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Antioxidant intake, GSTM1 polymorphism and pulmonary function in healthy young adults

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ABSTRACT: Dietary antioxidants may protect lung tissue against reactive oxygen species-induced injury, adverse respiratory effects and reduced pulmonary function. Genetic variability in antioxidant enzymes also determines response to oxidative stress in the lung. The current authors evaluated whether lung function levels are associated with dietary intake of antioxidants and the glutathione *S*-transferase M1 (GSTM1) polymorphism.

The current study cohort consisted of healthy, nonsmoking freshmen students who were lifetime residents in the Los Angeles or the San Francisco Bay areas (CA, USA). Participants completed comprehensive residential history, health history and food frequency questionnaires. Blood for genotyping was collected and forced expiratory volume measurements were obtained.

Dietary vitamin C, magnesium and daily fruit servings were associated positively with forced expiratory volume in one second in males and with maximum mid-expiratory flow, forced expiratory flow after 75% of expelled volume, and the ratio of maximum mid-expiratory flow to forced vital capacity in females. In multivariable regression, vitamin C (or fruit for male students) and magnesium showed a consistent, positive association with lung function.

Among healthy female adolescents, dietary intake of vitamin C is associated with increased levels of lung function. The current study does not support a role for the glutathione *S*-transferase M1-null genotype as an independent risk factor for decrements in lung function.

KEYWORDS: Dietary intake, genotype, lung function

The respiratory tract is subject to oxidative stress from exposure to air pollutants, cigarette smoke and inflammation related to infections; these exposures have been hypothesised to produce lung tissue damage [1]. An individual's total antioxidant capacity may confer a differential susceptibility to respiratory disease [2] and is a function both of dietary antioxidant intake and genetically determined antioxidant mechanisms [3]. A number of epidemiological studies suggest that individuals with lower antioxidant intake are at higher risk of adverse respiratory health effects; these include development of asthma [4] and reduced pulmonary function [5].

Antioxidant enzymes constitute the endogenous defence in the intra- and extracellular environments and are subject to genetic variability. Glutathione *S*-transferases (GSTs), a family of phase II detoxification enzymes, are important in the protection from toxic products, such as lipid and DNA hydroperoxides and aldehydes, generated by reactive oxygen species. The μ-class (GSTM1) is the most comprehensively studied to date, and is suggested to play an important role in the response to oxidative stress [3], particularly in the lung [6]. The high-prevalence GSTM1-null genotype (deletion of the entire gene and absence of the enzyme in homozygotes) has been associated with deficits in lung function growth in children [7], higher susceptibility to ozone-induced decrements of forced expiratory volume in asthmatic children [6] and increased sensitivity to nasal allergen challenge in the presence of diesel exhaust particles [8].

FRYER *et al.* [9] hypothesised that both dietary antioxidant intake and genetic determinants of the antioxidant capacity are involved in the determination of an individual's susceptibility to oxidative stress. Interaction between dietary

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and genetic antioxidant factors has been reported with respect to susceptibility to ozone-induced pulmonary function decrements in asthmatics [6]. Studies have also shown that individuals with genetic antioxidant deficiencies derive greater benefit from higher dietary antioxidant intake than those without such deficits [10].

As part of a study of the effects of long-term exposure to ambient ozone on lung function in healthy, nonsmoking adolescents, the current authors evaluated the hypothesis that lung function levels would be decreased in subjects who were homozygous for the GSTM1-null allele and that lung function would be positively associated with increases in dietary intake of antioxidants. Due to their importance in asthma [11], cigarette smoke-induced chronic obstructive lung disease [12] and ozone-induced airway damage [13], the current study focused on measures of small airways function.

MATERIALS AND METHODS

Study subjects

All protocols and procedures were approved by the Committee for the Protection of Human Subjects, University of California, Berkeley and by the Committee on Human Research, University of California, San Francisco (both CA, USA). Written consent was obtained for all subjects.

A convenience sample of University of California, Berkeley (UCB) freshmen was recruited. Students had to have never smoked and be lifetime residents either of the Los Angeles area or the San Francisco Bay area prior to coming to UCB. Based on sample size calculations from the present authors' previous work [13], the goal was to recruit ~300 subjects between the ages of 16–19 yrs.

