ポーツ・健康づくりのマネジメント理論が必要と成りうる。

一方，市場の発展と大学の教育プログラムは連動している[25]。身体活動を活用したビジネスには，スポーツビジネスがある。健康づくりのためには，科学的根拠の裏づけをもつ運動方法がある。この両者を活用して健康づくりの施策が，わが国において普及定着するためのマネジメントの理論が見いだせるのではと考えられる。近年，アメリカではこの実績として，プロスポーツ界におけるビジネスとスポーツマネジメント論が普及発展した。

ブランディングの進化の本質「消費者と経営者」の考察によれば，ブランドの進化には，6段階がある[26]。これらの段階は，世界的に著名なブランドがつくられてきた段階だけでなく，そのブランドのマインドシェアや株価，スポーツの場合は，ファン基盤に影響を及ぼしている。

スポーツのチームとリーグを含む大部分の組織は，以下に述べるブランディングの過程における5番目の同志としてのブランドを目指している。

すなわち，第1段階は，日用品として扱われている，ブランドを必要としない商品である。この段階では，商品は代替えが可能なため，顧客は商品の違いを区別することができない。例えば，コンビニの氷がそうである。

第2段階は，ブランド紹介の段階である。ブランド名が確認に使われる。ブランドがこの段階に達すると，消費者は記憶にあるブランド商品を，ブランドネームが付いているから品質が良いと信じて購入する。

第3段階は，ブランドの個性化である。ブランドネームは消費者の感性への追訴や，商品の優位性といったマーケティングの支援によって独り立ちしていく。この段階では，商品を消費者の個性と結び付けて購買のプロセスに新たな局面をもたらすようになる。

第4段階は，消費者はブランドを所有するまでになる。地域住民がすぐにローカル企業のロゴやマスコット，あるいはキャッチフレーズに気づけば，それ以上の説明は不要となる。

第5段階は，ブランドがアイデンティティをもつようになり，消費者はそれを注意深く評価するようになる。

そして，第6段階は，ブランドが政策になるとしている。このように，ビジネスマネジメントの分野では，マーケットに商品が受け入れられるようになるまでを体系化している[27]。この理論を健康づくりやそのための施策がマーケットに受け入れられるように図るためのノウハウとしていけば，科学的に得られた結果がマーケットに定着するのに有効な手法となるのではと考えられる。

別のメジャーな事例としてNBAは，1948年夏にバスケットボール・アソシエーション・オブ・アメリカ(BAA)とナショナル・バスケットボール・リーグ（NBL）が合体した結果17チームを商品とするリーグとなった。このNBAも第1段階から積み上げて市場に普及定着した成功事例である。

スポーツマネジメント普及の要因について原田[25]は，1980年代から1990年代にかけて北米を中心として急速に進展したスポーツのビジネス化とグローバル化という社会変化と，それに伴って起きたスポーツマネジメントに対する世界的な関心の高まりという二つがあると述べている。また，スポーツビジネスの発展とスポーツマネジメントへの関心の高まりは，やがて高等教育における専門家の養成と学問的知識体系の整備に拍車をかけることになった，としている。

また，一般に科学の発展とそのパラダイムの形成には，大学の学部学科構成およびその制度を支える支持集団としての職業集団，そして研究成果を開示する学会および研究業績を蓄積する学術雑誌が不可欠である。スポーツマネジメントが発展してきた過程は，他の学科領域の発展とも類似しており，社会経済現象としてのスポーツの進化が，新しい職業領域と研究領域を生み，大学の学部学科構成やカリキュラムを変え，研究者や大学院生，そして一般人から構成される支持集団を形成し，新しい学会を必要としたとしている。しかしながら，わが国の場合，スポーツのビジネス化と産業化はようやく緒についたばかりで，スポーツマネジメントの職業集団や研究成果を共有する専門家集団の形成は，欧米に比べると遅れていると述べている。

福林[7]は，ハンドボールが盛んなノルウェーにおいてオリンピック前に，あるスーパースターがACL損傷

を起こしたことを契機に，Engebretsen，Bahr ら[28]が予防の重要性を説き，予防プログラムの開発を実施し，それを担当大臣が了解して，国家予算でスポーツ外傷研究所をノルウェー大学体育学部に併設することになったと述べている。

この事例を，スポーツ障害予防の施策上の成功例として捉え，わが国における健康づくりのための障害予防策が科学的根拠を有し，かつ，国民に還元される参考例としたい。この場合に得られた根拠を市場に還元する専門領域との協働作業が必要と考えられる。

6. 今後の研究の展開と課題

わが国の市場の健康ニーズは，ますます高まっている。健康産業の一要素であるメタボリック症候群対策の関連市場規模だけでも，1 兆 6 千 6 百億円とも推測されている[29]。

戦後の健康づくりは，公衆衛生および西洋医療の整備を主に展開されてきた。体育・スポーツ・健康づくりのための運動の分野は，医療系資格を除いて，現時点で国家資格がない。健康運動指導士など設置当初は，将来国家資格になることが期待されたものもあるが昭和 63 年に厚生大臣の認定事業として創設されて以来，未だ国家資格に至っていない。しかしながら，その養成カリキュラムは進歩しており，今後の有資格者の活躍などにより，国の資格となることが期待されている。熊谷は，リサーチ・コアプロジェクトの中で研究成果を実社会へ還元するための高度専門職としての身体運動支援士（仮称）の必要性をあげており，健康づくり・福祉分野の国家資格が目指されている[30]。なお，身体活動を介した，健康づくり養成講座のテキストなどには，疾病予防および運動療法の両面において，体育学分野の研究成果に基づくカリキュラムが有効活用されている。

科学的に認められたことが市場に定着するためにはビジネスとして成り立つことが重要となる。すなわち社会においてその市場が確立されていくことやその中で専門家が必要とされ国家資格などが設置され，雇用が創出されることによると考えられる。わが国おける，障害予防と足底挿板の関係解明の歴史は浅く，かつ運動障害と足底挿板の関係となると，未だに未開のままである。競技者の下肢に関する障害予防および高齢者の変形性膝関節症などの下肢障害対策として，足底挿板の有効活用のための証拠の構築をすすめることと，解明された証拠が市場に受け入れられるまでをスポーツマネジメント学の領域と協働し検討することが今後の課題である。

7. まとめ

本資料では，わが国における下肢障害の現状とその対策として ACL 損傷にふれたうえで，障害予防策としての足底挿板の可能性をこれまでに成された研究成果より要約した。また，科学的裏付けを伴った根拠が市場に普及定着するための方策として，スポーツ・健康マネジメントの成功事例を示した。

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Effects of Aerobic Exercise on Lipid Profiles and High Molecular Weight Adiponectin in Japanese Workers

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Abstract

Background The metabolic syndrome is characterized by the accumulation of several metabolic risk factors. It is important to improve physical activity and dietary habits to reduce the risk of cardiovascular disease in humans.

Methods The study participants participated in a weekly aerobic exercise program that included a session composed of a brief meeting, warm-up exercises, and primary exercises (low and high impact, stretch, muscle training, and cooling down). To evaluate the effect of this intervention we measured body fat composition, holding power, and quality of life assessment. Blood tests were also carried out before and every 3 months during the study.

Results Of the 37 participants enrolled in the exercise group, 31 (83.8%) completed the 12-week program. The control group consisted of 42 subjects, 36 (85.7%) of whom were available for follow-up at the end of the 12-week study period. In the exercise group, weight, body fat percentage, waist circumference, the World Health Organization quality of life 26 (WHO-QOL 26) score, triglyceride, total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol had improved significantly at the end of three months. The high molecular weight adiponectin concentration of the participants in the exercise group increased during the 9-month period of the study, although this change did not reach statistical significance compared with pre-exercise.

Conclusion Aerobic exercise led to an improvement in body composition and lipid profiles. High molecular weight adiponectin concentrations tended to improve compared with pre-aerobic exercise levels.

Key words: aerobic exercise, high molecular weight (HMW) adiponectin

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Introduction

The metabolic syndrome is characterized by the accumulation of several metabolic risk factors, such as abdominal obesity, dyslipidemia, hypertension, glucose intolerance, and prothrombotic and proinflammatory states (1-5). It is well known that patients with the metabolic syndrome have an increased risk of cardiovascular disease (6). Recently, the prevalence of patients with the metabolic syndrome has increased in Japanese workers. According to the International Diabetes Federation (IDF) and the National Cholesterol Education Program (NCEP) III criteria, the prevalence of the metabolic syndrome in Japanese workers is reported to range from 25.8% to 33.0% (7).

It is important to improve physical activity and dietary habits to reduce the risk of cardiovascular disease in humans. Several earlier studies have shown that the beneficial effects of aerobic exercise on blood pressure, cholesterol levels, and insulin sensitivity occur regardless of whether weight loss is achieved or not (8, 9). In fact, the Pawtucket Heart Study group reported there is a close relationship between physical activity and the levels of high density lipoprotein (HDL) cholesterol (10). A study in 3,000 adult Japanese men also showed that the frequency of physical activity correlated positively with HDL cholesterol levels (11).

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The aims of the present study in Japanese workers were to assess the effects of long-term aerobic exercise on lipid profiles, glucose levels, and plasma high molecular weight (HMW) adiponectin, an anti-atherosclerotic adipokine.

Materials and Methods

Participants

Participants were enrolled in the study using newspaper and radio advertisements, and pamphlets. A total of 79 subjects aged 28-76 years (21 men and 58 women) were enrolled in the intervention group.

According to the Japanese diagnostic criteria, the metabolic syndrome is defined as a waist circumference of at least 85 cm in men, or 90 cm in women, plus at least two of the following characteristics: 1) triglycerides $\geq$1.69 mmol/L (150 mg/dL) or HDL cholesterol <1.03 mmol/L (40 mg/dL), 2) systolic blood pressure $\geq$130 mmHg or diastolic blood pressure $\geq$85 mmHg, and 3) fasting plasma glucose $\geq$6.11 mmol/L (110 mg/dL) (12). The exclusion criteria in this study were a history of cardiovascular diseases, other serious illnesses, and type 1 diabetes mellitus. After the participants were asked about their history of physical and psychiatric illnesses, a physical examination was carried out on each participant.

