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Effect of smoking reduction and cessation on the plasma levels of the oxidative stress biomarker glutathione – Post-hoc analysis of data from a smoking cessation trial

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Abstract

Cigarette smoke contains high concentrations of free radical components that induce oxidative stress. Smoking-induced oxidative stress is thought to contribute to chronic obstructive pulmonary disease, cardiovascular disease and lung cancer through degenerative processes in the lung and other tissues. It is uncertain however whether smoking cessation lowers the burden of oxidative stress. We used data from a randomized controlled cessation trial of 434 current smokers for a post-hoc examination of the effects of smoking cessation on blood plasma levels of total glutathione (tGSH), the most abundant endogenous antioxidant in cells, and total cysteine (tCys), an amino acid and constituent of glutathione. Smoking status was validated based on serum cotinine levels. Multivariate linear mixed models were fitted to examine the association of smoking cessation and change in cigarette consumption with tGSH and tCys. After 12 months follow-up, quitters (n=55) had significantly increased levels of tGSH compared to subjects who continued to smoke ($P<0.01$). No significant change in tGSH was found for subjects who continued to smoke but reduced their intensity of smoking. No significant effect of smoking cessation or reduction was observed on levels of tCys. These results suggest that smoking cessation but not smoking reduction reduces levels of oxidative stress.

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Keywords

Glutathione; cysteine; smoking; smoking cessation; oxidative stress

Introduction

Cigarette smoke is an abundant source of free radicals and aldehydes which cause oxidative stress and damage to the lung and other tissues in vivo. In clinical studies, long-term cigarette smoke exposure results in systemic lipid peroxidation and depletion of antioxidants such as vitamins A and C in plasma and elevation of inflammatory responses such as C-reactive protein, fibrinogen, and interleukin-6 [1]. Systemic oxidative stress observed in cigarette smokers can occur as a result of direct exposure to oxidants contained in cigarette smoke as well as indirectly through the activation of inflammatory responses resulting from exposure to cigarette smoke constituents [2]. Such effects in cigarette smokers may ultimately contribute to their increased risk of numerous diseases and disorders including chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), and cancer [3].

As the major endogenous antioxidant, glutathione (GSH) is thought to play a prominent role in protecting against oxidative stress in the lung and other tissues. GSH in the epithelial lining fluid of the lung is depleted by both acute and chronic exposure to tobacco smoke [4]. Plasma levels of both GSH and its rate-limiting precursor amino acid cysteine (Cys) are decreased in association with smoking [5,6]. These decreases were due, in part, to oxidation as increases in the major oxidized forms of GSH (GSH disulfide, GSSG, and GSH-protein mixed disulfides, GSSP) and Cys (cystine and Cys-protein mixed disulfides, CSSP) were observed in smokers [7]. However, decreases in total (reduced + oxidized) GSH (tGSH) and Cys (tCys) were also observed.

Surprisingly, there have been relatively few studies on the effects of smoking cessation on short-term and long-term changes in oxidative stress and GSH status [8], and especially large longitudinal studies are lacking. Knowledge of the effects of smoking abstinence would help clarify the role of oxidative stress in the etiology of smoking-induced diseases, help determine whether differences in redox status in smokers are due to smoking itself or other host or environmental factors associated with smoking, and provide information regarding a potential usefulness of dietary or supplemental antioxidants in smokers. The current study used data from a physician-based smoking cessation trial from 2002–2004 [9] for a post-hoc examination of the effects of smoking cessation on tGSH and tCys levels.

Materials and Methods

Study design and participants

The methods of the smoking cessation trial that was used for this study were previously described in detail [9,10]. In brief, a cluster-randomized smoking cessation trial was conducted in 82 general physician practices in south-west Germany from October 2002 through September 2004. The trial included 577 smokers randomized to one of 4

intervention arms. Patients aged 36 to 75 and who smoked at least 10 cigarettes per day were eligible for participation irrespective of their quit intentions. Participation in the study was conditional on written informed consent, and subjects were randomized to either a treatment-as-usual arm, a physician training and incentive intervention, a physician and training medication intervention, or a combined incentive and medication arm. A unique feature of the trial was that the interventions were physician-based. The training and incentive arm included a 2-hr cost free tutorial in smoking cessation and financial remuneration for each successful quitter. The training and medication arm included the same tutorial and full patient-reimbursement for nicotine or buproprion pharmacotherapy prescriptions. The study protocol was approved by the ethics committees of the Medical Faculty of the University of Heidelberg and of the State Chamber of Physicians of the federal state of Baden-Wuerttemberg.

