Physical activity is inversely associated with total homocysteine levels, independent of C677T MTHFR genotype and plasma B vitamins

Rachel Dankner · Angela Chetrit ·
Gie Ken Dror · Ben-Ami Sela

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Abstract The homocysteine level is considered to be a product of genetic and lifestyle interactions, mainly mutated methylenetetrahydrofolate reductase (MTHFR) and the intake of folate, vitamin B12 and pyridoxine, and their blood levels. Physical activity has been associated with lower homocysteine levels in some population studies, especially among elderly subjects. To further elucidate the observed association between homocysteine and physical activity, while accounting for the effect of the MTHFR C677T genotype, and of plasma levels of folate and B12 vitamins, a cross-sectional study of 620 males and females, aged 70.5 ± 6.8 years, was carried out. Information on lifestyle habits was collected and laboratory examinations of 12-h fasting total plasma homocysteine, folate, and vitamin B12, as well as DNA analysis for MTHFR C677T variant, were performed. Median total homocysteine values were $11.4 \mu\text{mol/l}$ for males and 9.4 for

females; $p < 0.001$. Smoking and ethnic origin were not found to be associated with homocysteine levels. Physically active subjects had significantly lower total homocysteine levels when adjusted for sex ($p = 0.01$). Significant inverse correlations were found between body mass index, plasma folate, B12 and homocysteine levels. Homocysteine levels of the CC, CT and TT genotypes were 9.7 , 10.6 and $10.2 \mu\text{mol/l}$, respectively ($p = 0.002$, controlling for sex). In a multiple linear regression model, a sedentary lifestyle increased homocysteine levels by 7% as compared to an active one ($p = 0.03$) controlling for sex, age, body mass index, folate, vitamin B12, and C677T genotype, all of which were also found to be significantly associated with homocysteine levels. Any level of physical activity was found to be independently associated with lower homocysteine levels in an elderly population, controlling for MTHFR genotype, plasma B-vitamins, age, sex, smoking and BMI. This study emphasizes the importance of maintaining a physically active lifestyle in the elderly.

R. Dankner · A. Chetrit
Unit for Cardiovascular Epidemiology, The Gertner
Institute for Epidemiology and Health Policy Research,
Tel Hashomer 52621, Israel

R. Dankner (✉) · G. Ken Dror · B.-A. Sela
Sackler School of Medicine, Tel Aviv University,
Tel Aviv, Israel
e-mail: racheld@gertner.health.gov.il

B.-A. Sela
Institute of Chemical Pathology, Sheba Medical Center,
Tel Hashomer, Israel

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Abbreviations

MTHFR	Methylenetetrahydrofolate Reductase
SNP	Single Nucleotide Polymorphism
PCR	Polymerase Chain Reaction
Hcy	Homocysteine

tHcy	Total homocysteine
BMI	Body Mass Index
CVD	Cardiovascular diseases

morphism MTHFR C677T common variant, as well as plasma levels of folate and B12 vitamins, in an elderly population.

Introduction

The homocysteine (Hcy) level is considered to be a product of genetic and lifestyle interactions, mainly mutated methylenetetrahydrofolate reductase (MTHFR) and the intake of folate, vitamin B12 and pyridoxine, and their blood levels (Gudnason et al. 1998, Simon et al. 1999, Saw et al. 2001). The common C677T polymorphism of the MTHFR gene has been established as an important genetic determinant of elevated Hcy (Engbersen et al. 1995). Moderately elevated Hcy levels are associated with a deficient dietary intake of B vitamins. Nutritional factors, i.e., complex B vitamin intake, seem to be most significant in terms of the levels of Hcy in the plasma (Simon et al. 1999), and supplementation with complex B vitamins lowers the Hcy plasma levels (Bonaa et al. 2006, Toole et al. 2004, Lonn et al. 2006).