The consent forms explained the purpose of this study, confidentiality of data, the possibility of declining to participate, and the contact numbers of the researchers. Documents containing explanations and precautions, and consent forms were distributed to the participants to obtain their written permission before the survey. They obtained and approval the written informed consent from 79 subjects in total. The study protocol was submitted to and approved by the Institutional Review Board (IRB) of the Faculty of Medicine at Saga University.

Intervention

The exercise program was undertaken 1 day each week for 3 months or more up to a maximum of 18 months. The three-month course therefore consisted of a total of 12 sessions. Each session was of 1.5 hours duration and consisted of a brief meeting (10 minutes), warm-up exercises (5 minutes), primary exercises (30-35 minutes), stretch exercises (10 minutes), muscle training (25 minutes), and cooling down (5 minutes). Participants were assigned training ranges using a pre-established heart rate equivalent to 70% to 85% of their maximum heart rate (13).

Physical assessment

Physical assessment including weight, body mass index (BMI), body fat percentage (% body fat) was performed for each participant before the aerobic exercise program and 3 months later using the TANITA TBF-305 (Tanita, Tokyo, Japan) body fat/composition monitor. Waist circumference was measured at the navel with the subject in the erect position after relaxed breathing. Duplicate measurements of holding power of the left and right hand were recorded using a grip dynamometer, and the average of the values recorded. During these measurements, the subjects were requested not to move the dynamometer.

QOL assessment

We evaluated the participants' quality of life using the World Health Organization Quality of Life scale (WHO-QOL) (14). The 26-item short form of the WHO-QOL is the brief version of the WHO-QOL 100, which was developed to assess subjects around the world, regardless of culture or local customs. It consists of 26 items classified into the following five domains: physical domain, psychological domain, social relationships, environmental domain, and general QOL. We evaluated the QOL assessment before the course and after three months of aerobic exercise.

Blood tests

The levels of triglyceride, total cholesterol, HDL cholesterol, low density lipoprotein (LDL) cholesterol, and fasting plasma glucose were measured during the study. Plasma HMW adiponectin level was measured using a sandwich ELISA kit (Fujirebio, Tokyo, Japan) that incorporated a monoclonal antibody to human HMW adiponectin, IH7 (15). A working standard of HMW adiponectin was prepared using human HMW adiponectin purified by passage through a Geratin-Cellulofine affinity column (Seikagaku Industrial Co., Tokyo Japan). The sensitivity and upper limit of the working range of HMW adiponectin levels were 0.18 to 22.05 μg/mL. The intra- and inter-assay variances were 2.4 to 3.0% and 4.2 to 5.1%, respectively.

Statistical analysis

The data were expressed as the mean ± standard error (SE). The Chi-square test and Mann-Whitney U test were used to compare the characteristics of the two groups. The Wilcoxon signed-rank test was used to compare the values before the program and after three months of aerobic exercise. A two-way repeated measures analysis of variance (ANOVA) (aerobic exercise duration × gender) was used to compare the values at 3, 6, 9, 12 and >12 months between the group performing exercises and pre-exercise. Pearson's correlation analysis and multiple regression analysis to examine the relationship between HMW adiponectin and the other factors and the Mann-Whitney U test was used to compare HMW adiponectin concentrations at 3, 6 and 9 months. The Statistical Package for the Social Sciences (SPSS, SPSS Japan Inc., Tokyo, Japan) software version 17 was used for the statistical analyses, with statistical significance being set at p values <0.05.

Results

Subjects

Thirty-seven subjects (10 men, 27 women, 73.0% women) who responded to the advertisement for participation in the present study were selected as the intervention group. We include 42 subjects as controls (11 men, 31 women, 73.8% women) who were matched to the subjects in the intervention group.

In the exercise group, 2 participants had a history of depression, 3 were dyslipidemia, and 3 had the metabolic syndrome. In the control group, 2 participants had a history of depression, 3 were dyslipidemia and 2 had the metabolic syndrome. The data were analyzed by the Chi-square test in order to confirm that the two groups were well matched and that our hypotheses were tenable (p=0.539).

Table 1. **Baseline Characteristics of Participants**

Variables	Exercise group	Control group	p
n	31	36	
Men/Women	8/23	10/26	0.539 [a]
Age (year)	45.5 ± 2.2	45.8 ± 1.2	0.934 [b]
Height (cm)	161.9 ± 1.4	160.4 ± 0.9	0.368 [b]
BMI (kg/m^2)	23.5 ± 0.7	22.4 ± 0.4	0.189 [b]
% body fat (%)	26.0 ± 1.2	26.6 ±1.1	0.708 [b]
Waist circumference (cm)	84.6 ± 2.0	80.6 ± 1.3	0.095 [b]
Weight (kg)	62.1 ± 2.2	58.5 ± 1.2	0.497 [b]

Values are expressed as means ± SE, a. using Chi-square test, b, using Mann-Whitney U test, BMI, body mass index, body fat percentage, % body fat.

Adherence to aerobic exercise

Of the 37 participants in the exercise group, 31 (83.8%) completed the 12-week program (8 men, 23 women, mean age 45.5±2.2 years, 74.2% women, mean BMI 23.5±0.7 kg/m^2), while 36 in the control group (85.7%) were available for follow-up at the end of the 12-week study period (10 men, 26 women, mean age 45.8±1.2 years; 72.2% women, mean BMI 22.4±0.4 kg/m^2) (Table 1). The attendance rate for the aerobic exercise program ranged between 43% to 84% for each period.

Effect of exercise training on outcome variable

In the exercise group, weight, % body fat, waist circumference, WHO-QOL 26 score, triglyceride, total cholesterol, HDL cholesterol and LDL cholesterol had improved significantly by the end of the third month (Table 2). Of the 12 variables measured in the aerobic exercise group, (5 body composite variables, WHO-QOL 26 score, and 6 blood test variables), 4 variables improved significantly (Fig. 1). The aerobic exercise caused significant improvements in weight (men; F=3.766, p=0.0152, women; F=3.834, p=0.0083), waist circumference (men; F=4.400, p=0.0075, women; F=2.830, p=0.0335), WHO-QOL 26 score (men; F=7.080, p=0.0005, women; F=6.120, p=0.0004), and HDL cholesterol (men; F=5.840, p=0.0017, women; F=3.732, p=0.0095).

Correlation with HMW adiponectin

As shown in Table 3, there was a significant negative correlation between HMW adiponectin and body composition (BMI; r=-0.509, p=0.037), LDL cholesterol (r=-0.578, p=0.015), and fasting plasma glucose (r=-0.559, p=0.020). In

Table 2. **The Effects of Aerobic Exercise on the Exercise Group and Control Group during 3 Months**

	Exercise group (n=31)				Control group (n=36)		
	Pre	3 months later	p		Pre	3 months later	p
Weight (kg)	62.1 ± 2.2	60.8 ± 2.1	0.001	*	58.5 ± 1.2	57.5 ± 1.1	0.381
BMI (kg/m^2)	23.5 ± 0.7	23.2 ± 0.7	0.249		22.4 ± 0.4	22.3 ± 0.5	0.825
% body fat (%)	26.0 ± 1.2	25.1 ± 1.2	0.008	*	31.3 ± 0.7	29.5 ± 1.0	0.169
Waist circumference (cm)	84.6 ± 2.0	80.1 ± 1.8	0.001	**	80.6 ± 1.3	81.1 ± 2.0	0.183
Left holding power (kg)	30.6 ± 1.9	31.3 ± 2.0	0.329		30.8 ± 1.7	31.0 ±1.9	0.945
Right holding power (kg)	33.2 ± 1.9	33.5 ± 1.9	0.455		31.9 ± 1.7	32.1 ± 1.8	0.455
WHO-QOL 26 score	2.9± 0.1	3.1 ± 0.1	0.001	**	2.9 ± 0.1	2.9 ± 0.1	0.878
Triglyceride (mg/dL)	135.3 ± 14.5	121.5 ± 19.4	0.019	*	110.1 ± 8.7	107.7 ± 8.7	0.523
Total cholesterol (mg/dL)	210.8 ± 6.3	194.7 ± 5.5	0.002	**	217.1 ± 5.8	217.4 ± 5.9	0.600
HDL cholesterol (mg/dL)	61.0 ± 3.0	71.8 ± 2.9	0.001	**	70.0 ± 2.2	72.3 ± 2.4	0.080
LDL cholesterol (mg/dL)	119.7 ± 5.7	112.9 ± 4.9	0.037	*	122.1 ± 5.1	120.8 ± 5.0	0.768
Fasting plasma glucose (mg/mL)	97.1 ± 4.5	95.2 ± 2.4	0.931		96.9 ± 3.7	102.9 ± 3.7	0.073

Values are means ± SE. * p<0.05 ** p<0.005, using Wilcoxon signed-rank test. BMI, body mass index, body fat percentage, % body fat.

