table 3. Test results (Endpoints): Female analysis.

	Group	n	Comparison of before and after						Group comparison (p value)
			0 w	4w	P value	8w	P value	8 w	
			Mean SE	Mean SE	rate of change	Mean SE	rate of change	rate of change	
Pentosidine (pmol/mL)	Total	20	26.38 ± 7.41	23.99 ± 6.59	**0.000**	23.47 ± 6.24	**0.000**		
	6min	14	26.24 ± 8.06	23.83 ± 6.86	**0.001**	23.67 ± 6.75	**0.011**	0.136	
	10min	6	26.72 ± 5.61	24.37 ± 5.88	**0.061**	22.98 ± 4.83	**0.000**		
IRI (μU/mL)	Total	20	6.38 ± 3.32	4.39 ± 2.18	**0.025**	4.56 ± 2.58	**0.001**		
	6min	14	6.07 ± 3.07	3.84 ± 1.61	**0.015**	4.36 ± 2.12	**0.009**	0.720	
	10min	6	7.08 ± 3.76	5.68 ± 2.71	0.662	5.02 ± 3.37	0.064		
HbA1c (%)	Total	20	5.80 ± 1.44	5.77 ± 1.34	0.763	5.65 ± 1.31	**0.002**		
	6min	14	5.99 ± 1.69	5.94 ± 1.57	0.574	5.81 ± 1.53	**0.004**	0.486	
	10min	6	5.35 ± 0.13	5.37 ± 0.11	0.748	5.27 ± 0.12	0.257		
FPG (mg/dL)	Total	20	96.35 ± 47.77	98.10 ± 54.11	0.545	90.75 ± 31.05	0.241		
	6min	14	99.93 ± 56.52	103.29 ± 63.91	0.250	94.64 ± 36.15	0.727	0.129	
	10min	6	88.00 ± 7.23	86.00 ± 4.40	0.533	81.67 ± 6.87	**0.005**		
Intensity of SAF	Total	20	2.11 ± 0.40	2.07 ± 0.35	0.501	2.02 ± 0.35	0.182		
	6min	14	2.09 ± 0.36	2.03 ± 0.30	0.051	1.96 ± 0.34	**0.008**	0.287	
	10min	6	2.15 ± 0.48	2.15 ± 0.43	0.837	2.15 ± 0.34	0.741		
IGF-I (ng/mL)	Total	20	143.80 ± 47.57	147.60 ± 58.33	0.626	139.00 ± 45.11	0.410		
	6min	14	149.79 ± 52.58	151.00 ± 64.48	0.805	142.07 ± 47.20	0.184	0.509	
	10min	6	129.83 ± 28.52	139.67 ± 39.37	0.381	131.83 ± 38.86	0.860		
DHEA-s (μg/dL)	Total	20	129.00 ± 62.18	146.00 ± 69.64	**0.012**	144.90 ± 60.75	**0.004**		
	6min	14	124.86 ± 48.88	150.93 ± 57.22	**0.001**	145.79 ± 53.50	**0.004**	0.139	
	10min	6	138.67 ± 84.72	134.50 ± 91.31	0.445	142.83 ± 74.94	0.507		
Cortisol (μg/dL)	Total	20	7.65 ± 2.52	8.44 ± 3.16	0.149	7.78 ± 2.11	0.229		
	6min	14	7.82 ± 2.56	8.71 ± 2.93	0.100	7.78 ± 2.44	0.460	0.685	
	10min	6	7.25 ± 2.35	7.78 ± 3.56	0.673	7.77 ± 0.96	0.274		
Cortisol/ DHEA-s	Total	20	0.07 ± 0.04	0.07 ± 0.04	0.865	0.06 ± 0.03	0.609		
	6min	14	0.08 ± 0.04	0.06 ± 0.02	0.353	0.06 ± 0.03	0.252	0.237	
	10min	6	0.07 ± 0.04	0.08 ± 0.06	0.423	0.07 ± 0.03	0.481		
Estradiol (pg/mL)	Total	20	59.67 ± 89.53	53.72 ± 62.29	0.232	65.82 ± 78.97	0.161		
	6min	14	75.26 ± 101.55	52.12 ± 59.85	0.655	49.04 ± 62.22	0.726	0.192	
	10min	6	23.28 ± 27.63	57.45 ± 67.49	0.283	104.97 ± 97.81	0.186		
Total testosterone (ng/mL)	Total	20	0.29 ± 0.10	0.27 ± 0.11	0.107	0.29 ± 0.09	0.606		
	6min	14	0.30 ± 0.10	0.30 ± 0.11	0.956	0.28 ± 0.09	0.644	**0.089**	
	10min	6	0.27 ± 0.09	0.22 ± 0.11	**0.018**	0.31 ± 0.08	0.104		
TG (mg/dL)	Total	20	94.65 ± 44.96	88.10 ± 40.60	0.976	75.85 ± 42.28	**0.010**		
	6min	14	101.71 ± 48.38	85.86 ± 36.83	0.181	76.71 ± 43.87	**0.014**	0.288	
	10min	6	78.17 ± 29.78	93.33 ± 47.85	0.240	73.83 ± 38.22	0.461		

See text for abbreviations.

