Effects of Long-term Regular Exercise on Cognitive Function, Lipid Profile and Atherogenic Biomarkers in Middle-aged Men

by

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Several studies on exercise and its effect on cognitive function in human and animal populations have documented the beneficial impact of regular physical activity on maintenance of good cognitive abilities and satisfactory health-related quality of life well into older age. The aim of the study was to evaluate the effects of long-term regular running on metabolic profile and cognitive function in middle-aged men.

A total of 24 regularly exercising, middle-age men (Group A), all being members of the Runners Club, and 22 age-matched sedentary subjects (Group B), as the control group, were enrolled in this study. The control group included 8 non-overweight (BMI=23.5±5.2) individuals (Group C) and 14 overweight/obese (BMI=30.7±1.6) subjects (Group D). Serum lipid profile, glucose and homocysteine concentrations were assessed by routine laboratory methods. Subjects' cognitive function was evaluated based on Trail Making Test (TMT) and Digit Symbol Test (DST) scores.

In a majority of runners (Group A), the BMI and the parameters of lipid profile (TC, HDL, LDL,TG, glycerol) were close to those recorded in non-overweight controls (Group C) and, in both groups, results were ideally within the reference ranges for healthy male subjects. However, as compared to the whole control group (Group B), which may be considered as an average population sample of sedentary middle-age men, significant differences were observed in BMI and concentrations of TC and LDL, as well as in the pro-atherogenic biomarkers (TC/HDL, LDL/HDL), which were lower in runners. A similar tendency was found in concentrations of TG (independent cardiovascular risk factor), glycerol and TG/HDL ratio (surrogate measure of insulin resistance), however the differences did not reach the level of significance. The level of homocysteine (pro-atherogenic biomarker) was comparable in all groups, and in most cases, within the reference range for male adults. Results of cognitive function tests did not reveal any significant between-group differences. The TMT score was found to be correlated positively (r=0.492, p<0.05), whereas DST score was correlated negatively (r=-0.549, p<0.005), with age. The DST performance, as evaluated in the group of runners (Group A), appeared to be strongly dependent (r=0.809, p<0.005) on the educational level of the subject.

These data provided evidence of beneficial effects of a long-term regular endurance running exercise on lipid profile and cardiovascular health in middle-aged men. However, we failed to confirm the findings of a favorable impact of regular physical activity on improvement in cognitive abilities.

Keywords: cognitive function, lipid profile, physical exercise

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Introduction

There is compelling evidence that regular physical activity may reduce the risk of chronic disease and premature death, especially from cardiovascular disease (Ergün et al., 2006; Warburton et al., 2006; Chakravarty et al., 2008, Blair et al., 2004, Berlin and Colditz, 1990, Kłapcińska, 2006, Mackey, 2008). It has been documented that a healthy lifestyle, including regular physical activity is associated with longer survival of men (Lee and Paffenbarger,2000) and women (Oguma et al., 2001). On the other hand, several large epidemiological studies have evidenced that low physical activity is a strong and independent risk factor for both cardiovascular disease (CVD) and all-cause mortality (Barengo et al., 2004). One of the growing public health problems is Type-2 diabetes and related disorders which increase with age and weight gain. A number of studies have provided evidence of a continuing increase in overweight, diabetes and obesity-related health risk factors among adults (Hu et al. 2007), although it is well documented that health risks of obesity, insulin resistance and Type-2 diabetes may be prevented through increased physical activity (Mokdad et al., 2003, Helmrich et al., 1991, Kriska, 2000). Moreover, recent studies have shown that insulin resistance may lead to cognitive decline and dementia, not only in older but also in middle-aged adults (Young et al., 2006). In view of these facts and of utmost importance are well documented findings on the association between physical activity and mental health (Paluska and Schwenk, 2000). Indeed, habitual, moderate physical activity may play an important role in the management of depression and anxiety, but excessive physical loads may lead to overtraining-related physical exhaustion and psychological symptoms that mimic depression. Evidence from animal studies (Cotman, 2002) indicate that physical activity, such as voluntary wheel running, supports brain function due to enhanced production of BDNF (brain-derived neurotrophic factor). BDNF is a molecule which promotes survival of hippocampal, striatal, and septal neurons, and thus enhances learning abilities and protects against cognitive decline. Interestingly, the increase in plasma concentration of BDNF was recently recorded in young healthy men after a five-week endurance cycling program, composed mainly of moderate levels of intensity (Zoladz et al., 2008). Several observational and interventional studies consistently reveal associations between regular physical exercise and better cognitive functioning of older human subjects (Ravaglia et al., 2008; O'Dwyer et al., 2007), although not all investigations support this finding (Madden et al., 1989; Hill et al., 1993).