Total homocysteine (tHcy) increases with age in both males and females, and is higher in males than in females. Positive associations between plasma Hcy and smoking, blood pressure, and cholesterol have been reported. De Bree et al. (2001) investigated the association of lifestyle factors including physical activity in a representative sample of the general Dutch population of 20–65 year olds (mean age of 40) and found a weak positive relationship between physical activity and tHcy in women only. This contrasts with the protective effect observed in the Hordaland Homocysteine Study (Nygard et al. 1995) of subjects aged 40–42 and 65–67 years, which demonstrated that plasma tHcy level is inversely related to level of physical activity, especially in the older age group. In a previous publication, we already reported that physical activity is associated with lower tHcy levels in an elderly population (Dankner et al. 2004), yet the levels of B vitamins in the plasma and the genotyping of MTHFR were unavailable at the time. To date, no conclusive data have been reported on the role of genetic, lifestyle and environmental factors on levels of tHcy in an elderly population.

The aim of this study was to further elucidate the observed association between tHcy and physical activity, while accounting for the effect of the poly-

Materials and methods

Study population

This cross-sectional study included 620 subjects of a 30-year cohort of the Jewish Israeli population, stratified according to sex, age and ethnic origin (Yemen, Middle East, North Africa, Europe/America) (Modan et al. 1985). Participants completed an interview and had their tHcy levels measured, in addition to all other laboratory examinations during 2001–2004.

Out of 948 survivors of the original cohort, 21 did not have a tHcy result, 96 individuals completed a telephone interview but not a laboratory examination, and of the 211 non-respondents (22%), 11.5% were not located, 7.4% refused to participate, 1.7% had communication problems, and 1.4% were ill.

Interview and laboratory examinations

The surveyed individuals were asked about demographic variables (age, ethnic origin), lifestyle characteristics (smoking and physical activity habits), and medical history and use of medications, during a personal interview performed by trained nurses in regional clinics. Three blood pressure measurements were taken during the interviews, and blood was drawn after 12 h of fasting for lipid, creatinine, B12, folic acid and tHcy measurements. DNA analysis was performed for MTHFR C677T common variant. Weight and height were measured with subjects wearing light clothing, without shoes, on a calibrated weight-scale, and body mass index (in kg/m²) was calculated. The study received the approval of the IRB at the Chaim Sheba Medical Center and each participant was asked to sign an informed consent form.

To evaluate leisure time physical activity, the following questions were asked: “Do you engage in any intentional physical activity? If so, what kind? How many times per week and how many hours each time?”. These questions were previously used in other studies in our department, but were not validated. Subjects who replied “no” were categorized as “sedentary”, while those who reported participating

in any type of intentional physical activity for any duration were categorized as “active”. Weekly physical activity of less than 3.5 h was categorized as “mildly active” and 3.5 h or more as “moderately active”.

Renal insufficiency was determined if one of the following conditions applied: a subject reported having a kidney disease, requiring a special diet due to the disease, or had a creatinine value of more than 1.5 mg dl^{-1} (based on the upper limit of the normal range for plasma creatinine values of our laboratory). All laboratory examinations were performed at the Institute of Biochemical Pathology laboratory in our medical center.

To measure tHcy, fasting blood samples were drawn in tubes containing EDTA. Plasma was separated immediately. Total Hcy levels were measured by fluorescent detection after separation by HPLC as described (Jacobsen et al. 1994), following labeling of plasma by monobromobimane. Normal values for adults range between 5.0 and $15.0 \mu\text{mol l}^{-1}$.

Venous blood samples were obtained after fasting overnight. Levels of total cholesterol, HDL-C and TG were determined using an automated enzymatic technique (Boehringer Mannheim, Germany), and standardized against reference materials supplied by the standardization program of the Center for Disease Control and Prevention. Folic acid and B12 assays were performed on the Access® Immunoassay system kit (AxSYM Abbott Laboratories, USA). Creatinine was measured in venous plasma using an Olympus AU2700 analyzer, by a spectroscopic method (Jaffé method). Creatinine forms a yellow-orange colored compound with picric acid in alkaline medium. The maximal absorbance at 520 nm monitors the concentration of plasma creatinine.

C677T Single Nucleotide Polymorphism (SNP) genotype was performed using PCR amplification of genomic DNA, a short extension reaction across the polymorphic site, and mass spectrometry to detect allele specific mass difference of the extension. Allele detection and genotype calling were performed using the MassARRAY system from Sequenom (Jurinke et al. 2005).