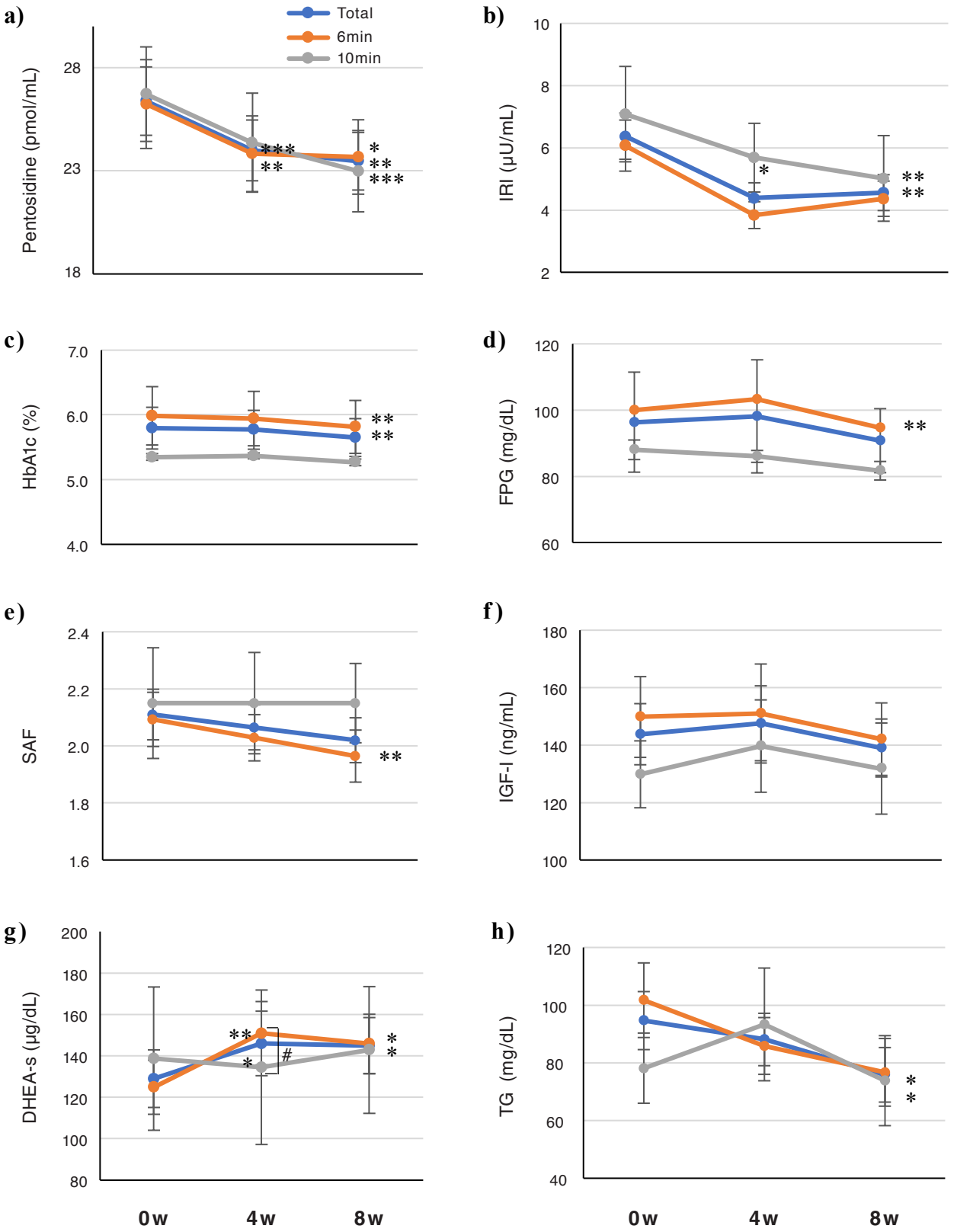

Fig. 1. **Changes of the endpoint items in the female analysis by the exercise intervention.**

(a) Pentosidine, **(b)** IRI, **(c)** HbA1c, **(d)** FPG, **(e)** SAF, **(f)** IGF-I, **(g)** DHEA-s, **(h)** TG.

Results are expressed as mean ± SEM, *p < 0.05, **p < 0.01, paired-t test vs pre-values. IRI, immune reactive insulin; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose; SAF, skin AGE fluorescence; IGF-I, insulin-like growth factor-I; DHEA-s, dehydroepiandrosterone-sulfate; TG, triglyceride; SEM, standard error mean.

DHEA-s

In the total analysis (***Table 2***), DHEA-s went from 142.20 ± 80.26 μg/dL to 171.47 ± 109.11 μg/dL in the 6M group (+20.6%, p = 0.015), from 141.86 ± 79.76 μg/dL to 164.68 ± 99.24 μg/dL (+16.1%, p = 0.010) over 8 weeks, while there was no significant change in the 10M group. In the femal analysis (***Table 3, Fig. 1-g***), the 6M group went from 124.86 ± 48.88 μg/dL to 145.79 ± 53.50 μg/dL (+16.8%, p = 0.004), the Total group from 129.00 ± 62.18 μg/dL to 144.90 ± 60.75 μg/dL (+12.3%, p = 0.004), while there was no significant change in the 10M group.

Secondary endpoints

Cortisol, cortisol/DHEA-s, estradiol, and Total testosterone

Total and female analyses showed no significant changes in any of the above 3 items after 8 weeks in any of the groups (***Table 2, 3***).

Triglycerides (TG)

In the total analysis (***Table 2***), TG significantly decreased from 97.80 ± 48.98 mg/dL to 75.00 ± 42.87 mg/dL (−23.3%, p = 0.037) in the 6M group and from 107.50 ± 82.92 mg/dL to 79.59 ± 46.48 mg/dL (−38.3%, p = 0.039) in the Total group after 8 weeks, while there was no significant change in the 10M group. In the female analysis (***Table 3, Fig. 1-h***), there was a significant decrease from 101.71 ± 48.38 mg/dL to 76.71 ± 43.87 mg/dL (−24.6%, p = 0.014) in the 6M group, from 94.65 ± 44.96 mg/dL to 75.85 ± 42.28 mg/dL (−19.9%, p = 0.010) in the Total group, while there was no significant change in the 10M group.

Safety endpoints

Hematological tests that showed significant differences over time or between groups included white blood cell count (WBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular pigment concentration (MCHC), and platelet count. hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular pigment concentration (MCHC), platelet count (PLT), and blood biochemical tests including total protein (TP), albumin level, creatinine (CRE), γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), C-reactive protein (CRP), high density-lipoprotein cholesterol (HDL-C), total bilirubin (TBil), sodium (Na), potassium (K), chlorine (Cl), and iron (Fe) (***Table 4, 5***). These changes were within the range of physiologic variability.

Safety

No adverse reactions that could be attributed to the test product or adverse events that could be attributed to the test product were observed in all subjects.

Discussion

Improvement of glucose metabolism and glucose tolerance

In terms of indices related to glucose metabolism and glucose tolerance, a significant decrease in FPG (10M group −7.4%), HbA1c (6M group −3.2%), and IRI (6M group −25.9%) were observed after 8 weeks (values in parentheses indicate values in the total male-female analysis). These results indicate that physical exercise improved glucose tolerance. Notably, the previous values of FPG, HbA1c, and IRI were all within the reference range, not at pathological levels, and there was little room for improvement beyond the current state. This finding implies that physical exercise is useful for health promotion.

Improvement of TG (−23.3% in the 6M group) was also observed in the present study; the decrease in TG is expected to reduce fatty acid-derived aldehyde production[6], which may have an additive effect on glycation stress.