Data and specimen collection

Participants were asked to fill in self-administered questionnaires both at baseline and at 12-months follow up, asking for socio-demographic information as well as for smoking-related information such as current smoking status and smoking intensity (in cigarettes per day). The follow-up questionnaire additionally asked for information on smoking cessation in those who had quit in the meantime. At both time points, the general practitioners collected blood samples (serum and EDTA plasma) from participants, and provided further medical diagnoses and anthropometric data (such as prevalent coronary heart disease, diabetes, hypertension and cancer, as well as height and weight). Blood samples were sent to the study center where plasma was stored at −80 °C.

Laboratory measurements

Subjects provided a blood specimen at baseline and at 12-months follow-up. Samples were analyzed for cotinine to determine and validate self-reported smoking status at baseline and follow-up. Serum cotinine levels were determined by radioimmunoassay at a commercial laboratory (Immundiagnostik, Bensheim, Germany).

Plasma samples were available from all subjects and were analyzed for plasma tGSH (GSH + GSSG + GSSP) and tCys (Cys + cystine + CSSP). The different redox forms of GSH and Cys were not measured individually because of the likelihood of oxidation occurring with long-term storage at −80 °C. Prior to analysis, 200 μl of blood plasma was added to 200 μl 8 M Urea in 1 mM EDTA (pH 7.5) and incubated for 10 minutes at 40 °C. After addition of 20 μl of octanol, 200 μl of aqueous 1.3 M potassium borohydride was added slowly to prevent foaming. This mixture was incubated at 40 °C for 1 hour, before cooling in an ice bath and slowly adding 600 μl of 20 % MPA. The samples were centrifuged, and the resultant supernatants were removed and analyzed for GSH or Cys.

GSH was determined using a 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB)/enzymatic recycling procedure previously described [11]. Briefly, to each well of a flat-bottomed 96-well microtiter plate (Greiner Bio-one, Frickenhausen, Germany) we added 50 μl of each calibrator or processed plasma sample, followed by 50 μl each of 0.5 mg/ml DTNB and 2.5 units/ml glutathione oxidoreductase (Sigma Aldrich, St. Louis, MO). Change in absorbance

at 540 nm was monitored for up to 5 min and quantitated by comparison with a calibration curve. tGSH concentrations were expressed in μM (μequiv GSH/L).

Measurements of Cys were performed using a modification of the acid ninhydrin spectrophotometric method of Gaitonde [12]. Briefly, processed plasma was reacted with ninhydrin and glacial acetic acid followed by incubation in boiling water for 10 minutes, cooling to room temperature, and addition of 95 % ethanol. The resulting Cys-ninhydrin conjugate was measured spectrophotometrically at 560 nm and quantified by comparison with a calibration curve. tCys concentrations in blood were expressed in μM (μequiv Cys/L).

Statistical analyses

Of 577 patients participating in the intervention, 574 had provided a blood sample at baseline. Of these, 437 both took part in the blood sample collection at 12 months-follow-up and provided self-reported smoking status at follow-up. Three subjects who reported being quit at follow-up had to be excluded because they indicated use of nicotine replacement therapy, which precluded a clear cotinine-based validation of their self-reported abstinence. Thus, 434 subjects formed the basis of this study.

Determination of quit status at follow-up was based on cotinine-validated self-reported smoking status, i.e. quitters were defined by self-reported quit status and a serum cotinine level < 15 ng/ml at follow-up. Self-reported continuing smokers or participants with a serum cotinine level ≥ 15 ng/ml were categorized as continuing smokers [13]. Smoking intensity at baseline was based on three categories of cigarettes per day (10–19, 20–29 and ≥ 30. Smoking intensity at follow-up was based on four categories of cigarettes per day (1–9, 10–19, 20–29 and ≥ 30.

Descriptive statistics included means and standard deviations. Cross-sectional differences between groups such as quitters versus continuing smokers were assessed with ANOVA, and within-group changes over time with paired t-tests. For multivariate analyses of change in tGSH and tCys values, linear mixed models were fitted with smoking-related variables (quit smoking versus continuing smoking, difference in cigarette consumption) as explanatory variables, controlling for the clustering of patients by general practitioner (random effect) as well as for sex, education and change in body mass index (BMI). The models were also adjusted for the baseline-level of the respective parameter in order to correct for autocorrelation.

All statistical tests were two-sided, with an alpha level of 0.05. SAS v9.3 was used throughout.

Results

Table 1 shows the basic demographic characteristics of the study sample. About 56 % were women and 79 % had less than 12 years of formal education. About 71 % of study participants smoked 20 or more cigarettes per day.