Statistical analysis

Median values of tHcy were presented for demographic (age, sex, ethnic origin) and lifestyle variables (smoking, physical activity), use of complex B-vitamin

supplement, renal insufficiency, and MTHFR C677T genotype. Since the tHcy values did not distribute normally in our sample according to the Shapiro–Wilk test, values were logarithmically transformed for statistical analysis. Differences in tHcy median values between categories of sex, age, ethnic origin, smoking, physical activity, vitamin B consumption, kidney insufficiency, and MTHFR genotype were assessed by the Wilcoxon and the Kruskal–Wallis non-parametric tests. The associations of the study parameters with the log-transformed values of tHcy were evaluated using the ANOVA-test, controlling for sex. Spearman Correlation coefficients were calculated to examine the association between tHcy and continuous covariates. The multiple linear regression model was used to examine the association between tHcy as the dependent variable and physical activity, after controlling for parameters known to be related to tHcy or found to be statistically significant in the univariate analyses. Estimates of the rate ratios and 95% confidence intervals (95% CI) were obtained by taking the antilog of the logarithmically-transformed values. The SAS software version 8 (SAS institute inc, Cary, NC) was used for all statistical analysis, and $p < 0.05$ was considered statistically significant. The figures were generated using the SPSS software.

Results

Table 1 shows demographic and other selected characteristics of the 620 study participants. In this fairly aged population averaging 70.6 ± 6.9 years, 53.5% were males.

About 18% of the participants were current smokers, and 57% reported being physically active during leisure time. Thirty-one percent reported regular use of B vitamin supplements (B6, Folic Acid or B12), and kidney insufficiency was found among 11.5% of the participants. BMI was higher among females as compared to males (29.2 ± 5.2 and 27.8 ± 4.0 , respectively; $p = 0.002$).

The frequencies of homozygosity for the C677T MTHFR common mutation (TT genotype), the heterozygous CT genotype and CC genotype without the mutation were 13.7%, 42.8% and 43.5%, respectively. The frequency of the T allele was 35.1% and the genotype distribution for men and women were as expected and did not deviate from the Hardy–Weinberg equilibrium ($p > 0.05$).

Table 1 Demographic and other selected lifestyle and clinical characteristics of study population

Background characteristics		<i>n</i> (%)
Mean age±SD (range)		70.6±6.9 (58–90)
Sex	Male	332 (53.5)
	Female	288 (46.5)
Ethnic origin	Yemen	120 (19.4)
	Middle East	170 (27.4)
	Europe/America	234 (37.7)
	North Africa	96 (15.5)
Smoking ^a	Never	336 (54.3)
	Past	170 (27.5)
	Current	113 (18.3)
Physical activity ^b	Sedentary	264 (42.6)
	Active	355 (57.4)
BMI mean±SD (range) ^c	Male	27.8±4.0 (18.7–40.1)
	Female	29.2±5.2 (17.6–49.9)
B vitamin supplement ^d	No	421 (68.6)
	Yes	193 (31.4)
Renal insufficiency	No	549 (88.6)
	Yes	71 (11.5)
B12 vitamin plasma level (pg/ml); median (range)		334 (68–1,875)
Folate (ng/ml); median (range)		4 (1.1–20)
MTHFR C677T ^d	CC	267 (43.5)
	CT	263 (42.8)
	TT	84 (13.7)

^aOne subject with missing data^bFor definition see text^cBody Mass Index = Weight/Height²^dSix subjects with missing data

The tHcy values for the total sample ranged from 2.6 to 47.1 $\mu\text{mol/l}$; the arithmetic-mean plasma level was $11.2 \pm 5.4 \mu\text{mol/L}$ (12.2 ± 5.9 and 10.1 ± 4.4 for males and females, respectively; $p < 0.001$).

Table 2 shows that, in each age group, tHcy median levels were higher for males than for females, and that they increased with age irrespective of sex ($p = 0.001$). The difference between sexes existed in each ethnic group, while no differences were observed in tHcy levels between the ethnic groups. There was no difference in tHcy level between current smokers and non-smokers (Table 2) nor between those who had never smoked and those who ever smoked (data not shown).

Among the sedentary population, median tHcy levels were significantly higher compared to the physically active participants, a finding which persisted when controlling for sex ($11.7 \mu\text{mol/l}$ vs $10.9 \mu\text{mol/l}$ for males and $9.7 \mu\text{mol/l}$ vs $9.1 \mu\text{mol/l}$ for females, respectively; $p = 0.01$; Fig. 1) and for age ($p = 0.002$; Fig. 2). In the total population, among the “active” group no differences in median tHcy level were found between mildly ($n = 188$) and moderately ($n = 165$) active subjects (9.7 and $9.8 \mu\text{mol/l}$, respectively). Therefore, we combined them into one category in subsequent analyses. A reduction in skewness of tHcy distribution was observed among the active subjects as compared to the sedentary ones.

