Total radical trapping antioxidant potential (TRAP) and exercise


From the Department of Clinical Biochemistry, Ulster Hospital, Dundonald, 1Department of Epidemiology and Public Health, Queen’s University, Belfast, 2Policy Planning and Research Unit, Department of Finance and Personnel, N. Ireland, 3Physical Education and Health Unit, Queen’s University, Belfast, and 4Northern Ireland Veterinary Research Laboratories, Stormont, UK

Received 30 July 1995; Accepted 22 August 1995

Summary

The relationship between physical activity, physical fitness and total radical trapping antioxidant potential (TRAP) was examined in the Northern Ireland Health and Activity Survey. This was a cross-sectional population study (n = 1600) using a two-stage probability sample of the population. TRAP was calculated using the sum of the individual serum antioxidant concentrations (urate, protein thiols, ascorbate, α tocopherol and bilirubin) multiplied by their respective stoichiometric values. Physical fitness was determined by estimation of VO₂max by extrapolation from submaximal oxygen uptake, and physical activity was recorded by computer-assisted interview. Mean serum TRAP concentrations were significantly higher in males (653 ± 8.2 μmol/l, mean ± SEM) compared to females (564 ± 8.0 μmol/l) (p< 0.0001). Both male and female smokers had significantly lower TRAP values than non-smokers (males p< 0.0001, females p = 0.02). In females, there was a positive relationship of TRAP with age (p< 0.001) and body mass index (p< 0.001) but a negative relationship with physical fitness (p<0.05). The known beneficial effects of exercise and activity do not appear to be directly mediated through increased antioxidant status.

Introduction

Exercise and physical activity are known to have beneficial effects1 and an active lifestyle protects against atherosclerosis, reducing the risk of both myocardial infarction and stroke.2,3 The health benefits of exercise depend on its frequency, duration and intensity, and recent recommendations suggest that every adult should accumulate 30 min or more of moderate intensity physical activity over the course of most days of the week.4 This is of particular importance in Northern Ireland, where the incidence of ischaemic heart disease is one of the highest in the world.5

In recent years, there has been considerable interest in the pathological role of free radicals in a variety of disease processes, including atherosclerosis. There is increasing evidence that antioxidants may have a protective function, in that antioxidant vitamins such as vitamin E inhibit the oxidation of low-density lipoprotein to a particularly atherogenic form and preserve endothelial function.6,7 It is therefore possible that some of the health benefits of exercise and physical activity could be directly or indirectly mediated through increased antioxidant defences. The human body has developed a defence strategy to minimize the damaging effects of oxidants which in extracellular fluids rests mainly with protein thiols (sulphydryl groups), bilirubin, urate, vitamin E (α tocopherol) and vitamin C (ascorbate).8 Since
antioxidants may act synergistically, for instance when ascorbate regenerates α tocopherol from the α tocopherol radical, it is important to evaluate the overall effectiveness of antioxidant defence systems in limiting peroxidative damage. Each antioxidant possesses a different ability to trap radicals, e.g. 1 mol of bilirubin can trap 2 mol of peroxyl radicals and has a stoichiometric value of 2. Knowing the serum concentration and stoichiometric value for each antioxidant, it is possible to determine a calculated total radical-trapping antioxidant potential (TRAP) value.

Although physical exercise is recognized to have many beneficial effects, there is concern that strenuous exercise of sufficient intensity and duration increases aerobic metabolism by a factor of up to 10, leading to an increase in the leakage of free radicals into the cytosol. This oxidative stress can lead to lipid peroxidation of polyunsaturated fatty acids in cell membranes and subsequent cell injury, if the rise in free radical activity exceeds the protective antioxidant capacity. Serum concentrations of antioxidants have been shown to fall in these circumstances and supplementation of subjects with combinations of antioxidant vitamins (α tocopherol, ascorbic acid and β carotene) reduces the exercise-induced increase of indicators of lipid peroxidation.

Most studies examining exercise and antioxidants have been in highly selected subjects, and to the best of our knowledge TRAP has not been measured in these circumstances. The aims of this study were to measure the serum antioxidants urate, bilirubin, protein thiols, α tocopherol and ascorbate, to calculate the TRAP (TRAPcalc) in a cross-section of the Northern Ireland population, and to examine the relationship between TRAPcalc and both physical activity and fitness. TRAP can also be measured directly (TRAPmeas) but, in general, TRAPcalc values show a good correlation with TRAPmeas although tending to be lower because of the effect of unidentified/unmeasured free-radical scavengers in the serum and synergistic relationships among the secondary antioxidants. As antioxidants and physical activity have protective effects against atherogenesis and coronary heart disease, we were interested in the hypothesis that TRAP values would be higher in those undertaking greater physical activity. However, if TRAP values were lower, this could be an indication that antioxidants were being consumed secondary to the increased oxidative stress of exercise, and that antioxidant supplementation may be required.