Study design

The objective of the present study was to evaluate lung function in relation to dietary intake of antioxidants and the GSTM1 polymorphism. At enrolment, participants (Supplementary data; E1) completed a comprehensive residential history (all lifetime residences), health history (Supplementary data; E2) and a food frequency questionnaire. Blood was collected to determine GSTM1 genotype. Participants were screened for any lifetime history of chronic respiratory disease or asthma and for acute respiratory symptoms for the 3 weeks prior to spirometry to measure pulmonary function.

Methods

Pulmonary function tests

Forced expiratory volumes were obtained in the sitting position with nose-clip (Collins Survey rolling seal spirometer; Plus software Warren E. Collins Co., Braintree, MA, USA), with two modifications from the standard criteria of the American Thoracic Society [14] (Supplementary data; E3). Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), forced mid-expiratory flow ($FEF_{25–75\%}$) and forced expiratory flow after 75% of expelled volume ($FEF_{>75\%}$) were recorded. The ratio $FEF_{25–75\%}/FVC$ was calculated. This ratio is interpreted as one-half the reciprocal of the time constant of the lung [15].

Biological sample collection and processing

Blood was collected *via* venepuncture in BD Vacutainer™ blood collection tubes (Becton Dickinson Inc., Franklin Lakes, NJ, USA). Clot was separated from serum and stored in aliquots at -80°C until use. Buccal cells were collected as previously described [16]. DNA was isolated from stored blood clot samples with a Qiamp Blood DNA Maxi kit (Qiagen Inc., Santa Clarita, CA, USA) after mechanical disruption with ClotSpin tubes (Gentra Systems Inc., Minneapolis, MN, USA), and from stored buccal cells with a mini Qiagen Blood DNA isolation kit (Qiagen Inc.).

Genotyping (Supplementary data; E4)

Genotyping for the GSTM1 polymorphism was carried out in a multiplex PCR with the albumin gene as an internal reference to verify DNA amplification in GSTM1-negative samples. Products were identified on 3.5% agarose gel. Quality control procedures included randomly distributed blanks, positive and negative controls, and duplicates and repeats of 5% randomly selected samples in separate assays.

Nutrient intake

Usual dietary intake over the prior 12 months was assessed using the Women's Health Initiative Food Frequency Questionnaire [17]. Nutrient analysis software (HHHQ-DietSys Analysis Software, version 4.0, 1999; National Cancer Institute, Bethesda, MD, USA) was used to calculate the daily mean values of 114 foods (10 vegetable and nine fruit items) and eight beverages, using the food composition database of the US Dept of Agriculture [18]. Data were collected on multivitamin and single antioxidant supplements used for vitamins C, E, β-carotene and selenium. As details on supplement dosage were limited, the current authors did not calculate nutrient intake from these sources; also, since few participants reported taking single antioxidant supplements, these individuals were combined with multivitamin users in a single bivariate, named ''any dietary supplement use'' in the descriptive and multivariate analyses.

Statistical methods and analysis

GSTM1 genotype was categorised as wild type (plus) or variant (null). Dietary variables were standardised to their corresponding nutrient density value [19] as:

$$\text{Nutrient density} = \text{mean daily nutrient intake} \times 1000\,\text{kcal}/\text{mean daily energy intake} \quad (1)$$

The adjusted dietary antioxidant variables and number of fruit and vegetable servings were examined as continuous variables for descriptive analysis. In regression models, the lowest quintile of intake was compared with the remaining quintiles of intake in relation to pulmonary function, on the basis of the current authors' hypothesis that pulmonary function may be negatively affected in individuals with inadequately low intake. As nutrient intake is sex dependent, sex-specific means were used for descriptive analysis and in all models of pulmonary function. The pulmonary function variables were examined with multiple linear regressions, based on natural logarithmic transformations for all but FEV_1. The base model for each lung function measure, stratified by sex, was fitted with the respondent's measured weight, height or height^2, and was based on the optimal model among several tested. The base models were used to create normalised lung function variables as follows:

$$\text{Normalised FEV}_1 = (\text{observed respondent FEV}_1 - \text{predicted FEV}_1)/\text{root mean square error} \quad (2)$$

Adjusted vitamin C and E, β-carotene, lycopene, lutein-zeaxanthin, magnesium, selenium (lowest quintile *versus* highest quintiles of intake), and daily servings of fruits and vegetables were added individually and then retained in multivariable dietary models, if the variable showed an association with pulmonary function ($p<0.25$). Lycopene intake was consistently associated with lower lung function in all models for both sexes. This unexpected finding could not be explained by negative correlation with other antioxidants or influential data outliers or obvious confounding with another nondietary variable; therefore, lycopene was not included in further analysis. The following dietary factors were also excluded from further models: vegetable intake (inconsistent effects across lung function models and sex); lutein-zeaxanthin and selenium (no association with any measure of lung function); and yes/no indicator variable of multivitamin or antioxidant supplement (no association between vitamin use and lung function, with or without other dietary variables).

