再取り込み阻害薬(SSRI：selective serotonin uptake inhibitor)が著効を示すことがわかった現在，本来「神経症」の治療法として発展してきた精神分析がどれほどの価値をもつのか，という疑問も生まれている。

だが，症状が薬物で除去されても，患者たちは例えば「私は自分の人生をもっていない」，「夫を尊敬しているがどうしても本当には愛せない」，「私は言葉がぴんときたことがなく，ものを考えたことがない，ただそのふりをしているだけだ」，「私は実は同性に惹かれているのではないだろうか」などというような苦しみに深く悩んでいる。こうした苦悩に薬物療法は無力である。そのような，いわば実存的な苦しみにふれる丁寧できめ細かい治療として，精神分析は潜在的需要をもっているが，わが国では訓練された臨床家があまりに少ないのが実情である。

精神分析が，ヒステリーや強迫神経症など症状として結晶化した病理の治療として出発したことは事実である。しかし，1950年代以後，精神分析は「偽りの自己」，「スキゾイド」のような「自分がない」病理を相手にし，彼らがパーソナルな人生の意味を生み出すことを援助することに関心を注いできた。おそらく，生物学的精神医学による治療法と社会的マネジメントが今後さらに進展していくにつれて，多くの症状はマネージでき，社会適応や社会復帰のための援助も有効なものになっていくだろう。しかし，それだけでは解決できないパーソナルな人生の意味を形にしたいという患者のニーズを満たす治療として，精神分析は役割を果たすであろう。

(藤山直樹)

文献

1) 土居健郎：精神分析と精神療法．金子書房，1961
2) 精神力動的精神医学 I II III．岩崎学術出版社，1997．

4. 自律訓練法

1) 理論

自律訓練法(AT：autogenic training)の起源は，1894～1903年の間に行われた，Vogt OとBrodmann Kの睡眠および催眠の研究に求めることができる。Vogtは，催眠を受けている知能の高い患者が，自由時間に催眠状態に非常によく似た状態へ自分自身で入り，この「自己催眠的」練習に関連して現れる〈重感〉および〈温感〉がある機能的意味をもっていることを発見した。この「自己催眠的休息」練習に顕著な回復効果があると報告し，「予防休息自己催眠」と名づけた。その後，ドイツのSchulzによって，自律訓練法が創始され，Lutheにより体系化された心理療法である。

2) 技法体系

ATの技法には標準練習(standard exercise)と特殊練習(special exercise)がある。標準練習はATの基本であり，最もよく用いられるため，その技法について以下に説明する。

まず，練習のための条件，工夫が重要である。

(1) 練習のための条件・工夫

1 姿勢，場所など

できる限り心身ともにリラックスできる環境が望ましいが，慣れてきたらどんな場所でもできるようにすると，応用範囲が広くなる。基本的な姿勢は，単純椅子姿勢(椅子に腰掛けて，足は軽く開き，両手を膝の上に載せる。全身の力を抜き，頭は軽く前屈し，口は固く閉じない)が一般的であるが，仰臥位(仰向けに寝た姿勢)など最もリラックスできる姿勢を選択してよい。服装は，ゆ

が，決してそのために身体の一部に力が入ったり，精神的に焦ったり(過剰に努力)しない状態を「受動的注意集中」という。自然に感じることのできる感覚を興味深く眺めるといった態度で行うことが大切である。

3 練習の回数と時間

自律訓練法の練習は，基本的に毎日，2～3回，1回につき3～5分ほどの時間を確保する(第1～2公式まで)。普通は，約4～8週間で「重感」あるいは「温感」をマスターできるようになる。この重感・温感練習が習得できれば，リラックスには十分な効果がある。

4 消去動作

終了時には必ず消去動作を行うようにする。消去動作とは，両手を強く握ってこぶしを作り，それをパッと開く。これを2～3回繰り返し，次に両手を曲げたり伸ばしたりする。次に，2～3回深呼吸をして目を開く。

5 訓練の記録

練習中は，いろいろな思い出や現在の心配事，将来の不安など(いわゆる雑念)が浮かんでくる。これらは浮かぶにまかせて，ただ公式の言葉(両腕が重いなど)を心のなかで繰り返していると，やがて気にならなくなる。

(2) 標準練習

ATの基本となる技法であり，最も多く用いられている標準練習は，背景公式と6段階の言語公式により構成されている。

1 背景公式(安静練習)：「気持ちがとても落ち着いている」

この公式は標準練習のなかで最も基本となるものである。呼気にあわせて公式語句をゆっくりと暗唱する。

2 第1公式(重感練習)：「両腕両足が重い」

この練習は，四肢の筋肉を弛緩させることを目的とする。まず利き腕から始め，四肢全部に重感を感じるようになったら，「両腕両足が重い」と暗唱する。

3 第2公式(温感練習)：「両腕両足が温かい」

「両腕両足が温かい」と暗唱すると，血管が拡張し，末梢の血流量が増大し，訓練中には指先の温度が1～1.5℃ほど上昇する。重感練習と同様に利き腕から始め，四肢全部に温感を感じるようになったら，「両腕両足が温かい」とまとめる。

この練習の流れは，背景公式→重感練習→温感練習→消去動作となる。このプロセスが最も基本的な練習である。重感，温感練習とも，下肢の感覚はあまりはっきりしないことが多い。

4 第3～6公式：以下のとおりである

第3公式(心臓調節)：心臓が静かに規則正しく打っている。

第4公式(呼吸調整)：楽に呼吸をしている。あるいは，呼吸が楽だ。

第5公式(腹部温感練習)：おなかが温かい。あるいは，胃のあたりが温かい。

第6公式(額部涼感練習)：額が(心よく)涼しい。

一般に第1・2公式をマスターするだけでも80%くらいの効果が期待されるため，第3公式以降を省略する簡易ATを行う指導者も少なくない。心臓・肺・腹部に疾患を有する人では，第3・4・5公式を行わないほうがよいとされている。

(佐藤　武)

文献

1) 佐々木雄二：自律訓練法の実際，pp1-229，創元社，2001

5. バイオフィードバック

1) バイオフィードバックの概念

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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.

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 x^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]:

Δsteps/day = ∑{(steps/day at Week X) – (steps/day at Week 0)} *(X=1-16)

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/m^2)	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.