The present study was aimed at the evaluation of the impact of long-term regular endurance running activity on metabolic profile and cognitive abilities in middle-age men.

Material and method

Participants

Twenty-four regularly exercising, middle-aged men (Group A, mean age 41.5±13.4 yrs), all

		Table 1

Physical characteristics (mean ± SD) of participants

Variable	Runners Group A (n=24)	Reference group of middle-aged men		
		Whole group (Group B, n=22)	Non-overweight men (Group C, n=8)	Overweight men (Group D, n=14)
Age (yr)	41.5±13.4	41.9±6.7	41.9±5.2	41.9±7.7
Height (cm)	175.0±6.6	178.1±5.2±	175.0±4.4	179.9±4.9
Body mass (kg)	73.9±12.0	90.1**±13.0±	73.5±5.6	97.6**$±9.2
BMI (kg/m²)	24.1±3.1	28.2**±3.8±	24.0±2.4	30.7**$±1.5

Data are means ± SD. The Mann-Whitney U test was used for between-group comparisons. **-significantly (p<0.005) different from the respective values in Group A; $-significantly (p<0.005) different from the respective values in Group C. Abbreviations: BMI = body mass index.

Table 2

Characteristics of the habitual physical activity of the runners (Group A)

Variable	N	mean±SD	min	max
Training experience (yr)	24	12.7±9.7	3.0	34.0
Monthly mileage (km/month)	24	174.6±92.0	49.6	415
Yearly mileage (km/y)	24	2095.6±984.5	595.0	4980.0
No. of marathons per year	19	1.6±1.9	0.0	5.0
No. of half-marathons per year	19	1.6±1.6	0.0	5.0
No. of 15 km runs per year	19	3.7±4.0	0.0	18.0
No. of 10 km runs per year	19	6.9±6.1	1.0	23.0
No. of 5 km runs per year	19	1.4±2.2	0.0	8.0

being recreational runners, were involved in this study. A total of twenty-two sex- and age-matched, apparently healthy sedentary subjects (Group B, mean age 41.9±6.7 yrs), randomly selected from a population of about 900 individuals previously examined in the frame of health screening programs organized by municipal public health services, and performed at the Geriatric Hospital, were invited to serve as a reference group. The controls were, subsequently, subdivided into two groups of non-overweight (Group C, N=8, BMI=24.0±2.4) and overweight (Group D, N=14, BMI=30.7±1.5) men. All subjects gave their informed consent to participate in the study conducted in accordance with the Helsinki Declaration (WMADH, 2000) with the approval of the Ethical Committee of the Academy of Physical Education. The basic characteristics of the participants are presented in table 1. Additional information about the habitual physical activity profile of the regularly exercising runners (Group A) is presented is table 2. Noteworthy, all subjects were non-smokers and were clinically healthy at the time of their visit in the laboratory to assess their cognitive function and to take their fasting blood samples. They were also asked to refrain from both strenuous physical activities on the day prior to their visit and the consumption of any stimulants (coffee, alcohol).

Assessment of Cognitive Function

Assessment of cognitive function was performed by a professional psychologist. The following tests were administered: Trail Making Test-part B (TMT – B), and the Digit Symbol Test.