A total of 64 subjects (14.7 %) reported having quit smoking by the end of the 12-month trial period. However, according to their cotinine levels, 9 of them were not abstinent; i.e. 55

subjects (12.7 %) reported having quit smoking and were cotinine-negative, and were thus defined as quitters. Among those subjects who did not quit and provided information about cigarette consumption levels at both timepoints, 88 maintained their current level of cigarette consumption (22.1 %), 33 increased their consumption (8.3 %), 119 reduced their consumption by 1 to 9 cigarettes per day (29.8 %), 80 by 10 to 19 cigarettes (20.1 %) and 24 by 20 or more cigarettes (6.0 %) (details not shown).

At baseline, tGSH and tCys concentrations did not differ statistically by smoking intensity (Table 2). At follow-up, those who had quit smoking during the trial had the highest tGSH and tCys concentrations, with differences in tGSH across smoking intensity categories being statistically significant.

When examining changes from baseline to follow-up, tGSH levels were significantly higher in quitters than non-quitters at the end of the follow-up (Table 3). tCys levels decreased within both groups from baseline to follow-up, but the extent of decrease did not significantly differ between groups.

In multivariate analysis, quitters had significantly higher mean tGSH levels at follow-up, after adjusting for age, sex, education, and baseline-levels of tGSH (Table 4). The β estimate of 0.45 (95% CL 0.14; 0.76) increased to 0.59 (0.24; 0.93) after additional adjustment for change in BMI and baseline history of diseases (of note, the change in the β estimate for smoking cessation on tGSH levels was almost entirely due to the adjustment for BMI). Levels of tCys also tended to increase after smoking cessation, but differences were not statistically significant in any model.

In a model further differentiating continuing smokers by their change in cigarette consumption (Table 5), there were little and insignificant differences in changes of tGSH after baseline for continuing smokers who decreased their cigarette consumption compared to subjects who did not change their cigarette consumption. No clear pattern was observed regarding changes of tCys. Adding change in BMI and baseline history of diseases as covariates in model 2 yielded basically similar estimates.

Conclusions

The current study demonstrates that smoking cessation but not reduction significantly increases tGSH in a large group of healthy smokers, suggesting that smoking cessation is not only a reduction in exposure to toxins in tobacco smoke, but also a systemic reduction in oxidative stress caused by tobacco smoke. For tCys, a GSH precursor and putative risk factor for cardiovascular disease [14], no significant change due to smoking cessation or reduction was seen.

Plasma levels of GSH measured in our study as well as our findings on the effects of smoking cessation on GSH are consistent with a cross-sectional study showing lower plasma tGSH levels in smokers (1.8 ± 1.3 μM) compared with non-smokers (2.4 ± 1.0 μM) [5]. Our findings are also in line with the only previous longitudinal study so far, in which tGSH levels in whole blood were significantly increased 3 weeks after smoking cessation in 22 individuals who were continuously abstinent [8]. The current study indicates that the benefit

of cessation is maintained after 12 months, when presumably many of the smokers are at lower risk of relapsing.

GSH in plasma is considered a reliable marker of body stores of GSH and represents a major source of GSH for tissues including the lung. GSH in lung tissue and in the alveolar lung epithelium lining fluid (ELF) protects lung cells against oxidants, which may be inhaled or produced endogenously by inflammatory cells. The present findings that smoking cessation enhances plasma GSH suggest that levels in the lung ELF may also be increased as a result of cessation. Future studies involving direct measurement of GSH in ELF would help establish the beneficial effect of smoking cessation on oxidative stress reduction, particularly for susceptible tissues such as the lung.

Previously, higher GSH levels have been observed in erythrocytes from smokers compared to non-smokers [15]. GSH is synthesized in all cells, including erythrocytes, by the sequential addition of precursor amino acids with the rate-limiting step being the formation of γ-glutamylcysteine by the heavily regulated enzyme γ-glutamylcysteine ligase (GCL) [16]. In response to cigarette smoke exposure, GCL is induced in erythrocytes and in lung tissue resulting in increased production of GSH in mice [4]. The higher erythrocyte levels of GSH in chronic smokers compared to non-smokers is thought to be an adaptive response to the continuous exposure to oxidants in tobacco smoke [15]. The impact of smoking reduction or cessation on erythrocyte GSH could not be determined in the present study as erythrocyte samples were not available.

The observed changes in plasma tGSH in the current trial are assumed to be due directly to smoking cessation. However, there are also significant dietary sources of GSH and it is possible that changes in diet following smoking cessation may account for the current findings. Smokers who successfully quit in cessation trials generally mostly gain weight [17], although less is known about changes in dietary composition following cessation. Female quitters have significantly higher energy intake but a somewhat smaller percent of fat calories [18]. Conflicting data have been reported on changes in calories from carbohydrates and sweets in smoking trial participants [19–21]. Little is known about changes in food-based or supplemental antioxidant consumption which could potentially affect GSH levels. Foods with high glutathione content include freshly prepared meats whereas dairy products and bread are low in GSH [22]. Smokers consume more meat than former smokers [23] although national representative data is lacking. Unfortunately, information on dietary habits was not collected in this study. But the increase in tGSH after smoking cessation was even more pronounced in our study after additionally adjusting for change in BMI, indicating that weight changes due to changes in smoking behavior might have confounded the association.