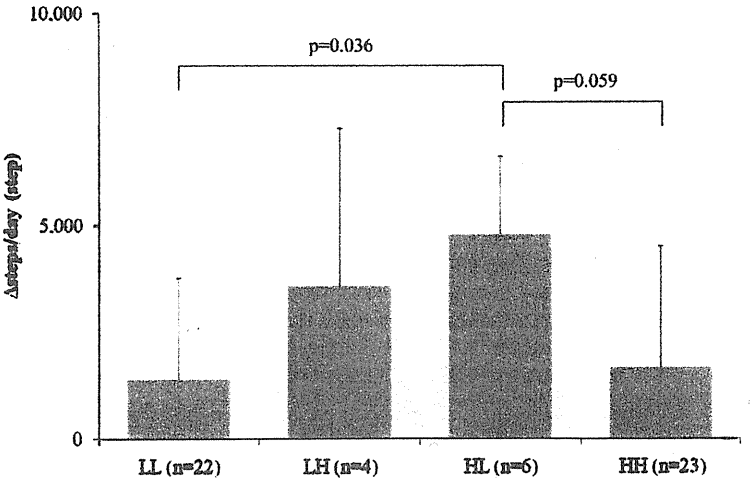

Fig. 1. **Comparison of the change in steps/day among four groups based on the cut-off value of baPWV**
Error bars show SD.

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.

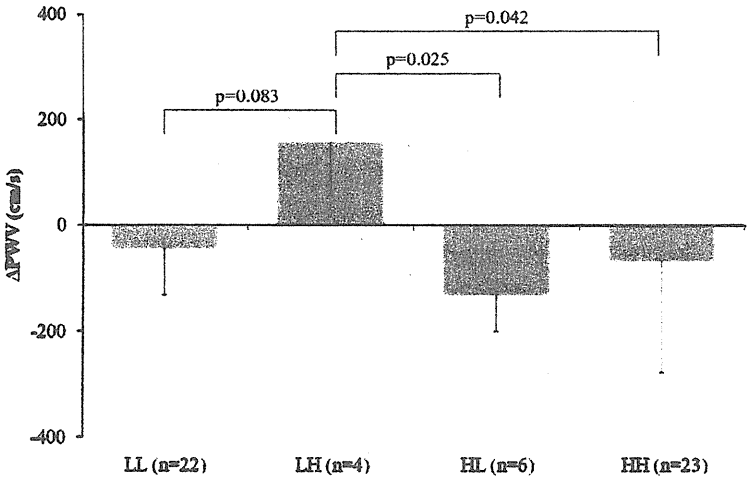

Fig. 2. **Comparison of the change in baPWV among four groups based on the cut-off value of baPWV**
Error bars show SD.

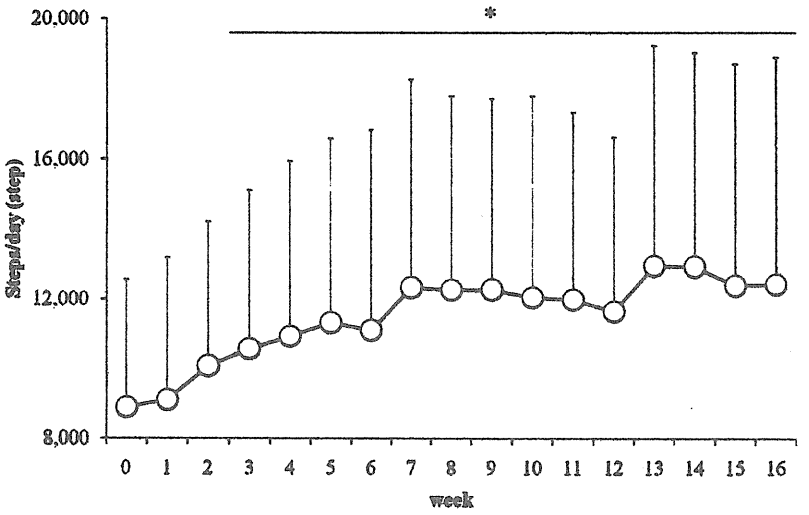

Fig. 3a. **The average steps/day of vascular age-older group during the study**
*p<0.05 compared with week 0. Error bars show SD.

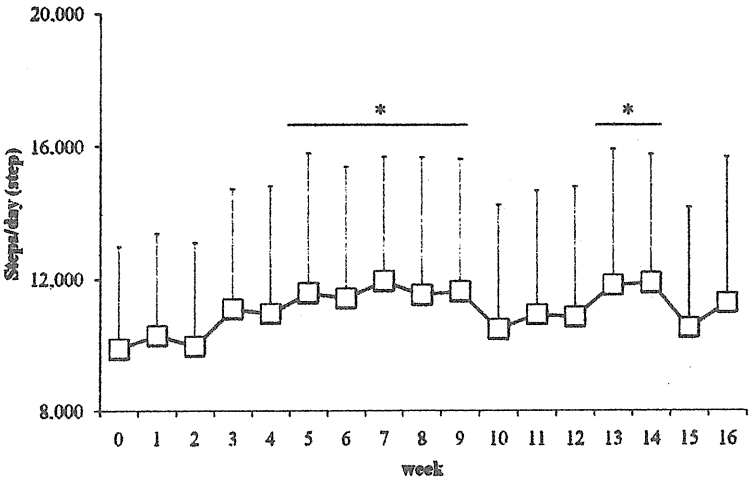

Fig. 3b. **The average steps/day of vascular age-younger group during the study**
*$p<0.05$ compared with week 0. Error bars show SD.

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.

