Biomarkers, September–October 2005; 10(5): 390–400

Preliminary studies on the effect of moderate physical activity on blood levels of glutathione

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Abstract

Molecular epidemiological approaches are being used to study how physical activity may protect against cancer. Prior epidemiological data suggest that physical activity protects against lung cancer; however, interpretation of these data is complicated by potential confounding by smoking. Glutathione (GSH) detoxifies cigarette smoke carcinogens and the paper tests whether physical activity levels are associated with blood GSH levels. Study subjects were enrolled in a chemoprevention trial testing whether antioxidant micronutrient supplementation reduces genetic damage from cigarette smoking. Physical activity data were collected by questionnaire from 178 subjects at 12 months of follow-up in the trial. Total GSH (tGSH), which is the sum of free and protein-bound GSH and glutathione disulfide levels, was measured using the 5,5′-dithiobis-(2-nitrobenzenoic acid) colormetric assay with red blood cell samples collected at the 12-month time point. In multivariate linear regression analyses that controlled for gender and cigarettes smoked per day, tGSH was positively associated with hours per week of moderate intensity activity ($\beta = 0.005$, $p = 0.02$). Hours per week of vigorous intensity activity were unassociated with tGSH and the effect of moderate activity remained after control for vigorous activity. The results are consistent with prior research showing differential effects of moderate and vigorous activity and suggest a mechanism through which physical activity may influence lung cancer risk.

Keywords: *Physical activity, glutathione, transitional study, smokers*

(Received 29 November 2004; accepted 19 July 2005)

Introduction

In light of the growing evidence that physical activity prevents certain cancers, researchers have recognized a need for biomarker studies that investigate potential mechanisms through which physical activity may exert its effect (McTiernan et al. 1999, Rundle 2005). Possible mechanisms include activity-induced changes in sex hormone levels, growth factors, immune function and endogenous antioxidants (McTiernan et al. 1998). There is epidemiological evidence to suggest that physical

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ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.1080/13547500500272663

activity is protective against lung cancer. However, the overwhelming impact of smoking and its likely association with activity levels makes the interpretation of these data difficult (Albanes et al. 1989, Sellers et al. 1991, Lee & Paffenbarger 1994, Thune & Lund 1997, Lee et al. 1999, Kubik et al. 2001, Petersen et al. 2001). To help clarify this relationship, we are performing biomarker studies of physical activity among a cohort of smokers. Here we report on the association between activity levels and the endogenous antioxidant, glutathione, measured in red blood cells.

Glutathione (GSH) is a low molecular weight tripeptide found in great abundance in cells, and it is a key endogenous antioxidant (Kelly 1999). GSH acts as a non-enzymatic free radical scavenger, and as a substrate for glutathione peroxidase mediated reduction of H_2O_2 and other hydroperoxides (Balakrishnan & Anuradha 1998, Kelly 1999, Rahman et al. 1999). The glutathione S-transferase family of enzymes also uses GSH as a conjugating group in the Phase II detoxification of polycyclic aromatic hydrocarbons and other xenobiotics (Bartsch et al. 1991, Rebbeck 1997). Thus, GSH, and the enzymes for which it serves as a substrate, defends cells against a multitude of carcinogenic insults. Consistent with this protective role, a recent case-control study of lung cancer found increased dietary intake of glutathione to be protective. In comparison with the lowest quartile of consumption, the highest quartile of intake had an odds ratio of 0.42 (95% CI 0.27 to 0.63) (Stefani et al. 1999).

