

## Original article

## High carotenoid values in people with flexibility training

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## Abstract

**Background:** Free radicals play essential roles in aging and various pathological processes, and antioxidants may help prevent these conditions. Therefore, the body should have high antioxidant levels and carotenoids can be a representative of antioxidants.

**Objectives:** This study aimed to use the BioPhotonic Scanner S3 and resonant Raman spectroscopy technology for comparing the skin carotenoid values between those who exercise frequently and those who did not within 6-months.

**Methods:** Four equal groups of 600 men and women volunteers, aged 20 - 60 years, who resided in Bangkok Metropolitan Region were formed, with 150 subjects in each group: cardiorespiratory training, resistance training, flexibility training, and nonexercising.

**Results:** The flexibility training group had the highest carotenoid values than the other groups ( $36,847 \pm 1,053$  vs.  $32,413 \pm 535$  units, respectively). Women had higher carotenoid values than men ( $36,182 \pm 773$  vs.  $29,245 \pm 694$  units). People who are obese (body mass index  $25 \text{ kg/m}^2$ ) showed lower carotenoid values than the others ( $28,519 \pm 844$  vs.  $32,413 \pm 535$  units) Finally, advancing age was positively correlated with the carotenoid values ( $r = 0.257, P < 0.001$ ). Furthermore, all four factors showed significant correlations with the carotenoid levels in a multivariate analysis.

**Conclusion:** In this study, flexibility training was associated with the highest carotenoid, followed by cardiorespiratory training, resistance training and nonexercising, respectively. Furthermore, high carotenoid levels were related to adolescence, female gender, and lower body mass index.

**Keywords:** Antioxidants, cardiorespiratory training, carotenoid, flexibility training, free radical, resistance training.

Antioxidants can inhibit or slow down oxidation reactions, which generate free radicals.<sup>(1)</sup> Instability electron from free radicals will pull electrons from biological molecules leading to cellular damage.<sup>(2)</sup> The body can make enzymes to eliminate free radicals, such as superoxide dismutase, catalase and glutathione peroxidase; however, they are not enough to prevent cellular injury. Moreover, Lobo V, *et al.*<sup>(3)</sup> found that when people get older, the levels of antioxidants in the body are reduced, whereas the generation of free radicals remains the same. These ambulations are plausible pathobiochemical explanation for the development and progression of

many diseases.<sup>(4)</sup>

Factors that increase free radicals include air pollution, ozone, food containing unsaturated fatty acids, sunlight, heat, and gamma radiation.<sup>(4)</sup> Viroonudomphol D, *et al.*<sup>(5)</sup> found that amino acids from proteins can increase free radical levels and inflammation in arterial walls. In addition, moderate to intense exercise can increase the rate of oxygen consumption, particularly in skeletal muscles generating free radicals during oxidative phosphorylation in the mitochondria and inducing the release of NADPH oxidases (NOX enzymes) contributing to ROS production.<sup>(6)</sup> Moreover, Kawamura T and Muraoka I.<sup>(7)</sup> found that abrupt exercises can increase ROS production, resulting in biomolecular oxidative stress. In addition, regular exercise appears to increase the resistance against oxidative stress.<sup>(8)</sup>

Carotenoids are naturally occurring pigments and a type of antioxidant. They can be found in various plants, such as papaya, mango, grapefruit<sup>(9)</sup>,

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potatoes, and chili<sup>(10)</sup>, and marine animals.<sup>(11)</sup> Moreover, Nieman DC, *et al.*<sup>(12)</sup> found that consumption of a tomato-based supplements containing carotenoids (lycopene, phytoene, phytofluene, and others) for 4 weeks could reduce muscle damage during exercise. Moreover, Weber D, *et al.*<sup>(13)</sup> found a positive relationship between carotenoids and age.

Recent studies unveiled that exercise may affect the body's ability to produce more antioxidants. Konongbua P, *et al.*<sup>(14)</sup> reported that a 10 weeks aerobic exercise program increased the level of antioxidants in the blood, consistent with the finding of Kokong H.<sup>(15)</sup> showing the effects of regular sports activities for 8 weeks on increasing plasma antioxidant levels.