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References

1) van Popele NM, Grobbee DE, Bots ML, et al: Association between arterial stiffness and atherosclerosis: the Rotterdam Study. Stroke 32(2); 454-60: 2001
2) Yamashina A, Tomiyama H, Takeda K, et al: Validity, reproducibility, and clinical significance of noninvasive branch-ankle pulse wave velocity measurement. Hypertension Res 25; 359-64: 2002
3) The Japanese Society of Hypertension: Hypertension Treatment Guidelines (JSH2009). Life Science Publishing Co., Ltd. 2009 (in Japanese)
4) Seals DR, Desouza CA, Donato AJ, et al: Habitual exercise and arterial aging. J Appl Physiol 105(4); 1323-32: 2008
5) Gregg EW, Cauley JA, Stone K: Relationship of changes in physical activity and mortality among older women. JAMA 289(18); 2379-86: 2003
6) Stessman J, Hammerman-Rozenberg R, Cohen A, et al: Physical Activity, Function, and Longevity Among the Very Old. Arch Intern Med 169(16); 1476-83: 2009
7) Morris JN. Hardman AE: Walking to health. Br J Sports Med 31(4); 277-84: 1997
8) Hakim AA, Curb JD, Petrovitch H, et al: Effects of walking on coronary heart disease in elderly men: the Honolulu Heart Program. Circulation 100(1); 9-13: 1999
9) Okamoto T, Masuhara M, Ikuta K: Home-based resistance training improves arterial stiffness in healthy premenopausal women. Eur J Appl Physiol 107(1); 113-7: 2009
10) Tanaka H, Dinenno FA, Monahan KD, et al: Aging, habitual exercise, and dynamic arterial compliance. Circulation 102(11); 1270-5: 2000
11) Lazarevic G, Antic S, Cvetkovic T, et al: A physical activity programme and its effects on insulin resistance and oxidative defense in obese male patients with type 2 diabetes mellitus. Diabetes Metab 32(6); 5835-90: 2006
12) Casey DP, Pierce GL, Howe KS, et al: Effect of resistance training on arterial wave reflection and brachial artery reactivity in normotensive postmenopausal women. Eur J Appl Physiol 100(4); 403-8: 2007
13) Miura H, Nakagawa E, Takahashi Y: Influence of group training frequency on arterial stiffness in elderly women. Eur J Appl Physiol 104(6); 1039-44: 2008
14) Yoshizawa M, Maeda S, Miyaki A, et al: Effect of 12 weeks of moderate-intensity resistance training on arterial stiffness: a randomised controlled trial in women aged 32-59 years. Br J Sports Med 43(8); 615-8: 2009
15) Miyazaki R, Ishii K, Ichikawa H, et al: Community medicine and Anti-Aging: effects of combining a long-term pedometer-based physical activity program with Anti-Aging medical checkups on health and Anti-Aging medical indicators in community-dwelling older adults (Yurin Study 1). Anti-Aging Med 7(12); 143-52: 2010
16) Japan Health Promotion & Fitness Foundation, Healthy Japan 21, National health promotion movement in the 21st century, Tokyo, Japan Health Promotion & Fitness Foundation, 2000
17) Tudor-Locke C, Bassett DR Jr.: How many steps/day are enough? Preliminary pedometer indices for public health. Sports Med 34(1); 1-8: 2004
18) Tsuchikura S, Shoji T, Kimoto E, et al: Brachial-ankle pulse wave velocity as an index of central arterial stiffness. J Atheroscler Thromb 17(6); 658-65: 2010
19) Arai T, Koji Y, Tsuda H, et al: Cardiovascular diseases and pulse wave velocity. Mebio 18; 135-9: 2001 (in Japanese)
20) Nakae S, Chiba H, Ishii K: Effects of 1-year intervention using pedometers and newsletters on the body composition, blood pressure and serum lipids in the elderly. Journal of Japan Society for the Study of Obesity 13; 130-6: 2007 (in Japanese)
21) Matsui H, Taniguchi N, Kario K, et al: Measurements of pulse wave velocity as a new marker of atherosclerosis. The Journal of Japanese Medical Instruments 80(4); 343-9: 2010 (in Japanese)
22) Turin TC, Kita Y, Rumana N, et al: Brachial-ankle pulse wave velocity predicts all-cause mortality in the general population: findings from the Takashima study, Japan. Hypertens Res 33(9); 922-5: 2010
23) Aso K, Miyata M, Kubo T, et al: Brachial-ankle pulse wave velocity is useful for evaluation of complications in type 2 diabetic patients. Hypertens Res 26(10); 807-13: 2003
24) Kim DH, Kim J, Kim JM, et al: Increased brachial-ankle pulse wave velocity is independently associated with risk of cerebral ischemic small vessel disease in elderly hypertensive patients. Clin Neurol Neurosurg 110(6); 599-604: 2008
25) Tomiyama H, Koji Y, Yambe M, et al: Brachial - Ankle Pulse Wave Velocity is a Simple and Independent Predictor of Prognosis in Patients With Acute Coronary Syndrome. Circ J 69(7); 815-22: 2005
26) Burattini R, Campbell KB: Comparative analysis of aortic impedance and wave reflection in ferrets and dogs. Am J Physiol Heart Circ Physiol 282(1); H244-255: 2002
27) Tomiyama H, Yamashina A, Arai T, et al: Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement -a survey of 12517 subjects. Atherosclerosis 166(2); 303-9: 2003
28) Fujiwara Y, Chaves P, Takahashi R et al, Relationships between brachial-ankle pulse wave velocity and conventional atherosclerotic risk factors in community-dwelling people. Prev Med 39(6); 1135-42: 2004
29) Maruyama Y: Aging-related arterial-cardiac interaction in Japanese men. Heart Vessels 24(6); 406-12: 2009
30) Hokkaido Health Promotion Foundation: Hokkaido Health Promotion Basic Guidelines, 2006 (in Japanese)
31) Grover SA, Lowensteyn I, Joseph L, et al: Patient knowledge of coronary risk profile improves the effectiveness of dyslipidemia therapy: the CHECK-UP study: a randomized controlled trial. Arch Intern Med 167(21); 2296-303: 2007
32) Parkes G, Greenhalgh T, Griffin M, et al: Effect on smoking quit rate of telling patients their lung age: the Step2quit randomised controlled trial. BMJ 336(7644); 598-600: 2008
33) Bravata DM, Smith-Spangler C, Sundaram V, et al: Using pedometers to increase physical activity and improve health: a systematic review. JAMA 298(19); 2296-304: 2007
34) Amoh-Tonto CA, Malik AR, Kondragunta V, et al: Brachial-ankle pulse wave velocity is associated with walking distance in patients referred for peripheral arterial disease evaluation. Atherosclerosis 206(1); 173-8: 2009
35) Ochi M, Kohara K, Tabara Y, et al: Arterial stiffness is associated with low thigh muscle mass in middle-aged to elderly men. Atherosclerosis 212(1); 327-32: 2010

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原著論文

高強度身体活動はメンタルヘルス低下の防御因子である：大学体育の場を活用した6ヶ月の縦断研究

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High-intensity physical activity reduces the risk of future onsets in poor psychological well-beings Japanese adolescents : Longitudinal study in university health-related physical education

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Abstract

PURPOSE: To determine whether physical activity and/or sedentary behavior reduces the risk for future onsets in poor mental health across a 6-month period among university students.

METHOD: Participants (N=559) completed the International Physical Activity Questionnaires short version (Murase et al., 2002), the exercise self-efficacy (Oka, 2003), and the General Health Questionnaires 12 items (Honda et al., 2001) in the May of 2010 (baseline data) and October of 2010 (follow-up data).

RESULTS: Among 559 university students, 27.0% (male: 21.2%, female: 33.3%) of them had a poor mental health at baseline. Using logistic regression analyses that controlled for several covariates, we found that high levels of high-intensity physical activity significantly reduced the risk for future developments of poor mental health in males (OR [Odds Ratio]=0.487, 95% CI [confidence interval]：0.247-0.962). Also, high levels of exercise self-efficacy significantly reduced the risk for future developments of poor mental health in males (OR=0.510, 95% CI：0.261-0.996). However, there were not significant relationships between them in females. No predictive effects were for total physical activity and sedentary behavior time in both males and females.