Comparing the rates of change in FPG, HbA1c, and IRI, the rate of IRI decrease was greater than that in FPG and HbA1c, indicating that insulin resistance was improved. Since less insulin is secreted from pancreatic β-cells due to the stimulation of glucose rise, the β-cell load associated with insulin biosynthesis is reduced.

Insulin is synthesized in β-cells via preproinsulin and proinsulin (***Fig. 2***)[5]. Proinsulin is cleaved at the C-terminal side of Arg 31-Arg 32, which connects the insulin B chain to the C peptide, and Lys 64-Arg 65, which connects the C peptide to the A chain, by the action of prohormone convertase in the secretory granules. Subsequently, these basic amino acids (Arg, Lys) are removed by the action of carboxypeptidase E to produce insulin and C-peptide[8]. During increased glycative stress, the amount of short-chain aldehydes in the blood increases and is transferred into the β-cell, increasing intracellular glycated proinsulin. Arg and Lys, which have two amino residues (-NH_2), are susceptible to carbonylation modification, resulting in resistance to carboxypeptidase E. Therefore, insulin production is decreased during increased glycative stress.

In addition to this, the composition of IRI is presumed to change during increased glycative stress. As the glycative stress rises, the ratio of glycated insulin increases and active insulin decreases. Conversely, if glycative stress is reduced, the amount of glycated proinsulin and glycated insulin in beta cells will decrease, resulting in increased insulin production, and the compositional ratio of IRI will decrease glycated insulin and increase the ratio of insulin.

The IRI value used is measured using antigenicity to insulin peptide specific sites, and thus indicates immunological cross-reactivity and represents the total amount of insulin (glycated and non-glycated) and proinsulin (glycated and non-glycated). The fasting proinsulin content in normal subjects is 0.05 ~ 0.4 ng/mL, with a ratio to IRI value of 5 ~ 48%[9].

Fig. 3 shows a schematic diagram of the speculated changes in IRI during hyper-glycative-stress and amelioration. Physical exercise reduces the ratio of proinsulin to IRI and decreases IRI. The ratio of glycation modification in proinsulin and insulin is reduced. Speculatedly, as a result, insulin activity increases and insulin resistance improves despite the decrease in IRI. To prove this hypothesis, it is necessary to measure glycated and non-glycated insulin and proinsulin in IRI. This is a subject for future work.

Table 4. Test results (Safety endpoints): Total male-female analysis.

	Group	n	Comparison of before and after						Group comparison (p value)
			0 w	4w	P value	8w	P value	8 w	
			Mean SE	Mean SE	rate of change	Mean SE	rate of change	rate of change	
WBC (/μL)	Total	22	6100.00 ± 1232.88	5222.73 ± 1350.06	**0.003**	5945.45 ± 1545.59	0.869		
	6min	15	6086.67 ± 1238.74	5173.33 ± 1295.36	**0.003**	6200.00 ± 1645.60	0.585	0.053	
	10min	7	6128.57 ± 1219.74	5328.57 ± 1454.76	0.273	5400.00 ± 1126.31	**0.000**		
RBC (×10^4/μL)	Total	22	439.05 ± 33.65	445.09 ± 34.21	0.100	447.18 ± 29.12	0.078		
	6min	15	440.60 ± 34.39	444.13 ± 31.43	0.335	445.87 ± 32.57	0.358	0.334	
	10min	7	435.71 ± 31.75	447.14 ± 39.43	0.202	450.00 ± 19.49	0.068		
Hb (g/dL)	Total	22	12.95 ± 1.32	13.17 ± 1.46	0.097	13.22 ± 1.20	0.055		
	6min	15	12.97 ± 1.42	13.07 ± 1.42	0.440	13.14 ± 1.31	0.320	0.279	
	10min	7	12.91 ± 1.07	13.39 ± 1.52	0.140	13.39 ± 0.89	**0.030**		
Ht (%)	Total	22	40.56 ± 3.55	40.77 ± 3.81	0.545	41.87 ± 3.12	**0.008**		
	6min	15	40.62 ± 3.75	40.73 ± 3.73	0.740	41.65 ± 3.51	0.096	0.351	
	10min	7	40.44 ± 3.08	40.86 ± 3.97	0.600	42.34 ± 1.98	**0.022**		
MCV (fL)	Total	22	92.50 ± 4.87	91.55 ± 4.35	**0.011**	93.68 ± 4.19	**0.000**		
	6min	15	92.20 ± 4.93	91.60 ± 4.36	0.213	93.47 ± 4.27	**0.002**	0.599	
	10min	7	93.14 ± 4.67	91.43 ± 4.30	**0.003**	94.14 ± 3.98	**0.040**		
MCH (pg)	Total	22	29.48 ± 1.65	29.54 ± 1.73	0.482	29.55 ± 1.64	0.364		
	6min	15	29.40 ± 1.74	29.37 ± 1.80	0.712	29.45 ± 1.71	0.501	0.825	
	10min	7	29.66 ± 1.43	29.89 ± 1.50	0.155	29.76 ± 1.48	0.582		
MCHC (%)	Total	22	31.90 ± 0.64	32.25 ± 0.88	**0.010**	31.54 ± 0.74	**0.000**		
	6min	15	31.88 ± 0.72	32.03 ± 0.88	0.290	31.51 ± 0.75	**0.002**	0.865	
	10min	7	31.93 ± 0.40	32.71 ± 0.67	**0.004**	31.60 ± 0.72	0.109		
PLT (×10^4/μL)	Total	22	29.74 ± 6.57	27.64 ± 6.43	**0.004**	28.75 ± 6.53	0.183		
	6min	15	31.47 ± 6.73	29.29 ± 6.60	**0.048**	30.01 ± 6.96	0.185	0.197	
	10min	7	26.03 ± 4.27	24.10 ± 4.25	**0.022**	26.04 ± 4.42	**0.039**		
TP (g/dL)	Total	22	7.39 ± 0.37	7.49 ± 0.38	0.147	7.43 ± 0.34	0.555		
	6min	15	7.43 ± 0.39	7.61 ± 0.35	**0.035**	7.49 ± 0.26	0.457	0.755	
	10min	7	7.30 ± 0.29	7.21 ± 0.27	0.434	7.30 ± 0.43	0.978		
ALB (g/dL)	Total	22	4.52 ± 0.32	4.52 ± 0.32	0.836	4.49 ± 0.27	0.747		
	6min	15	4.51 ± 0.34	4.60 ± 0.31	0.135	4.53 ± 0.26	0.716	0.215	
	10min	7	4.53 ± 0.28	4.36 ± 0.28	**0.017**	4.40 ± 0.26	0.213		
BUN (mg/dL)	Total	22	14.68 ± 3.65	14.04 ± 4.19	0.652	13.25 ± 3.38	0.110		
	6min	15	14.72 ± 3.46	14.47 ± 3.94	0.890	13.63 ± 3.67	0.207	0.780	
	10min	7	14.59 ± 4.03	13.11 ± 4.54	0.093	12.44 ± 2.48	0.371		
CRE (mg/dL)	Total	22	0.71 ± 0.15	0.70 ± 0.13	0.262	0.64 ± 0.15	**0.000**		
	6min	15	0.68 ± 0.14	0.67 ± 0.13	0.851	0.60 ± 0.14	**0.000**	0.317	
	10min	7	0.78 ± 0.13	0.75 ± 0.14	0.120	0.72 ± 0.12	**0.022**		
UA (mg/dL)	Total	22	4.30 ± 1.03	4.19 ± 1.07	0.544	4.25 ± 1.20	0.789		
	6min	15	4.19 ± 1.06	4.15 ± 1.17	0.911	4.20 ± 1.31	0.886	0.364	
	10min	7	4.53 ± 0.94	4.29 ± 0.80	0.260	4.36 ± 0.92	0.145		
AST (U/L)	Total	22	19.82 ± 5.70	22.23 ± 7.97	**0.013**	21.32 ± 6.84	0.052		
	6min	15	18.87 ± 5.29	19.60 ± 3.26	0.147	19.13 ± 3.81	0.336	0.187	
	10min	7	21.86 ± 6.01	27.86 ± 11.41	**0.046**	26.00 ± 9.17	0.097		