Carotenoids can be measured in a various ways, such as taking a blood sample and retinal or skin measurements. However, blood testing is costly and invasive. Currently, the BioPhotonic Scanner, which uses resonance Raman spectroscopy (RRS) technology can measure skin carotenoids levels. Conrady CD, *et al.*<sup>(16)</sup> found a correlation between skin carotenoid levels in lymph RRS and zeaxanthin concentrations in the lymphatic fluid. In addition, Zidichouski JA, *et al.*<sup>(17)</sup> reported that RRS accurately measured carotenoids in human skin with less intraindividual variability than lymphatic carotenoid measurements by high performance liquid chromatography. Janse van Rensburg A, *et al.*<sup>(18)</sup> also proved the accuracy and reliability of three portable RRS instruments. Scarmo S, *et al.*<sup>(19)</sup> observed a positive correlation of carotenoids with fruit/vegetable consumption which was lower in young children than in adults. Ermakov IV, *et al.*<sup>(20)</sup> used a portable RRS instruments to measure the carotenoids levels in the stratum corneum of hands of 1,375 subjects in a field study showing that carotenoid levels were a good indicator of antioxidant levels. Rerksupphaphol S. and Rerksupphaphol L.<sup>(21)</sup> used RRS to demonstrate that carotene levels were directly related to fruit and vegetable intake. Smidt CR, *et al.*<sup>(22)</sup> and Joseph JC, *et al.*<sup>(23)</sup> concluded that the BioPhotonic Scanner was a good alternative to measuring carotenoid levels in the blood with only 10.0% deviation and 95.0% reliability. Chattanachotikul W, *et al.*<sup>(24)</sup> confirmed that skin carotenoid determinations with BioPhotonic Scanner S3 strongly correlated with the concentrations measured by liquid chromatography tandem mass spectroscopy in Thai volunteers. Furthermore, the subcutaneous fat thickness and skin color did not effect on the measurements.

Exercise can be divided into three types: 1) cardiorespiratory training with repetitive and rhythmic movements of large muscles to increase cardiovascular efficiency and prevent cardiovascular diseases; 2) resistance training for the strength and endurance of muscles to increase the efficiency of energy use and muscle mass; and 3) flexibility training with stretching of muscles, tendons, and joints to enhance flexibility. This type improves flexibility and reduces the risk of muscle and joint injury.

So far, no studies have reported the relationship between different types of physical exercise and antioxidant (carotenoid) values. Thus, this study aimed to investigate the exercise type that maximizes the antioxidant (carotenoid) values as determined by a BioPhotonic scanner, which does not require blood from healthy Thai volunteers.

## Materials and methods

The target population was from the general population aged 20 - 60 years, male and female, in Bangkok Metropolitan Region. Volunteers were selected from people who were exercising in parks and fitness centers in Bangkok Metropolitan Region. The places were selected using purposive sampling of four areas, namely Benjakitti Park, Suan Luang Rama IX Park, FBT Fitness Center Ramkhamhaeng branch, and FBT Fitness Center Lad Krabang branch. For nonexercizing individuals, volunteers were recruited from those visiting shopping malls. The Chamchuri Square Department Store was selected using purposive sampling.

A total of 600 subjects were selected and divided into four equal groups (150 each) as follows: 1) cardiorespiratory training to increase the strength of the cardiovascular system regularly at least 150 min per week) for at least 6 months, for example, running, swimming, cycling, and aerobic dancing; 2) resistance training to increase muscle endurance by the contraction of muscles against an external resistance regularly at least 90 min per week for at least 6 months. For example, weight training, free weights, and elastic band training; 3) flexibility training by low-intensity exercises to increase muscle flexibility and increase the total range of joints motion regularly (at least 70 min per week) at least 6 months, for example, yoga, stretching, pilates, and tai chi; and 4) nonexercizing at least 6 months.

Those who did not qualify for any exercise group, and took carotenoid supplements, drugs or stimulants, or vitamin supplements that accelerate antioxidants or substances related to measurement in research were excluded. Those who were not getting enough rest (sleeping  $\leq 8$  h in the previous night) or exercised for  $\leq 6$  months were also excluded.

This cross-sectional observational study compared the antioxidant values between the exercising and nonexercising groups using RRS technology. A questionnaire survey was performed, and skin carotenoid values were obtained using BioPhotonic Scanner S3. This method was found to be highly correlated with the blood carotenoid levels, with a correlation coefficient of 0.987.<sup>(24)</sup>

This research proposal was reviewed by the Ethics Review Committee for Research involving Human Research Subjects of Chulalongkorn University for approval (Certificate of approval no. 035/65).

