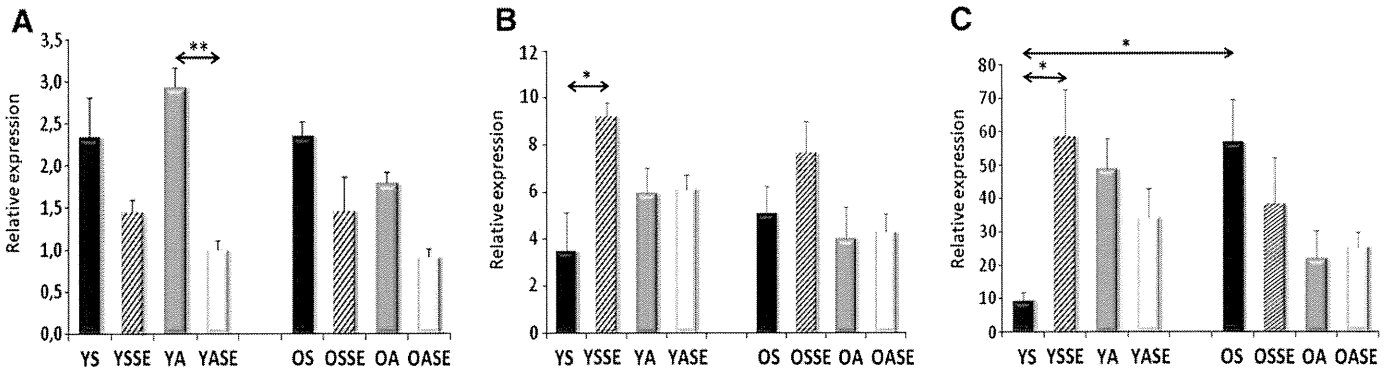

Fig. 3. Effect of SEB on expression of (A) Ku70, (B) Cu,Zn-superoxide dismutase (SOD), and (C) Mn-SOD. RNA was isolated from muscle biopsies excised before and 24 h after SEB. Quantitative RT-PCR was carried out as described under Materials and methods. YS, young sedentary; YSSE, young sedentary after 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. $^{*}p<0.05$, $^{**}p<0.01$.

preexercise levels in physically active individuals, both OA and YA, its level in DNA remained high in sedentary young and old subjects after a 24-h recovery period (Fig. 1A). For example, 8-oxoG levels were approximately four times higher in untrained older (Fig. 1A) compared to younger individuals without SEB (Fig. 1A). Importantly, there was no change in genomic 8-oxoG levels in muscle biopsies of OA individuals after SEB (Fig. 1A).

The subphysiological level of genomic 8-oxoG in physically active subjects suggested an efficient repair of DNA. We observed that OGG1 levels did not significantly change in younger subjects, but they increased in the older subjects in response to SEB (Fig. 1B). In contrast, Ac-OGG1 levels were significantly increased in younger individuals, whereas in the older subjects no significant change was observed in response to SEB. Ac-OGG1 level was approximately threefold higher in active compared to older, sedentary individuals (Figs. 1E and C). SEB did not change Ac-APE1 (Fig. 2A), which was similar to APE1 levels (data not shown), suggesting that neither Ac-APE1 nor APE1 is limiting in the repair of 8-oxoG.

In response to SEB, the expression of p300/CBP increased approximately fivefold in the younger subjects, but unexpectedly, it significantly decreased in older subjects (Fig. 3A). If indeed p300/CBP is the acetyltransferase in muscle, these results are in line with the levels of Ac-OGG1 (Figs. 1C and E). In physically active subjects SEB did not significantly alter p300/CBP levels (Fig. 2B). Expression of the deacetylase SIRT1 showed a significant increase only in younger sedentary subjects in response to SEB (Fig. 2C). The expression of SIRT3, which has no deacetylase activity, was the highest in muscle biopsies of active, younger subjects (Fig. 2D), and its expression did change upon SEB (Fig. 2D). SIRT6 expression (Fig. 2E), along with Ku70 (Fig. 3A), decreased in both young and old muscles after SEB. Together these data suggest that a physically active lifestyle induces an adaptive response by generating mild oxidative stress and prevents the age-associated increase in genomic 8-oxoG levels possibly due to the age-independent increase in OGG1's acetylation.