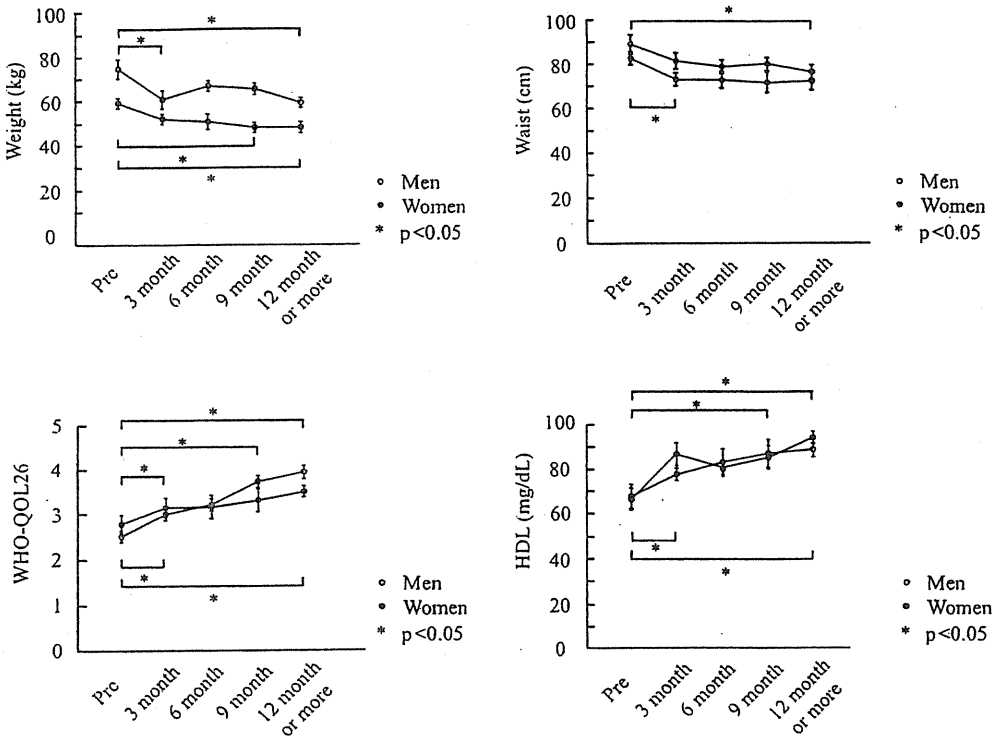

Figure 1. **Changes in body composition and blood with the exercise group. Values are means ± SE. *p<0.05, using two-way repeated measures analysis of variance (ANOVA). Numbers of men were as follows: pre, (n=8) 3 months, (n=8), 6 months, (n=8), 9 months, (n=7), 12 months or more, (n=5). Numbers of women: pre, (n=23), 3 months, (n=23), 6 months, (n=20), 9 months, (n=17), 12 months or more, (n=11).**

Table 3. **Pearson's Correlation Analysis and Multiple Regression Analysis of HMW Adiponectin and Other Factors**

	Pearson's correlation analysis (n=17)		Multiple regression analysis (n=17)	
	Pearson's Y	p	ß-values	p
BMI	-0.509	0.037 *	-0.174	0.018 *
% body fat	-0.031	0.907	-0.088	0.453
Waist circumference	-0.180	0.488	0.101	0.244
WHO-QOL26	0.207	0.426	0.040	0.213
Triglyceride	-0.093	0.722	0.024	0.361
Total cholesterol	-0.309	0.228	0.014	0.114
HDL cholesterol	0.665	0.004 **	0.389	0.002 **
LDL cholesterol	-0.578	0.015 *	-0.443	0.008 *
Fasting plasma glucose	-0.559	0.020 *	-0.306	0.010 *
R^2				0.761
Adjusted R^2				0.454

Values are means ± SE. * p<0.05, ** p<0.005, using pearson's correlation analysis and multiple regression analysis. R^2 = coefficient of determination. BMI, body mass index, body fat percentage, % body fat.

contrast, there was a significantly positive correlation between HMW adiponectin and HDL cholesterol (r=0.665, p=0.004). There was no relationship between HMW adiponectin and WHO-QOL 26 score, triglyceride, total cholesterol levels, % body fat, and waist.

Multiple regression analysis examined the relationship between HMW adiponectin and the other factors. There was a significant correlation between HMW adiponectin and body composition (BMI; β=-0.174, p=0.018), HDL cholesterol (β=0.389, p=0.002), LDL cholesterol (β=-0.443, p=0.008),

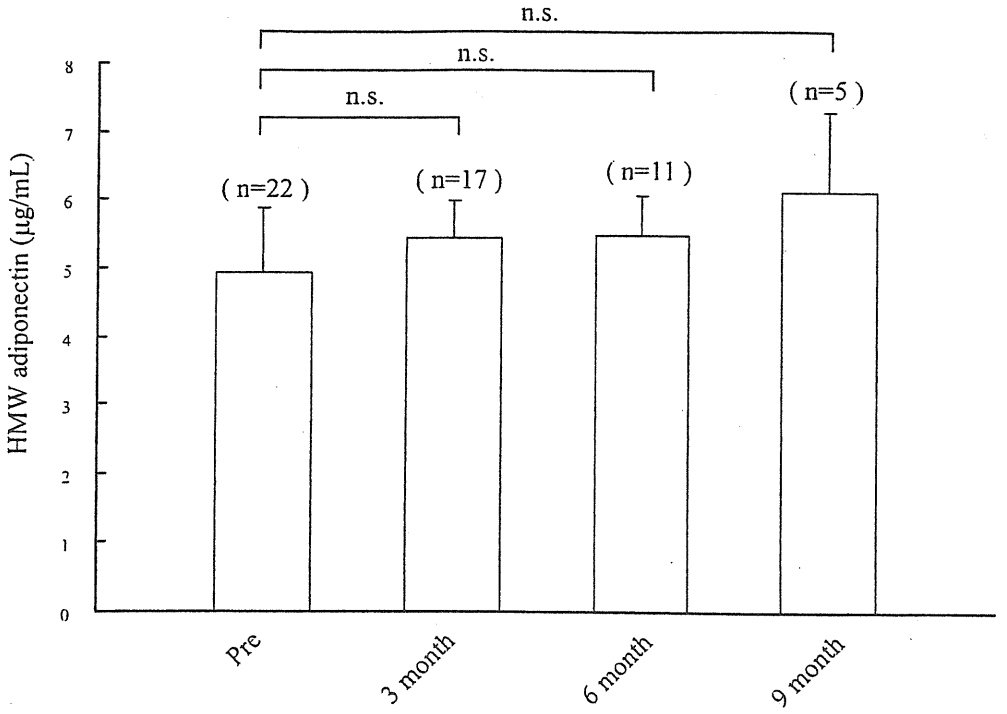

Figure 2. **Changes in HMW adiponectin responses with the exercise group during the exercise and post exercise. Values are means ± SE. n.s., p>0.05, using the Mann-Whitney U test. Numbers of participants were as follows: pre, (n=22), 3 months, (n=17), 6 months, (n=11), 9 months, (n=5).**

and fasting plasma glucose (β=-0.306, p=0.010, Coefficient of determination R^2=0.761, and adjusted R^2=0.454). There was still no relationship between HMW adiponectin and WHO-QOL 26 score, triglyceride, total cholesterol levels, % body fat, and waist.

Adiponectin responses

The concentration of HMW adiponectin increased in the exercise group, although the difference between pre- and post-exercise levels did not reach statistical significance (Fig. 2). The HMW adiponectin concentration was increased by 24% after 9 months of exercise compared with pre-exercise levels.

Discussion

Aerobic exercise improved body weight, waist circumference, WHO-QOL 26 score, and HDL cholesterol. The concentration of HMW adiponectin increased during the aerobic exercise program, with a significant negative correlation being observed between HMW adiponectin and body composition (BMI). HMW adiponectin also showed a significant positive correlation with HDL cholesterol and a significant negative correlation with LDL cholesterol. We found that the 31 (83.8%) participants who completed the exercise program had significant improvements in weight, % body fat, waist circumference, WHO-QOL 26 score, triglyceride, total cholesterol, HDL cholesterol and LDL cholesterol at the end of the third month. These findings are in agreement with a previous study on endurance exercise training that reported positive changes in lipid and lipoprotein metabolism (16).

It is generally considered that aerobic exercising has several beneficial effects on health. For example, regular exercise may promote chronic positive effects such as an improvement in lipid profiles, insulin resistance, BMI, and % body fat, as well as basal metabolic state (17). As the metabolism of lipoproteins occurs mainly during the aerobic exercise (18), the positive changes in anthropometric variables observed in the present study indicate that periodical aerobic exercising has the potential to improve the lipid profile. The pathological changes in the lipid and glucose metabolism in metabolic syndrome are closely related to the state of insulin resistance, unfortunately we have no data of fasting insulin concentration (IRI); further studies are needed to rectify the relation between IRI and exercise.

A loss of body mass and fat has also been associated with decreases in total and LDL cholesterol and an increase in HDL cholesterol. The improvement in total cholesterol may result from the exchange of cholesterol esters between tissues and lipoproteins to HDL cholesterol. Furthermore, exercise training usually decreases the concentration of fasting plasma triglycerides. As a consequence, the levels of very low density lipoprotein (VLDL) decrease and HDL cholesterol levels tend to increase (19). It is considered beneficial to increase the clearance of VLDL and triglyceride, in order to decrease the mean residence time of these lipoproteins in

the circulation. The concentration of LDL cholesterol is regulated by the balance between synthesis in the liver and removal from the plasma by lipoprotein receptors, while HDL concentration is determined by both HDL cholesterol and apolipoprotein A-I (apoA-I) concentrations. HDL cholesterol concentration increases frequently in response to a decrease in triglyceride, although in this study exercise training did not change the triglyceride levels. Therefore, another possible mechanism for the exercise-induced rise in HDL cholesterol rise we observed may be induction of apoA-I synthesis. There is further evidence that lecithin and cholesterol acyltransferase (LCAT) activity are changed by exercise training, and may therefore also affect HDL cholesterol levels before and after exercise training (20-22).