### Statistical analysis

Continuous data were presented as the mean and standard error of the mean (standard error, SE). The carotenoid values of the four exercise groups, gender, age range, and body mass index (BMI) were analyzed. Post-hoc analysis was performed for intergroup differences. Multivariate analysis was also performed to study the relationship between carotenoid values and factors such as types of exercise, gender, age group, and (BMI) range. A  $P < 0.05$  was considered statistically significant.

## Results

### Baseline characteristics

The total number of the sample was 600 including 326 men, 54.3% and 274 women, 45.7%. The mean age was 43 ( $\pm 12.2$ ) years. The mean BMI was 23.4

( $\pm 3.5$ ) kg/m<sup>2</sup>. The 450 exercising subjects were selected from those exercising in four places, namely Benjakitti Park, Suan Luang Rama IX Park, FBT Fitness Center Ramkhamhaeng branch, and FBT Fitness Center Lad Krabang branch. The ages, gender, and BMI for each exercising types are shown in **Table 1**. The cardiorespiratory and resistant exercising groups were male predominantly; however, there were more female in the flexibility training (63.3%) and nonexercising categories groups (66.7%). The flexibility training group was older and had lower BMI levels than the other groups.

### Mean carotenoids values of classified by exercise types

The mean carotenoid values were different between the four exercising groups ( $P < 0.001$ ). In the post-hoc analysis, the carotenoid score in the flexibility training group was significantly higher than those in cardiorespiratory training ( $P = 0.003$ ), resistance training ( $P < 0.001$ ) and nonexercising group ( $P < 0.001$ ).

In the comparisons of the skin carotenoid scores in the cardiorespiratory training, resistance training, flexibility training and nonexercising groups, the mean value of all samples was  $32,413 \pm 535$  units. Flexibility training showed the highest carotenoid value of  $36,847 \pm 1,053$  units, and the mean carotenoid value in the resistance training group was the lowest, with  $30,173 \pm 991$  units, as shown in **Figure 1A**.

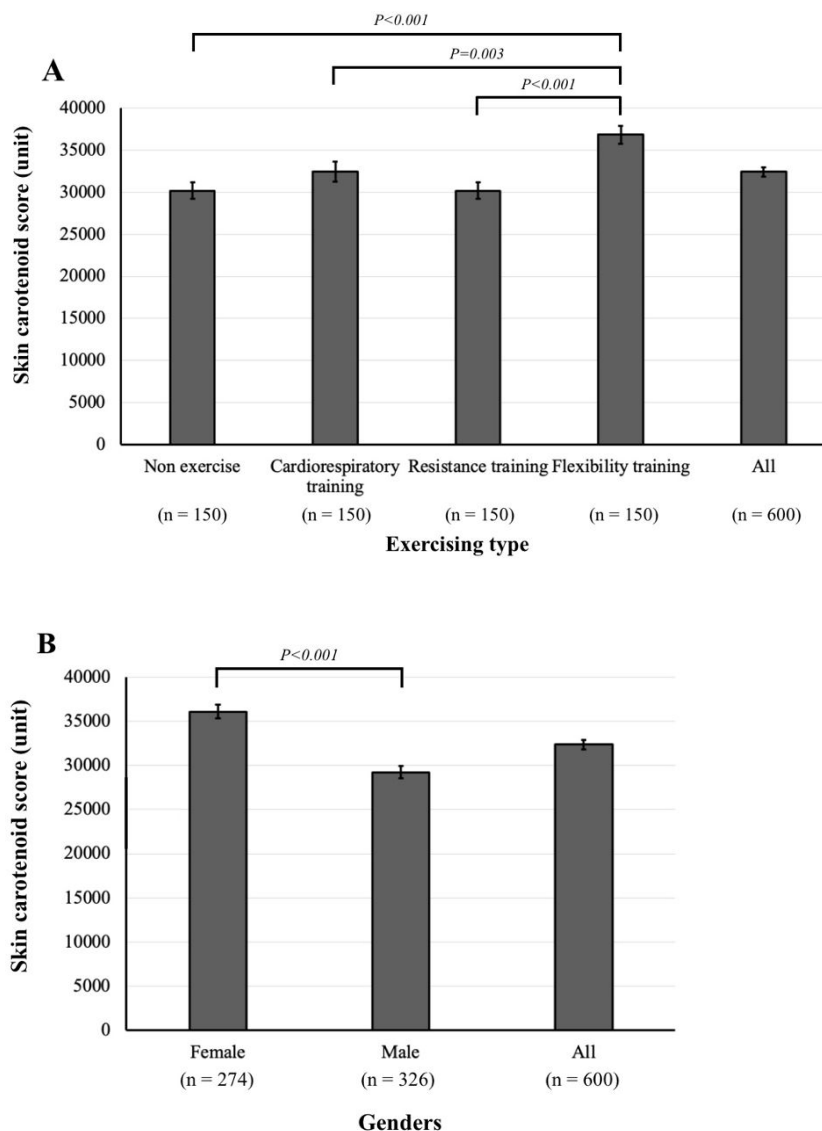
### Mean carotenoid value classified by gender