ALT (U/L)	Total	22	17.73 ± 13.86	20.50 ± 21.92	0.202	20.18 ± 19.95	0.134	0.284
	6min	15	14.13 ± 7.46	13.07 ± 5.21	0.977	13.47 ± 4.18	0.473	
	10min	7	25.43 ± 19.94	36.43 ± 32.87	0.060	34.57 ± 30.16	0.211	
γ-GT (U/L)	Total	22	22.27 ± 17.52	20.55 ± 15.13	0.228	20.18 ± 12.66	0.646	0.268
	6min	15	21.60 ± 17.87	17.27 ± 8.73	**0.014**	18.53 ± 10.38	0.171	
	10min	7	23.71 ± 16.67	27.57 ± 21.99	0.083	23.71 ± 15.96	0.524	
ALP (U/L)	Total	22	66.64 ± 17.44	64.95 ± 17.37	0.409	63.59 ± 16.97	0.108	0.055
	6min	15	67.67 ± 17.09	64.40 ± 17.46	0.195	62.33 ± 16.74	**0.002**	
	10min	7	64.43 ± 17.95	66.14 ± 17.10	0.091	66.29 ± 17.14	0.433	
LDH (U/L)	Total	22	227.45 ± 29.42	226.91 ± 41.07	0.953	216.82 ± 29.14	**0.009**	0.659
	6min	15	228.20 ± 30.05	229.67 ± 44.49	0.829	218.47 ± 25.02	**0.026**	
	10min	7	225.86 ± 27.94	221.00 ± 31.76	0.383	213.29 ± 36.18	0.172	
CPK (U/L)	Total	22	99.45 ± 45.57	100.27 ± 61.36	0.959	112.82 ± 74.20	0.073	0.464
	6min	15	95.60 ± 45.98	99.73 ± 65.72	0.630	111.60 ± 82.32	0.125	
	10min	7	107.71 ± 43.57	101.43 ± 50.74	0.473	115.43 ± 52.66	0.330	
CRP (mg/dL)	Total	22	0.09 ± 0.15	0.22 ± 0.21	**0.013**	0.05 ± 0.07	0.269	0.066
	6min	15	0.12 ± 0.17	0.20 ± 0.23	0.060	0.06 ± 0.09	**0.002**	
	10min	7	0.04 ± 0.05	0.25 ± 0.18	**0.090**	0.03 ± 0.02	0.332	
TC (mg/dL)	Total	22	212.18 ± 32.41	208.64 ± 34.02	0.331	210.45 ± 37.58	0.647	0.338
	6min	15	209.80 ± 35.79	206.80 ± 34.18	0.561	205.93 ± 41.82	0.405	
	10min	7	217.29 ± 22.77	212.57 ± 33.34	0.449	220.14 ± 23.50	0.585	
LDL-c (mg/dL)	Total	22	123.36 ± 24.91	121.64 ± 25.18	0.776	122.36 ± 27.12	0.843	0.244
	6min	15	121.73 ± 28.53	122.13 ± 25.30	0.573	118.33 ± 30.02	0.480	
	10min	7	126.86 ± 13.75	120.57 ± 24.90	0.361	131.00 ± 16.48	0.366	
HDL-c (mg/dL)	Total	22	69.45 ± 14.19	66.68 ± 12.79	0.081	72.68 ± 16.80	0.080	0.694
	6min	15	69.40 ± 14.83	68.13 ± 11.93	0.721	73.00 ± 17.65	0.158	
	10min	7	69.57 ± 12.72	63.57 ± 13.97	**0.012**	72.00 ± 14.79	0.284	
Bil (mg/dL)	Total	22	0.76 ± 0.26	0.68 ± 0.30	0.105	0.86 ± 0.42	0.108	0.390
	6min	15	0.74 ± 0.26	0.71 ± 0.32	0.686	0.86 ± 0.43	0.114	
	10min	7	0.80 ± 0.26	0.60 ± 0.23	**0.029**	0.86 ± 0.39	0.724	
Na (mEq/L)	Total	22	140.18 ± 1.82	138.77 ± 1.65	**0.000**	139.14 ± 1.58	**0.001**	0.067
	6min	15	139.87 ± 2.03	138.93 ± 1.84	**0.021**	139.20 ± 1.64	**0.038**	
	10min	7	140.86 ± 0.99	138.43 ± 1.05	**0.000**	139.00 ± 1.41	**0.010**	
K (mEq/L)	Total	22	4.69 ± 0.39	5.10 ± 0.56	**0.001**	5.23 ± 0.45	**0.000**	0.107
	6min	15	4.77 ± 0.36	5.11 ± 0.64	**0.029**	5.17 ± 0.40	**0.004**	
	10min	7	4.51 ± 0.41	5.09 ± 0.31	**0.006**	5.36 ± 0.51	**0.011**	
Cl (mEq/L)	Total	22	101.45 ± 1.97	100.41 ± 2.04	**0.003**	102.00 ± 1.93	0.151	0.122
	6min	15	101.13 ± 2.28	100.47 ± 2.19	0.078	102.07 ± 1.95	0.053	
	10min	7	102.14 ± 0.64	100.29 ± 1.67	**0.011**	101.86 ± 1.88	0.649	
Ca (mg/dL)	Total	22	9.40 ± 0.34	9.40 ± 0.39	0.912	9.28 ± 0.21	0.081	0.832
	6min	15	9.41 ± 0.32	9.46 ± 0.37	0.495	9.28 ± 0.20	0.152	
	10min	7	9.37 ± 0.39	9.27 ± 0.40	0.143	9.27 ± 0.23	0.370	
Fe (μg/dL)	Total	22	87.95 ± 34.61	80.45 ± 37.58	0.710	94.14 ± 41.52	0.195	0.320
	6min	15	82.47 ± 34.25	85.67 ± 37.18	0.392	93.00 ± 41.37	0.134	
	10min	7	99.71 ± 32.38	69.29 ± 35.96	0.063	96.57 ± 41.72	0.955	