In this study we showed that continued exercise training led to further improvements in weight, waist circumference, WHO-QOL 26 score and HDL cholesterol levels (Fig. 1) while the other variables remained stable. We also demonstrated that HMW adiponectin correlated positively with HDL cholesterol, and negatively with LDL cholesterol and fasting plasma glucose. It has been reported that HMW adiponectin is not only the more active form of the adipokine (23, 24), but is also related closely to insulin sensitivity in the metabolic syndrome (25, 26). The present study investigated the role of aerobic exercise and improvement in lipid profiles in relationship to changes in HMW adiponectin concentration. Blüher et al (27) found that HMW adiponectin concentration increased with exercise treatment even in subjects with normal glucose tolerance. In contrast, Bobbert et al (28) showed that HMW adiponectin concentration was similar before and after 6 weeks of marathon training without a reduction in body weight. In the present study, HMW adiponectin concentration increased in the exercise group, although this increase did not reach significance during the aerobic exercise program (Fig. 2). The effect of aerobic exercise on HMW adiponectin concentration therefore remains to be established conclusively. However, HMW adiponectin concentrations tended to be higher than in the pre-exercise period. Further studies are needed to determine whether HMW adiponectin concentration changes significantly during longer periods of aerobic exercise or with different intensity of exercises.

Conclusion

Aerobic exercise leads to an improvement in body composition and lipid profile. The concentration of HMW adiponectin also showed a tendency to improve with regular aerobic exercise compared with pre-exercise levels.

The authors state that they have no Conflict of Interest (COI).

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Original Article

Relationship Between the Change in Daily Step Count and Brachial-Ankle Wave Velocity During a Pedometer-Based Physical Activity Program for Older Adults

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Abstract

Objective: To study the relationship between the change in the number of steps taken and brachial-ankle pulse wave velocity (baPWV) during a long-term pedometer-based physical activity program in healthy older adults.
Methods: Sixty older adults participated in this 17-week program. Each subject was provided with a pedometer and was given a goal to walk a set number of steps/day. After five subjects were excluded because of insufficient step data, data from 55 subjects (19 men and 36 women; age range: 65–79 years, mean age: 71.3±3.7 years; mean body mass index [BMI]: 24.1±8.8 kg/m^2) were analyzed. Subjects were checked before and after the study. Each subject was informed of his or her vascular age, calculated from baPWV, at the start of the study.
Results: Subjects were divided into four groups based on the results of baPWV. The group in which baPWV improved above a selected cut-off value (1,700 cm/s) revealed the largest increase in steps/day among groups. This increase (4837.7±1868.7 steps) was larger than in groups in which baPWV remained low (1406.7±2402.1 steps, p=0.036) and high (1678.2±2871.4 steps, p=0.059). In any group, age or initial steps/day did not influence the change in steps. Subjects classified as having an older vascular age than the actual age on the basis of initial baPWV walked further.
Conclusion: An increase in steps/day might improve baPWV. Although walking is a low intensity physical activity, it can have an anti-atherosclerosis effect.

KEY WORDS: walking, atherosclerosis, arterial stiffness, baPWV, aging

Introduction

Pulse wave velocity has been used as an indicator of atherosclerosis and arterial stiffness [1]. Recent studies have focused on the use of brachial-ankle pulse wave velocity (baPWV) as a clinical tool for screening atherosclerosis [2]; this variable was recognized in 2009 as a tool for the measurement of arterial stiffness in guidelines for the diagnosis of hypertension by the Japanese Society of Hypertension (JSH2009) [3]. Habitual Physical activity has been reported to effectively prevent atherosclerosis [4] even when begun at an older age [5,6]. This emphasizes the importance of appropriate physical activity programs for older people. Such physical activity programs should be effective, safe and easy, and walking is one activity suitable for older people [7]. A quantitative increase of physical activity has been reported to prevent atherosclerosis [8]. Accordingly, an increase in the number of steps taken may improve baPWV. However, most previous studies assessed the effectiveness of physical activity programs on baPWV either using resistance training [9] or exercise at special facilities [10-14]. Such exercise programs are difficult for older people to complete at home without supervision. We are not aware of any investigation the relationship between the number of steps taken and baPWV among older adults.

In the present study, we established a long-term pedometer-based physical activity program for healthy older adults and analyzed the relationships between change in steps/day and change in baPWV. We hypothesized that the change in steps/day would lead to an improvement in baPWV.

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Methods

1. Subjects

A total of 60 healthy older adults (65 years old or older), living in the central district of S City, Hokkaido participated in this study. Subjects were recruited either from the city news of S City or bulletin boards in the city center (the Central Health Center or the M Community Development Center). During analysis, four subjects were excluded because of insufficient step data during the study period and one subject missed the final checkup for personal reasons and was excluded from the study. Therefore, data from a total of 55 subjects (19 men and 36 women; age range: 65–79 years, mean age: 71.3±3.7 years; mean body mass index [BMI]: 24.1±8.8 kg/m^2) were included in the analyses. All subjects provided written informed consent. The present study was approved by the Ethics Committee of the Graduate School of Education at Hokkaido University.

2. Pedometer-based physical activity program

The procedures of the physical activity program are described elsewhere [15]. Briefly, the program consisted of pedometers and newsletters. Each subject was provided a pedometer (Walking Style HJ-720IT, Omron Healthcare Co. Ltd., Ukyo-ku, Kyoto) and instructed to walk everyday during the study. Each subject was given a goal to walk a set number of steps/day. At least once a month, subjects were instructed to bring their pedometers to the assigned center. Step data were entered by health nurses or staff into a personal computer using BI-Link Professional Edition 2.0 software (Omron Healthcare Co. Ltd., Ukyo-ku, Kyoto). Newsletters were delivered to each subject's house every four weeks. Newsletters for each subject showed the average steps/day achieved for the current month as well as the goal number of steps/day for the upcoming months, determined based on the individual's average steps/day in the current month using the following criteria. Step goals for each month were decided as follows: increase of 1,000 steps/day for subjects below 5,000 [16], increase to 7,500 steps/day for 5,000-7,500 [17], increase to 10,000 steps/day for 7,500-10,000 [17], and maintenance steps/day over 10,000 [16]. In step data analysis, the average steps/day for the first week (Week 0) of the study were treated as baseline data for "start of the study". Only data obtained during a wearing period of >12 hours per day was included in analysis. This excluded low steps/day data if a subject forgot to wear his or her pedometer.

3. Measurements of anthropometrics, blood pressure and baPWV

Each subject was given a medical checkup before and after the study, after an overnight fast. Anthropometrics, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured before the program was started; baPWV was measured separately on a different day during 0900-1500 h at rest using an automatic oscillometric device (form PWV/ABI; Omron Colin Co. Ltd., Bunkyo-ku, Tokyo). The validity and reproducibility of baPWV measurements have been described elsewhere [18]

4. Estimation of vascular age from baPWV

The instrument used in the study is calibrated so that vascular age can be calculated from baPWV [19]. At the beginning of the study, each subject was informed of his or her vascular age via mail. When a subject's calculated baPWV was within the range average to average + ½ standard deviation (SD) of the standard baPWV [19] for their actual age, their vascular age was set as equal to their actual age. If baPWV differed by more than average + ½ SD from the standard, his or her vascular age was calculated from the value average +½ SD. The subjects were divided into two groups to analyze the effect of differences between estimated vascular age and actual age; subjects were allocated to a vascular age-older group if vascular age was estimated as ≥2 years older than actual age; otherwise, the subject was enrolled into the vascular age-younger group.

5. Statistical analysis

All data were expressed as mean±SD, and $p<0.05$ was considered statistically significant. Statistical tests were performed using SPSS for Windows Ver. 15.0 (SPSS Inc., Chicago, IL, USA). Differences within groups were estimated by paired t-test or Wilcoxon's signed rank test. The differences between groups were estimated using one-way ANOVA, ANCOVA and the χ^2-test. Dunnett's test was used to compare weekly average steps/day (Week 1-16) with baseline steps/day (Week 0). To estimate the effect of increasing steps/day during the study, Δsteps/day was calculated using the following formula [20]:

$$\Delta\text{steps/day} = \sum\{(\text{steps/day at Week } X) - (\text{steps/day at Week 0})\} * (X=1\text{-}16)$$

To evaluate change in baPWV, we set a cut-off baPWV = 1,700 cm/s. Although there is no clear cut-off value of baPWV that defines organ disorders [21], this value is a predictor of several health disorders such as all mortality [22], type 2 diabetes mellitus [23], cerebral ischemic small vessel disease [24] and acute coronary syndrome [25]. Each subject was categorized as either 'high' or 'low' according to his or her starting baPWV above or below the cut-off value.

At the end of the study, subjects were divided into four groups: the high-high (HH) group remained above the cut-off value baPWV throughout the study; the low-low (LL) group remained below the cut-off value; and the low-high (LH) and high-low (HL) groups showed a change in baPWV across the cut-off value.

Results

Characteristics of the subjects

Fifty-five subjects completed the study. At the start of the study (baseline), actual age, SBP, DBP and baPWV of the HH group was higher than other groups ($p<0.05$) The average steps/day in the first week (Week 0) and the sex ratio was similar in all groups (*Table 1*).

Change in steps/day (Δsteps/day)

The change in number of steps/day (Δsteps/day) in each group is shown in *Fig. 1*. The HL group (4837.7±1868.7 steps) changed more than the LL group (1406.7±2402.1 steps, $p=0.036$) and tended to be larger than HH group (1678.2±2871.4 steps, $p=0.059$). The difference between groups in Δsteps/day was unchanged after age and steps/day at baseline were adjusted using ANCOVA. This indicates that neither age nor steps/day at baseline affected Δsteps/day among groups.