CONCLUSIONS: These results suggested that increasing high-intensity physical activity and/or exercise self-efficacy reduce the risk for future developments of poor mental health among university students.

キーワード：身体活動、メンタルヘルス、心理的良好状態、縦断研究

Key Words : Physical activity, Mental health, Psychological well-being, Longitudinal study

1. 緒言

大学生のメンタルヘルス低下が懸念されている（一宮ら，2003）。大学生を含めた青年期における重度のメンタルヘルス不良は、自殺、学業不振、対人トラブル、未就職、薬物依存や非行行動を増加させるとの報告（Klein et al.,2008）もあり、大学生のメンタルヘルスを良好に保つことは重要である。

青年期のメンタルヘルスを維持・改善させる手

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段のひとつとして、身体活動や運動が注目されている。身体活動・運動が抑うつ傾向に影響するメカニズムとしては生物学的仮説と心理学的仮説が提唱されている。生物学的仮説としては、身体活動・運動がエンドルフィンやモノアミン（セロトニンや、ノルエピネフリン、ドーパミン）を増加させた結果として快感情の増加や抑うつ症状を低減させるというエンドルフィン仮説やモノアミン仮説である（O'Neal et al.,2000)。心理学的仮説としては、身体活動・運動が不快感情から気をそらすことで抑うつ傾向を抑えるという気晴らし仮説（Just and Alloy,1997)や、身体活動・運動はセルフエスティームを高め抑うつを減少させるという仮説（Ekeland et al.,2005)が有力視されている。実際に、青年期を対象者としたトレーニング研究では、身体活動・運動がメンタルヘルスを改善できると考えられている。Calfas and Talor(1994)の総説では、身体活動による抑うつ低減効果は比較的高いことが示され、中等度の有酸素運動を週3回計60分以上行うことが推奨されている。同様の結論が、次々に報告されている(Field,2012)。

一方、青年期における身体活動とメンタルヘルスの観察疫学に基づく研究の結果は、その見解が一致しているとはいいがたい。いくつかの横断研究では、低水準の身体活動と抑うつ症状に有意な関連性が認められている（荒井ら，2005；Motl et al.,2004；Norris et al.,1992)ものの、関連性が認められないという報告（Haarasilta et al.,2004)もあり、身体活動と抑うつの関連性の有無の結果は一貫していない。また、横断研究の知見をいくら積みあげても、身体活動とメンタルヘルスに関する因果関係には言及できない。

青年期における身体活動の実施が将来のメンタルヘルス低下を予防できるかを検討した縦断研究は少ない。数は少ないものの、海外で行われた縦断研究では、青年女性における身体活動は将来のメンタルヘルス低下を抑制するという報告(Jerstad et al.,2010)がある一方で、そのような結果は認められないという報告（Rothon et al.,2010；Birkeland et al.,2009)もあり、こちらの結果も一貫性がない。観察疫学研究における結果の相違を生じさせる原因のひとつは、身体活動の測定指標の精度の問題が想定される。先述の3つの縦断研究においても、簡便な測定指標が用いられているため、測定の信頼性が担保されているとはいいがたい。標準化された指標を利用した縦断研究を行う必要がある。また、身体を動かさない不活動に関する研究も注目されつつあるが、身体不活動とメンタルヘルスの関連性を縦断的に検討した研究（Cao et al.,2011)は少ない。

そこで、本研究の目的は、標準化された信頼できる身体活動指標を用い、大学体育の場を活用した6ヵ月の縦断研究により、1)大学1年生のメンタルヘルスの短期の変化を明らかにするとともに、2)身体活動および不活動が将来のメンタルヘルス不良を予測できるかを検証することであった。

2. 方法

1）調査対象者およびデータ収集

本研究の対象者は九州北部の国立4年制大学にて開講される「健康スポーツ科目」を受講し、計2回の質問紙調査に回答した大学1年生559名（女性47.8%、年齢18.3±0.8歳)であった。本研究で扱うデータは、大学1年時の必修科目として開講されている「健康スポーツ科目」の授業時に実施された調査によって得られたものである。

本研究の調査時期は5月と10月であった。初回調査は入学後1ヵ月が経過した連休明けの5月で、629名(男性338名、女性291名)が回答した。2回目の追跡調査は夏季休暇明けの10月で、「健康スポーツ科目」の後学期開始時に行った。追跡調査の回答者は570名（男性297名、女性273名)で回収率は90.6%であった。

本研究の解析対象者は計2回の調査に参加し回答不備のなかった559名(男性292名、女性267名)であった。全対象者には初回調査時に研究の趣旨を説明し、研究参加の同意を得られた者から調査用紙を回収した。

2）調査項目

(1) 身体活動・不活動

村瀬ら(2002)によって加速度計との比較により日本語版の妥当性と信頼性が検証されている International Physical Activity Questionnaire 日本語短縮版（I-PAQ）を用いた。I-PAQは世界保健機関のワーキンググループによって、身体活動を評価し国際比較するために作成された。過去1週間または平均的な1週間において高強度および中等度の身体活動について実施した日数ならびに時間を質問し、8 METs以上の高強度、4～7 METsの中等度、そして歩行という3種の活動量を算出することができる。本研究では上記3指標を合計し「総身体活動量（METs・時/週）」を算出した。また、不活動の指標として平日と休日の座位を中心とした不活動時間を平均し「不活動時間（時間/日）」を算出した。I-PAQ短縮版における不活動時間とは「毎日座ったり寝転んだりして過ごしている時間(仕事中、自宅で、勉強中、余暇時間など)についてです。すなわち、机に向かったり、友人とおしゃべりをしたり、読書をしたり、座ったり、寝転んでテレビを見たり、といった全ての時間を含みます。なお、睡眠時間は含めません」と定義されている。

(2) 運動セルフエフィカシー

岡(2003)によって信頼性と妥当性が検証された運動セルフエフィカシー尺度を用いた。運動セルフエフィカシーは、運動の実施・継続に関連する指標であり、異なる状況や障害におかれても、逆戻りすることなく運動を継続して行うことができる見込み感を測定する尺度である。本尺度は5項目（うち1項目は無関項目）で構成されており、回答の際は「全くそう思わない（1点）」から「かなりそう思う（5点）」の5つからあてはまるものを選択させた。本尺度の得点範囲は4点から20点であり、得点が高いほど運動を実施・継続する効力感が高いことを示す。