The Trail Making Test (TMT) is a part of the Halstead-Reitan Test Battery (Lezak, 2004; Reitan, 1958). In this study, part B of the TMT was used to measure attentional acute visual (visual scanning, visual-motor coordination and visual-spatial ability). The test consists of 25 circles, including numbers (1 – 13) and letters (A – L), distributed over a sheet of paper. The subject's task is to connect numbers and letters in an ascending pattern alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.) in as little time as possible, without lifting the pen or pencil from the paper. The results are given in seconds spent on performing the task; thus a higher score is indicative of worse visual-spatial function (Gaudino et al., 1995).

The Digit-Symbol test is one of the performance subtests of Wechsler Adult Intelligence Scale – Revised WAIS-R (Wechsler, 1997). In this study, the Polish standard version of WAIS-R was employed (Brzeziński et al., 2004) to assess processing speed, complex attention, psychomotor speed, cognitive-motor translation, concentration and learning abilities. In this test the respondent learns a code in which each digit is represented by a symbol (e.g., 1 might be represented by a square, 2 by a triangle, 3 by a star, and so on). The subject's task is to ascribe correct symbols to a series of randomly presented digits (to complete a total of 93 pairs) as accurately as possible within a defined time limit (90 s). The results are given in a number of correctly completed squares; thus a higher DST score is indicative of better learning abilities.

Analytical procedures

Fasting blood samples were collected between 8:00 to 10:00 am in heparin- or EDTA-

Table 3

Biochemical parameters and lipid ratios measured in fasting blood samples collected from the participants

Variable	Reference range	Runners Group A (n=24)	Reference group of middle-aged men		
			Whole group (GroupB,n=22)	Non-overweight men (Group C, n=8)	Overweight men (Group D, n=14)
Hemoglobin (g/dL)	14-18	17.2±0.59	15.8**±1.0	15.9**±1.0	15.8**± 1.0
Glucose(mg/dL)	70-105	91.5±9.7	88.6±11.5	85.4±6.6	90.4±13.4
T-C (mg/dL)	<200	183.2±32.3	220.2**±49.1	205.9±35.4	228.4**±55.0
TG (mg/dL)	<150	130.2±57.8	176.8±103.2	110.1±26.2	214.9*$±112.0
HDL-C (mg/dL)	>40	59.0±15.7	53.5±9.3	58.3±5.2	50.7$±10.2
LDL-C (mg/dL)	<100	98.2±34.7	135.8**±35.8	123.0±36.3	143.1**±34.7
Glycerol (mmol/L)	28-108	68.8±18.8	88.4±44.7	71.4±23.6	98.1±51.5
T-C/HDL-C	<4.0	3.32±1.16	4.21**±1.06	3.56±0.69	4.59**$±1.06
LDL-C/HDL-C	<4.0	1.81±0.88	2.60**±0.73	2.13±0.69	2.87**$±0.63
TG/HDL-C	<3.5	2.56±1.74	3.69±2.96	1.91±0.54	4.70*$±3.31
CK, U/L	24-195	109.1±43.0	91.0±14.1	85.4±6.6	94.2±16.4
Hcy (μmol/l)	<15	12.1±0.5	10.8±3.5	10.7±3.9	10.9±3.4
Uric acid (mg/dL)	3-5,7	4.85±1.24	5.90**±0.90	5.16±0.59	6.33±0.76
MDA (μmol/L)	-	5.98±2.06	6.17±2.29	5.61±2.38	6.50±2.27
TAS (mmol/L)	1.30-1.77	1.53±0.24	1.18**±0.12	1.10**±0.10	1.23**±0.12

Data are means ± SD. The Mann-Whitney U test was used for between-group comparisons. $P<0.05$ was considered significant. *-significantly ($p<0.05$) different from the respective values in Group A; $-significantly ($p<0.05$) different from the respective values in Group C. Abbreviations: T-C = total cholesterol, TG = triacylglycerol, HDL-C = high-density lipoprotein, LDL-C = low-density lipoprotein, Hcy- homocysteine, MDA- malondialdehyde, TAS-total antioxidant status