Methods

This study formed part of the Northern Ireland Health and Activity Survey, the field work of which took place between February and November 1992. The purpose of the survey was to obtain a detailed assessment of physical activity in all aspects of daily living and to measure physical fitness. This was the first population measure of fitness in Northern Ireland. The other major purpose was to study relationships between physical activity, fitness and blood measurements with a particular emphasis on coronary heart disease.

The survey was designed to yield a representative sample of adults aged 16 years or over in Northern Ireland, and involved two-stage probability sampling. A randomized sample of 1600 addresses was taken from the Northern Ireland rating and valuation lists, stratified by region to ensure proportional sampling across the Province. A Kish grid random selection procedure was used to identify one person over the age of 16 living at the particular address, and the sample was subsequently weighted to take account of household size.

The fieldwork comprised three parts: a computer-assisted interview, physical appraisal and fasting blood samples. The questionnaire and physical appraisal were similar to that used in the Allied Dunbar National Fitness Survey with some minor modifications to clarify ambiguities and some additions of relevance to Northern Ireland. The fieldwork was conducted in 14 centres throughout Northern Ireland. The computer-assisted interview included, amongst other aspects, questions with regard to lifestyle, e.g. smoking history and physical activity.

A dietary score was calculated based on the response to a number of dietary questions about consumption of meat, dairy products, vegetables and fibre so that a low score represented a good diet. While this was a relatively crude scoring system, it allowed a comparison between physical activity and diet in all subjects. Physical activity was classified under three headings:

1. The highest level of activity in the previous 4 weeks independent of duration (Actmax). There were four categories. (a) Vigorous: active at a vigorous level on at least one occasion (energy cost greater than 7.5 kcal/min or 60% VO2max). (b) Moderate: active at a moderate level on at least one occasion (energy cost 5–7.5 kcal/min or 40–60% VO2max). (c) Light: active at a light level on at least one occasion (energy cost 2–5 kcal/min or <40% VO2max). (d) None: not active at vigorous, moderate or light level in previous 4 weeks.

2. The frequency and intensity of bursts of physical activity (habitual activity) lasting 20 min in the previous 4 weeks (Actlevel). Level 5, 12 or more occasions of vigorous activity; level 4, 12 or more occasions mixed between vigorous and moderate activity; level 3, 12 or more occasions of at least
moderate activity; level 2, 5–11 occasions of at least moderate activity; level 1, 1–4 occasions of at least moderate activity; level 0, no moderate or vigorous activity lasting 20 min.

3. Past participation (Pastpar): Lifetime participation since the age of 14 years was calculated by counting the number of years in which each respondent participated in vigorous or moderate activities on a regular basis (at least once per week for a few months of the year or more). This included walks of over 2 miles and cycling. Dividing the number of years of participation by total years from age 14 until the present created a ratio of lifetime participation. The % of life active was subdivided into five categories (0%, 1–24%, 25–49%, 50–74%, 75% +).

Physical appraisal included the measurement of height, weight, body fat, blood pressure, muscle strength and power. Aerobic fitness was measured by $O_2$ uptake on treadmill and maximum $O_2$ uptake ($VO_2\text{max}$) was predicted by extrapolation from performance at 85% of maximum predicted heart rate.

Venous blood was collected from the subjects following a 12 h fast. The sample was taken either following centrifugation the supernatants were stored at $-40^\circ$C prior to analysis. Serum ascorbate concentrations were stabilized by adding 0.5 ml serum to 1 ml freshly prepared 6% metaphosphoric acid, and following centrifugation the supernatants were stored at $-40^\circ$C.

Serum urate and bilirubin concentrations were measured using routine laboratory methods. Serum total ascorbate was measured following reaction with 2,4-dinitrophenylhydrazine, and the resulting dinitrophenylhydrazone was measured as $A_{320}$. Plasma sulphydryl (SH) groups were assayed with 5,5'-dithio-bis-(2-nitrobenzoic acid)(DTNB). Vitamin E (a tocopherol) was measured by high-performance liquid chromatography (HPLC) with spectrofluorescence detection. The method was standardized with the National Institute of Standards reference material 968A. Mean recovery was 108%.