(3) メンタルヘルス

本田ら(2001)が妥当性と信頼性の検証を行ったGeneral Health Questionnaire 12項目版(GHQ 12)を用いた。GHQ 12は抑うつや不眠などの精神医学的症状に関する12の質問項目について、以前に比べ最近1ヶ月間の症状の頻度を4段階の中から選び回答するものである。各項目に対し「特に多い」または「いつもより多い」など抑うつ度が高いと判断される最後2つのカテゴリーを選んだ場合に1点、その他を0点とする。12項目の合計得点が高いほどメンタルヘルスが不良であることを示す。また、本田ら(2001)が提案している4点をカットオフ値とし、4点以上を精神医学的な問題あり(メンタルヘルス不良)と見なした。

(4) その他の調査項目

性、年齢、および運動部または運動系サークル活動の実施状況を尋ねた。

3）分析

初回調査時の対象者特性の比較では、カテゴリー変数に対してはχ^2検定を、他はすべて対応のないt検定を用いた。追跡調査時のメンタルヘルス不良の発現に影響する身体活動・不活動の関連要因を検証する解析では、メンタルヘルスに対する性差を考慮し男女別に以下の検討を行った。すなわち、各独立変数の中央値以下を低群、中央値より大きいものを高群とし、初回調査時のメンタルヘルス不良の有無と年齢を調整したロジスティック回帰分析を行った。

エクササイズガイド2006(運動所要量・運動指針の策定検討会，2006)の運動基準の充足状況とメンタルヘルス不良の関連性を検討するために、初回または追跡調査時の総身体活動量が23 METs・時/週以上を1、未満を0とし解析に用いた。また、スポーツ系の運動部やサークル所属とメンタルヘルス不良との関連性を検討するために、スポーツ系の運動部やサークル所属を1、所属していない場合を0として解析を行った。

全ての統計解析は統計ソフトSPSS 17.0 Jを用い、有意水準は5％未満とした。

3. 結果

(1) 初回調査時の対象者の特性

調査対象者の初回調査時の平均年齢は18.3±0.8歳で、性差は認められなかった（男性18.3±1.0歳、女性18.2±0.5歳）。I-PAQで評価された総身体活動量は38.2±50.2 METs・時/週であり、その内訳は高強度が21.8±38.6 METs・時/週、中等度が8.7±17.1 METs・時/週、歩行が7.7±13.0 METs・時/週であった。総身体活動量と高強度身体活動量に男女差が認められた。男性の総身体活動量は49.7±58.6 METs・時/週で女性の25.7±35.0 METs・時/週より有意に高く、また男性の高強度活動量は32.8±46.3 METs・時/週で女性の9.9±23.0 METs・時/週より有意に高いという結果であった($p<0.05$)。

不活動時間の平均値は7.9±4.3時間/日で、内訳は平日が7.5±4.7時間/日、休日が8.2±4.4時間/日であった。不活動時間には有意な男女差が認められ、女性の8.5±4.1時間/日が男性の7.3±4.3時間/日より長いという結果であった（$p<0.05$）。

運動セルフエフィカシー得点の平均は11.5±4.1点であり、男性が12.4±4.0点で女性の10.7±3.9点より有意に高かった（$p<0.05$）。

GHQ 12の得点は2.3±2.8点で、女性が2.7±3.1点と男性の1.9±2.5点より有意に高値であった（$p<0.05$）。

運動系の部活動およびサークル活動所属者は40.6%(231名)であり、非運動系サークルの所属者は17.0%(97名)、所属なしが40.6%(231名)であった。運動系の部活動およびサークル活動所属者の割合は男女差が認められ、男性が52.7%に対し女性が28.9%であった($p<0.05$)。また、所属なしは女性の50.2%が男性の33.4%より高率であった($p<0.05$)。

(2) 初回調査および追跡調査時のメンタルヘルス不良の発現状況

初回調査時(5月)にGHQ 12が4点以上でメンタルヘルス不良状態にあった大学1年生は27.0%(151名)であり、女性のメンタルヘルス不良者が33.3%で男性の21.2%より高率であった($p<0.05$)。

追跡調査時(10月)のメンタルヘルス不良者は22.7%(127名)であり、女性が28.1%で男性の17.8%より高率であった（$p<0.05$）。

また、初回調査時から追跡調査時のメンタルヘルスの変化を検討した結果、初回調査時も追跡調査時もメンタルヘルス不良が認められなかった者は63.3%(354名)、初回調査時にメンタルヘルス不良が認められたが追跡調査時には解消された者は14.0%(78名)、初回調査時にはなかったが追跡調査時にメンタルヘルス不良が認められた者は9.7%(54名)、初回調査時も追跡調査時もメンタルヘルス不良が認められた者は13.1%(73名)であった。すなわち、本集団のメンタルヘルス不良の新規発現率は9.7%で、解消率は14.0%であった。

(3) 追跡調査時のメンタルヘルス不良の発現に関連する要因の検討（表1、表2）

追跡調査時のメンタルヘルス不良の発現に影響する要因を明らかにするために、初回調査時のメンタルヘルス不良の有無と年齢を調整したロジスティック回帰分析を行った。その結果、男性では高強度の身体活動量（オッズ比0.487、95% 信頼区間0.247-0.962）と運動セルフエフィカシー（オッズ比0.510、95% 信頼区間0.261-0.996）に有意な関連性が認められた（表1）。

女性では有意な関連性を認める項目はなかった（表2）。

4. 考察

本研究では、大学1年生のメンタルヘルスの実態とその予測因子についての検証を、大学体育の場を活用して縦断的に行った。その結果、入学後1ヵ月程度の5月時点で、GHQ 12得点が4点以上でメンタルヘルス不良と考えられる大学1年生が27.0%も存在していた。大学1年生にGHQ 12を用いた他の報告がみあたらないため、本研究のメンタルヘルス不良者27.0%という結果が高いかどうかを判断することは難しい。荒井ら(2005)が

表1　男子大学生の追跡調査時のメンタルヘルス不良の発現に影響する身体活動・不活動関連要因

	モデル1（調整なし）			モデル2（初回調査時の抑うつと年齢を調整）		
独立変数（初回調査時）	オッズ比	95%信頼区間		オッズ比	95%信頼区間	
不活動時間[*1]	0.983	0.540 - 1.792		0.907	0.480 - 1.714	
総身体活動量[*1]	0.663	0.362 - 1.216		0.686	0.361 - 1.303	
高強度の身体活動量[*1]	0.491	0.259 - 0.933	*	0.487	0.247 - 0.962	*
中等度の身体活動量[*1]	0.811	0.442 - 1.485		0.757	0.398 - 1.440	
歩行活動量[*1]	1.186	0.650 - 2.164		1.038	0.550 - 1.959	
エクササイズガイド2006運動基準[*2]	0.681	0.308 - 1.510		0.636	0.336 - 1.202	
運動セルフエフィカシー[*1]	0.458	0.243 - 0.862	*	0.510	0.261 - 0.996	*
部活動[*3]	0.666	0.381 - 1.162		0.738	0.389 - 1.401	