Exercise physiology studies and small cross-sectional studies of athletes and controls suggest that GSH levels are influenced by physical activity levels (Robertson et al. 1991, Evelo et al. 1992). However, due to the small size of the prior studies, potential confounding and interacting factors were not assessed. Furthermore, these studies accessed the impact of training programmes, such as training for a half marathon, and it is not clear to what extent activity, within the normal range engaged in by the general population, impacts on GSH levels (Evelo et al. 1992). A larger cross-sectional study of the general population also observed increased GSH levels in those who exercised, but did not access potential confounders or effect modifiers, such as diet or smoking (Michelet et al. 1995). Similarly, a study of 31 men aged 65 years or more found a positive correlation between activity level and blood GSH levels but also did not take into account possible confounding factors (Karolkiewicz et al. 2003). Before activity-related biomarkers can be included in molecular epidemiological studies of physical activity and cancer, transitional studies are required to identify potential confounders and to describe the dose–response curve between activity and the biomarker (Hulka & Margolin 1992). In this preliminary transitional biomarker study, we have investigated the association between GSH levels and physical activity taking into account other potential determinants of GSH levels, and the potential differing effects of moderate and vigorous activity.

Materials and methods

Cross-sectional analyses of GSH and physical activity were conducted in a population taking part in randomized double-blind placebo controlled study of whether antioxidant micronutrient supplementation reduces genetic damage induced by cigarette smoking. From 1997 to 2000, smokers ($n = 320$) were randomized to receive treatment with 500 mg vitamin C and 400 IU vitamin E daily or a placebo. Men and women aged 18 years and older who attended the New York State

Psychiatric Institute Smoking Clinic or who responded to a newspaper advertisement were recruited to participate in the chemoprevention trial. Subjects were determined eligible to participate if they smoked more than ten cigarettes per day, had no prior history of cancer or liver disease, had not taken vitamin supplements within the past year, had normal liver enzyme function tests, lived at a permanent address, owned a telephone, were willing to participate for the total duration of the trial (24 months), returned 1 month after the baseline visit and completed the placebo run-in. Participants received monetary compensation for their participation. Written informed consent was obtained from all subjects. Consent forms and recruitment procedures were approved by the IRBs of Columbia Presbyterian Medical Center (CPMC), the Herbert Irving Cancer Center, and the New York State Psychiatric Institute. The cohort was followed for 2 years, during which the intervention arm received vitamin treatment for 15 months and then placebo for another 9 months.

At baseline, the study subjects completed extensive questionnaires on demographic and lifestyle characteristics. Study subjects visited the CPMC at 3-month intervals and gave blood samples and filled out study questionnaires. At the 12-month time point, study subjects completed the Paffenbarger Physical Activity Questionnaire and at the 15-month time point study subjects completed the Block 95 Food Frequency Questionnaire. The Block 95 questionnaires were processed by the Berkeley Nutrition Systems (Berkeley, CA, USA) to derive estimates of cysteine and methionine intake contained in protein and GSH intake. GSH levels were measured at the Institute for Cancer Prevention (Valhalla, NY, USA) in frozen red blood cells collected at the 12-month time point. Of the 320 subjects who entered the cohort, 203 remained at the 12-month time point and had red blood cell samples available for analyses. Of these, 178 subjects (79 women and 99 men) had also completed the physical activity questionnaire administered at the 12-month time point. Of these 178 subjects, data on dietary intake at 15 months of follow-up were available from 163 subjects.

Total GSH (tGSH), which is the sum of free and protein-bound GSH and glutathione disulfide (GSSG) levels, was measured in red blood cells using the 5,5′-dithiobis-(2-nitrobenzenoic acid) (DTNB) colormetric assay (Richie et al. 1996b) after reduction with potassium borohydride, as described (Mills et al. 1994). Briefly, packed red blood cell samples were incubated in 8 M urea, 1 mM EDTA, 0.43 M potassium borohydride at 40°C for 1 h, followed by addition of 20% metaphosporic acid. GSH was assessed by incubating resulting acid extracts in 96-well microtiter plates with DTNB and glutathione oxidoreductase. The rate of thiobis nitrobenzoic acid (TNB) formation was monitored by measuring absorbance at 410 nm in a plate reader. Each sample was run in duplicate in adjacent columns on the same plate and the results for each sample were averaged. While this method measures tGSH, oxidized forms (protein-bound GSH and GSSG) represent a small percentage of the total glutathione pool. Use of tGSH measurements, expressed as units of GSH equivalents, was necessary since, during storage, GSH can be oxidized to GSSG and bind to cellular proteins. To test the reliability of the assay 30 randomly selected samples were re-analysed on a different day. Also, in six randomly selected samples, GSH (free and protein-bound) and GSSG levels were analysed separately by HPLC with electrochemical detection (Kleinman & Richie 1995).