CC homozygotes had significantly lower median tHcy values in both males and females as compared to the CT and the TT genotypes. When we looked at tHcy levels according to physical activity categories among the three MTHFR C677T genotypes, only in the TT homozygotes was there a negative correlation between median tHcy levels and the amount of hours spent weekly in sports-related physical activity, although not statistically significant (10.5 , 10.1 and $9.3 \mu\text{mol/l}$ for sedentary, mildly and moderately active subjects; $p = 0.18$) (not shown).

For the total group and for each sex, users of B vitamin supplements had lower tHcy levels ($p = 0.05$). No differences were found in tHcy levels with regard to the presence of reported renal insufficiency or having a creatinine level greater than 1.5 mg/dL ($p = 0.5$).

Homocysteine was significantly and negatively correlated with B12 plasma levels, folate plasma levels and BMI, in both males and females. Spearman correlation coefficients were: -0.22 , -0.20 and -0.10 for B12, folate and BMI, respectively ($p < 0.001$ for B12 and folate, $p = 0.01$ for BMI).

In a multiple linear regression model, controlling for age, sex, ethnic origin, smoking status, BMI, folate and B12 vitamin plasma levels, and MTHFR genotype, physical activity was found to be significantly associated with lower tHcy levels ($p = 0.03$). A sedentary lifestyle increased the level of tHcy by 7% as compared to an active lifestyle when controlling for the remaining parameters included in the model. Males and older age were also found to be significantly associated with elevated tHcy levels ($p < 0.001$ for both). In comparison to females, males had a 15% increase in tHcy plasma levels (95%CI 1.07 – 1.23), and each increment of 1 year of age was associated

Table 2 Total homocysteine levels ($\mu\text{mol/l}$) by sex and possible influencing factors

	Total		Males		Females		p^a
	<i>n</i>	Median	<i>n</i>	Median	<i>n</i>	Median	
Total	620	10.1	332	11.4	288	9.4	<0.001
Age group							
< 65	129	8.7	74	9.5	55	8.1	0.001
65–74	306	9.9	152	11.4	154	9.0	
75+	185	11.2	106	12.5	79	10.3	
Ethnic origin							
Yemen	120	10.1	64	10.8	56	9.5	0.9
Middle East	170	10.4	92	10.7	78	9.5	
North Africa	96	10.0	49	11.9	47	9.5	
Europe/America	234	10.0	127	10.9	107	9.1	
Smoking							
Never and past	506	10.1	258	11.3	248	9.4	0.6
Current	113	10.3	73	11.4	40	8.8	
Physical activity							
Sedentary	264	10.5	142	11.7	122	9.7	0.01
Mildly and moderately active	355	9.8	189	10.9	166	9.1	
Complex B vitamin supplement							
No	421	10.4	260	11.7	161	9.4	0.05
Yes	193	9.5	67	9.8	126	9.2	
Renal insufficiency							
No	547	10.1	277	11.6	270	9.4	0.5
Yes	73	10.1	55	10.1	18	9.2	
MTHFR C677T							
CC	267	9.7	145	10.7	122	8.6	0.002
CT	263	10.6	146	12.1	117	9.6	
TT	84	10.2	39	11.1	45	10.0	

^a Based on ANOVA test controlling for sex (in all except the total group).

with a 2% increase in tHcy plasma levels (95%CI 1.01–1.02). Current smokers exhibited an 8% increase in tHcy plasma levels as compared to those who never smoked or to past smokers (95%CI 0.99–1.18; $p=0.07$). In this elderly population, each 1 unit increment in the BMI was associated with a 1% decrease in tHcy plasma levels ($p=0.04$). Every 50-unit increment in the plasma B₁₂, and 1-unit increment of plasma folate conferred a 1% and 2% decrease in the plasma tHcy, respectively, $p=0.01$ for both. In comparison to the TT homozygosity to the MTHFR C677T genotype, CC wild-type was associated with a 16% decrease in tHcy levels ($p=0.001$), controlling for all the confounding factors included in the model, i.e., age, sex, ethnic origin, smoking status, physical activity, BMI, plasma B12 vitamin and plasma folate (Table 3).