Discussion

Age-related and physical exercise-associated changes in DNA damage levels in skeletal muscle of experimental animals have been reported previously [13,14,48]. This study analyzed levels of 8-oxoG in DNA and the abundance of rate-limiting BER enzymes in human muscle biopsies before and after a single exercise bout. We also examined expression of acetyltransferases and deacetylases linked to DNA repair pathways and antioxidant genes that could reflect on cellular redox conditions. We show that the genomic 8-oxoG level is lastingly elevated in sedentary young and old subjects, but it returned rapidly to preexercise levels in physically active individuals independent of age upon a single exercise bout. The 8-oxoG level in DNA inversely correlated with the abundance of Ac-OGG1, but not with total OGG1, APE1, or Ac-APE1. Importantly, our data also demonstrate a physical activity-dependent increase in the acetylated forms of OGG1 in human skeletal muscle. Accordingly, it is possible that an exercise-induced acetylation pathway would enhance OGG1 activity, not only in muscles, but in other tissues, and thereby exercise may decrease the incidence of various pathological conditions, such as inflammation, that have been linked to carcinogenesis, cardiovascular diseases, strokes, or Alzheimer disease.

8-oxoG is arguably one of the important forms of DNA base damage induced by ROS, and it has been proposed to play a role in the aging process and is also linked to age-associated diseases [1–3,5]. This hypothesis is consistent with the severalfold increase in 8-oxoG (and possibly of other oxidized bases) content in nuclear and mtDNA from aged tissues [1–3,5]. A single bout of exercise has been shown to cause mild oxidative stress [32,49,50], and thus we applied a SEB and determined cellular oxidative states, changes in 8-oxoG levels, and abundance of selected repair enzymes. Because of a limited amount of muscle biopsies, we used quantitative fluorescence analysis [36,38,41] to assess 8-oxoG levels, as the quantity of DNA isolated did not allow us to use HPLC with electrochemical detection [7,8], which would provide a better estimates. By using a highly specific, anti-8-oxodG-specific antibody, we observed significantly higher levels of genomic 8-oxoG in human skeletal muscle of sedentary, older individuals compared to the levels in younger subjects, in line with previous observations [13,14,43,44]. In response to SEB-induced ROS, 8-oxoG levels increased further and were not repaired, even after a 24-h period, in sedentary individuals, independent of age. In contrast, 8-oxoG levels returned to preexercise levels in physically active individuals, a finding that may mean regular physical activity could prevent accumulation and/or increase repair efficacy of 8-oxoG and possibly other bases in DNA human skeletal muscle.

The observed increase in 8-oxoG levels in sedentary individuals points to a possible age-dependent decrease in levels of OGG1. In contrast, our data show a significantly increased OGG1 level in elderly subjects and, interestingly, SEB furthered its level. Unexpectedly, the 8-oxoG level was also enhanced. These paradoxical observations suggested to us that OGG1 may have a low DNA glycosylase/AP lyase activity or that BER activities are significantly lower in aged human muscle. Indeed, a recent publication documents decreased overall BER activities in both the nuclei and the mitochondrial extracts from skeletal muscles, compared to those from liver or kidneys of the same mice [51]. Although decreased overall BER activity could be a possibility, our data also imply that a lack of or delayed repair of 8-oxoG could be linked to a deficiency in posttranslationally modified OGG1 in aged muscles. Indeed, OGG1's glycosylase/AP-lyase activity is

modulated via acetylation, phosphorylation, and redox [23,25]. For example, OGG1 is acetylated on lysines 338 and 341 and has an approximately 10-fold increase in its 8-oxoG excision activity compared to unacetylated OGG1 [23]. To explore this possibility we show that approximately one-fifth of OGG1 is in an acetylated form in younger individuals and, importantly, Ac-OGG1 was nearly undetectable in the sedentary elderly. This observation is a feasible possibility, as 8-oxoG level in DNA was inversely correlated with levels of Ac-OGG1 in muscles of young and old individuals.

Repair of 8-oxoG is initiated by OGG1 during the BER pathway, followed by APE1-mediated cleavage of the DNA strand at the abasic site. After removal of this 3′-blocking group, the single-nucleotide gap is filled in by a DNA polymerase, and DNA ligase seals the nick to restore DNA integrity [17]. It has also been shown that OGG1 remains tightly bound to its AP product after base excision, and APE1 prevents its reassociation with its product, thus enhancing OGG1 turnover [45]. Accordingly, APE1 is considered to be rate-limiting in the BER of 8-oxoG [17,39]. However, neither APE1 nor Ac-APE1 showed significant changes with aging and/or physical activity. Therefore, it may be proposed that the Ac-OGG1 is limiting in the repair of 8-oxoG lesions in human skeletal muscle during BER processes. As modification by phosphorylation substantially alters the incision activity of only OGG1 [24], our earlier observations of an exercise-induced increase in OGG1 activity in skeletal muscles of human and experimental animals [14,43] may be attributed to Ac-OGG1.