To assess the individual *GSTM1* genotype, DNA was extracted from blood leukocytes and analysed by polymerase chain reaction (PCR) as described (Bell et al. 1993). The primers used in the PCR mix were G5, 5′-GAA CTC CCT GAA

AAG CTA AAG C; G6 5′-GTT GGG CTC AAA TAT ACG GTG G (Bell et al. 1993). As a control to detect PCR failures, the assay included primers for the beta-globin gene. Subjects homozygous (+/+) or heterozygous (−/+) for *GSTM1* were classified as *GSTM1* positive, and those who were homozygous deleted (−/−) were classified as *GSTM1* null.

To assess the reliability of the GSH data, 30 randomly selected red blood cell samples were analysed twice and the data were analysed by paired *t*-tests, Pearson correlation coefficients, and intra-class correlation analyses (Shrout & Fleiss 1979).

Demographic and lifestyle characteristics previously shown or hypothesized to be associated with GSH levels were assessed as potential confounders, including cigarette smoking (Cantin et al. 1987, Michelet et al. 1995, Richie et al. 1996b), dietary intake of GSH (Jones et al. 1992, Flagg et al. 1994), dietary intake of GSH precursors (Richie et al. 1996a, Kleinman & Richie 2000, Lyons et al. 2000), and antioxidant micronutrient treatment (Costagliola et al. 1985, Henning et al. 1991, Brown et al. 1996, Monget et al. 1996). Additionally, the deletion polymorphism in GSTM1 was assessed as a predictor of GSH because the deletion may alter GSH utilization. For continuous predictor variables, study subjects were categorized by quartiles or tertiles of the predictor variable. Mean GSH levels were calculated for each category of the predictor variables and ANOVA was used to determine whether GSH levels varied by category. Since physical activity patterns differed by gender analyses were conducted separately by gender, with subjects categorized using gender specific quartiles or tertiles of the predictor variables. For dichotomous predictor variables, *t*-tests were used to determine whether GSH levels varied by level of the predictor.

Multivariate linear regression analyses were used to determine whether physical activity was associated with tGSH levels after control for potential confounders. Because of the potential for differential effects of moderate and vigorous activity, two measures of physical activity were used; hours of activity per week engaged in at a moderate intensity and hours of activity per week engaged in at vigorous activity (Hartmann et al. 1994, Niess et al. 1996, Asami et al. 1998, Kasai et al. 2001, Tsai et al. 2001, Rundle 2005). Each potential confounding variable was added singularly to a model that included a variable for physical activity level (Miettinen 1974, Kleinbaum et al. 1988). When the addition of a variable to the model appreciably altered the effect estimate for the physical activity variable, the added variable was considered to be a confounder (Rothman 1986, Kleinbaum et al. 1988). Smoking related variables were of primary concern and were assessed as potential confounders first. Since the data on diet in the past year were collected later in follow-up, at the 15-month time point, dietary data were not available from all of the subjects. Statistical analyses that included dietary data had a smaller sample size than analyses that did not, thus effect estimates for the tGSH-physical activity associations might have differed between models with and without the dietary variables due to changes in sample size or due to confounding effects of the dietary factor. Thus, to evaluate whether diet was a confounder, analyses with and without diet in the models were performed on the subset of individuals from whom dietary data was collected. The final model included all variables that were identified as being confounders of GSH–activity relationships. In these analyses continuous predictor variables were not categorized, but were entered into the models in their natural scale.