TRAP was calculated using the following formula:

$$\text{TRAPcalc (\mu mol/l)} = \Sigma \text{antioxidant concentration} \times \text{stoichiometric value.}$$

$$\text{TRAPcalc (\mu mol/l)} = 2[a \text{ tocopherol}] + 1.7[\text{ascorbate}] + 1.3[\text{urate}] + 0.2[\text{sulphydryl groups}] + 2[\text{bilirubin}]^{22,23}$$

Statistical analysis

Data were analysed using SPSS (Statistical Package for Social Sciences). Analysis of variance was used for comparison of means between activity groups and two-way analysis of variance was used to adjust for age. The relationship between TRAP and activity (or fitness) was also examined using multiple linear regression to adjust for influence of age, body mass index, mean blood pressure, social class, education, smoking and alcohol consumption.

Results

Out of 1456 effective addresses, 1020 (70%) achieved interviews; of those interviewed 62% had physical appraisal, representing 43.4% of the eligible sample and 43.5% had blood tests taken. Those who had blood tests were representative of those who completed the questionnaire in respect of age, weight, physical activity and health but more were male and more non-smokers ($p<0.05$).

TRAPcalc distribution in the Northern Ireland population

The total number of males and females who had TRAP blood samples taken were 194 and 212, respectively (for age range 16–74). The mean TRAPcalc was significantly higher in males ($653 \pm 82 \mu \text{mol/l}$, mean $\pm$ SEM) compared to females ($564 \pm 80 \mu \text{mol/l}$) ($p<0.0001$). The distribution of results within the population is shown in Figure 1. The explanation for this difference lies in the findings that serum urate, bilirubin and protein thiols were significantly higher in males (urate $0.30 \pm 0.005 \mu \text{mol/l}$ vs. $0.22 \pm 0.005 \mu \text{mol/l}$, $p<0.0001$; bilirubin $10.5 \pm 0.41 \mu \text{mol/l}$ vs. $7.5 \pm 0.3 \mu \text{mol/l}$, $p<0.0001$; protein thiols $513 \pm 9.3 \mu \text{mol/l}$ vs. $478 \pm 9.6 \mu \text{mol/l}$, $p=0.01$). However, serum ascorbate was significantly higher in the female population ($66.1 \pm 2.0$ vs. $53.9 \pm 2.1 \mu \text{mol/l}$, $p<0.0001$) and $\alpha$ tocopherol did not differ ($26.6 \pm 0.57$ vs. $27.6 \pm 0.64 \mu \text{mol/l}$, $p=0.24$). Both male and female cigarette smokers had lower TRAPcalc values than non-smokers (males, $597 \pm 14.3 \mu \text{mol/l}$ vs. $676 \pm 9.4 \mu \text{mol/l}$, $p<0.0001$; females, $536 \pm 13.6 \mu \text{mol/l}$ vs. $576 \pm 9.8 \mu \text{mol/l}$, $p=0.02$). Smokers represented 32% of the sample population. The only statistically different result in the individual components of the TRAP equation between smokers and non-smokers of both sexes was in serum ascorbate (males: smokers $45.1 \pm 4.4 \mu \text{mol/l}$, non-smokers $57.5 \pm 2.2 \mu \text{mol/l}$, $p=0.01$; females: smokers $56.1 \pm 3.7 \mu \text{mol/l}$, non-smokers $70.8 \pm 2.3$, $p<0.0001$). However, in male smokers serum urate and bilirubin were also signific-
Table 1 TRAP and Actmax

<table>
<thead>
<tr>
<th>Activity (Actmax)</th>
<th>Male (μmol/l)</th>
<th>Female (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>606.5 ± 127.5</td>
<td>623.8 ± 119.8</td>
</tr>
<tr>
<td>Light</td>
<td>638.1 ± 132.2</td>
<td>579.6 ± 133.6</td>
</tr>
<tr>
<td>Moderate</td>
<td>641.8 ± 102.5</td>
<td>561.6 ± 111.0</td>
</tr>
<tr>
<td>Vigorous</td>
<td>671.1 ± 122.0</td>
<td>556.1 ± 127.0</td>
</tr>
<tr>
<td>( p ) (corrected for age)</td>
<td>0.26</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Data are means ± S.D.