GSTM1 genotype was examined with and without the addition of dietary antioxidant variables and as an interaction with single dietary variables.

RESULTS

Participants who enrolled in the study were aged between 17–21 yrs, and included 102 males and 141 females (58%). All participants completed a food frequency questionnaire, a residential history and performed spirometry (table 1). The majority of participants were Asian (53%) or non-Hispanic White (32%), and reported living in the Los Angeles area since birth (60%). Students comprising the study population were from similar socio-economic as well as geographical backgrounds. Due to the small variability in socio-economic background, as measured by household income, no effect on either lung function or dietary intake was found.

Female students in the study population reported consuming a greater number of fruits and vegetables per day, and had a higher average intake of dietary antioxidant nutrients per 1,000 kcal than male students (table 2). Females also tended to meet or exceed the US Dept of Agriculture 2000 dietary guidelines for at least two fruit and three vegetable servings per day, and meet the recommended daily allowance (RDA) for individual nutrients more often than males (data not shown). However, fewer than half of both sexes met the RDA for vitamin E, magnesium and the guideline for fruit and vegetables servings. The majority of males also did not meet the RDA for vitamin A.

When dietary variables were evaluated individually in regressions with pulmonary function (table 3), dietary vitamin C, magnesium and daily fruit servings were positively associated (p-value <0.25) with FEV_1 for male students and with $FEF_{>75\%}$, $FEF_{25-75\%}$ and $FEF_{25-75\%}/FVC$ for female students. These nutrients were included in further analysis. When fruit and vitamin C both reached statistical significance for a given sex–lung function equation, both of the correlated nutrients were entered into a simultaneous variables model and the nutrient with the stronger association was retained in further analysis (Spearman correlation for fruit and vitamin C intake=0.70).

GSTM1-null genotype varied according to ethnic group, with students of Asian descent having the highest prevalence (56%) and of Hispanic descent having the lowest (37%). Caucasian students had a prevalence of 44% for the GSTM1-null genotype. In sex-specific univariate models, no consistent association between GSTM1 genotype and lung function was found. Contrary to the current authors' hypothesis, most parameter estimates indicated a positive association between

TABLE 1 Characteristics of participants

Variable	Males	Females
Subjects n	102	141
Age yrs	18.41±1.8	18.22±2.1
Height cm	176.55±7.3	162.51±7.1
Weight kg	80.22±3.3	73.90±3.3
FEV_1 L	4.35±0.6	3.23±0.5
$FEF_{>75\%}$ mL·s^{-1}	2.40±0.7	2.02±0.6
$FEF_{25-75\%}$ mL·s^{-1}	4.65±1.0	3.94±0.9
$FEF_{25-75\%}/FVC$ s^{-1}	0.93±0.2	1.09±0.3
Asian	49.0	55.3
Caucasian	38.2	27.0
Hispanic	5.9	10.6
Other	6.9	7.1
Los Angeles area residents	58.2	61.2

Data are presented as mean±SD or %, unless otherwise stated. FEV_1: forced expiratory volume in one second; $FEF_{>75\%}$: forced expiratory flow after 75% of expelled volume; $FEF_{25-75\%}$: forced mid-expiratory flow; FVC: forced vital capacity.

TABLE 2 Sex-specific dietary nutrient intake distributions

	Males	Females
Subjects n	102	141
Calories	2210±934*	1867±947
Vitamin C mg	65±35*	78±40
Total vitamin A μg retinol activity equivalent	432±200	471±219
β-Carotene μg	1665±1405	1909±1350
Lycopene μg	5585±2400	5732±2840
Vitamin E mg α-tocopherol equivalent	4.6±1.6	4.9±1.4
Magnesium mg	139±33*	151±33
Selenium μg	57±13	59±13
Fruit servings·day^{-1}	1.96±1.4	2.16±1.3
Vegetables servings·day^{-1}	1.73±1.4	1.98±1.5
Multivitamin/antioxidant supplement use %	43	44

Data are presented as mean±SD, unless otherwise stated. Nutrient means are expressed as nutrient intake per 1,000 kcal. *: unpaired t-test for difference between sexes, $p<0.05$.