Table 1 ***Characteristics of the subjects at baseline***

	LL (n=22)	LH (n=4)	HL (n=6)	HH (n=23)	*p* -value	post-hoc
Male sex (No.)	5	3	3	8		
Weight (kg)	56.2 ± 7.0	61.1 ± 11.5	60.1 ± 9.4	60.8 ± 9.6	0.323	
BMI (kg/m²)	23.2 ± 2.1	24.1 ± 2.9	23.7 ± 1.8	24.7 ± 2.5	0.203	
Waist circumference (cm)	86.0 ± 6.3	82.8 ± 10.0	88.9 ± 2.5	90.4 ± 7.9	0.097	
Hip circumference (cm)	95.9 ± 5.2	96.1 ± 2.6	97.6 ± 4.2	98.4 ± 4.7	0.371	
SBP (mmHg)	132.7 ± 14.2	135.8 ± 14.6	141.7 ± 18.0	154.6 ± 19.0	0.001	LL<HH
DBP (mmHg)	76.3 ± 7.4	76.2 ± 3.4	79.2 ± 8.6	82.9 ± 12.1	0.019	LL<HH
baPWV (cm/s)	1,520.9 ± 121.9	1,666.8 ± 14.3	1,793.8 ± 94.0	2,068.7 ± 284.7	0.000	LL<HL<HH
Steps/day at week 0 (step)	10,114.2 ± 3,062.9	9,509.2 ± 3,882.3	8,506.1 ± 2,303.6	8,801.0 ± 3,940.4	0.573	

Values are Means ± SD.

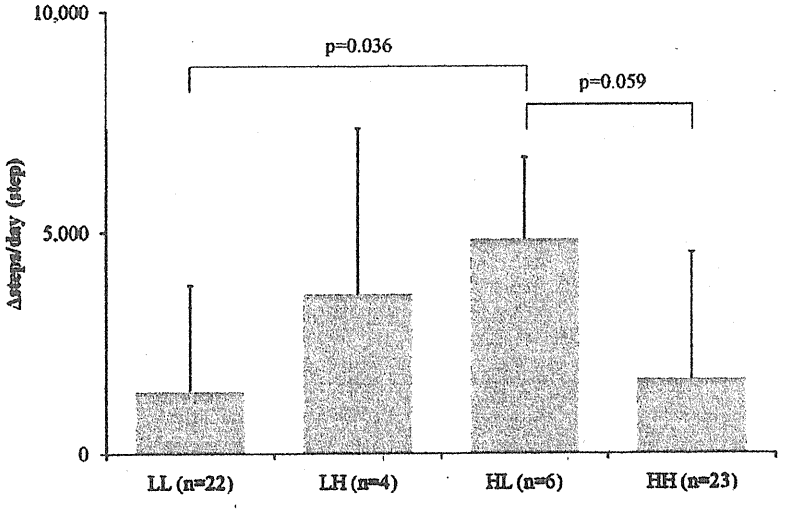

Fig. 1. **Comparison of the change in steps/day among four groups based on the cut-off value of baPWV**
Error bars show SD.

Change in anthropometrics and resting blood pressure

The changes in weight, waist circumference, hip circumference, SBP and DBP are shown in *Table 2*. Weight, waist circumference and hip circumference decreased in all groups except the LH group ($p<0.05$, *Table 2*).

Change in baPWV

The baPWV of the two groups above or below the baseline cut-off value are compared in *Fig. 2*. The change in baPWV tended to be larger in the LH group than the LL group ($p=0.083$) but there was no significant difference in change in baPWV between the HH and HL groups.

Difference in steps/day between vascular age-older and age-younger groups.

The average steps/day in the age-older group increased every week except week 1 and 2 ($p<0.05$, *Fig. 3a*); in the vascular age-younger group, the average steps/day only increased in week 5–9 and 13–14 ($p<0.05$, *Fig. 3b*).

Discussion

The present study examined whether change in baPWV was related to the number and change in number of steps/day. We found the HL group (indicating baPWV changed from high level at the start of the study to low level at the end of the study) exhibited greatest increase in steps/day. This implies that subjects with a higher initial baPWV who increased steps/day tended to decrease baPWV by the end of the program.

It is important to note that this program consisted of daily walking and all subjects were older adults. As far as we know, the effect of walking alone and quantitative investigation of physical activity on baPWV have not been previously reported. Most previous studies [10-14] measured the effect of supervised physical activity sessions using exercise facilities. For older people, visiting exercise facilities appears more difficult than walking. Given that walking is easily included in everyday routine, the results in the present study are clinically meaningful.

BaPWV is strongly influenced by SBP [26]. However, when the baPWV was adjusted for baseline SBP the change of baPWV in the HL group was greater than that in the LH group. As Δsteps/day in HL group was the largest of the four groups, the result suggests the improvement in baPWV was related to the increase in steps.

Table 2 The change in anthropometrics, blood pressure and baPWV in each group

	LL (n=22)	*p* -value	LH (n=4)	*p* -value	HL (n=6)	*p* -value	HH (n=23)	*p* -value
Weight (kg)	−1.6 ± 2.1	0.002 **	−0.6 ± 2.1	0.583 **	−2.3 ± 1.8	0.026 *	−1.8 ± 1.6	0.000 ***
BMI (kg/m^2)	−0.7 ± 0.9	0.002 **	−0.3 ± 0.8	0.553 **	−0.9 ± 0.6	0.023 *	−0.7 ± 0.6	0.000 ***
Waist circumference (cm)	−2.4 ± 3.6	0.005 **	−2.2 ± 2.1	0.126 **	−3.4 ± 4.0	0.090	−1.7 ± 3.9	0.043 *
Hip circumference (cm)	−3.6 ± 5.1	0.003 **	−0.5 ± 5.3	0.854 **	−5.3 ± 3.5	0.014 *	−3.0 ± 3.5	0.001 **
SBP (mmHg)	−11.4 ± 13.3	0.001 ***	8.8 ± 17.6	0.394 ***	−11.7 ± 13.6	0.090	−2.7 ± 16.8	0.450
DBP (mmHg)	−4.4 ± 7.6	0.014 *	8.0 ± 6.9	0.104 *	−6.0 ± 4.1	0.016 *	−2.9 ± 12.0	0.258
baPWV (cm/s)	−46.2 ± 87.6	0.022 *	157.5 ± 103.6	0.056 *	−134.0 ± 70.1	0.005 **	−69.5 ± 211.9	0.130

Values are Means ± SD. * p <0.05, ** p <0.01, *** p <0.001 vs. baseline.

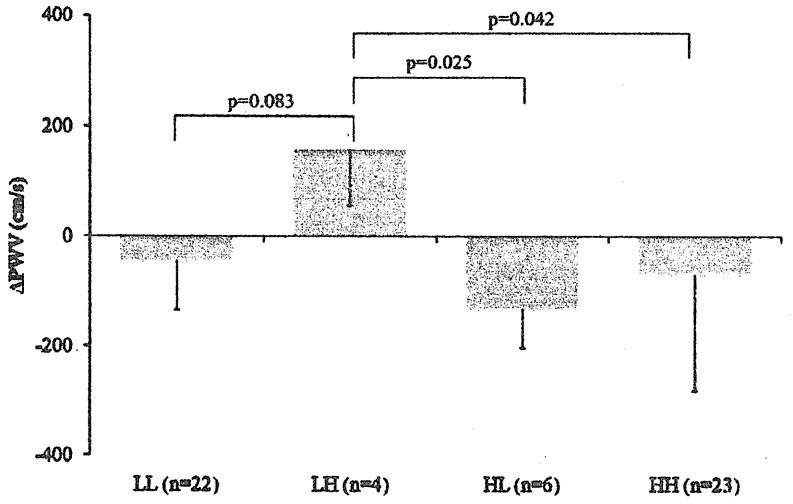

Fig. 2. **Comparison of the change in baPWV among four groups based on the cut-off value of baPWV**
Error bars show SD.

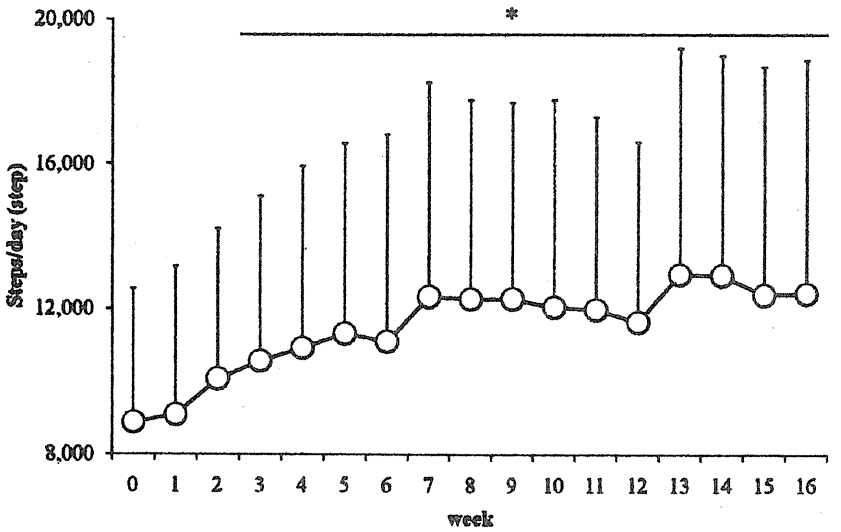

Fig. 3a. **The average steps/day of vascular age-older group during the study**
*p<0.05 compared with week 0. Error bars show SD.

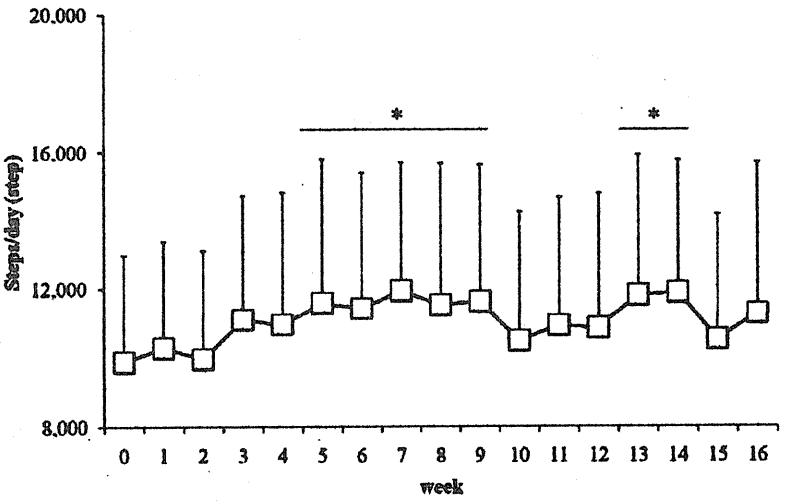

***Fig. 3b*. The average steps/day of vascular age-younger group during the study**
*$p<0.05$ compared with week 0. Error bars show SD.