Table 2 TRAP and habitual activity (Actlevel)

<table>
<thead>
<tr>
<th>Activity (Actlevel)</th>
<th>Male (μmol/l)</th>
<th>Female (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 0</td>
<td>667.3 ± 155.6</td>
<td>601.5 ± 127.7</td>
</tr>
<tr>
<td>Level 1</td>
<td>636.5 ± 118.5</td>
<td>550.9 ± 108.6</td>
</tr>
<tr>
<td>Level 2</td>
<td>703.6 ± 115.0</td>
<td>567.0 ± 126.2</td>
</tr>
<tr>
<td>Level 3</td>
<td>633.8 ± 95.1</td>
<td>551.5 ± 103.8</td>
</tr>
<tr>
<td>Level 4</td>
<td>658.4 ± 76.9</td>
<td>534.6 ± 87.2</td>
</tr>
<tr>
<td>Level 5</td>
<td>656.1 ± 105.8</td>
<td>571.7 ± 159.5</td>
</tr>
<tr>
<td>( p ) (corrected for age)</td>
<td>0.42</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Table 3 TRAP and Pastpar

<table>
<thead>
<tr>
<th>% of life active (Pastpar)</th>
<th>Male (μmol/l)</th>
<th>Female (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>620.0 ± 84.0</td>
<td>579.2 ± 128.6</td>
</tr>
<tr>
<td>1–24</td>
<td>588.3 ± 116.1</td>
<td>529.8 ± 119.7</td>
</tr>
<tr>
<td>25–49</td>
<td>626.3 ± 114.7</td>
<td>605.0 ± 96.3</td>
</tr>
<tr>
<td>50–75</td>
<td>686.2 ± 159.4</td>
<td>517.5 ± 80.2</td>
</tr>
<tr>
<td>75+</td>
<td>665.2 ± 105.4</td>
<td>568.1 ± 122.3</td>
</tr>
<tr>
<td>( p ) (corrected for age)</td>
<td>0.02</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Figure 1. TRAP distribution in the (a) male and (b) female populations.

Antithymosynthesized lower (urate 0.28 ± 0.004 vs. 0.31 ± 0.005 mmol/l, \( p < 0.01 \); bilirubin 8.1 ± 0.34 vs. 11.5 ± 0.43 μmol/l, \( p < 0.0001 \)). Multiple regression analysis showed no relationship between TRAPcalc and age in males but in females there was a positive relationship (coeff = 2.3, \( p < 0.001 \)). There was a significant relationship between log α tocopherol and age in both sexes (coeff = 0.01, \( p < 0.001 \)) and between ascorbate and age in females (coeff = 0.02, \( p < 0.01 \)).

TRAPcalc and physical fitness/physical activity

These results are summarized in Tables 1–3. The survey found that only 21% of males and 6% of females took part in 12 or more occasions of vigorous activity lasting at least 20 minutes in the 4 weeks before the interview. Seven out of 10 men and 8/10 women fell below the recommended activity levels considered to be the minimum to confer a cardioprotective benefit. Using analysis of variance to compare the mean TRAPcalc between the activity levels in the three different activity groups (Actmax, Actlevel and Pastpar), the only statistically significant result was in the male Pastpar group after adjustment for age (\( p = 0.02 \)). This was also significant using multiple regression analysis (coeff = 75.2, \( p < 0.01 \)), but not after adjustment for confounding factors. Multiple regression in males showed no relationship between TRAPcalc and age, body mass index (BMI), VO2max, Actmax and Actlevel. In females there was a relationship with age (coeff = 2.3, \( p < 0.001 \)), BMI (coeff = 5.49, \( p < 0.001 \)), VO2max (coeff = -2.26, \( p < 0.05 \)) and with Actlevel (coeff = -53.9, \( p < 0.05 \)). However, the significant negative relationship with Actlevel did not persist following adjustment for confounding factors. There was no relationship between diet and physical activity or physical fitness in either sex.
Discussion

The finding of higher TRAP values in the male population has been noted previously and is explained on the basis of higher serum urate concentrations in this population. Differences in urate concentrations between the sexes have also been documented previously. Urate is the major contributor to TRAP, accounting for between 38–47% of the total. In contrast, the antioxidant vitamins C and E contribute between 13–17% and 2–8%, respectively. Protein thiols (sulphydryl groups) have the highest plasma concentration but act as relatively ineffective antioxidants. Bilirubin can act as an efficient antioxidant, with a stoichiometric value of 2, but normally contributes only relatively minor amounts to the total radical trapping ability of serum in healthy subjects. The vitamins E (α tocopherol) and C (ascorbate) have important differences in the contribution they make to antioxidant potential, in that α tocopherol is the major lipid-soluble chain-breaking antioxidant in cell membranes, and ascorbate is an important aqueous phase antioxidant. We found serum ascorbate to be significantly higher in the female population, which is in keeping with other studies and is explained by ovarian hormone activity. The increased protein thiols (sulphydryl groups) and serum bilirubin in males, perhaps secondary to higher serum albumin concentrations, would only contribute to small increases in calculated TRAP values.