TABLE 3 Regression coefficients (β) for association between normalised[#] pulmonary function and single dietary variables[¶]

Model	Sex	Vitamin C			Magnesium			Fruit			β-Carotene			Vitamin E		
		β	SE	p-value	β	SE	p-value	β	SE	p-value	β	SE	p-value	β	SE	p-value
FEV_1	Males	0.313	0.21	0.14	0.420	0.21	0.05	0.386	0.22	0.09	-0.162	0.21	0.45	0.051	0.22	0.82
	Females	-0.019	0.25	0.94	0.131	0.26	0.61	-0.044	0.23	0.85	0.294	0.26	0.27	-0.010	0.24	0.96
$FEF_{>75\%}$	Males	0.199	0.21	0.35	0.261	0.26	0.23	0.128	0.23	0.57	-0.017	0.21	0.94	-0.289	0.22	0.19
	Females	0.464	0.25	0.06	0.460	0.26	0.07	0.343	0.23	0.14	0.221	0.26	0.40	0.072	0.24	0.77
$FEF_{25–75\%}$	Males	0.232	0.21	0.28	0.248	0.22	0.25	0.119	0.23	0.60	-0.034	0.21	0.87	-0.170	0.22	0.44
	Females	0.390	0.25	0.12	0.396	0.26	0.12	0.281	0.23	0.22	0.129	0.26	0.63	0.045	0.24	0.85
$FEF_{25–75\%}$/FVC	Males	0.063	0.22	0.77	0.120	0.22	0.96	-0.130	0.23	0.57	0.038	0.21	0.86	-0.259	0.22	0.24
	Females	0.565	0.25	0.02	0.467	0.26	0.07	0.405	0.23	0.08	0.070	0.27	0.79	0.038	0.24	0.87

FEV_1: forced expiratory volume in one second; $FEF_{>75\%}$: forced expiratory flow after 75% of expelled volume; $FEF_{25–75\%}$: forced mid-expiratory flow; FVC: forced vital capacity. [#]: Pulmonary function values are normalised (observed–predicted/root mean SE): normalised FEV_1 adjusted for height (males), height^2 and weight (females); normalised $FEF_{>75\%}$ adjusted for height^2 and weight (males), height^2 (females); normalised $FEF_{25–75\%}$ adjusted for height^2 (males), height^2 and weight (females); normalised $FEF_{25–75\%}$/FVC adjusted for height^2 and weight (males), height^2 (females). [¶]: Dietary variables are entered as upper quintiles of adjusted nutrient intake *versus* lowest quintile.

null genotype and lung function. This positive association was significant in the $FEF_{25–75\%}$/FVC model for males only (data not shown).

The results of multivariable regression with simultaneous contribution of dietary variables and GSTM1 are presented in table 4. Results show that vitamin C and magnesium (and fruit for male students) have a consistent, positive association with lung function. For female students, increased intake of vitamin C-containing foods is associated with a 0.48 $L \cdot s^{-1}$ increase (95% confidence interval (CI) -0.02–0.97) in $FEF_{>75\%}$, 0.41 $L \cdot s^{-1}$ increase (95% CI -0.10–0.91) in $FEF_{25–75\%}$ and 0.54 s^{-1} increase (95% CI 0.04–1.04) in $FEF_{25–75\%}$/FVC ratio compared with females who were in the lowest quintile of vitamin C intake. For male students, eating more fruit was associated marginally with an increase in FEV_1. Moreover, magnesium, on its own, was not associated with FEV_1, but higher magnesium intake appears to benefit males with GSTM1-null genotype ($p=0.084$ for interaction between magnesium intake and GSTM1). However, the lack of any other precisely estimated interactions makes the interpretation of this latter observation uncertain.

DISCUSSION

In the current study of healthy, nonsmoking adolescents, increased levels of vitamin C were associated with higher levels of $FEF_{>75\%}$, $FEF_{25–75\%}$ and $FEF_{25–75\%}$/FVC for female students. Magnesium and fruit servings were also positively associated with female lung function ($FEF_{>75\%}$, $FEF_{25–75\%}$ and $FEF_{25–75\%}$/FVC). Reported dietary antioxidant intake was not associated with lung function measures in males. Reported intake of multivitamins or vitamin supplements showed no association with lung function in either sex.