treated vacutainer tubes for selected biochemical analyses. Blood plasma and serum were separated according to routine procedures, and either processed immediately or kept frozen at -80oC until analysis. Fresh plasma samples were assayed for activity of creatine kinase (CK; E.C. 2.3.7.2) and glucose using diagnostic kits (CK 522 and GL 2623) from Randox Laboratories (UK). Total plasma cholesterol (T-C), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), and glycerol, were enzymatically determined in duplicate using commercially available kits from Randox (CH 200, CH 203, TR 1697, GY 105, FA115, respectively). Concentrations of LDL cholesterol (LDL-C) were calculated from T-C, HDL-C, and TG with the use of the Friedewald equation (Friedewald, 1972). In order to evaluate the risk for vascular disease,

the lipid ratios (T-C/HDL-C, LDL-C/HDL-C and TG/HDL-C) were calculated. Plasma total homocysteine (Hcy) was determined according to the procedure of Young et al. (Young et al., 1994), using a reverse-phase-high-performance-liquid-chromatography (LaChrom® HPLC; Merck-Hitachi) with fluorescence detection.

Lipid peroxides in plasma were assayed by the thiobarbituric acid test (Buege and Aust, 1978) with extraction of the chromogen formed with n-butanol and reading the absorbance of the organic layer at 532 nm. The level of lipid peroxides were expressed as μmol of malondialdehyde (MDA) per liter of plasma, which was calculated from the calibration curve prepared with 1,1,3,3-tetraethoxypropane (Sigma) as an external standard. Plasma uric acid concentration was measured by using a commercially

Table 4

Trail Making Test (TMT) and Digit Symbol Test (DST) scores

Variable	Runners Group A (n=24)	Reference group of middle-aged men		
		Whole group (Group B, n=22)	Non-overweight men (Group C, n=8)	Overweight men (Group D,n=14)
TMT (sec)	87.1±50.7	81.5±32.1	72.8±14.7	86.5±38.4
DST	50.1±13.3	50.8±8.8	53.2±9.9	49.4±8.2

available diagnostic kit UA230 (Randox Laboratories, Ltd.). Plasma Total Antioxidant Status (TAS) was assessed spectrophotometrically by using a commercially available diagnostic kit (NX 2332, Randox).

Statistical analysis

The data are presented as means ± standard deviations (SD). Between-group differences were identified with the non-parametric Mann-Whitney U-test. Significance level was set at $p<0.05$. In addition, Spearman rank order correlation coefficients were computed to reveal associations between the variables. All statistical analyses were performed using Statistica 6.0 (StatSoft, Inc.) software.

Results

There were no significant differences in mean age between the groups. However, compared to regularly exercising runners (Group A), both the whole reference group (Group B) and the overweight subjects (Group D) were characterized by a significantly higher BMI (Table 1). A comparison of blood test results provided evidence of a more favorable metabolic profile in runners, as compared to the whole reference group. The most important differences were significantly higher concentrations of hemoglobin and total antioxidant status (TAS), as well as lower concentration of LDL-cholesterol and a marked tendency toward lower level of triacyglycerols (TG), uric acid, glycerol and malondialdehyde (MDA) (Table 3).

The differences were even more pronounced when the measures recorded in runners were compared with those found in the overweight controls. Significant differences were also found in the case of the lipid ratios (T-C/HDL-C, LDL-C/HDL-C, TG/HDL-C) that were within the reference ranges in the runners, but significantly higher in the whole reference group. Moreover, the highest values, mostly beyond the upper borderline levels, were recorded in the overweight group of control subjects. In contrast, except for concentration of hemoglobin and TAS, no significant differences in any other parameters studied were found between the runners and the non-overweight controls. As predicted, the activity of creatine kinase (CK) tended to be higher in runners, but the difference did not reach the significance level. The level of homocysteine (Hcy), considered as independent predictor for cardiovascular disease (CVD), was within the normal range (5-15 µmol/L) (Nurk et al., 2004) in both runners and controls.