There is a dramatic, exponential [27] increase in baPWV with increasing age [28,29]. In the present study, the average baPWV of the subjects at baseline (men, 1,820.1±321.5 cm/s; women, 1,774.7±323.7 cm/s) was slightly higher than previously reported [28]. Previous studies found PWV increased 7.5–11.8 cm/s per year [29] and the decrease of 46.0 cm/s found in the present study (data not shown) is equivalent to an Anti-Aging effect of approximately 4–6 years. This demonstrates the potential of programs that encourage older people to increase daily steps to prevent atherosclerosis.

However, the physical activity level of the subjects at baseline may also affect the results. Subjects in the present study were highly active; the average steps/day at baseline was 9,389.1±3,412.1, markedly higher than that reported in other studies of men and women in their 60s (7,961 for men and 6,666 steps for women) [30]. Further study needs to explore the effects of physical activity in more sedentary people.

Further, the steps/day of subjects in the two groups based on vascular age assessed by baPWV differed. The vascular age-older group (indicating vascular age > actual age at start of the study) exhibited greatest increase in steps/day, although there was no difference between the two groups at the start of the study (***Fig. 3a,b***). That is, subjects whose vascular age was diagnosed >2 years older than actual age, walked more during the study. This result suggests that informing subjects of their vascular age encouraged the age-older group to increase physical activity. Previous studies have also suggested that telling subjects their cardiovascular age [31] or lung age [32] yielded better results than traditional therapies. Similarly, it was reported that interventions that used a pedometer and provided a goal induced an increase in steps/day [33]. Taken together, showing older people simple indexes such as "goal steps/day" or "vascular age" may be an effective tool for increasing physical activity among the elderly.

The limitation of the present study was that we could not show any relationship between baPWV and other indicators except steps/day. This may be a result of small sample size or individual differences. However, previous studies reported that higher baPWV is related to slower walking speed among older people [34] and an increase of 100 cm/s baPWV increases sarcopenia risk among older people by 1.14 times [35]. Higher PWV may affect other physical measures other than atherosclerosis. These facts imply the decrease of PWV in this study may improve health and physical functions of older people.

Conclusions

The present study examined the effects of a 17-week pedometer-based physical activity program for healthy older adults and the relationship between change in steps/day and change in baPWV. The findings are:

1. Even low intensity physical activity such as walking may decreased baPWV.
2. A group, in whom baPWV was above 1,700 cm/s at baseline and decreased baPWV during the study, showed the largest increase in steps/day during the study
3. A group, in whom vascular age at baseline was diagnosed older than his/her actual ages, increased steps/day during the study.

These findings suggest that high individual baPWV may be decreased by increasing number of steps/day although no dose-response relationship was found between number of steps/day and baPWV. Providing simple indicators such as vascular age and target number of steps can encourage older people to increase physical activity.

Acknowledgements

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Original Contribution

Age-dependent changes in 8-oxoguanine-DNA glycosylase activity are modulated by adaptive responses to physical exercise in human skeletal muscle

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ABSTRACT

8-Oxo-7,8-dihydroguanine (8-oxoG) accumulates in the genome over time and is believed to contribute to the development of aging characteristics of skeletal muscle and various aging-related diseases. Here, we show a significantly increased level of intrahelical 8-oxoG and 8-oxoguanine-DNA glycosylase (OGG1) expression in aged human skeletal muscle compared to that of young individuals. In response to exercise, the 8-oxoG level was lastingly elevated in sedentary young and old subjects, but returned rapidly to preexercise levels in the DNA of physically active individuals independent of age. 8-OxoG levels in DNA were inversely correlated with the abundance of acetylated OGG1 (Ac-OGG1), but not with total OGG1, apurinic/apyrimidinic endonuclease 1 (APE1), or Ac-APE1. The actual Ac-OGG1 level was linked to exercise-induced oxidative stress, as shown by changes in lipid peroxide levels and expression of Cu,Zn-SOD, Mn-SOD, and SIRT3, as well as the balance between acetyltransferase p300/CBP and deacetylase SIRT1, but not SIRT6 expression. Together these data suggest that that acetylated form of OGG1, and not OGG1 itself, correlates inversely with the 8-oxoG level in the DNA of human skeletal muscle, and the Ac-OGG1 level is dependent on adaptive cellular responses to physical activity, but is age independent.

Age-associated increases in levels of reactive oxygen species (ROS), especially during the last quarter of life, result in excessive oxidative damage to macromolecules, including DNA [1–5]. Among DNA and RNA bases, guanine is predominantly prone to oxidation because of its lowest reduction potential [6]. It is modified primarily by hydroxyl radicals at or near diffusion-controlled rates (reviewed in [7–9]). More than 20 oxidation products of the guanine base have been identified [10] and among them one of the most abundant is 8-oxo-7,8-dihydroguanine (8-oxoG) [7–9]. In DNA, the 8-oxoG level increases upon radiation, ischemia/reperfusion, acute exercise, and aging [4,11–14]. 8-OxoG is excised from DNA by formamidopyrimidine-DNA glycosylase (Fpg) in *Escherichia coli* and by its functional homolog 8-oxoguanine-DNA glycosylase (OGG1) in mammals in the base excision repair (BER) pathway [15–18]. Whereas Fpg is well known to excise 4,6-diamino-5-formamidopyrimidine (FapyA), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), and 8-oxoG with nearly similar excision kinetics [18,19], the mammalian and yeast OGG1 is specific for 8-oxoG and FapyG, but not FapyA [20,21]. When 8-oxoG is not repaired, it is mutagenic, as it has been shown to pair with adenine (A) instead of cytosine (C) and thereby induces G:C→T:A transversions [15,22].

It is documented that in covalent modifications of DNA repair proteins, e.g., by acetylation, phosphorylation plays a significant role, particularly in their repair activity, which consists of the removal/repair of oxidative base lesions [23,24]. In fact, it has been shown that OGG1 and human apurinic/apyrimidinic endonuclease 1 (APE1) activities are primarily regulated by p300/CBP-mediated acetylation reactions, processes that significantly influence their repair activities and hence cell fate [23–25]. The role of sirtuin family deacetylases has gathered considerable attention [26], as SIRT1 and SIRT6 have been shown to be

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involved in DNA repair [27–29]. An increased deacetylase activity of sirtuins may lead to a decrease in acetylation levels of proteins, which, in turn, would result in a decline in enzymatic activities, including those of OGG1 and APE1.

Although it is well documented that acetylation increases OGG1 activity in cell cultures and in vitro assays, the existence of acetylated OGG1 (Ac-OGG1) and APE1 (Ac-APE1) under in vivo conditions is still unknown. The goals of this investigation were (a) to determine changes in Ac-OGG1 and Ac-APE1 in human skeletal muscle, (b) to study the effects of aging and acute as well as regular physical conditioning on acetylation levels of these DNA repair enzymes, and (c) to evaluate the possible roles of SIRT1, SIRT3, and SIRT6 in the adaptability of human skeletal muscle. This report shows that the level of acetylated OGG1 changes as a function of age, and exercise training increases this posttranslational modification independent of age in human muscles.

Materials and methods

Subjects

Forty-eight healthy men volunteered to participate in this study. A written informed consent was signed by all participants regarding their participation after they were told of all risks, discomforts, and benefits involved in the study. Procedures were in accordance with the Helsinki Declaration of 1975 and were approved by the ethics committee of the University of Thessaly.

Participants were assigned to one of four groups according to a cross-over, repeated-measures design: (a) young sedentary (YS; 26.0 ± 4.5 years), (b) young physically active (YA; 30.2 ± 7.9 years), (c) old sedentary (OS; 63.4 ± 4.7 years), and (d) old physically active (OA; 62.4 ± 2.9 years). Subjects were exposed to a single bout of the exercise protocol and muscle biopsies were taken. Participants were assigned to the young or old sedentary group based on a maximal oxygen uptake (VO_{2max}) of below 25 ml/kg/min for old participants and below 35 ml/kg/min for young participants, and the young and old physically active groups were based upon the ACSM description [30], VO_{2max} over 45 ml/kg/min for young participants and over 35 ml/kg/min for old (YS, 35.9 ± 4.7; OS, 25.1 ± 3.0; YA, 51.8 ± 7.9; OA, 37.1 ± 2.9 ml/kg/min).

Participants visited the laboratory on three occasions. During their first visit, participants were examined by a trained physician for limiting health complications; in their second visit, participants had their body height/weight and skin-folds measured and underwent a Graded Exercise Testing (GXT) to evaluate their VO_{2max}. During their third visit, a week later, participants underwent a submaximal exercise bout to exhaustion on the treadmill, and muscle biopsies were collected before and after exercise.

Measurement of peak oxygen uptake (VO_{2peak})

VO_{2peak} was determined during a GXT on a treadmill to voluntary exhaustion as previously described [31].

Exercise protocol

A single bout of exercise included initially 45 min of running on a treadmill at 70–75% of the subject's VO_{2max}. After 45 min, the speed increased to 90% of VO_{2max}, and exercise was terminated at exhaustion [32].