Analyses were conducted separately by gender and treatment status to assess potential effect modification by these variables. If the effect estimate for any of the

predictors of GSH appeared to vary by gender or treatment a formal test for interaction was conducted by fitting a model in the entire study population that included an interaction term for the variables of interest. If the interaction term was non-significant, the strata for that variable were collapsed in the final multivariate model (Kleinbaum et al. 1988). Lastly, a model was implemented that included all identified confounders and interaction terms and separate variables for hours of moderate physical activity and hours of vigorous physical activity.

Results

The analyses of the data from the 30 samples which were re-analysed for tGSH levels demonstrated that the assay is highly reliable. There was no significant difference in the first and second analytical runs. The tGSH levels resulting from the first analysis (mean = 1.08 meq. l^{-1}) were not significantly different from those of the second analysis (mean = 1.06 meq. l^{-1}) ($p = 0.21$). Further, the results from the two analytical runs were highly correlated, with a correlation coefficient of 0.97 ($p < 0.001$). A formal analysis of reliability by intra-class correlation analysis resulted in a reliability of 0.97 (95% CI 0.94–0.99). Also, for the six samples which were re-analysed by HPLC with electrochemical detection, values for GSH and GSSG combined (mean 1.09 meq. l^{-1}) were nearly identical with tGSH values determined by the micro-plate method (1.07 meq. l^{-1}). Regression analyses showed that tGSH measured by the microplate method was highly associated with tGSH measured by HPLC ($\beta = 0.97$, $R = 0.99$, $p < 0.001$).

In univariate analyses, tGSH levels were not associated with demographic characteristics such as gender, age and race (Table I). Nor were levels associated with personal characteristics or behaviours such as body mass index (BMI), smoking or physical activity. Furthermore, tGSH levels were not associated with dietary intake of glutathione, cysteine, or methionine and were unassociated with antioxidant micronutrient treatment and GSTM1 genotype.

However, multivariate analyses revealed some important associations between lifestyle factors and tGSH levels. Across the entire population hours of moderate activity per week were positively associated with tGSH levels ($\beta = 0.005$, $p = 0.02$). Among women cigarettes smoked per day at baseline entry into the cohort were negatively associated with tGSH levels ($\beta = -0.01$, $p = 0.01$). However, in men there was no association between cigarettes smoked per day and tGSH levels ($\beta = 0.003$, $p = 0.55$). Gender was associated with tGSH levels with men having lower levels than women ($\beta = -0.383$, $p = 0.01$). The interaction between gender and smoking was significant ($p = 0.04$) and the final multivariate model included an interaction term for the joint effects of gender and smoking intensity (Table II).

In multivariate analyses hours of vigorous physical activity remained unassociated with blood tGSH levels. In the model that included separate variables for both hours of moderate and hours of vigorous physical activity, vigorous activity was unassociated with tGSH levels while moderate activity remained associated with tGSH levels (Table II). Furthermore, in comparison to the model that included hours of moderate activity as the only measure of physical activity, the addition of a variable for vigorous activity did not alter the parameter estimates for any of the other variables in the model (Table II).

Table I. Associations between demographic, lifestyle, dietary and genetic variables and blood tGSH levels.