We examined the possible interactions between the variable of interest, i.e. physical activity, and plasma

B12 vitamin, plasma folate, BMI, and MTHFR genotype. A three-factor interaction between MTHFR genotyping, physical activity and sex was also examined. None of them were found to be statistically significant ($p>0.2$). A statistically significant interaction was found between plasma folate and B12 vitamin ($p=0.007$) on plasma level of tHcy, indicating that the effect of plasma folate on the tHcy plasma level is modified by the plasma level of B12 vitamin. A constant value of plasma folate would be associated with different values of tHcy, depending on plasma B12 vitamin values. No significant interaction was found between MTHFR genotype and plasma folate.

Discussion

We found physical activity to be independently associated with lower tHcy plasma levels, when

Fig. 1 Median total homocysteine values by physical activity level for males and females

* p adjusted for sex

adjusting for the confounding effect of the MTHFR polymorphism, and for plasma levels of both B vitamins and folate, as well as demographic and lifestyle parameters. No interactions were found between physical activity and tHcy-related factors, indicating that the main effect of physical activity on tHcy plasma levels was not modified by the above factors. While previous studies investigated the association between lifestyle factors and Hcy, the current report addresses the genetic–lifestyle associa-

tions as well, i.e. the presence of the MTHFR C677T polymorphism. It is postulated that subjects with the genetic variant for the common C677T polymorphism (TT genotype) are more susceptible to the develop-

Table 3 Factors associated with total plasma homocysteine levels (μmol/l)

	Estimate (rate ratio) ^a	95% CI	p-value
Physical activity			
Active (versus sedentary)	0.93	0.85–0.99	0.03
Sex			
Male (versus female)	1.15	1.07–1.23	<0.001
Age (increment of 1 year)	1.02	1.01–1.02	0.002
Smoking			
Current smokers (versus never/past smokers)	1.08	0.99–1.18	0.07
BMI (1-unit increment)	0.99	0.86–1.0	0.04
B ₁₂ vitamin (50-unit increment)	0.99	0.98–1.00	0.004
Folate (1-unit increment)	0.99	0.98–1.00	0.01
MTHFR (versus TT)			
CC	0.83	0.75–0.92	0.001
TC	0.92	0.84–1.02	0.1

* Based on the ANOVA test

Fig. 2 Median total homocysteine values by physical activity level according to age groups

^a Adjusted for ethnic origin.

Multiple linear regression analysis results

ment of intermediate hyperhomocysteinemia despite normal folate and B12 levels (Kang et al. 1991). In our study, homozygotes for the TT common mutation (occurring in 14% of the subjects) were found to have tHcy concentrations that were 16% higher than those of the CC wild type individuals, controlling for B12 and folate plasma levels as well as demographic and lifestyle characteristics.

In this elderly population, tHcy increased with age, yet in any age group lower tHcy levels were observed in the active subjects as compared to the sedentary ones (Fig. 2). This finding is supported by Kuo et al. (2005), who reported an inverse association between fitness level and Hcy independent of age among women without significant CVD in the NHANES 1999–2002 population. Nygard et al. (1997) also reported that physical inactivity was positively associated with plasma Hcy levels and that this association remained significant after adjustment for dietary intake of fruit, vegetables and B vitamin supplements. Schneede et al. (2000) reported that the difference in Hcy between subjects with a sedentary lifestyle and those exercising on a daily basis was most prominent in the elderly. In a relatively young (mean age 41) and active population, De Bree et al. (2001) found a weak positive association between physical activity and plasma Hcy levels in women only. Factors previously reported to influence Hcy levels in the general population are diet, in particular folate intake, blood levels of folate, vitamin B12, and the MTHFR C677T polymorphism (Refsum et al. 2006). A positive association was reported between physical inactivity and plasma Hcy concentration, although it disappeared after adjustment for plasma folate concentration (Saw et al. 2001). In our study, the positive association between tHcy and a sedentary lifestyle remained significant after adjusting for plasma folate concentration. In the Inter99 study, no association was observed between changes in Hcy levels in a 1-year follow up and lifestyle changes, including physical activity, smoking and dietary habits (Husemoen et al. 2004).