*1 各指標の中央値以下を0．それを越える群を1として解析　　* $p<0.05$

*2 23METs・時未満を0、23METs・時以上を1として解析

*3 部活動については非運動部・所属無を0、運動部やスポーツ系サークル所属を1として解析

表2　女子大学生の追跡調査時のメンタルヘルス不良の発現に影響する身体活動・不活動関連要因

	モデル1（調整なし）		モデル2（初回調査時の抑うつと年齢を調整）	
独立変数（初回調査時）	オッズ比	95%信頼区間	オッズ比	95%信頼区間
不活動時間[*1]	0.777	0.456 - 1.323	0.885	0.487 - 1.607
総身体活動量[*1]	1.150	0.678 - 1.952	1.389	0.762 - 2.535
高強度の身体活動量[*1]	0.831	0.462 - 1.495	0.913	0.476 - 1.749
中等度の身体活動量[*1]	0.984	0.578 - 1.675	1.089	0.599 - 1.979
歩行活動量[*1]	1.037	0.611 - 1.759	0.903	0.499 - 1.635
エクササイズガイド2006運動基準[*2]	1.228	0.711 - 2.121	1.453	0.782 - 2.699
運動セルフエフィカシー[*1]	0.712	0.418 - 1.213	0.771	0.424 - 1.402
部活動[*3]	0.774	0.431 - 1.389	0.725	0.375 - 1.401

*1 各指標の中央値以下を0．それを越える群を1として解析　　* $p<0.05$

*2 23METs・時未満を0、23METs・時以上を1として解析

*3 部活動については非運動部・所属無を0、運動部やスポーツ系サークル所属を1として解析

報告しているHospital Anxiety and Depression Scale質問表（HADS）にて工科系大学1年生の男子を対象とした横断的研究では、HADS 11点以上で抑うつ傾向にある者は16.6%であった。測定指標の違いにより単純に比較はできないが、本研究における初回調査時のメンタルヘルス不良者は全体で27.0%、男子学生に限っても21.2%と荒井らが報告した16.6%より多いものであった。海外からの報告（Rothon et al.，2010）では、11歳から14歳までの英国の集団3322名のうち抑うつ傾向者は24.5%と、対象者の年代や測定指標が異なるものの本研究の結果とほぼ同等であった。

本研究では2回の調査を縦断的に行うことで、大学1年生のメンタルヘルスの変化を観察することができた。その結果、6ヵ月という短期間の中でもメンタルヘルスが変化することが明らかとなった。また、メンタルヘルス不良の新規発現は9.7%、メンタルヘルス不良状態から解消された者が14.0%であることが示された。今後、異なる年度の入学生、上級学生、異なる大学での検討が不可欠である。

本研究では身体活動や不活動がメンタルヘルス不良の予測因子であるかを検証することができた。その結果、男子学生において、高強度身体活動量と運動セルフエフィカシーに有意な関係が認められた。すなわち、初回調査時の高強度身体活動量が中央値より高い群ではそれ以下の群に対して51%も追跡調査時のメンタルヘルス不良者が少なかった。運動セルフエフィカシーも同様に、中央値より高い群ではそれ以下の群に対して追跡調査時のメンタルヘルス不良者が49%も少なかったのである。これらの結果は、男子学生では高強度身体活動量や運動セルフエフィカシーが将来のメンタルヘルス低下の防御因子となりうることを示している。

青年男子に限られるものの、高強度の身体活動が特異的にメンタルヘルス低下の防御因子となりうることを示した研究は我々が知る限り皆無である。本研究は高強度の身体活動が男子学生のメンタルヘルス低下を特異的に防ぐことを示した初めての研究といえよう。海外の先行研究では、強度別ではなく、全体または中等度以上の身体活動量を独立変数とした縦断研究（Motl et al.,2004；Sagatun et al.,2007；Ströhle et al.,2007；Jerstad et al.,2010）が報告されており、いずれも身体活動量が高い者で将来の抑うつ傾向者が少ないという結果がでており本研究の結果と一致していた。しかし、身体活動がメンタルヘルス低下の防御因子として認められたという点では一致するものの、それらの関係は男女共に認められたという報告（Ströhle et al.,2007, Motl et al.,2004）がある一方で、明らかな性差（青年男性のみ有意；Sagatun et al.,2007）が認められているという報告もある。Ströhle et al.(2007)は身体活動の抑うつ傾向に対する予防効果は男女共に認められるが、その効果は女性より男性で大きいと考察しているように、身体活動の効果は男性で強く認められやすいのかもしれない。

本研究では運動セルフエフィカシーもメンタルヘルス低下の防御因子となりうることが示された。運動セルフエフィカシーがメンタルヘルス低下の防御因子となりうることを説明しうるメカニズムは存在しない。同様の結果が他集団でも認められるかの検証を進めるとともに、運動セルフエフィカシーがメンタルヘルス低下防御の原因なのか媒介変数なのかを明らかにしていくことが必要である。

本研究で注目した身体不活動や他の身体活動関連指標には有意な結果が認められなかった。身体不活動とメンタルヘルス低下の関係が有意でなかった理由を明らかにすることは難しいが、身体不活動の指標として不活動時間を用いた影響も考えられる。本研究における対象者は座位中心の生活時間を正確に思い出せていないかもしれない。また、テレビ視聴やゲームなどの大学生がよく行う特定の不活動時間を指標として用いることを検討すべきかもしれない。

本研究の長所として、標準化された身体活動指標を用いたこと、さらに縦断研究であったことがあげられる。特に縦断調査を行ったことで因果関係に近づけた点から、因果関係への言及ができない従来の先行研究の知見を進めることができたといえよう。

本研究の限界として、まず本研究のフィールドが1つの大学に限られたことがあげられる。本研究の結果を他大学生にまで一般化できるかは不明である。また、身体活動測定には標準化された質問紙を利用したものの、質問紙法の正確性は種々の生物心理社会的要因に影響されることが知られている（山津ら、2003）。今後の研究でより信頼できる結論を得るには、加速度計などのより客観的な身体活動指標の利用が望まれる。さらに、より

長期の追跡を実施していくこと、異なる研究デザインでの検討、他のライフスタイルやライフイベントなどの影響について検討する必要がある。

今後の課題として、本結果の再現性を異なる集団で確認する必要がある。本結果が他大学や入学年度の異なる男子大学1年生に一般化できれば、教養教育における大学体育の中でメンタルヘルス低下の予防対策が展開できる可能性が生じるからである。また、必修科目と位置づけられている大学入門科目の中でスポーツ活動を通じた高強度身体活動の実践や交友関係の強化により、大学1年生のメンタルヘルス対策を展開できる可能性がある。

付記

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引用文献

荒井弘和，中村友浩，木内敦詞，浦井良太郎(2005) 男子大学生における身体活動・運動と不安・抑うつ傾向との関係．心身医学，45:865–871.