	Women Mean tGSH meq. l^{-1} (SD) *n*		Men Mean tGSH meq. l^{-1} (SD), *n*
tGSH ($p=0.21$)	1.16 (0.42) 89		1.08 (0.44) 114
Ethnicity ($p=0.19$)		Ethnicity ($p=0.69$)	
African American	1.16 (0.47) 49	African American	1.13 (0.50) 48
Caucasian	1.21 (0.34) 31	Caucasian	1.08 (0.41) 43
Hispanic	0.89 (0.21) 7	Hispanic	1.03 (0.32) 18
Age (years) ($p=0.58$)		Age (years) ($p=0.21$)	
≤31	1.19 (0.42) 23	≤33	1.14 (0.43) 29
>31 to ≤38	1.21 (0.49) 24	>33 to ≤39	1.08 (0.46) 29
>38 to ≤44	1.18 (0.43) 21	>39 to ≤45	0.94 (0.40) 28
>44	1.05 (0.32) 21	>45	1.17 (0.43) 28
BMI ($p=0.41$)		BMI ($p=0.12$)	
≤22.68	1.21 (0.47) 21	≤22.11	1.24 (0.47) 28
>22.68 to ≤25.82	1.14 (0.28) 23	>22.11 to ≤25.89	1.067 (0.43) 29
>25.82 to ≤31.28	1.23 (0.49) 22	>25.89 to ≤28.77	1.07 (0.48) 29
>31.28	1.04 (0.41) 22	>28.77	0.96 (0.33) 28
Cigarettes per day at baseline ($p=0.16$)		Cigarettes per day at baseline ($p=0.70$)	
<20	1.27 (0.50) 32		1.06 (0.47) 44
20	1.09 (0.32) 29		1.14 (0.40) 37
>20	1.10 (0.40) 28		1.07 (0.43) 32
Cigarettes per day at 12 months ($p=0.51$)		Cigarettes per day at 12 months ($p=0.80$)	
<20	1.19 (0.44) 48		1.09 (0.47) 64
20	1.19 (0.41) 23		1.12 (0.38) 26
>20	1.06 (0.37) 18		1.04 (0.41) 24
Hours of vigorous activity per week ($p=0.97$)		Hours of vigorous activity per week ($p=0.45$)	
<4.67	1.18 (0.43) 25	<7	1.02 (0.47) 24
≥4.67 to <14.67	1.19 (0.36) 28	≥7 to <21	1.12 (0.38) 41
≥14.67	1.16 (0.48) 26	≥21	1.01 (0.41) 34
Hours of moderate activity per week ($p=0.03$)		Hours of moderate activity per week ($p=0.92$)	
<17.67	1.16 (0.36) 26	<14	1.05 (0.36) 22
≥17.67 to <33	1.03 (0.37) 26	≥14 to <28	1.04 (0.42) 36
≥33	1.33 (0.48) 27	≥28	1.08 (0.44) 41
Methionine in the diet ($p=0.30$)		Methionine in the diet ($p=0.27$)	
≤1.24 g	1.17 (0.32) 21	≤1.72 g	1.17 (0.44) 30
>1.24 to ≤1.76 g	1.23 (0.47) 22	>1.72 to ≤2.27 g	1.05 (0.41) 31
>1.76 g	1.05 (0.32) 21	>2.27 g	1.01 (0.35) 28
Cysteine in the diet ($p=0.73$)		Cysteine in the diet ($p=0.38$)	
≤0.77 g	1.15 (0.29) 23	≤0.95 g	1.14 (0.46) 30
>0.77 to ≤1.05 g	1.20 (0.47) 20	>0.95 to ≤1.28 g	1.09 (0.41) 30
>1.05 g	1.10 (0.38) 21	>1.28 g	1.00 (0.34) 29
Total GSH in the diet ($p=0.45$)		Total GSH in the diet ($p=0.32$)	
≤34.093 μg	1.21 (0.33) 21	≤42.89 μg	1.13 (0.45) 30
>34.093 to ≤45.093 μg	1.07 (0.39) 22	>42.89 to ≤58.46 μg	1.12 (0.40) 30
>45.093 μg	1.17 (0.41) 21	>58.46 μg	0.98 (0.37) 29

Table I (*Continued*)

	Women Mean tGSH meq. l^{-1} (SD) *n*		Men Mean tGSH meq. l^{-1} (SD), *n*
Vitamin C and E treatment ($p=0.30$)		Vitamin C and E treatment ($p=0.76$)	
No	1.12 (0.40) 49		1.10 (0.45) 59
Yes	1.21 (0.44) 40		1.07 (0.42) 55
GSTM1 genotype ($p=0.11$)		GSTM1 genotype ($p=0.21$)	
Null	1.28 (0.39) 26		1.02 (0.41) 43
+/+, +/−	1.12 (0.43) 63		1.13 (0.46) 68