Acetylation levels of OGG1 and APE1 are dependent on the level/activity of the acetyltransferase p300/CBP [23,25] and possibly on a deacetylase(s) such as some of the sirtuins [52]. Results from our studies show that p300/CBP's expression was increased in young individuals by SEB, independent of whether they were sedentary or active. However, we were not able show such consistency in the elderly. SIRT1, a NAD-dependent histone deacetylase [53], has been shown to interact with p300/CBP to regulate its acetyltransferase activity [52]. SIRT1 levels increased in both young and elderly muscles in response to exercise. These observations are in line with the general role of SIRT1 in the DNA damage response and maintenance of genomic integrity, as it promotes proper chromatin structure and DNA damage repair foci formation for repair of DNA base lesions [27,28]; however, the patterns of change in SIRT1 expression in young vs old or sedentary vs physically active suggest an inverse correlation between SIRT1 and the level of Ac-OGG1.

Among sirtuins, only SIRT3 expression correlates with the life span of humans [54]. Interestingly, SIRT3 expression was increased with physical fitness level only in young subjects in this study. SIRT3 has two isoforms with different molecular masses (44 and 28 kDa), which are localized in mitochondria and nucleus, respectively [55]. The translocation of SIRT3 from the nucleus to the mitochondria has been shown to be induced by oxidative stress [55]. SIRT3 is also a modulator of apoptosis [56]. Recent findings also indicate that SIRT3 is a downstream target of PGC-1α and one of the regulators of mitochondrial ROS production [57].

Exercise has been shown to cause mild oxidative stress [32,49,50,58]. Although the 8-oxoG level is a documented measure of such an oxidative insult [14], MDA levels and expression of superoxide dismutase(s) were used to evaluate further SEB-induced oxidative stress. An increase in MDA levels in plasma correlated with genomic 8-oxoG level in both young and old subjects in response to SEB. Interestingly, only the expression of Cu,Zn-SOD showed age-independent and exercise-associated changes, and Mn-SOD expression was increased only in the younger sedentary group. Based on these observations, it appears that Cu,Zn-SOD expression is a better measure of an adaptive response to ROS than that of mitochondrial Mn-SOD. These data also imply a decline in adaptive response with age at the level of Mn-SOD. These observations are in line with those showing that the adaptive capability of an organism to withstand oxidative stress challenge(s) is markedly decreased as a function of age [59,60]. Based on our data, however, we propose that adaptive responses to ROS are not age dependent, but decided by the physical status of an individual.

In conclusion, this investigation offers insight into interactions between aging processes, exercise, and regulation of the repair of oxidized DNA base lesions in human skeletal muscle. We show for the first time that (1) acetylated forms of OGG1 and APE1 are present in human tissues, but (2) only Ac-OGG1 seems to be rate limiting in the BER processes of 8-oxoG, and (3) repair of 8-oxoG seems to be independent of age, but (4) is dependent on the physical state of muscles. Our data also imply that regular exercise induces an adaptive response that involves an improved, more efficient antioxidant and DNA repair machinery.

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－研究資料－

疫学的アプローチによる学生のメンタルヘルス支援に向けた システム構築：身体活動量，食物摂取量 九州大学 P&P 研究 EQUSITE Study 3

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Epidemiological study toward constructing a mental health care system on campus: physical activity and dietary intake

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要 旨

目的：EQUSITE study の一環として，大学生の日常生活における身体活動量および栄養摂取量の実態を明らかにすること。**方法**：2010 年に九州大学に入学した 1 年生を対象に，1 週間の身体活動量の計測，および栄養調査を行った。**結果**：1 日あたりの歩行数の中央値は，男性が 7447 歩，女性が 7488 歩であった。3METs 以上の身体活動量は，男女ともに 4 エクササイズ（EX）であった。摂取エネルギーは，男性が 1839kcal（たんぱく質: 13.2%, 脂質: 26.6%, 炭水化物: 60.0%），女性が 1443kcal（たんぱく質: 13.6%, 脂質: 30.0%, 炭水化物: 56.1%）であった。一人暮らしの学生は実家暮らしの学生と比較して，全ての栄養素において栄養摂取量が有意に少なかった。

キーワード：身体活動量，エクササイズ，栄養摂取量

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1. はじめに

九州大学 P&P プロジェクト（教育研究プログラム・研究拠点形成プロジェクト）EQUSITE Study は，学生のメンタルヘルス改善のための支援システムを構築することを最終目標として 2010 年に発足した疫学研究である。本研究では，学生のメンタルヘルス悪化の危険因子となる生活習慣を明らかにし，その具体的な支援策を講じるために，前向き研究を行う。本年度はベースライン調査を行った。以下に，身体活動および栄養調査の結果をまとめる。