The carotenoid levels were then classified by gender. Male subjects (54.3%) had the mean carotenoids of to  $29,245 \pm 694$  units, and female subjects (45.7%)  $36,182 \pm 773$  units. Female subjects showed significantly higher skin carotenoid scores than their male peers ( $P < 0.001$ ) (**Figure 1B**).

**Table 1.** Age, gender, and body mass indices divided by the exercising types and non-exercising group.

Exercising types	Gender		AGE*	Body mass index*
	Male (%)	Female (%)		
Cardiorespiratory	70.7	29.3	$41.4 \pm 12.7$	$23.2 \pm 3.3$
Resistance	76.7	23.3	$41.1 \pm 13.3$	$23.9 \pm 3.2$
Flexibility	36.7	63.3	$45.2 \pm 15.9$	$23.0 \pm 3.5$
Non-exercising	33.3	66.7	$41.1 \pm 13.7$	$23.6 \pm 4.1$
<b>Total</b>	<b>54.3</b>	<b>45.7</b>	<b><math>43.0 \pm 12.2</math></b>	<b><math>23.4 \pm 3.5</math></b>

The data are expressed as means  $\pm$  standard deviations.



**Figure 1.** The skin carotenoid score of the total subjects (A) and was classified by gender (B). The data are expressed as means  $\pm$  standard errors of mean.

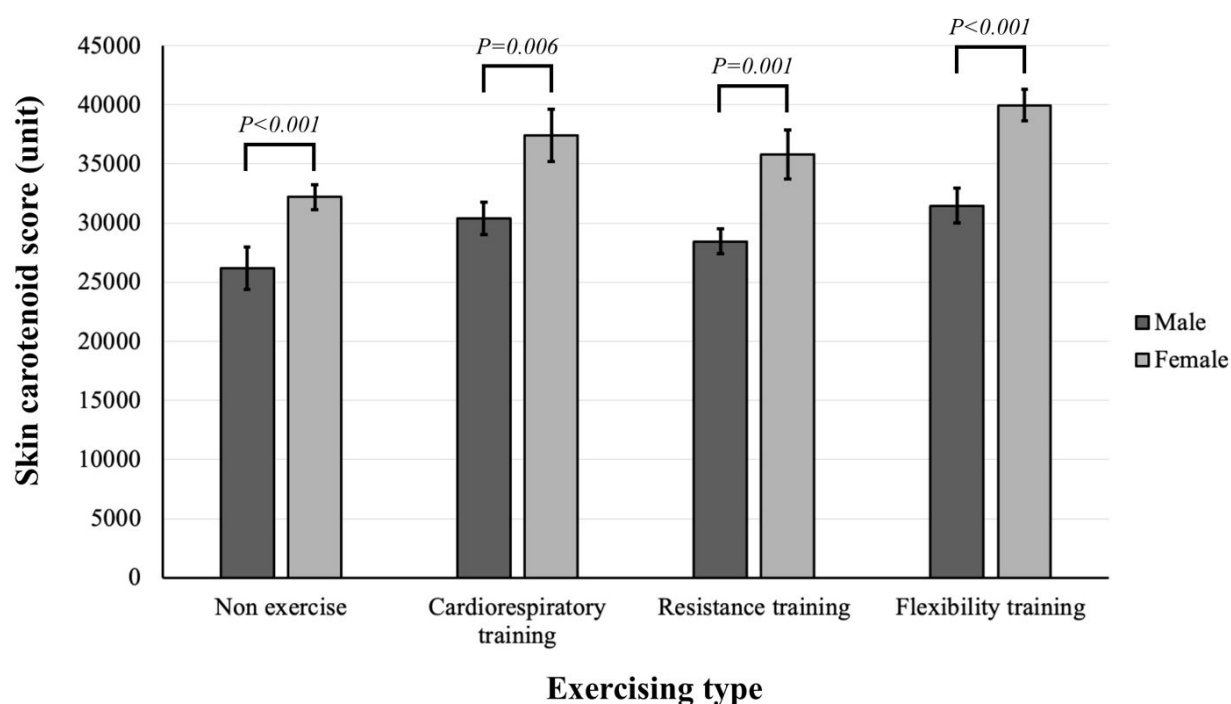
#### **Mean carotenoid values of male and female subjects classified by exercise type**