See text for abbreviations.

Table 5. Test results (Safety endpoints): Female analysis.

	Group	n	Comparison of before and after						Group comparison (p value)
			0 w	4w	P value	8w	P value	8 w	
			Mean SE	Mean SE	rate of change	Mean SE	rate of change	rate of change	
WBC (/μL)	Total	20	6075.00 ± 1290.30	5050.00 ± 1242.78	**0.000**	5880.00 ± 1574.99	0.817		
	6min	14	6064.29 ± 1279.29	5128.57 ± 1329.57	**0.004**	6100.00 ± 1658.74	0.697	0.084	
	10min	6	6100.00 ± 1315.29	4866.67 ± 987.70	0.074	5366.67 ± 1213.35	**0.000**		
RBC (×10^4/μL)	Total	20	437.90 ± 33.91	441.20 ± 31.81	0.305	444.80 ± 29.50	0.123		
	6min	14	441.93 ± 35.22	443.64 ± 32.48	0.586	444.00 ± 32.93	0.643	0.087	
	10min	6	428.50 ± 28.49	435.50 ± 29.40	0.403	446.67 ± 19.12	**0.035**		
Hb (g/dL)	Total	20	12.88 ± 1.33	12.99 ± 1.34	0.304	13.09 ± 1.18	0.112		
	6min	14	12.99 ± 1.47	13.03 ± 1.46	0.694	13.06 ± 1.32	0.569	0.109	
	10min	6	12.63 ± 0.88	12.90 ± 1.02	0.293	13.17 ± 0.77	**0.032**		
Ht (%)	Total	20	40.37 ± 3.59	40.31 ± 3.61	0.934	41.55 ± 3.08	**0.014**		
	6min	14	40.65 ± 3.88	40.57 ± 3.81	0.917	41.37 ± 3.46	0.190	0.100	
	10min	6	39.70 ± 2.68	39.70 ± 3.00	0.993	41.95 ± 1.87	**0.014**		
MCV (fL)	Total	20	92.30 ± 5.06	91.35 ± 4.49	**0.019**	93.45 ± 4.32	**0.001**		
	6min	14	92.00 ± 5.04	91.36 ± 4.42	0.214	93.21 ± 4.31	**0.005**	0.707	
	10min	6	93.00 ± 5.03	91.33 ± 4.64	**0.010**	94.00 ± 4.28	0.076		
MCH (pg)	Total	20	29.39 ± 1.71	29.41 ± 1.75	0.834	29.43 ± 1.67	0.610		
	6min	14	29.34 ± 1.78	29.31 ± 1.85	0.773	29.39 ± 1.75	0.502	0.697	
	10min	6	29.52 ± 1.50	29.62 ± 1.46	0.233	29.50 ± 1.44	0.944		
MCHC (%)	Total	20	31.87 ± 0.65	32.19 ± 0.81	**0.014**	31.48 ± 0.71	**0.000**		
	6min	14	31.89 ± 0.75	32.06 ± 0.91	0.245	31.52 ± 0.77	**0.003**	0.639	
	10min	6	31.83 ± 0.34	32.48 ± 0.40	**0.005**	31.38 ± 0.52	**0.031**		
PLT (×10^4/μL)	Total	20	30.35 ± 6.58	27.96 ± 6.64	**0.002**	29.22 ± 6.66	0.159		
	6min	14	32.03 ± 6.63	29.53 ± 6.78	**0.022**	30.36 ± 7.08	0.141	0.114	
	10min	6	26.43 ± 4.48	24.30 ± 4.56	**0.026**	26.57 ± 4.57	**0.039**		
TP (g/dL)	Total	20	7.42 ± 0.36	7.51 ± 0.39	0.175	7.46 ± 0.34	0.534		
	6min	14	7.48 ± 0.36	7.64 ± 0.35	**0.065**	7.50 ± 0.27	0.713	0.781	
	10min	6	7.27 ± 0.30	7.20 ± 0.29	0.604	7.35 ± 0.45	0.636		
ALB (g/dL)	Total	20	4.52 ± 0.31	4.51 ± 0.33	0.889	4.48 ± 0.28	0.624		
	6min	14	4.54 ± 0.34	4.60 ± 0.33	0.268	4.53 ± 0.27	0.988	0.457	
	10min	6	4.45 ± 0.21	4.28 ± 0.23	**0.040**	4.35 ± 0.24	0.373		
BUN (mg/dL)	Total	20	14.82 ± 3.76	13.99 ± 4.32	0.457	13.16 ± 3.49	**0.044**		
	6min	14	14.95 ± 3.47	14.29 ± 4.02	0.774	13.47 ± 3.75	**0.031**	0.927	
	10min	6	14.52 ± 4.35	13.30 ± 4.88	0.193	12.43 ± 2.68	0.504		
CRE (mg/dL)	Total	20	0.68 ± 0.12	0.67 ± 0.10	0.277	0.61 ± 0.12	**0.000**		
	6min	14	0.66 ± 0.12	0.65 ± 0.09	0.852	0.57 ± 0.10	**0.000**	0.163	
	10min	6	0.75 ± 0.10	0.71 ± 0.10	0.135	0.69 ± 0.12	0.055		
UA (mg/dL)	Total	20	4.09 ± 0.83	3.96 ± 0.76	0.453	3.96 ± 0.74	0.451		
	6min	14	4.04 ± 0.94	3.91 ± 0.81	0.643	3.91 ± 0.78	0.675	0.736	
	10min	6	4.18 ± 0.44	4.05 ± 0.60	0.471	4.07 ± 0.63	0.273		
AST (U/L)	Total	20	19.15 ± 5.16	21.30 ± 7.49	**0.030**	19.85 ± 4.44	0.169		
	6min	14	18.79 ± 5.47	19.21 ± 3.03	0.237	18.57 ± 3.29	0.640	0.257	
	10min	6	20.00 ± 4.24	26.17 ± 11.48	0.071	22.83 ± 5.27	0.201		