Despite the smaller size of the current study, data are consistent with those reported recently from the much larger Children's Health Study [20], which observed an association among young females, aged 11–19 yrs, between low vitamin C and lower $FEF_{25–75\%}$, FVC and FEV_1 (lowest *versus* highest decile). No such consistent associations were seen for males of the same age, although reported low intake of orange and ''other'' juices was associated with deficits in FVC and FEV_1. It was also found that both the number of fruit servings per day and vitamin C intake were associated with FEV_1 in male students, although the estimates were somewhat imprecise. Similar positive associations were observed between vitamin C and several measures of flow among the female students. Other studies of antioxidant intake vary in terms of the antioxidant nutrient identified or the source of intake (supplemental *versus* dietary) and associations with lung function. Among studies of children, vitamin C [2, 21], vitamin A [20] and vitamin E [2, 20] intake have been reported to be associated positively with pulmonary function.

Recent studies of adults have found that respondents who reported rarely or never eating fruit were more likely to have lower FEV_1 values relative to those who reported consumption of fruit at least once a day [22]. In the present study, daily fruit consumption was associated only with FEV_1 in male students. Although fruit servings were positively associated in univariate models with several measures of lung function, for both males and females, the small number of subjects and inherent measurement error in the food frequency questionnaire resulted in poor precision of the effect estimate.

There are several explanations as to why fruits, rather than vegetables, were more consistently associated with increased levels of lung function. First, more fruits are high in vitamin C, which may be the more important dietary antioxidant, a suggestion supported by both the current study's data and the Children's Health Study data [20]. Secondly, one serving of fruit is generally equal to one piece of fruit consumed as a whole unit, *i.e.* one banana, or one glass of orange juice, which facilitates recall of fruit consumed throughout the day. Respondents may recall less readily their daily vegetable servings, because it is more common to mix vegetables with other food components, instead of eating one whole carrot or

TABLE 4 Multiple linear regression coefficients for regression on normalised[#] forced expiratory volume in one second (FEV_1), forced expiratory flow after 75% of expelled volume ($FEF_{>75\%}$), forced mid-expiratory flow ($FEF_{25–75\%}$), and forced mid-expiratory flow $FEF_{25–75\%}$/forced vital capacity (FVC)

Variable[¶]	Males			Females		
	β	SE	p-value	β	SE	p-value
FEV_1 R^2		0.11			0.001	
Fruit	0.36	0.24	0.13	0.05	0.24	0.83
Magnesium	0.036	0.29	0.90	0.05	0.26	0.84
GSTM1 null	-0.507	0.36	0.16	-0.04	0.17	0.83
GSTM1-null magnesium interaction	0.751	0.75	0.08			
$FEF_{>75\%}$ R^2		0.04			0.05	
Vitamin C	0.07	0.25	0.79	0.475	0.25	0.06
Magnesium	0.17	0.25	0.50	0.262	0.26	0.32
GSTM1 null	0.32	0.21	0.13	-0.100	0.17	0.56
$FEF_{25–75\%}$ R^2		0.04			0.03	
Vitamin C	0.10	0.25	0.68	0.408	0.25	0.11
Magnesium	0.13	0.25	0.60	0.237	0.27	0.38
GSTM1 null	0.31	0.21	0.14	-0.027	0.18	0.88
$FEF_{>25–75\%}$/FVC R^2		0.05			0.05	
Vitamin C	0.01	0.26	0.97	0.539	0.25	0.04
Magnesium	-0.12	0.26	0.63	0.303	0.27	0.26
GSTM1 null	0.45	0.21	0.038	0.003	0.67	0.98

SE: standard error; GSTM1: glutathione *S*-transferase M1. [#]: Pulmonary function values are: (observed–predicted)/root mean SE. Normalised FEV_1 adjusted for height (males), height^2 and weight (females); normalised $FEF_{>75\%}$ adjusted for height^2 and weight (males), height^2 (females); normalised $FEF_{25–75\%}$ adjusted for height^2 (males), height^2 and weight (females); and normalised $FEF_{25–75\%}$/FVC adjusted for height^2 and weight (males), height^2 (females). [¶]: Dietary variables are entered as upper quintiles of adjusted nutrient intake *versus* lowest quintile.

broccoli crown as identifiable, distinct units. For this reason, fruits and their respective principal micronutrients may be more precisely estimated with dietary recall methods than vegetables.