The carotenoid levels were then classified by exercise types. Male subjects showed significantly lower carotenoid values than female subjects in all exercise groups, including the nonexercizing group ( $P < 0.001$ ). Male subjects who did not exercise showed the lowest mean carotenoid value of  $26,200 \pm 1,802$  units (Figure 2). Female subjects in the flexibility training group presented the highest mean carotenoid value of  $39,958 \pm 1,333$  units. Female subjects who did flexibility training group showed the highest in the carotenoid values compared with those of subjects (Figure 2). Moreover, female subjects in the nonexercizing group ( $32,190 \pm 1,062$

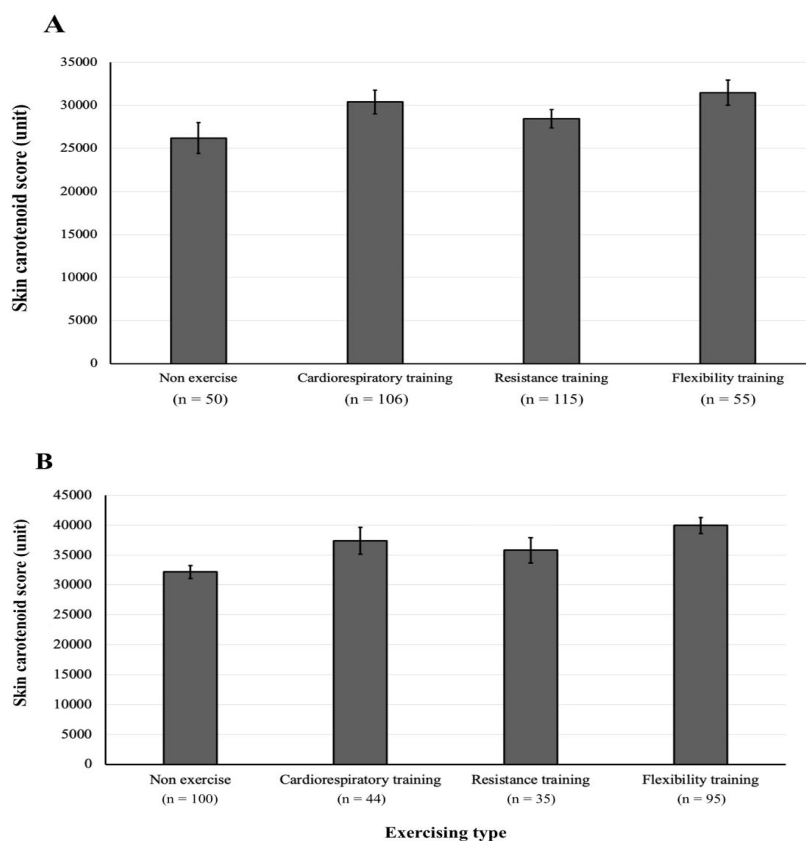
units) had nearly similar carotenoid levels as male subjects in the flexible training group ( $31,473 \pm 1,470$  units).