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2. 方法

(1) 対象者

平成22年度に九州大学に入学した1年生2,633名のうち，研究参加への同意が得られた2,119名（80.5%）を本研究の調査対象者とした。このうち，活動量計の装着日が1日未満であった365名および摂取エネルギーが500kcal未満であった2名を除外した1,752名（男性1185名，女性567名，対象者の66.5%，調査同意者の82.7%）を解析対象者とした。

(2) 測定方法

1) 身体活動量

身体活動量計は，3軸加速度センサー活動量計（Active Style Pro HJA-350IT, オムロン社製，以降活動量計と略す）を用いた。本活動量計の特徴は，身体の動きと姿勢の変化を捉え様々な活動を識別することで，歩行時の活動量だけでなく，従来の活動量計では過小評価していた生活活動時の活動量についても精度良く計測できる点にある。活動量計の信頼性および妥当性については，二重標識水（DLW）法により計測された消費エネルギー量との比較により確認されている（$r=0.859$, $p<0.05$）。活動量計の装着は，入浴および入水時以外の起床から就寝までとし，装着期間は1週間とした。解析には，活動量計を8時間以上装着した日のデータを使用した。測定期間中，参加者が計測値を閲覧しないように画面表示は日時のみとした。

2) 栄養調査

栄養調査には,簡易型自記式食事歴調査紙(brief-type self-administered diet history questionnaire: BDHQ)を使用し，過去1ヶ月間の栄養摂取量を推定した。

3) 統計解析

一人暮らしと実家暮らしにおける栄養摂取量の比較には，Wilcoxonの順位和検定を用いた。有意水準は5%未満とした。統計解析にはSAS（var 9.2）を使用した。

3. 結果

1) 対象者の特性

対象者の特性を表1に示す。

表1. 対象者の特性

	男性 N=1185	女性 N=567
年齢, 才	18 (1)	18 (1)
身長, cm	171.0 (8.1)	158.1 (6.9)
体重, kg[a)]	61.8 (11.8)	51.2 (8.7)
Body Mass Index, kg/m^2	21.2 (3.6)	20.4 (2.9)

中央値（四分位偏差）で表す

[a)] 平均値（標準偏差）

2) 身体活動量

1日あたりの平均身体活動量を表2に示す。

表2. 1日あたりの平均身体活動量

	男性 N=1185	女性 N=567
歩行数, 歩/日	7447 (3121)	7488 (2895)
歩行時間		
2≤METs<3, 分/日	45 (28)	40 (19)
3≤METs<6, 分/日	55 (28)	51 (24)
6≤METs , 分/日	2 (4)	1 (3)
歩行以外の活動時間		
2≤METs<3, 分/日	49 (27)	61 (29)
3≤METs<6, 分/日	6 (6)	8 (6)
6≤METs , 分/日	0 (0)	0 (0)
EX, METs*h/日	4 (2)	4 (2)
総消費カロリー, kcal/日	2491 (336)	1973 (237)
運動による消費カロリー, kcal/	649 (213.2)	517 (161)

中央値（四分位偏差）で表す

次に，1日のうち各運動強度が占める時間の割合を図1に示す。2METs未満の活動を不活動，2METs以上3METs未満の活動を低強度，3METs以上6METs未満の活動を中等度，6METs以上の活動を高強度と定義した。

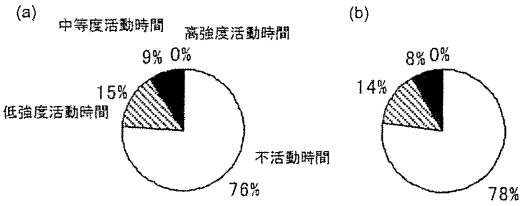

図1. 活動時間の内訳

(a)男性 (b)女性

厚生労働省が提唱した「エクササイズガイド2006」[1)]では，週23EX以上の身体活動が生活習慣病の予防に効果的であるとされている。本研究では，対象者のうち男性では74%，女性では73%がこの基準値を満たしていた。

3) 栄養摂取量

表3に1日あたりの栄養素摂取量を示す。摂取エネルギーは，男性が1839kcal（たんぱく質: 13.2%，脂質: 26.6%，炭水化物: 60.0%），女性が1443kcal（たんぱく質: 13.6%，脂質: 30.0%，炭水化物: 56.1%）であった。