Using multivariate analyses the other variables of interest were also assessed as potential confounders of the moderate activity–GSH relationship. Analyses were first performed in men and women separately and then were repeated in men and women combined, using models that included variables for gender, cigarettes smoked per day and an interaction term for gender and cigarettes smoked per day. In these multivariate models, dietary components, age, ethnicity, cigarettes smoked per day at 12 months, body mass index, antioxidant micronutrient treatment, vigorous activity, blood haemoglobin levels and GSTM1 genotype remained unassociated with tGSH levels. Addition of these variables to the basic model described above did not appreciably alter the parameter estimates reported in Table II, indicating that they did not have confounding effects.

Discussion

The findings that increased hours of moderate intensity activity per week are positively associated with blood tGSH are consistent with past research. Robertson et al. (1991) showed that among runners, blood GSH levels were correlated with weekly training levels and that runners had higher levels than sedentary controls. Evelo et al. (1992) showed that after training sedentary individuals for 20 weeks to run a half marathon, blood GSH levels were increased. In a cross-sectional study of 265 subjects, Michelet et al. (1995) found that the practice of regular physical activity was associated with increased blood GSH levels. Karolkiewicz et al. (2003) showed that among older men, increased activity was associated with increased levels of oxidized GSH in red blood samples. We have extended this literature by considering a multitude of potential confounding factors and covariates, and we showed that the effect of activity is

Table II. Multivariate linear regression model[1] predicting tGSH levels.

Variable	Parameter estimates	Parameter estimates
Hours of moderate activity per week	$B=0.005$, $p=0.02$	$\beta=0.005$, $p=0.02$
Cigarettes smoked per day at entry into the clinical trial	$B=-0.009$, $p=0.01$	$\beta=-0.01$, $p=0.01$
Gender (women as the referent group)	$B=-0.383$, $p=0.01$	$\beta=-0.368$, $p=0.01$
Gender * cigarettes smoked per day at entry into the clinical trial	$B=0.013$, $p=0.04$	$\beta=0.013$, $p=0.04$
Hours of vigorous activity per week	–	$\beta=-0.002$, $p=0.40$

[1]Each variable's parameter estimate adjusted for the other variables in the table.

independent of these factors. One concern with exercise physiology studies is that the effects observed with formal training programmes may not be seen at activity levels reported in epidemiological studies. Here we show that increased activity performed within the context of daily living has a positive effect on GSH levels.

To place the results in context, the standard deviation of the GSH levels was 0.42 meq. l^{-1}. Thus, all else being equal, the effect of gender (-0.38 meq. l^{-1}) was quite strong, being approximately equivalent to 1 SD difference in GSH levels. The effect of physical activity was much more modest. All else being equal, the effect of engaging in 1 h of moderate activity a day for 5 days a week, activity compatible with going to the gym five times a week, was associated with a 0.03 meq. l^{-1} increase in GSH, or 7% of a 1 SD change in GSH levels. An active occupation, that might generate 35 h of moderate activity a week, was estimated to be associated with a 0.18 meq. l^{-1} increase in GSH, or 42% of 1 SD increase in GSH levels.