Muscle biopsy sampling

Participants had been instructed to refrain from physical activity and caffeine consumption for 48 h before exercise. Both muscle specimens (pre- and postexercise), of approximately 100–120 mg each, were obtained from the vastus lateralis of the same leg of each participant by using the needle biopsy technique [33]. The first biopsy was obtained approximately 20 cm away from the midpatella of the right (dominant) leg with the application of suction [34].

Assessment of malondialdehyde levels

Blood samples were collected from an antecubital arm vein into evacuated tubes containing ethylenediaminetetraacetic acid. Plasma was separated by centrifugation (1500 *g*, 4 °C, for 15 min). Samples were stored at −80 °C. Malondialdehyde (MDA) levels were measured by reverse-phase, high-performance liquid chromatography (HPLC) with fluorimetric detection (excitation 532 nm and emission 550 nm) as described [35].

Real-time quantitative RT-PCR

Total RNA from skeletal muscle samples (~30 mg) was extracted with NucleoSpin RNA/protein (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. Analyses of the real-time quantitative PCR data were performed using the comparative threshold cycle (C_t) method, as suggested by Applied Biosystems (User Bulletin 2). The primers used are listed in Table 1.

Fluorescence imaging and quantification

At optimal cutting, temperature-fixed, paraffin-embedded muscles were sectioned into 5-μm sections. The measurement of 8-oxoG levels in nuclear DNA of muscles was assessed by quantitative microscopic imaging, as we previously described [23,36]. Briefly, sections were deparaffinized, air-dried, and fixed in acetone:methanol (1:1), rehydrated in PBS for 15 min, and then sequentially treated with RNase (100 μg/ml) for 15 min followed by 100 μg/ml pepsin in the presence of 0.1 N HCl for 30 min at 37 °C. The sections were washed and then incubated with affinity-purified, nonimmune IgG (100 μg/ml) for 30 min and washed in PBS containing 0.5% bovine serum albumin and 0.1% Tween 20 (PBS-T). After incubation with anti-8-oxoG antibody (Trevigen, Gaithersburg, MD, USA; 1:300 dilution) [37] for 30 min, the sections were washed for 15 min three times with PBS-T and then binding of primary antibody was detected with conjugated secondary antibody.

Table 1
Primers used in RT-PCR.

	Primer sequence
Reference gene	
β-Actin	Forward: 5′-GCTCGTCGTCGACAACGGCTC-3′
β-Actin	Reverse: 5′-CAAACATGATCTGGGTCATCTTCT-3′
RP28S	Forward: 5′-AGCCGATCCATCATCCGCAATG-3′
RP28S	Reverse: 5′-CAGCCAAGCTCAGCGCAAC-3′
Target gene	
OGG1	Forward: 5′-GTGGACTCCCACTTCCAAGA-3′
OGG1	Reverse: 5′-GAGATGAGCCTCCACCTCTG-3′
EP300	Forward: 5′-TCATCTCCGGCCCTCTCGGC-3′
EP300	Reverse: 5′-GCTCTGTTGGGCCTGGCTGG-3′
SIRT1	Forward: 5′-TGCGGGAATCCAAAGGATAATTCAGTGTC-3′
SIRT1	Reverse: 5′-CTTCATCTTTGTCATACTTCATGGCTCTATG-3′
SIRT3	Forward: 5′-GTCGGGCATCCCTGCCTCAAAGC-3′
SIRT3	Reverse: 5′-GGAACCCTGTCTGCCATCACGTCAG-3′
SIRT6	Forward: 5′-GAGGAGCTGGAGCGGAAGGTGTG-3′
SIRT6	Reverse: 5′-GGCCAGACCTCGCTCCTCCATGG-3′
SOD1	Forward: 5′-AGGGCATCATCAATTTCGAG-3′
SOD1	Reverse: 5′-ACATTGCCCAAGTCTCCAAC-3′
SOD2	Forward: 5′-GCAGAAGCACAGCCTCCCCG-3′
SOD2	Reverse: 5′-CCTTGGCCAACGCCTCCTGG-3′
XRCC6 (Ku70)	Forward: 5′-CTGTCCAAGTTGGTCGCTTC-3′
XRCC6 (Ku70)	Reverse: 5′-CTGCCCCTTAAACTGGTCAA-3′

OGG1 and Ac-OGG1 levels were also determined via quantitative microscopic imaging [36,38]. Purified mouse anti-OGG1 antibody (human OGG1 reactive) generated against a synthetic peptide (C-DLRQSRHAQEPPAK-N) representing the C-terminus of OGG1 was acquired from Antibodies-Online (Atlanta, GA, USA). The immunogen affinity-purified, human-reactive rabbit polyclonal antibody to Ac-OGG1 was generated against an Ac-Lys-containing peptide (PAKRR^{Ac}KG G^{Ac}KGPEC) [23] obtained from AbCam (Cat. No. ab93670) [23,36]. Antibody reactive with human APE1 [39] and rabbit anti-Ac-APE1 antibody were characterized previously [40]. Binding of primary antibodies was visualized with fluorochrome-labeled secondary antibodies. Confocal microscopic evaluations were performed on a Zeiss LSM510 META system using the 488-nm line of the argon laser for excitation of FITC and the helium–neon 543-nm line for excitation of rhodamine, combined with appropriate dichroic mirrors and emission band filters to discriminate between green and red fluorescence. Images were captured at a magnification of 60 (60× oil immersion objective; numerical aperture 1.4). To objectively quantify fluorescence intensities morphometric analyses were done by using MetaMorph software version 9.0r (Universal Imaging Corp., Downingtown, PA, USA) as we have described [38]. Specifically, images were obtained from >15 fields per muscle section containing 160–180 nuclei and reassembled using the montage stage stitching algorithm of the Metamorph software [41]. Colocalization was visualized by superimposition of green and red images using MetaMorph software version 9.0r.

Statistical analyses

Statistical significance was assessed by three-way ANOVA (age × physical activity status × time), followed by Tukey's post hoc test. The significance level was set at $p<0.05$.

Results

Changes in 8-oxoG level in DNA as a function of age and physical activity in human skeletal muscle

DNA glycosylase/apurinic/apyrimidinic (AP) lyase activity of OGG1 declines with age [42–44]. Here, first we investigated the association between abundance of 8-oxoG in DNA and OGG1, as well as Ac-OGG1 in nuclei of skeletal muscle of OS and YS individuals. Results from quantitative fluorescence intensity analysis showed that there was a significant ($p<0.01$) increase in genomic 8-oxoG (8-oxodG; Fig. 1A) and total OGG1 ($p<0.01$) levels in skeletal muscle