Another important part of the study protocol included the assessment of the cognitive function of participants. With the aim of determining whether a long-term physical activity may influence cognitive abilities, all participants were subjected to cognitive testing based on assessment of the TMT and DST scores. The results are listed in table 4. Additionally, in order to reveal which factors specific to the whole group of participants or to the physical activity profile of runners are associated with cognitive function, the non-parametric Spearman rank

Table 5

Spearman rank-correlation coefficients between selected variables

	Variables	R	P
All participants	TMT and DST	-0.606	<0.005
	TMT and age	0.492	<0.005
	TMT and TAS	-0.355	<0.05
	DST and age	-0.549	<0.005
	DST and TAS	0.425	<0.05
	BMI and age	0.280	NS (p=0.059)
	TAS and age	-0.398	<0.05
Runners	TMT and age	0.488	<0.05
	TMT and TAS	-0.535	<0.05
	DST and age	-0.660	<0.005
	DST and education level	0.809	<0.005
	DST and TAS	0.608	<0.005
	DST and years of training	-0.472	<0.05
	TAS and no.of 10 km runs	-0.463	<0.05

correlation analysis was used to reveal the associations between selected variables. The results of this analysis are summarized in table 5.

Discussion

The major findings of the present study are as follows: (1) long-term regular endurance running is associated with better health outcomes and a reduced risk for cardiovascular disease among middle-aged men as evidenced by a more favorable lipid profile and higher plasma antioxidant capacity compared with age-matched sedentary population, (2) cognitive function of the subjects was found to depend on age, education level and total plasma antioxidant capacity, (3) long-term endurance running activity had little effect on improving cognitive function in this middle-aged population sample.

Effects of long-term regular exercise on cardiovascular health outcomes

Cardiovascular disease (CVD) is a leading cause of mortality worldwide; therefore, effective prevention requires the accurate identification of individuals at risk for CVD. Traditional cardiovascular risk factors include age, dyslipidemia, smoking, diabetes mellitus, obesity, and hypertension. Based on experience derived from numerous prospective studies, several classical and novel CVD risk biomarkers have been proposed to be incorporated into clinical practice (Wang, 2008). In the present study the risk for CVD was evaluated based on traditional biomarkers measurable in the plasma or serum, including parameters of lipid profile, lipid ratios and homocysteine. Noteworthy, plasma lipids include both atherogenic (triglycerides, total cholesterol, LDL-cholesterol) and antiatherogenic (HDL-cholesterol) components. It is generally assumed that hypertriglycidemia is an independent CVD risk factor, but if combined with elevated LDL-cholesterol and high LDL-C/HDL-C ratio, it may increase the risk for major coronary events by approximately six-fold (Cullen, 2000). Abnormal blood lipid profile, frequently associated with obesity, precedes the onset of Type-2 diabetes, whereas physical activity, known to increase sensitivity to insulin, appeared to be effective in preventing this dis-

ease (Hu et al., 2007). Furthermore, there is a growing body of evidence that physical activity beneficially influences most of the atherosclerotic risk factors and has a favorable impact on lipid and lipoprotein profiles.

In the present study, several favorable features of the metabolic profile were identified in the group of runners. The most important are a lack of obesity (BMI<25.0), normal glycemia level and optimal concentrations of TG, T-C, LDL-C, and HDL-C, all within the optimal ranges established in recently revised NCEP Cholesterol Guidelines released by U.S. National Institute of Health (NIH Publication No. 05-3290). As compared with the whole control group and the overweight men, the levels of all lipid profile parameters were lower, although the differences were significant only in the case of T-C and LDL-C. Noteworthy, in the whole reference group, the concentrations of T-C, TG and LDL-C were borderline high, whereas in the overweight controls, they reached the highest levels. Interestingly, as compared to the runners, all lipid profile measures recorded in the non-overweight controls were only slightly worse. Interestingly, there were no between-group differences in the plasma homocysteine content. However, this biomarker is known to be strongly dependent on age and gender (Nurk et al., 2004), so in the case of age- and sex-matched groups, as those enrolled in the present study, this could be predicted.