#### **Mean carotenoid values of male subjects classified by exercise type**

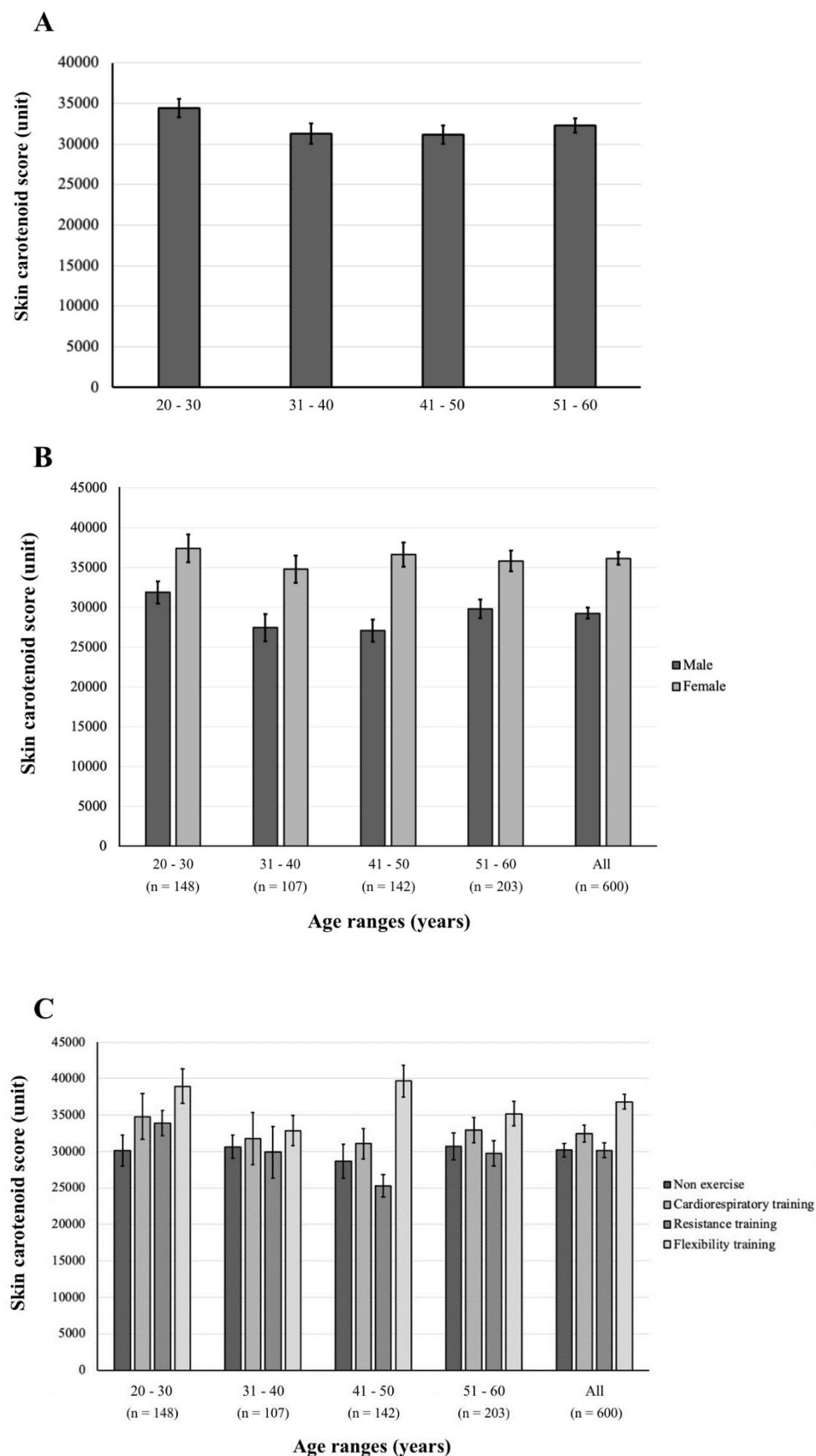
In the comparisons of skin carotenoid scores in 326 male subjects, the flexibility training group showed the highest carotenoid level of  $31,473 \pm 1,470$  units, followed by the cardiorespiratory training ( $30,377 \pm 1,354$  units) and resistance training ( $28,461 \pm 1,079$  units) groups. Male subjects in the nonexercizing group showed the lowest carotenoid level of  $26,200 \pm 1,802$  units Figure 3A.



**Figure 2.** The means of carotenoid values of male and female subjects classified by type of exercise. The data were presented as means  $\pm$  standard errors of mean.



**Figure 3.** The means of carotenoid values of male subjects (A) and female (B) classified by type of exercise. The data were presented as means  $\pm$  standard errors of mean.



**Figure 4.** The mean skin carotenoid scores divided by age ranges (A) and by gender (B) and by exercising types (C) The data are presented as means  $\pm$  standard errors of mean.

### **Mean values of carotenoids of female subjects classified by type of exercise**

In the comparisons of skin carotenoid scores in 274 female subjects, the flexibility training group showed the highest carotenoid level of  $39,958 \pm 1,333$  units, followed by the cardiorespiratory training ( $37,409 \pm 2,232$  units) and resistance training ( $35,800 \pm 2,101$  units) groups. Female subjects in the nonexercizing group showed the lowest carotenoid level of  $32,190 \pm 1,062$  units (**Figure 3B**).