ALT (U/L)	Total	20	15.20 ± 9.39	16.65 ± 16.02	0.390	16.25 ± 10.56	0.202	0.417
	6min	14	13.71 ± 7.54	12.14 ± 4.03	0.752	13.00 ± 3.93	0.475	
	10min	6	18.67 ± 12.00	27.17 ± 25.69	0.124	23.83 ± 15.93	0.328	
γ-GT (U/L)	Total	20	20.15 ± 16.43	17.80 ± 10.54	0.156	17.80 ± 9.40	0.657	0.342
	6min	14	21.00 ± 18.35	16.79 ± 8.84	**0.027**	17.86 ± 10.42	0.208	
	10min	6	18.17 ± 10.43	20.17 ± 13.42	0.175	17.67 ± 6.42	0.584	
ALP (U/L)	Total	20	66.20 ± 18.21	64.00 ± 17.90	0.305	63.25 ± 17.72	0.162	0.046
	6min	14	67.21 ± 17.61	63.43 ± 17.68	0.156	61.71 ± 17.16	**0.003**	
	10min	6	63.83 ± 19.33	65.33 ± 18.35	0.171	66.83 ± 18.46	0.307	
LDH (U/L)	Total	20	226.80 ± 30.69	227.80 ± 42.18	0.863	216.80 ± 30.19	**0.014**	0.382
	6min	14	227.29 ± 30.90	232.36 ± 44.86	0.536	219.64 ± 25.49	**0.050**	
	10min	6	225.67 ± 30.17	217.17 ± 32.77	0.122	210.17 ± 38.19	0.144	
CPK (U/L)	Total	20	91.85 ± 40.47	88.40 ± 46.14	0.641	93.55 ± 31.66	0.136	0.882
	6min	14	90.64 ± 43.55	86.36 ± 44.09	0.830	90.71 ± 26.78	0.224	
	10min	6	94.67 ± 31.98	93.17 ± 50.26	0.655	100.17 ± 40.06	0.438	
CRP (mg/dL)	Total	20	0.09 ± 0.16	0.24 ± 0.22	**0.012**	0.05 ± 0.08	0.474	0.048
	6min	14	0.12 ± 0.18	0.21 ± 0.23	0.056	0.06 ± 0.09	**0.004**	
	10min	6	0.04 ± 0.05	0.29 ± 0.17	0.086	0.04 ± 0.02	0.195	
TC (mg/dL)	Total	20	210.40 ± 33.47	206.20 ± 34.71	0.293	208.55 ± 38.78	0.653	0.239
	6min	14	208.29 ± 36.58	204.50 ± 34.24	0.477	203.57 ± 42.31	0.349	
	10min	6	215.33 ± 24.05	210.17 ± 35.45	0.480	220.17 ± 25.38	0.451	
LDL-c (mg/dL)	Total	20	121.50 ± 25.19	121.10 ± 24.46	0.872	119.90 ± 27.24	0.729	0.398
	6min	14	119.57 ± 28.32	119.57 ± 24.23	0.644	116.21 ± 29.97	0.515	
	10min	6	126.00 ± 14.67	124.67 ± 24.61	0.731	128.50 ± 16.52	0.590	
HDL-c (mg/dL)	Total	20	70.60 ± 13.97	67.90 ± 11.68	0.129	73.50 ± 16.79	0.152	0.746
	6min	14	69.43 ± 15.35	67.93 ± 12.32	0.658	72.57 ± 18.19	0.238	
	10min	6	73.33 ± 9.46	67.83 ± 10.02	0.025	75.67 ± 12.70	0.426	
Bil (mg/dL)	Total	20	0.73 ± 0.24	0.67 ± 0.32	0.239	0.83 ± 0.42	0.113	0.125
	6min	14	0.71 ± 0.23	0.71 ± 0.33	0.939	0.86 ± 0.45	0.071	
	10min	6	0.77 ± 0.27	0.58 ± 0.25	0.069	0.77 ± 0.35	0.783	
Na (mEq/L)	Total	20	140.15 ± 1.85	138.70 ± 1.71	**0.000**	139.00 ± 1.58	**0.001**	0.095
	6min	14	139.93 ± 2.09	138.93 ± 1.91	**0.021**	139.14 ± 1.68	**0.016**	
	10min	6	140.67 ± 0.94	138.17 ± 0.90	**0.000**	138.67 ± 1.25	**0.018**	
K (mEq/L)	Total	20	4.72 ± 0.40	5.10 ± 0.59	**0.003**	5.23 ± 0.47	**0.001**	0.159
	6min	14	4.79 ± 0.36	5.10 ± 0.67	0.056	5.16 ± 0.42	**0.008**	
	10min	6	4.55 ± 0.43	5.10 ± 0.33	**0.020**	5.38 ± 0.54	**0.029**	
Cl (mEq/L)	Total	20	101.50 ± 2.04	100.50 ± 2.06	**0.002**	102.05 ± 2.01	0.187	0.164
	6min	14	101.21 ± 2.34	100.43 ± 2.26	**0.044**	102.14 ± 1.99	0.071	
	10min	6	102.17 ± 0.69	100.67 ± 1.49	**0.017**	101.83 ± 2.03	0.655	
Ca (mg/dL)	Total	20	9.40 ± 0.35	9.40 ± 0.40	0.953	9.28 ± 0.22	0.090	0.437
	6min	14	9.42 ± 0.32	9.44 ± 0.38	0.768	9.27 ± 0.21	0.101	
	10min	6	9.35 ± 0.42	9.28 ± 0.43	0.302	9.30 ± 0.24	0.681	
Fe (μg/dL)	Total	20	83.05 ± 32.30	77.55 ± 37.81	0.873	92.25 ± 42.76	0.139	0.350
	6min	14	79.29 ± 33.25	85.21 ± 38.44	0.290	92.79 ± 42.82	0.099	
	10min	6	91.83 ± 28.08	59.67 ± 29.34	0.085	91.00 ± 42.59	0.964	

See text for abbreviations.