The sex differences in the associations between reported dietary intake and pulmonary function in the current data could be explained, in part, by differences in reported intake of a number of nutrients between males and females (table 2). A much greater proportion of females than males reported intake of vitamins C and A that met the RDA for these vitamins. In particular, the fact that airflows (as measured and expressed in FEV_1, $FEF_{25–75\%}$, $FEF_{>75\%}$/FVC) and the time constant appeared to be associated with reported intake in females and not males is of particular interest, given their associations with respiratory symptoms in children and adolescents [23].

Few studies of healthy, nonasthmatic individuals have investigated the potential between dietary consumption of magnesium and measures of lung function. Dietary magnesium was strongly associated with levels of FEV_1 in male students and to a lesser extent with $FEF_{25–75\%}$, $FEF_{>75\%}$ and $FEF_{25–75\%}$/FVC (as noted above, an indirect estimate of the time constant of the lung) in female students in the current study. Magnesium is a known cofactor in >300 enzyme activation reactions [24] and has been found to have a beneficial effect on asthma symptoms, possibly through bronchodilation of smooth muscle in airways [5, 25]. An epidemiological study of 2,633 adults in the UK reported that higher magnesium intake was associated with higher FEV_1 and a reduction in airway hyperreactivity [26].

The students who participated in this study were healthy, relatively well-nourished, nonsmoking 17–21 yr olds. The influence of dietary antioxidants on the mitigation of the harmful exposure to exogenous oxidative stress among healthy nonsmokers may be difficult to detect. Several studies that have reported positive associations between genetic or dietary factors and lung function were based on subjects with lung cancer and asthma among current smokers [27], or asthma alone [2, 6]. As the subjects of this study were selected to be healthy, no associations between past history of chronic respiratory symptoms and illness were found (no subjects had active asthma after 12 yrs of age; data not shown); therefore, oxidative stress from chronic respiratory disease is not a factor in this group of subjects. In the current analysis, the effects of exposure to ambient air pollutants, an important source of oxidative stress [2] with effects on levels of lung function, were not assessed; this will be considered in a separate paper.

Measurement error inherent in food frequency data [28] undoubtedly resulted in some misclassification of dietary intake, with bias to the null being most likely. Categorisation of continuous data with measurement error may lead to additional misclassification error, the direction of whose bias can be unpredictable [28]. The current authors attempted to mitigate any potential underreporting of total intake by use of adjusted nutrient intake values (nutrient per 1,000 kcal) in the current analysis [19]. Elimination of ''suspicious'' reporting errors from analysis did not change the results of the current findings.

No consistent association was found between the GSTM1-null genotype and decreased levels of any measure of lung function; however, there was little possibility of detecting such interactions in the present study. In fact, the parameter estimates for GSTM1 null often suggested increased levels of lung function in persons with this genotype, although the estimates were imprecise. The explanation for this finding could be chance or unmeasured confounding and/or unmeasured interactions. In other studies, the GSTM1-null genotype has been associated with evidence of increased oxidative stress following ozone exposure [29], a higher susceptibility to ozone-induced decrements of forced expiratory volume in asthmatic children [6], increased sensitivity to nasal allergen challenge in the presence of diesel exhaust particles in sensitised individuals [8] and deficits in lung function growth in children [7], particularly in asthmatic children.

While GSTM1 may play a role in a gene–environment interaction context [8], and/or as a risk modifier in asthmatic individuals [6], the GSTM1 deletion may not be a sufficient

stand-alone risk factor for decreased levels of lung function in healthy young subjects, regardless of dietary profile. The observations that only combinations of GST gene polymorphisms show significant associations with rapid lung function decline in persons with chronic obstructive pulmonary disease [30] are consistent with previous reports that single genes are unlikely to confer a significant relative risk in what is a polygenic complex trait [31, 32]. In addition, the high prevalence of the null genotype (up to 50% in Caucasians [30], up to 65% in Asians [33], 40% in Hispanics [7], and 25% in African-Americans [7, 30]) makes it an unlikely influential genetic factor for significant health disadvantage, particularly in the lung, where GSTP1, and not GSTM1, is the main GST isoform [34].

In summary, the present study adds some evidence to support an association between dietary intake of vitamin C and increased levels of lung function in healthy female adolescents. The study does not support a role for the glutathione *S*-transferase M1-null genotypic as an independent risk factor for decrements in any measure of lung function derived from spirometry in healthy adolescents.

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