### **Carotenoid values classified by age range**

Carotenoid levels showed slight variations. The Carotenoid values for the group aged 20 - 30 (24.7%), 31 - 40 (17.8%), 41 - 50 (23.7%), and 51 - 60 (33.8%) are shown in Figure 4. The group aged 41 - 50 year showed the lowest mean carotenoids values ( $31,162 \pm 1,098$  units) whereas the group aged 20 - 30 year had the highest mean carotenoids values ( $34,507 \pm 1129$  units) (**Figure 4A**).

The analysis of carotenoid values showed difference in at least one age pair ( $P < 0.001$ ). The post-hoc test revealed differences in mean carotenoids in all age groups ( $P < 0.05$ ), except for the group aged 20 - 30 years and 41 - 50 years and between the group aged 31 - 40 and 41 - 50 years.

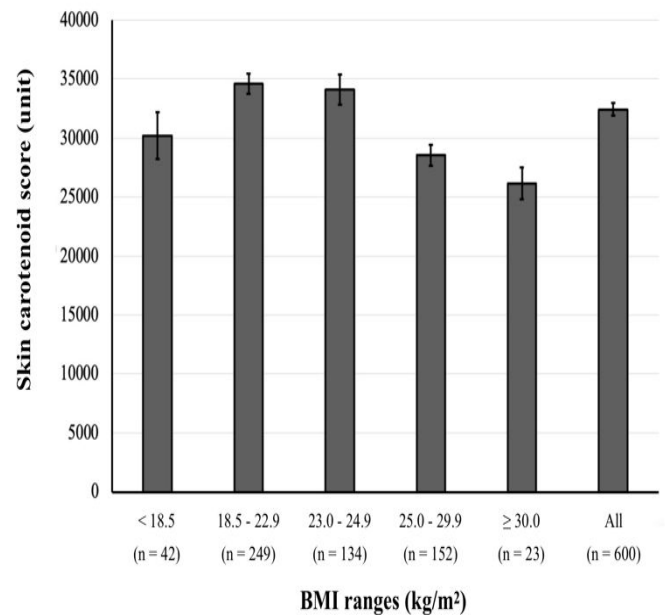
### **Mean carotenoids values of male and female subjects classified by age group**

Comparisons of carotenoid levels between the two genders showed that female subjects had significantly higher skin carotenoid levels in all age groups including "all" categories. The highest carotenoid score in female subjects aged 20 - 30 years was to  $37,380 \pm 1744$  units. Male subjects age 41 - 50 years showed the lowest carotenoid values equal to  $27,040 \pm 1395$  units (**Figure 4B**).

The analysis of carotenoid values in male and female subjects showed difference in at least one age pair ( $P < 0.001$ ) and ( $P < 0.001$ ), respectively.

### **Mean carotenoid values of all exercise types including classified by age group**

Comparisons of skin carotenoid scores in various age groups presented that all exercise groups tended to have higher skin carotenoid scores than the nonexercizing group, with flexibility training showing the highest scores across age groups, and the highest carotenoid levels were recorded in the group aged 41 - 50 years ( $39,676 \pm 2160$  units) (**Figure 4C**).



**Figure 5.** The carotenoid values divided by body mass index (BMI) ranges. The data are presented as means  $\pm$  standard errors of mean.

Individuals aged 41 - 50 years who did resistance training had the lowest carotenoid levels compared with the other exercise types and age groups ( $25,297 \pm 1,584$  units). Accordingly, individuals who did resistance training across all age groups had the lowest skin carotenoid score compared with the other exercise types ( $30,173 \pm 991$ ) units. The all-age group provided a summary of the scores across all age groups, indicating that flexibility training had the highest overall mean score of  $36,847 \pm 1,053$  units.

### **Mean carotenoid values classified by BMI**

The analysis of mean carotenoid values of the four BMI ranges revealed a difference in at least one pair ( $P < 0.001$ ). The post-hoc test revealed that the carotenoid values of BMI ranges of 18.5 - 22.9 vs. 25.0 - 29.9, 18.5 - 22.9 vs. 30, 23.0 - 24.9 vs. 25.0 - 29.9, and 23.0 - 24.9 vs.  $> 30 \text{ kg/m}^2$  were significantly different.

The carotenoid values of carotenoids were divided into five BMI groups:  $< 18.5$  (7.0%, 42), 18.5 - 22.9 (41.5%, 249), 23.0 - 24.9 (22.5%, 134), 25.0 - 29.9 (26.7%, 152), and more than  $30 \text{ kg/m}^2$  (3.7%, 23). The BMI range of 18.5 - 22.9  $\text{kg/m}^2$  had the highest mean carotenoid values ( $34,594 \pm 845$  units) whereas BMI of  $30 \text{ kg/m}^2$  had the lowest mean carotenoid values ( $26,125 \pm 1,377$  units) (**Figure 5**).