次に，住居形態別に栄養素摂取量を比較検討した。一人暮らしの学生は，実家暮らしの学生と比較して，表3に挙げた全ての栄養素において摂取量が有意に少なかった。エネルギー，カルシウム，鉄，食物繊維摂取量の比較を図2に示す。

表3. 1日あたりの栄養素摂取量

	男性		女性	
エネルギー,kcal	1839	(782)	1443	(535)
たんぱく質,g	60.7	(29.6)	48.1	(21.4)
脂質,g	53.6	(28.3)	46.7	(22.2)
炭水化物,g	260.6	(112.2)	196.8	(70.6)
カリウム,mg	1978	(1194)	1719	(1014)
カルシウム,mg	413	(297)	362	(207)
マグネシウム,mg	196	(109)	161	(83)
リン,mg	874	(469)	717	(325)
鉄,mg	6.2	(3.6)	5.4	(3.0)
亜鉛,mg	7.7	(3.7)	6.1	(2.6)
銅,mg	1.05	(0.53)	0.84	(0.39)
ビタミンA,μgRE	490	(460)	475	(375)
ビタミンD,μg	6.0	(7.5)	5.0	(5.5)
ビタミンK,μg	228	(204)	205	(181)
ビタミンB1,mg	0.69	(0.35)	0.57	(0.3)
ビタミンB2,mg	1.08	(0.62)	0.96	(0.43)
ナイアシン,mgNE	12.4	(7.3)	10.1	(6.0)
ビタミンB6,mg	1.00	(0.58)	0.83	(0.49)
ビタミンB12,μg	4.7	(5.2)	3.9	(4.0)
葉酸,μg	257	(180)	243	(153)
ビタミンC,mg	85	(67)	82	(64)
パントテン酸,mg	5.95	(3.06)	4.79	(2.2)
コレステロール,mg	326	(235)	315	(187)
総食物繊維,g	9.5	(5.8)	8.4	(5.1)
食塩,g	9.3	(4.2)	7.4	(3.2)

中央値（四分位偏差）で表す

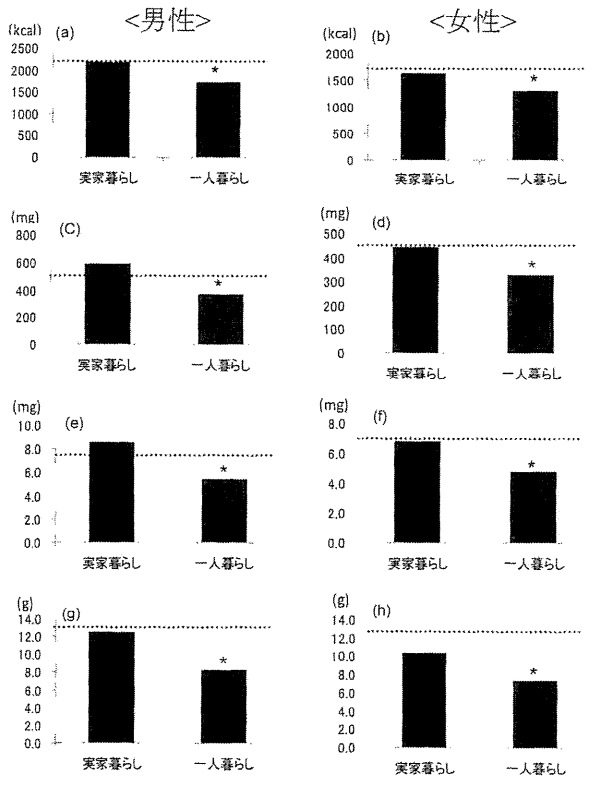

図 2. 住居形態別栄養素摂取量

a),b) エネルギー

c),d) カルシウム

e),f) 鉄

g),h) 食物繊維

*; P>0.05 vs 実家暮らし

破線：平成 19 年度の 18～29 歳の全国平均

（国民健康・栄養調査報告より）[2)]

4. 研究の限界

本研究の限界として以下の 2 点が考えられる。1）水中での身体活動量（入浴，水泳など）および自転車による身体活動量が含まれていないため，実際の身体活動量よりも過小評価している可能性がある。2）質問紙により栄養摂取量を調査したため，思い出しバイアスにより実際の摂取量よりも過小評価している可能性がある。

5. おわりに

九州大学 1 年生の学生を対象に，身体活動および栄養摂取の実態を把握した。今後，学生のメンタルヘルスの状態と身体活動や栄養摂取との間にいかなる関連性があるかについて検討を行っていく。

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