Birkeland MS,Torsheim T,Wold B(2009) A longitudinal study of the relationship between leisure–time physicalactivity and depressed mood among adolescents. Psychology of Sport and Exercise,10(1):25–34.

Calfas KJ,Taylor WC (1994) Effects of physical activity on psychological variables in adolescents.Pediatric Exercise Science, 6: 406–423.

Cao H,Qian Q,Weng T,Yuan C,Sun Y,Wang H, Tao F(2011) Screen time,physical activity and mental health among urban adolescents in China. Preventive Medicine ,53:316–320.

Ekeland E,Heian F,Hagen KB.(2005) Can exercise improve self–esteem in children and young people? A systematic review of randomized controlled trials . British Journal of Sports Medicine ,39:792–798.

Field T.(2012) Exercise research on children and adolescents. Complementary Therapies in Clinical Practice ,18(1):54–59.

Haarasilta LM,Marttunen MJ,Kapiro JA,Aro HM (2004) Correlates of depression in a representative nationalwide sample of adolescents (15–19 years)and young adults (20–24 years).European Journal of Public Health, 14(3):280–285.

本田純久，柴田義貞，中根充文(2001) GHQ–12項目質問紙を用いた精神医学的障害のスクリーニング，厚生の指標，48(10):5–10.

一宮厚，馬場園明，福盛英明，峰松修(2003) 大学新入生の精神状態の変化：最近14年間の質問紙による調査．精神医学，49(9)，959–996.

Jerstad SJ,Boutelle KN,Ness KK,Stice E(2010) Prospective reciprocal relations between physical activity and depression in female adolescents.Journal of Consulting and Clinical Psychology, 78(2), 268–272.

Just N,Alloy LB(1997)The response styles theory of depression:tests and an extension of the theory.Journal of Abnormal Psychology, 106 (2):221–229.

Klein DN, Torpey DC, Bufferd SJ, Dyson MW. (2008)Depressive disorders. In:Beauchaine, TP, ; Hinshaw SP, editors. Child and adolescent psychopathogy. Hoboken, NewJersey:John Wiley & Sons: pp.477–509.

Motl RW,Birnbaum AS,Kubik MY,Dishman RK (2004) Naturally occurring changes in physical activity are inversely related to depressive symptoms during early adolescence. Psychosomatic Medicine,66:336 –342.

Norris R,Carroll D,Cochrane R(1992)The effects of physical activity and exercise training on psychological stress and well–being in an adolescent population. Journal of Psychosomatic Research, 36(1):55–65.

村瀬訓生，勝村俊仁，上田千穂子，井上茂，下光輝一(2002) 身体活動量の国際標準化：IPAQ日本語版の信頼性，妥当性の評価．厚生の指標，49(11):1–9.

岡浩一郎(2003) 中高年者における運動行動変容の段階と運動セルフエフィカシーの関係．日本公衆衛生雑誌，50:208–215.

O'Neal HA, Dunn AL, Martinsen EW (2000) Depression and exercise. International Journal of Sport Psychology, 31:110–135.

Rothon C,Edwards P,Bhui K,Viner RM,Taylor S, Stansfeld SA(2010)Physical activity and depressive symptoms in adolescents : a prospective study. BMC Medicine, 8 : 32. (http://www.biomedcentral.com/1741–7015/8/32)

Sagatun A, Søgaard AJ, Bjertness E, Selmer R (2007) The association between weekly hours of physical activity and mental health:A three–year follow–up study of 15–16–year–old students in the city of Oslo, Norway. BMC Public Health 2007,7 : 155 (doi:10.1186/1471–2458–7–155)

Ströhle A,Höfler M,Pfister H,Müller AG,Hoyer J, Wittchen HU,Lieb R(2007)Physical activity and prevalence and incidence of mental disorders in adolescents and young adults. Psychological Medicine. 37(11):1657–1666.

運動所要量・運動指針の策定検討会(2006) 健康づくりのための運動基準2006, pp.1–26. (http://www.mhlw.go.jp/bunya/kenkou/undou02/pdf/data.pdf)

山津幸司, 山口幸生, 足達淑子(2003) 質問紙法による身体活動量の正確性に影響を及ぼす要因：社会的望ましさと測定間隔の交互作用について. 健康支援, 5:9–17.

英文抄録の和訳

【目的】本研究の目的は、大学生の身体活動や不活動時間が6ヵ月間のメンタルヘルス不良の発現率を低下させるかを検証することである。

【方法】研究対象（559名）は2010年5月（ベースラインデータ）と2010年10月（追跡データ）にInternational Physical Activity Questionnaire短縮版（村瀬ら, 2002)、運動セルフエフィカシー尺度（岡, 2003)、およびGeneral Health Questionnaire 12項目版（本田ら, 2001)に回答した。

【結果】559名の大学生のうち、ベースライン時にメンタルヘルス不良が認められたのは27.0%(男性21.2%、女性33.3%)であった。ロジスティック回帰分析の結果から、交絡因子の調整後も、男子学生の高強度身体活動は将来のメンタルヘルス不良が発現する危険性を低下させていた（オッズ比0.487、95%信頼区間0.247−0.962)。また、運動セルフエフィカシーも将来のメンタルヘルス不良が発現する危険性を低下されることが認められた（オッズ比0.510、95%信頼区間0.261−0.996)。しかし、女子学生ではそれらの関係は認められなかった。総身体活動量と不活動時間には有意な予測効果は男女共に認められなかった。

【結論】以上の結果から、高強度身体活動や運動セルフエフィカシーは大学生の将来のメンタルヘルス不良の発現を低下させることが示された。

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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