**Table 2.** Multivariate regression coefficients of factor that correlated with carotenoids.

	Unstandardized	Coefficients	Standardized	P - value
	B	SE	Beta	
Constant	22336.311	4309.881		<0.001
Exercising types	1317.077	442.421	0.257	0.003
Gender	5821.853	1004.718	0.257	<0.001
Age range	254.290	37.440	0.257	<0.001
BMI range	-536.294	142.171	-0.144	<0.001

### Multivariate analysis

A multivariate regression analysis found that each of the four factors, namely age ( $r = 0.257$ ,  $P < 0.001$ ), gender ( $r = 0.221$ ,  $P < 0.001$ ), BMI ( $r = -0.144$ ,  $P < 0.001$ ), and exercise types ( $r = 0.112$ ,  $P < 0.005$ ), was independently related to the skin carotenoid scores (Table 2).

### Discussion

In this study, subjects who did flexibility training regularly, female subjects, older subjects, and the subjects with BMI between 18.5 and 25.0 kg/m<sup>2</sup> were associated with carotenoid values higher than those of the average. The high antioxidant values after flexibility training might be related to the reduction of muscle stiffness because the exercise was not strenuous, leading to likely produce fewer free radicals from cellular respiration than the other exercise groups<sup>(25)</sup> and might have high antioxidant values, according to Wang F, *et al.* that acute exercises can contribute to excessive ROS production, which causes an imbalance in the oxidation-antioxidant homeostasis in cells.<sup>(26)</sup> Flexibility training group had higher carotenoid values than the non-exercise group. This may be because the generation of antioxidants during flexibility exercise outweigh the free radicals produced in the cellular respiration process.<sup>(7)</sup> It is possible that non-exercising people are unable to produce antioxidants as much as exercising people; however, the body still generate free radicals.<sup>(27, 28)</sup>

Female subjects had higher carotenoid values than male volunteers. The possible explanation is that women usually prefer flexibility training more than

men, whereas men prefer cardiorespiratory and resistance training. In addition, cardiorespiratory and resistance training in women may not be as heavy as those in men, resulting in less free radical production in woman.

The group with a BMI of 18.5 - 25.0 kg/m<sup>2</sup> had higher antioxidant (carotenoid) values than the other groups. This may be due to the appropriate amounts of fats in this group. Because adipose tissue is a source of free radicals in the body<sup>(29)</sup>, excessive amounts of fats can cause an imbalance between free radicals and antioxidants. Moreover, She C, *et al.*<sup>(30)</sup> revealed a significant relationship among age, gender, BMI, smoking, and physical activity with serum carotenoids.

This our study supports the role of exercise in increasing antioxidant values. However, vigorous exercises may generate free radicals from cellular respiration and consumption of carotenoid antioxidants. Additional flexibility exercise, such as yoga or other stretching exercises, is encouraged to increase antioxidant levels. In addition, the body fat levels should be kept in balance, because the adipose tissue may accumulate free radicals that can deplete antioxidants (carotenoids). In the future, prospective randomized studies are needed to determine whether flexibility training can increase the values, of carotenoids and other antioxidants.

The strength of this study was related to its a large sample size to achieve an acceptable level of credibility from the target population in Bangkok Metropolitan Region. In addition, this study focused on a Thai general population in the community. However, this study was limited by the cross-sectional analysis of carotenoid values correlating with other factors of interest. Therefore, the results cannot confirm cause-and-effect relationships.



## Conclusion

This study examined the relationship between different exercise types, and various related factors (age, gender, and BMI), with antioxidant (carotenoid) values. Flexibility training was associated with the highest carotenoid values, followed by cardiorespiratory training, resistance training and nonexercizing. Furthermore, high carotenoid levels were associated with female gender, adolescence, and a BMI of 18.5 - 24.9 kg/m<sup>2</sup>. Future prospective studies are warranted to investigate effects of exercise types to prove that these lifestyle modifications may influence antioxidants in the body.

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## Conflicts of interest statement

All authors have completed and submitted the International Committee of Medical Journal Editors Uniform Disclosure Form for Potential Conflicts of Interest. None of the authors disclose any conflict of interest.

## Data sharing statement

All data generated or analyzed in this study are included in this published article and the citations herein. Further details, opinions, and interpretation are available from the corresponding author on reasonable request.

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