The Hordaland study demonstrated back in 1995 that plasma Hcy level is inversely related to physical activity (Nygard et al. 1995). The most pronounced difference was found between the physically inactive and active subjects in the older age group of 65–67 years old. In accordance with our observation, Nygard et al. (1995) found that with increasing activity levels a reduction in skewness of the Hcy distribution was

observed, suggesting that exercise exerts its most favorable effect in subjects with hyperhomocysteinemia.

Our finding of a negative association between plasma folate and vitamin B12 and tHcy was also found by Nurk et al. (2004) who reported that each unit of increase in plasma folate and vitamin B12 was associated with a reduction in Hcy concentration of 0.2 $\mu\text{mol/l}$ and 0.1 $\mu\text{mol/l}$, respectively.

While we found no association between smoking and tHcy in either sex, when controlling for B vitamin complex intake (Dankner et al. 2004) or for their plasma level, most studies reported a positive association between smoking and Hcy levels (de Bree et al. 2001; Nygard et al. 1995; Jacobsen et al. 1994; Nygard et al. 1997; Ward et al. 1997) which is more pronounced in women. However, in some studies (de Bree et al. 2001; Jacobsen et al. 1994; Bar On et al. 2000), this association disappeared after adjustment for B vitamins. This discordance with other results may be explained by the relatively low prevalence of current smokers, especially in women (14%), in our study.

The importance of folic acid supplementation for the reduction of plasma Hcy as a means for primary and secondary prevention of atherosclerosis and cardiovascular events has been studied during the last decade. Ten years ago, Guttormsen et al. (1996) concluded from their follow up case control study of the Hordaland cohort that daily supplementation of low dose folic acid will reduce and often normalize Hcy plasma levels of homozygotes for the C677T mutation in the MTHFR gene. The debate about whether to recommend folic acid and vitamin B supplementation for high risk populations has increased since the publication of clinical trials which failed to reduce CVD events (Bonaa et al. 2006; Toole et al. 2004; Lonn et al. 2006) or improve cognitive performance while reducing Hcy levels via those supplementations (McMahon et al. 2006). The first three trials recently reported that there is no clinical benefit of the use of folic acid and vitamin B12 for lowering Hcy levels in patients with established vascular disease (Bonaa et al. 2006; Toole et al. 2004; Lonn et al. 2006). This finding might be explained by a change in the cell phenotype that promotes the development of plaque, by the use of folic acid and vitamin B12 in the setting of mild hyperhomocysteinemia due to alteration in the methylation potential in vascular cells (Loscalzo 2006). Due to this potential adverse effect of folic acid and

B12 supplementation, one should consider an alternative approach to reduce Hcy concentrations.

Epidemiologic evidence is sufficient to recommend that most persons should increase their level of physical activity. Elevated Hcy values are related to a poor lifestyle, and an improvement might lower them. We propose physical activity as an alternative approach to reduce Hcy levels without the potential of adversely affecting the development of the atherogenic plaque. Physically active lifestyles in the elderly could be a surrogate to healthier habits, associated with better nutrition and a lower exposure to oxidative stress. The biological mechanism through which physical activity and lower Hcy plasma levels are associated has yet to be determined. However, in this and in our previously reported study (Dankner et al. 2004), we have shown that physical activity itself, or as a proxy for additional factors which could not be accounted for in this study, is associated with lower tHcy, independently of the confounding factors of sex, age, ethnic origin, BMI, smoking, vitamin B supplementation in the diet, vitamin B12 and folic acid levels in the plasma, renal insufficiency, and MTHFR C677T genotype.

This cross-sectional analysis of survivors from a randomly stratified cohort makes assessment of associations possible, but inferences regarding causality of decreased Hcy levels as a result of physically active lifestyle cannot be made. Further controlled intervention trials, which examine the effect of increasing the level of physical activity in a group of elderly patients with hyperhomocysteinemia, are needed to assess the causality of the association of physical activity on Hcy levels.

In conclusion, any level of physical activity was found to be an independent lifestyle habit associated with lower tHcy levels in an elderly population, controlling for MTHFR C677T genotype, plasma B-vitamins, and demographic characteristics. This study emphasizes the importance of maintaining a physically active lifestyle in the elderly.

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