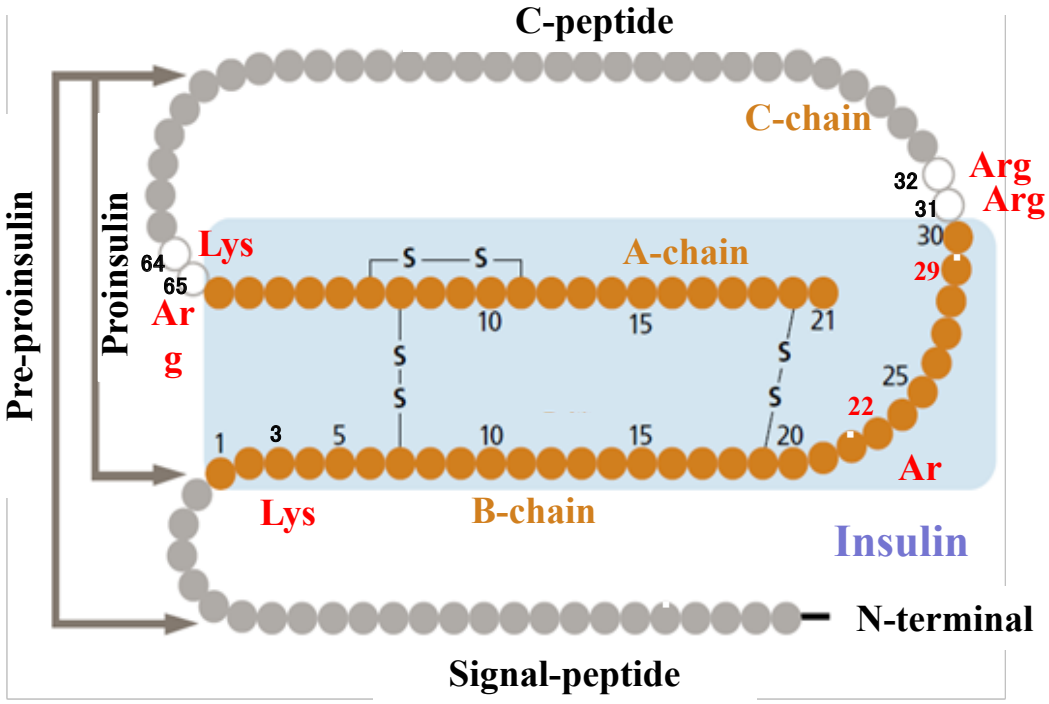

Fig. 2. **The structure of insulin, proinsulin and pre-proinsulin.**
The figure quoted from Reference 5).

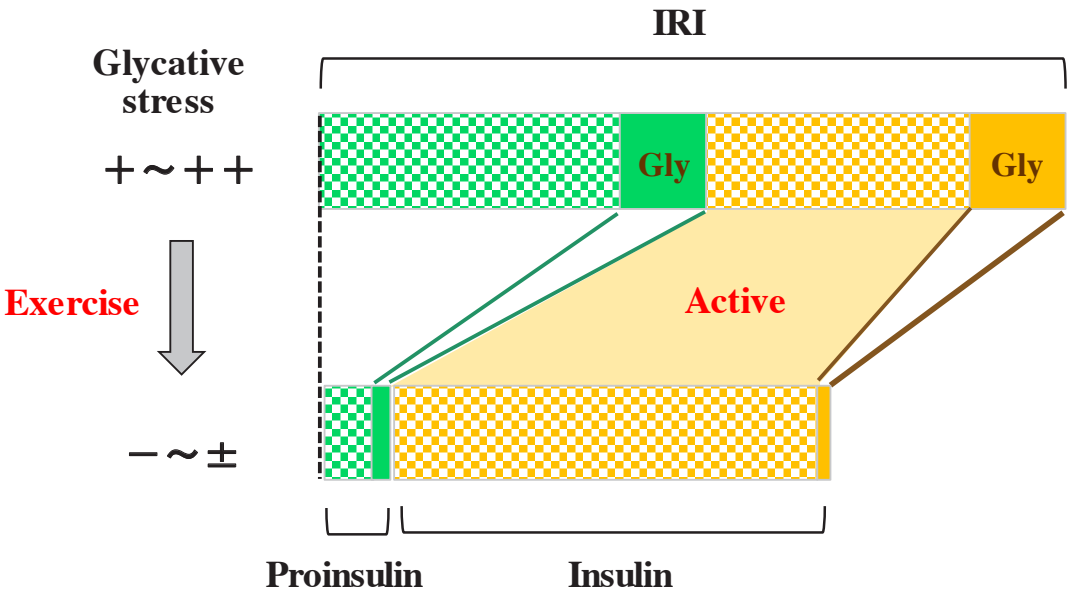

Fig. 3. **IRI compositional changes by exercise (speculated schematic).**
If physical exercise decreases glycative stress, expectedly insulin action would increase despite the decrease in IRI. IRI, immune reactive insulin; Gly, glycated proinsulin or insulin.

Reduction of glycative stress

In this study, pentosidine was significantly reduced in the 6M and 10M groups. Regarding the amount of fluorescent AGEs, the mean age of the subjects was 45 years, and the mean SAF values for men and women of this age ranged from 2.0 to 2.2 when checked against population data [10, 11], and the subjects' previous values (2.08 ± 0.35) were within this range. The subjects' glycation stress levels were average, or at least not pathological, and there was little room for further improvement. Nevertheless, a significant reduction was observed in the 6M group (−5.3% change). This physical exercise may be effective in reducing glycative stress, even in apparently healthy subjects.

Mechanism of insulin resistance improved by physical exercise

Since approximately 70% of glucose is consumed in skeletal muscle, glucose metabolism in skeletal muscle greatly influences whether insulin acts efficiently. When insulin binds to insulin receptors on the surface of skeletal muscle cells, glucose transportor 4 (GLUT4) moves from cytoplasma to the cell surface (translocation) and glucose enters the cell via GLUT4 [12]. Whereas, contractile movements of skeletal muscle (myosin and actin sliding) stimulate GLUT4 gene expression, resulting in increased GLUT4 production and high intracytoplasmic storage [12, 13]. Thus, insulin-stimulated GLUT4 translocation increases quantitatively and the amount of GLUT4 on the cell surface increases. This results in increased efficiency of insulin action and glucose uptake. In this study, muscle contraction exercise may have increased GLUT4 production in the muscle cells.