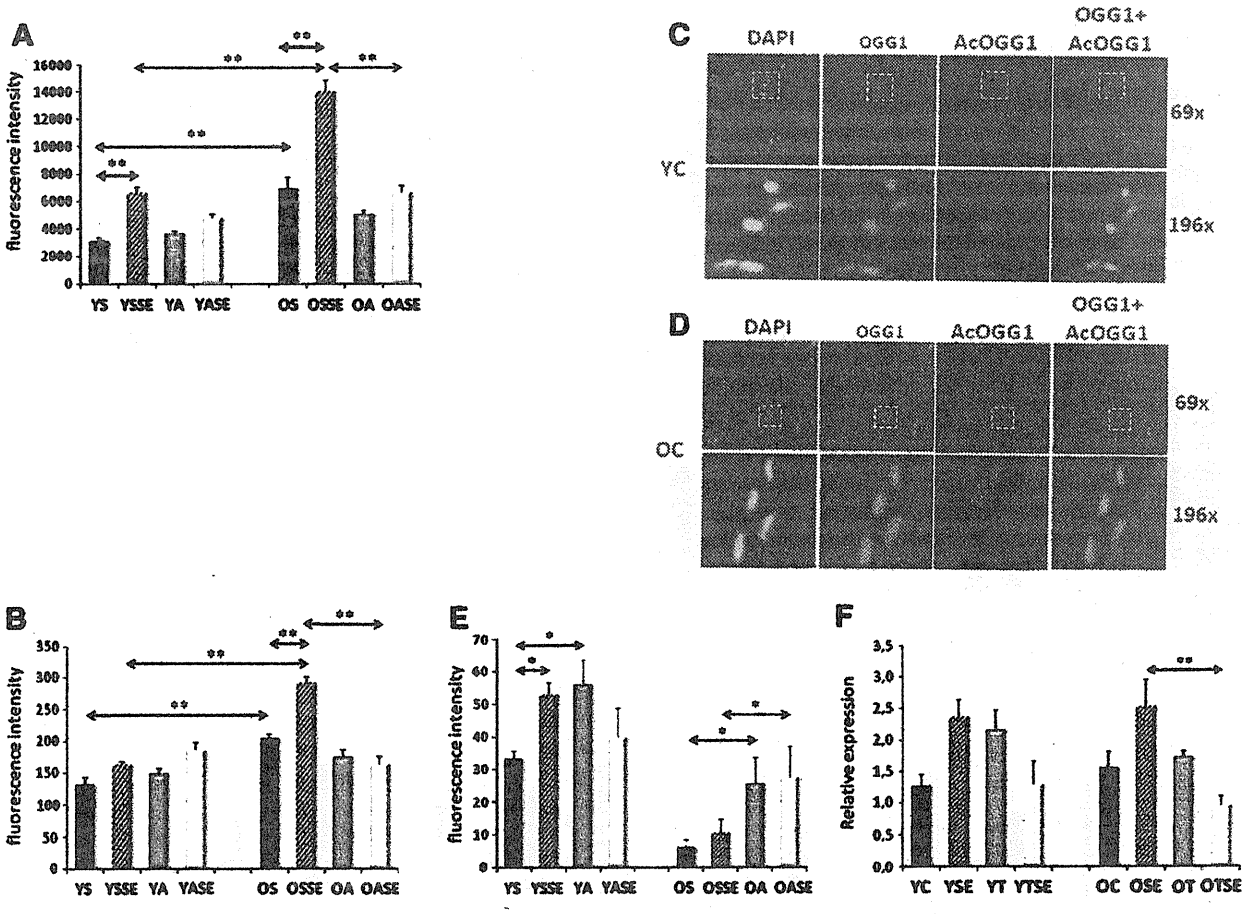

Fig. 1. 8-OxoG, OGG1, and Ac-OGG1 levels in skeletal muscle (SkM) before and after single exercise bout (SEB). (A) Increase in 8-oxoG level in genomic DNA of aged muscles and in response to SEB. (B) Total OGG1 level in SkM of sedentary and physically active subjects. In (A) and (B), sections were stained and fluorescence intensities were analyzed using a montage stage stitching algorithm of the MetaMorph software (Materials and methods). (C) Representative fluorescence images of OGG1 and Ac-OGG1 in sections from the muscles of young individuals. Top: original magnification 69×. Bottom: original magnification 196×. Leftmost images are DAPI, the rightmost images are the superimposition of the OGG1- and Ac-OGG1-mediated fluorescence images. (D) Representative fluorescence images of OGG1 and Ac-OGG1 in muscle sections of old volunteers. Top: original magnification 69×. Bottom: original magnification 196×. Leftmost images are DAPI-stained, the rightmost images are the superimposition of OGG1- and Ac-OGG1-mediated fluorescence images. (E) Changes in Ac-OGG1 levels in skeletal muscle of young and elderly subjects in response to SEB. (F) The relative expression of OGG1 mRNA is shown. DAPI, 4′,6′-diamino-2-phenylindole; YS, young sedentary; YSSE, young sedentary after a single bout of exercise; YA, young active; YASE, young active after a single bout of exercise; OS, old sedentary; OSSE, old sedentary after a single bout of exercise; OA, old active; and OASE, old active after a single bout of exercise. Values are means ± SE for six subjects per group. $^{*}p<0.05$, $^{**}p<0.01$.

of elderly compared to young participants (Fig. 1B). This paradoxical observation suggests an increase in oxidative stress and/or decrease in OGG1 activity; the latter may be due to altered OGG1 posttranslational modification(s), such as acetylation [23]. The acetylated form of OGG1, compared to the unacetylated form, shows an approximately 10-fold increase in repair activity [23]. Immunohistochemical analysis shows that the level of Ac-OGG1 was significantly higher in the skeletal muscle of young individuals (Fig. 1C, top and bottom) compared to that of older subjects. Ac-OGG1 was nearly undetectable in the skeletal muscle of the elderly (Fig. 1D, top and bottom). As calculated from fluorescence intensities, only 5.1 ± 2.5% of total OGG1 was acetylated in the old, whereas 24.5 ± 6% of total OGG1 reacted with anti-Ac-OGG1 antibody in the young individuals (Fig. 1E). APE1 is a multifunctional and abundant protein [39] and has been shown to stimulate 8-oxoG repair initiated by OGG1 during BER [45]. Because of APE1's abundance, it was not surprising to observe that its level was not different in the muscle of the young and old groups (data not shown). Ac-APE1 [46] levels were substantially higher only in skeletal muscle of YS individuals compared to that of OS subjects (Fig. 2A); not the APE1 level but the Ac-APE1, together with Ac-OGG1, plays a role in the repair of 8-oxoG. These results support the hypothesis that an increase in the genomic 8-oxoG level is associated with an inability of aged skeletal muscle to posttranslationally modify OGG1 [25].

OGG1's acetylation level is altered by the activity of acetyltransferase p300/CBP [23,25] and deacetylases such as sirtuins [27]. Our results show that expression of p300/CBP is increased ($p<0.01$) in skeletal muscle of OS subjects compared to that in younger counterparts (Fig. 2B). On the other hand, expression of SIRT1 and SIRT6 (Figs. 2C and E) was not affected by age, whereas SIRT3 expression was significantly lower in the OS compared to the YS group (Fig. 2D). In controls, there were no differences in the expression of Ku70 (binds directly to free DNA ends) in the muscles of young and old individuals (Fig. 3A), an indication that the repair efficiency of 8-oxoG is unaffected by age and level of unrepaired AP sites, and DNA single-strand breaks are not sufficient to alter the expression of Ku70.

Oxidative stress induced by physical activity mediates an adaptive response for efficient oxidative DNA damage repair

Old and young physically inactive and active individuals were subjected to a single exercise bout (SEB). SEB-induced changes in oxidative stress levels were determined indirectly by measuring the levels of the lipid peroxidation product MDA in plasma (YS, 0.176 ± 0.02; YSSE, 0.262 ± 0.03*; YA, 0.143 ± 0.01; YASE, 0.181 ± 0.02; OS, 0.254 ± 0.04; OSSE, 0.338 ± 0.06*; OA, 0.188 ± 0.03; OASE, 0.233 ± 0.03 μmol/L; *$p<0.05$). It is obvious that the MDA level was significantly increased only in the plasma of physically inactive old and young subjects. Although we recognize the limitations of MDA measurements [47], the strong match between MDA and 8-oxoG ($p=0.001$) levels suggests that indeed aging and SEB elevate the level of oxidative damage. These results are supported by the observed increase in the expression of Cu,Zn-SOD (Fig. 3B) in the muscle of physically inactive (old and young) subjects. Mn-SOD expression is increased in response to SEB only in young subjects (Fig. 3C). Surprisingly, Mn-SOD expression was not affected by SEB in active/trained old and young individuals (Fig. 3C). Together these data imply an adaptive response of the skeletal muscle to SEB in trained/active individuals.

An increase in MDA level predicts enhanced genomic 8-oxoG levels upon exercise. Thus we asked if regular physical exercise-induced antioxidant responses protect guanine from oxidation in the DNA from muscle biopsies of sedentary vs trained and young vs old subjects. In response to a SEB, the 8-oxoG level was doubled in the muscle of all individuals regardless of whether they were sedentary or physically active. Importantly, whereas 8-oxoG levels returned to

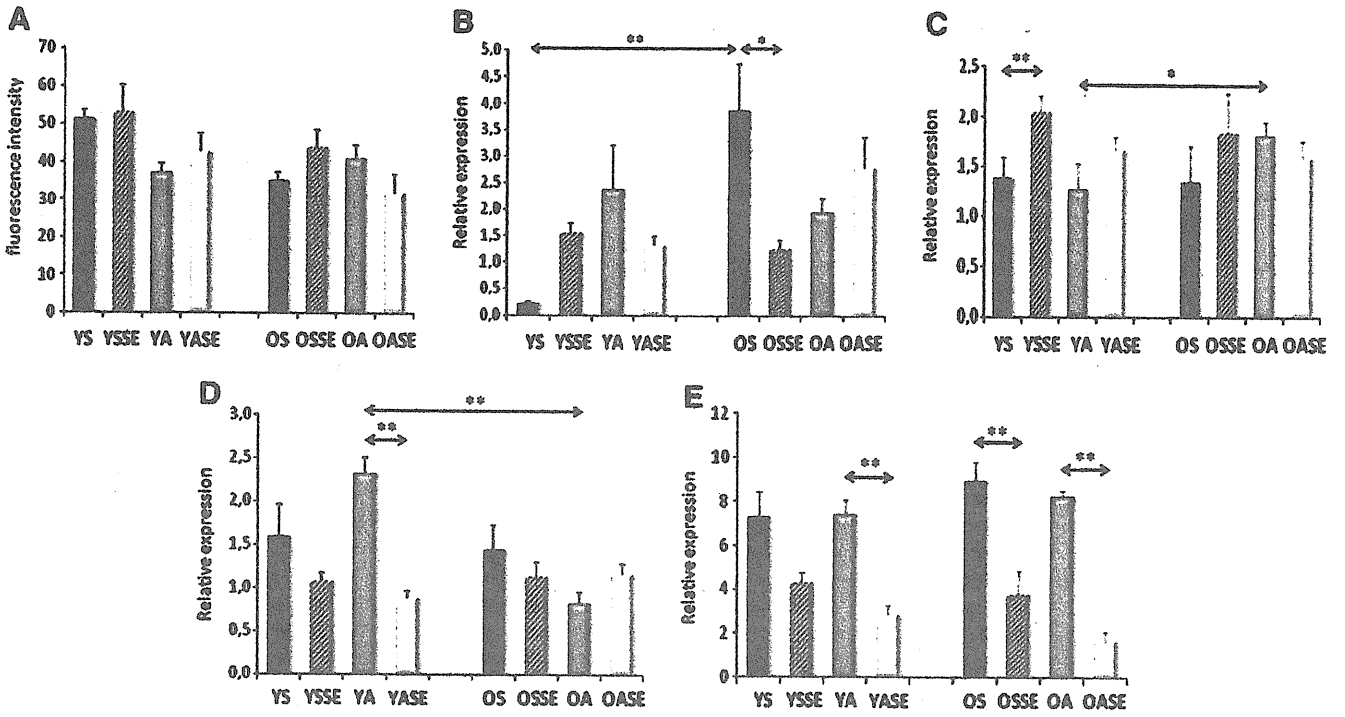

Fig. 2. Ac-APE1 level and expression of p300/CBP, SIRT1, SIRT3, and SIRT6 before and after physical exercise in skeletal muscle. (A) Level of Ac-APE1 as assessed by fluorescence imaging (analyzed as for Fig. 1A). (B–E) Expression at the mRNA level of (B) p300/CBP, (C) SIRT1, (D) SIRT3, and (E) SIRT6. RNA was isolated from muscle biopsies excised before and 24 h after SEB. Quantitative RT-PCR was undertaken as described under Materials and methods. YS, young sedentary; YSSE, young sedentary after a single bout of exercise; YA, young active; YASE, young active after a single bout of exercise; OS, old sedentary; OSSE, old sedentary after a single bout of exercise; OA, old active; and OASE, old active after a single bout of exercise. Values are means ± SE for six subjects per group. *$p<0.05$, **$p<0.01$.