In view of recent findings on the importance of lipid ratios (LDL-C/HDL-C, T-C/HDL-C), considered as better predictors of cardiac disease than levels of total cholesterol or LDL-C alone (Natarajan et al., 2003), the most important finding of our study was that these indices were not elevated in the physically active runners, but significantly higher in the whole group of controls, and especially in the overweight men. Insignificant differences in lipid ratios, as compared to Group A, were only recorded in non-overweight controls. Assuming the usefulness of the ratio of the plasma concentrations of triglyceride to high-density lipoprotein cholesterol as a good predictor of insulin resistance (McLauglin et al., 2005), we have found that the physically active runners and non-overweight controls were characterized by relatively low TG/HDL values, not exceeding

the cutoff point (<3.5), while this measure, assessed in the whole reference group, and especially in the overweight controls, was significantly higher. All these findings support the view of the association of obesity with dyslipidemia and higher risk for CVD in obese individuals (Mokdad et al., 2003) and of the beneficial impact of a long-term endurance running on cardiovascular health outcomes (Berlin & Colditz, 1990; Barengo et al., 2004; Lee at al., 1997; Ergün et al., 2006). It should be stressed that our reference group may be considered as being representative of a community population sample of middle-aged men, in which more than a half is characterized as being overweight or obese.

Other favorable features of the metabolic profile of regularly exercising runners were significantly higher values of plasma total antioxidant status (TAS) and a tendency toward lower levels of malondialdehyde. Assessment of the total antioxidant status (TAS) is used to measure the cumulative action of all antioxidants present in plasma, thus providing a parameter suitable for evaluation the intrinsic balance between pro-oxidants and antioxidants in the plasma (Ghiselli et al., 2000). Plasma content of malondialdehyde (MDA), as one of the by-products of lipid peroxidation, is one of the most frequently used biomarkers of oxidative stress (Nielsen et al., 1997). Higher level of TAS and lower MDA content are indicative of a higher capacity of antioxidant defense in regularly exercising runners, compared to the sedentary population sample. These findings support previous observations (Finaud et al., 2006 ; Carlsohn et al., 2008) that endurance training leads to improvement of the total antioxidant capacity due to exercise-induced increase in antioxidant enzyme activities in plasma, muscle and other tissues.

Effects of long-term regular exercise on cognitive function

The next goal of the present study was to evaluate whether long-term regular exercise is associated with improvement in cognitive abilities in middle-aged subjects. Cognitive impairment and dementia represent major health problems in aging societies. Over the past several decades, increasing attention has been paid

to the identification of interventions such as diet and lifestyle habits which could be efficient in prevention of cognitive decline and dementia in elderly persons. The potential of physical activity to prevent weight gain, alleviate depression and enhance cognition has been previously well-supported by numerous studies (Kramer et al., 2006; Fabre et al.,2002; Laurin et al., 2001; Ravaglia et al., 2008; Paluska, 2000). Our results have supported the view that regular exercise is beneficial for preservation of a good health status and might be an effective strategy to prevent development of cardiovascular disease. However, we failed to find association between physical activity and better cognitive performance. There were no significant between-group differences in TMT and DST scores. The Spearman rank correlation analysis revealed that the TMT scores, as assessed by all participants, were significantly and positively related with age and negatively associated with plasma total antioxidant status. Obviously, the inverse character of interrelationships was identified between DST and age or TAS.

We are aware that the present study had some limitations. First, a relatively low number of subjects of a different age in each group, although generally being age-matched, limits the possibility of finding significant between-group differences. Second, the results of cognitive tests could be modulated by educational level of the participants. Moreover, we had not gathered information about education level in individuals from the control group. It may be presumed that higher education may be associated with higher cognitive scores (Koepsell et al., 2008). This presumption seems to be supported by results of correlation analysis of the data collected from the runners. Therefore, the most important finding derived from this analysis is the positive impact of regular aerobic training on the plasma total antioxidant status (TAS), which, in turn, positively affects cognitive functioning, but deteriorates with age.

In conclusion, these data provided evidence of beneficial effects of long-term regular endurance running exercise on lipid profiles and cardiovascular health in middle-aged men. However, we failed to confirm the findings of a favorable impact of regular physical activity on improvement in cognitive abilities.

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