Prior research has suggested that the effects of physical activity, particularly on oxidative stress and oxidative defences, depend greatly on the context of activity. Contextual factors that need to be considered include the intensity of activity, whether activity is conducted as part of training or an active lifestyle or is an acute incident and whether activity is performed to exhaustion (Niess et al. 1996, Asami et al. 1998, Poulsen et al. 1999, Miyazaki et al. 2001, Rundle 2005). Past work has suggested that sustained, regular activity, rather than acute bouts of vigorous activity, positively affects endogenous antioxidant systems (Niess et al. 1996, Asami et al. 1998, Poulsen et al. 1999, Miyazaki et al. 2001). In our study population, patterns of moderate rather than vigorous activity seem to best reflect sustained, regular, activity patterns. Some participants appeared to be 'weekend warriors' with one or two episodes of vigorous activity, while others engaged in regular exercise or sports of moderate intensity. As such the activity data were analysed using two measures of activity: hours of activity per week performed at a moderate intensity and hours of activity per week performed at a vigorous intensity. The observed difference in the effects of moderate and vigorous activity demonstrate that the modelling of activity–biomarker dose–response relationships may not be as straightforward as in molecular epidemiological studies of chemical carcinogens (Rundle 2005).

One of the goals of transitional studies of biomarkers is to identify other variables that are determinants of the biomarker, so that confounding and effect modifying relationships can be correctly modelled in aetiological studies that use the biomarker (Hulka & Margolin 1992). A strength of this study was the ability to investigate a wide array of variables that may influence tGSH levels. An interaction was observed between cigarettes smoked per day at baseline and at gender. Cigarettes smoked per day were inversely associated with blood tGSH levels in women, but not in men. Although not extensively studied, past studies have found smoking to be positively associated with blood GSH levels. In prior studies by Richie and colleagues, smokers were found to have higher tGSH levels than non-smokers. However, no dose–response relationship was observed between smoking intensity and tGSH levels (Richie et al. 1996b, Muscat et al. 2004). Michelet et al. (1995) found smokers had higher levels of GSH than non- or ex-smokers; and among smokers there was a positive correlation between cigarettes smoked per day and blood GSH levels. The current study only includes smokers so comparisons with non-smokers are not possible. It has been suggested that the higher levels of GSH seen in smokers represents an adaptive response to smoking induced oxidative stress. Supporting this

supposition is the observation that respiratory tract lining fluid from healthy smokers has higher levels of GSH than fluid from non-smokers (Cantin et al. 1987). Our study subjects were predominantly of lower socio-economic status: over 40% of the subjects earned less than US$10 000 per year. In addition, the study subjects appeared to be heavier smokers than the smoking subjects in prior studies (Michelet et al. 1995, Richie et al. 1996b, Muscat et al. 2004). It is possible that this population is exposed to other stressors, such as a poor diet, that prevent a smoking related adaptive response in GSH levels.

GSH measurements in the current study used tGSH measurements as an indicator of GSH status. This was based upon the high correlation of tGSH levels with levels of free GSH in individuals. Measurement of individual forms of glutathione including GSH, GSSG and protein-bound GSH were not possible due to the artefactual oxidation of GSH during sample storage. Future studies aimed at assessing the effects of physical activity on individual reduced and oxidized forms of glutathione are warranted, but will require the immediate processing and analysis of blood samples for GSH and its related oxidized forms. The present focus on glutathione in red cells as opposed to plasma is based on the low and highly variable and unstable nature of plasma levels, and inaccuracies inherent in its measurement (Kleinman & Richie 2000).

In conclusion, these preliminary cross-sectional analyses show that patterns of increased physical activity performed at moderate intensity are associated with increased blood levels of tGSH. The study is limited in that all the subjects were smokers. However, the goal was to generate physical activity-related biomarker data relevant to assessing whether physical activity protects against lung cancer. Thus, the study was conducted in a population at high risk for this disease. The major strength of this study is that it is the first to assess a wide range of possible confounding factors and to demonstrate an association between physical activity and tGSH levels in multivariate analyses. The study also demonstrated that relationships between physical activity and tGSH might not be straightforward and attention should be paid to both the extent of activity and whether activity is performed acutely or consistently. These results extend previous work and suggest a plausible mechanism through which physical activity might influence lung cancer risk.

Acknowledgements

Research was supported by grants from the National Cancer Institute (K07 CA92348 and M01RR000645-320825). The authors acknowledge the thoughtful comments of Dr Alex Halim.

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