This study showed that TRAP increased with age in females (coeff = 2.3, p < 0.001) but there was no relationship with age in males. This may be accounted for by an increase in serum α tocopherol and ascorbate with age noted in the female population, although a similar increase in α tocopherol in males did not lead to a statistically significant increase in TRAP. As α tocopherol is a lipid-soluble antioxidant, this increase is secondary to increasing serum cholesterol concentrations that occur in older individuals. However, the increase in serum ascorbate in females is unusual, in that most studies have found a decrease with age based on poorer dietary intake.

Smokers of both sexes display lower TRAP values than non-smokers—this is most probably related to the increased oxidative stress imposed by cigarette smoke and the subsequent consumption of antioxidants and/or a decreased dietary intake of antioxidant vitamins. Most studies have shown decreased ascorbate concentrations in smokers but to the best of our knowledge there have been no reports of lower serum urate and bilirubin concentrations. Our findings, which are confined to the male subpopulation, require further investigation.

Previous studies assessing oxidant status and exercise have involved quite strenuous activity in highly selected subjects. Antioxidants were measured during or shortly after the completion of the exercise activity, and showed reduced concentrations. In contrast this study measured activity levels and antioxidants in a random sample of the population. We found a positive relationship between TRAP and past participation in exercise and physical activity in men, suggesting that antioxidant status may be increased in those who are more active over the long term. However, this relationship disappeared following adjustment for confounding factors. In the female population, we found a negative relationship between TRAP and habitual activity (Actlevel) although again this relationship did not persist following adjustment for confounding factors. However, in addition we found a negative relationship between TRAP and physical fitness as measured by VO_{2max} such that an increase of 1 ml/kg/min in VO_{2max} was associated with a decrease of 2.26 μmol/l in TRAP (p < 0.05). We are unsure of the implications of this finding and no particular component of the TRAP calculation appeared to be responsible for this decrease. This finding raises the issue of increased oxidative stress and consumption of antioxidants in those with greater physical fitness and could point towards a possible role for antioxidant supplementation in females. However, it is important to recognise that aerobic fitness does not necessarily indicate that an individual has been active, although in this study VO_{2max} was significantly higher in the more active subgroups. Regular training can improve aerobic fitness but natural endowment is the greatest determinant of aerobic capacity and although active people tend to be fitter, some untrained individuals may have greater aerobic fitness compared to the physically active.

We do not have a satisfactory explanation for the positive relationship between TRAP and body mass index (BMI) in females and though it might be expected that those who were active would show more health awareness and be more likely to consume a healthier diet, there was no relationship between activity and diet in either sex in the study.

We are aware of a potentially confounding factor in this longitudinal study. It is well recognized that there is a seasonal variation in serum ascorbate related to decreased consumption of fresh fruit and vegetables in the winter months. As this study encompassed all four seasons, the possibility of seasonal influence is present but we believe the effect of this to be small and to be balanced out over the time period. In conclusion the distribution of TRAP in the population of Northern Ireland is similar to other studies. Males display higher values than females (p < 0.0001), due predominantly to higher urate concentrations (p < 0.0001). Smokers
displayed lower values than non-smokers. There was no suggestion that antioxidant status is altered with physical activity and therefore the recognized health benefits of exercise do not appear to be mediated through increased antioxidant status. There was also no evidence that those who participate in regular exercise require antioxidant supplementation. The negative correlation between TRAP and physical fitness in females may be due to chance but requires further intensive investigation.

Acknowledgements

The Northern Ireland Health and Activity Survey was supported by the Sports Council for Northern Ireland, Northern Ireland Chest, Heart and Stroke Association, The Department of Education (NI), the Department of Health and Social Services (NI), Save and Prosper and a number of local councils. We also thank Miss Annette Henry for her assistance with the manuscript, and the laboratory staff at Ulster Hospital.

References