Increased DHEA secretion

The mean age of the subjects was 45 years, and the mean DHEA-s values for men of this age, when checked against Japanese population data [14, 15], were 175 μg/dL for men and 160 μg/dL for women, and the subjects' previous values (men: 142.20 ± 80.26 μg/dL, women: 129.00 ± 62.18 μg/dL) were lower than these values. The subjects' DHEA-s values were approximately 20% lower than the average value for the age-matched group. The major cause is thought to be inadequate physical activity and inactivity. In such a population, a significant increase was observed in the 6M group (+20.6% change), which can be interpreted as a recovery to the average level in 8 weeks.

DHEA is biosynthesized in DHEA-producing cells in the glomerular zone of the adrenal cortex. The accumulation of waste products (lipofuscin) in the cytoplasm is a factor in the decline of DHEA production with aging [16]. Lipofuscin are denatured proteins and lipids formed by oxidation and glycation, and the presence of AGEs has also been confirmed. Since physical exercise reduced AGEs, namely glycative stress, in this study, it is possible that the amount of DHEA production and secretion increased as a result of reduced ER stress in DHEA-producing cells as well.

Changes in testosterone

The test results showed no significant changes in total testosterone levels; however, its composition may have changed. What was measured in this study was total testosterone, which is composed of free testosterone, albumin-bound testosterone, and SHBG (sex hormone-binding globulin)-bound testosterone. Of these, free testosterone and albumin-bound testosterone have androgenic activity (***Fig. 4***) [17]. SHBG is a glycoprotein synthesized mainly in the liver and secreted into the blood, where it functions as a sex hormone-binding transporter. Recently, SHBG has been found to enhance lipolysis in adipocytes and shrink adipocytes, attracting attention from the perspective of lipid metabolism. SHBG is decreased in obesity, diabetes, and metabolic syndrome (MS). In particular, SHBG decreases when insulin resistance is increased [18, 19] and increases when insulin sensitivity is restored by diet and exercise therapy. These reactions are common in both men and women.

In the present study, physical exercise improved insulin resistance, which would be expected to increase SHBG. Of note, total testosterone behavior differs between men and women. In men, total estestosterone and SHBG are negatively correlated, while in women they are positively correlated [20]. Higher total testosterone correlates with lower MS incidence in men and higher MS incidence in women.

In women, increased androgen activity leads to an increased risk of developing polycystic ovarian syndrome (PCOS) and decreased fertility [21, 22]. In the results of the female analysis, there is no change in total estestosterone, so an increase in SHBG would result in a decrease in active testosterone. Decreased androgen activity acts in the direction of decreased PCOS and MS risk. Increasing physical activity is presumed to have a positive effect on women's health because it increases SHBG production and leads to the regulation of androgenic activity.

Study limitations

In this measurement, serum insulin levels were measured as IRI, so both insulin and proinsulin were detected, and moreover, glycated insulin and glycated proinsulin cannot be excluded. In order to analyze blood insulin dynamics in terms of glycative stress, it is necessary to analyze glycated and non-glycated insulin/proinsulin. The test results showed no change in total testosterone, but androgenic activity may have changed. To evaluate androgenic activity, free testosterone and SHBG should be measured concurrently. This is a subject for future study.

Safety

Exercise using this test product was mildly loaded, no adverse events were observed, and the safety of the product was judged to pose no major problems.

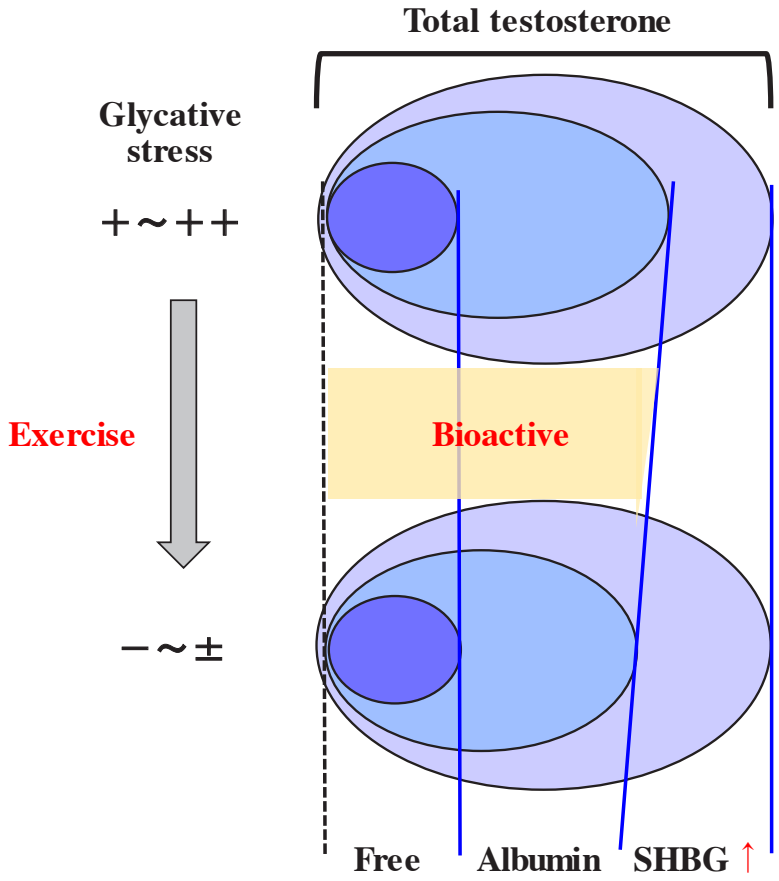

Fig. 4. **Total testosterone compositional changes by exercise (speculated schematic in female).**
If physical exercise decreases glycative stress, expectedly SHBG would increase and bioactive testosterone may decrease in the female. SHBG, sex hormone-binding globulin.

Conclusion

Exercise with this test product improved glucose tolerance, insulin resistance, and AGEs (pentosidine and SAF), and also increased DHEA-s, which is important for maintaining youth and health. It should be noted that these parameters further improved from normal ranges. The results suggest that this exercise using ultra-soft rubber bands, which can be performed in a short period of time, is a safe and effective health promotion method.

Declaration of Conflict of Interest

In conducting this study, we received research support from Kyowa Rubber Co.

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