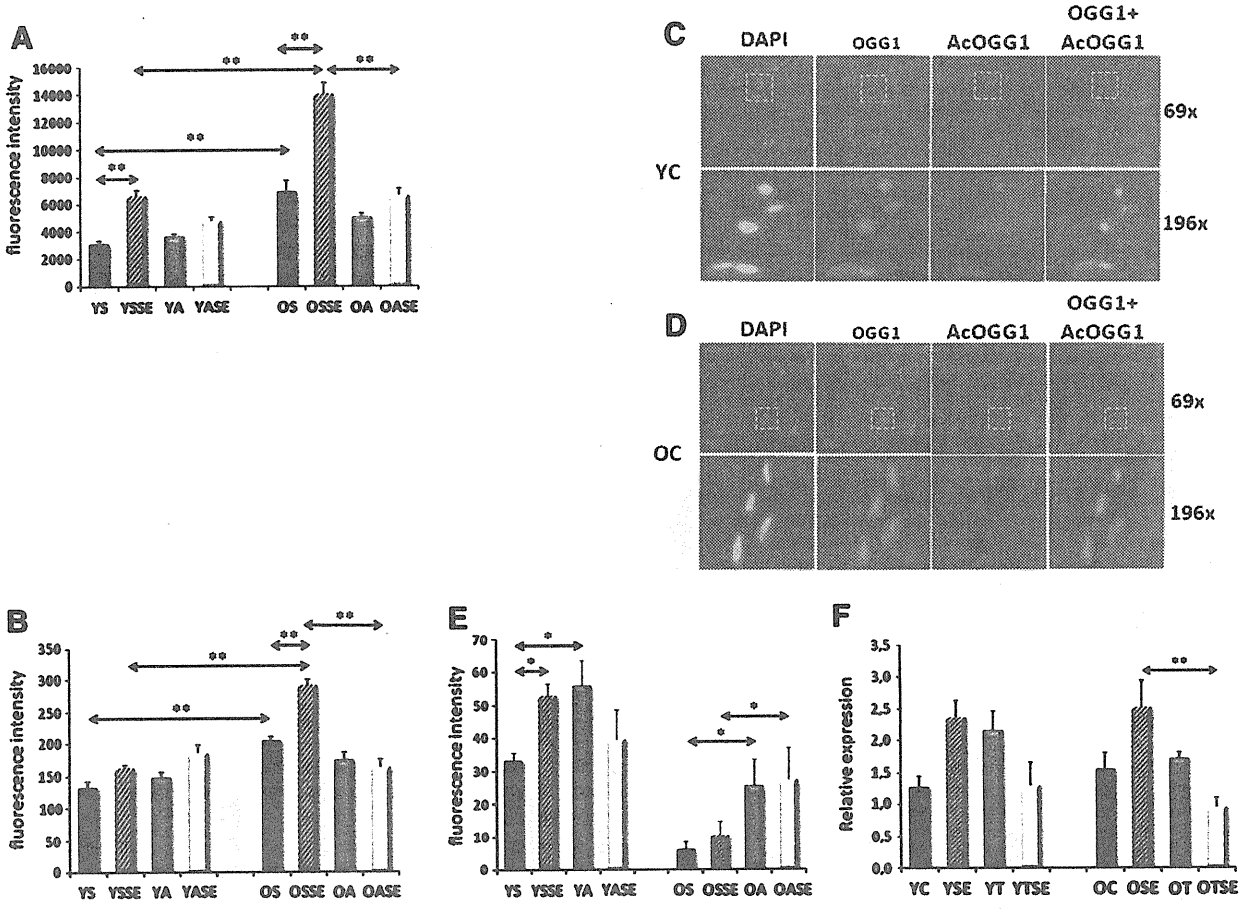

Fig. 1. 8-OxoG, OGG1, and Ac-OGG1 levels in skeletal muscle (SkM) before and after single exercise bout (SEB). (A) Increase in 8-oxoG level in genomic DNA of aged muscles and in response to SEB. (B) Total OGG1 level in SkM of sedentary and physically active subjects. In (A) and (B), sections were stained 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

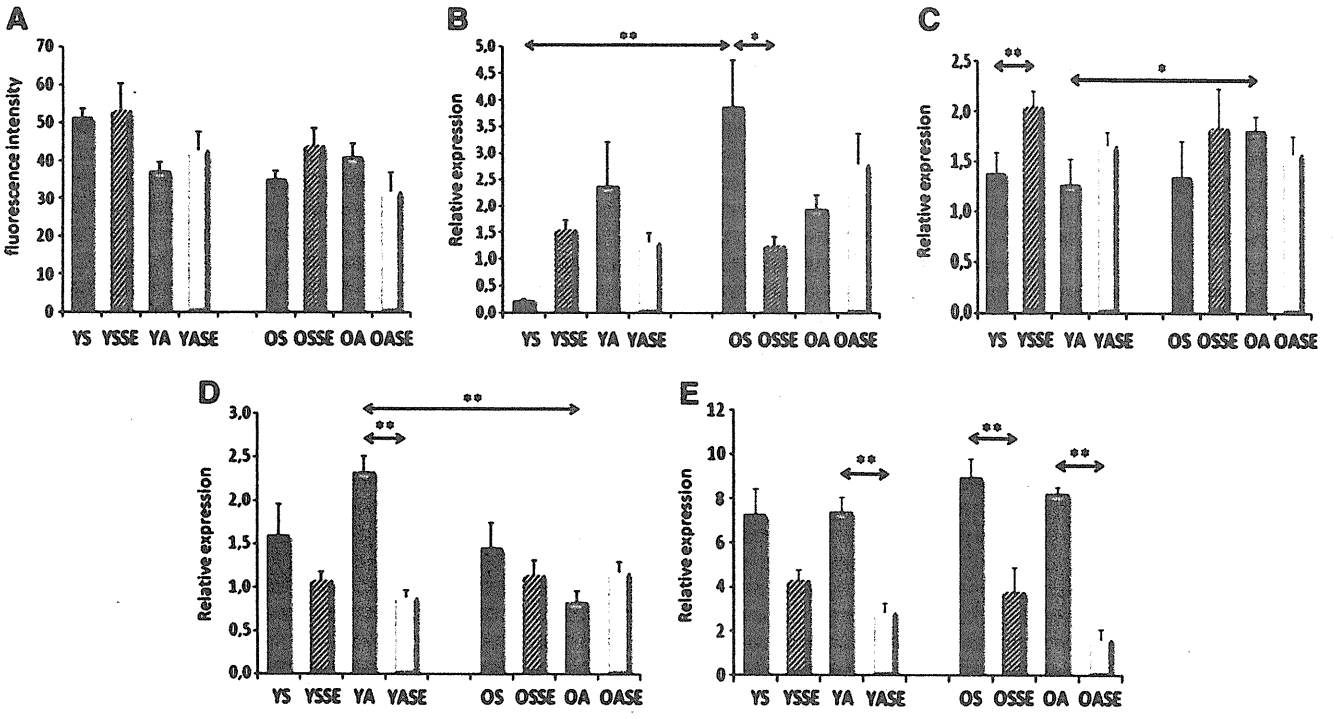

Fig. 2. Ac-APE1 level and expression of p300/CBP, SIRT1, SIRT3, and SIRT6 before and after physical exercise in skeletal muscle. (A) Level of Ac-APE1 as assessed by fluorescence imaging (analyzed as for Fig. 1A). (B–E) Expression at the mRNA level of (B) p300/CBP, (C) SIRT1, (D) SIRT3, and (E) SIRT6. 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. *$p<0.05$, **$p<0.01$.