We found no significant changes in plasma levels of tCys after smoking cessation. Cysteine combines with glutamate and glycine to form GSH and thus is the rate-limiting substrate for GSH synthesis. Although it is a GSH precursor, tCys does not appear to be affected as much by smoking cessation based on the findings from this study. Like GSH, cysteine can be obtained in the diet from meats, grains and vegetables. It is possible that dietary changes in cysteine consumption may differ between quitters and continuing smokers. In contrast to

GSH, Cys is easily oxidized which can result in increased levels of oxidative stress [24]. Cys can dramatically accelerate the oxidation of homocysteine [25], and high plasma levels of tCys, but not GSH have been linked to endothelial dysfunction [26]. High plasma tCys levels have been closely linked with obesity in both preclinical investigations and large population studies [27]. Further, increased plasma tCys has been associated with pathologic condition including CVD [28,29].

A limitation of our study is that individual redox forms of GSH and Cys could not be examined, due to the likelihood of oxidation occurring with long-term storage of blood samples at −80 °C. Biomarkers of inflammation such as neutrophil counts were also not measured, so that we were unable to explore potential mechanisms by which oxidative stress levels were reduced.

A particular strength of this study is the cotinine-validation of smoking status, minimizing misclassification bias through misreporting of quit status and thus rendering our estimates of the effect of quitting on oxidative stress markers quite reliable. A validation of self-reported cigarette consumption was not possible though because cotinine is not a very sensitive marker for smoking intensity [30] probably owing to differences in individual puff profiles, and the results for categories of change in cigarette consumption on tGSH and tCys levels are thus prone to misclassification bias.

Low GSH levels are associated with increased risks for cancer [31], cardiovascular diseases, arthritis and diabetes [32,33]. Smoking cessation by reduction of oxidative stress is likely an important mechanism for risk reduction in healthy smokers. The results however may not necessarily be generalizable to all smokers. Smoking cessation does not reduce inflammation in the bronchial airway tissue of patients with COPD [34] and it is possible that GSH levels in the lungs of COPD patients are not optimal after cessation. In asymptomatic smokers with normal lung function, most markers of inflammation are reduced [34].

With oxidative stress being a significant factor in the etiology of many diseases, antioxidants have long been proposed as potential chemopreventive agents. Some clinical investigations indicate the potential efficacy of dietary antioxidants at protecting against smoking-induced oxidative stress. Depletion of vitamin C levels in smokers was ameliorated within 3 months by supplementation with a vitamin cocktail containing vitamin C, alpha-tocopherol and folate [35]. Vitamin C supplementation was also effective at reducing levels of lipid peroxidation byproducts in smokers' urine [36]. Using a metabolic approach, antioxidant supplementation in smokers was found to alter lipid profiles in plasma in a manner consistent with reductions in oxidative stress [37]. However, disease outcome antioxidant clinical trials have provided less-than enthusiastic results and the ability of oral antioxidants to be absorbed and effectively reach target organs remains an area of active investigation [38]. Such studies have not been conducted in former smokers, who are at lower risk than smokers but still maintain a higher risk than nonsmokers. The current study would indicate that former smokers would likely not benefit from antioxidant supplementation since GSH levels increased in these subjects. Continuing smokers who only decreased cigarette

consumption might however benefit from dietary or supplemental interventions to bolster their levels of antioxidant defense systems.

In summary, the current post-hoc analysis of data from a randomized trial shows that smoking cessation but not smoking reduction results in a significant increase in tGSH levels, providing further support that oxidative stress could be an important mechanism of smoking-induced diseases.

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HIGHLIGHTS

- We exploit data from a randomized controlled cessation trial of 434 current smokers.
- We examine effects of smoking cessation on plasma levels of tGSH and tCys.
- tGSH-levels significantly increased after cessation, but not after smoking reduction.
- No significant effect of smoking cessation was observed on levels of tCys.
- Results suggest that smoking cessation but not reduction reduces levels of oxidative stress.

Table 1

Baseline characteristics and follow-up smoking status of subjects in the study sample

	N (%)	tGSH [μM]	tCys [μM]
		Mean ± SD	**Mean ± SD**
N	434	1.51 ± 0.93	265.6 ± 85.9
Sex			
Male	193 (44.5)	1.46 ± 0.66	261.2 ± 89.7
Female	241 (55.5)	1.56 ± 1.10	271.0 ± 80.7
Age at interview			
< 45 years	168 (38.7)	1.50 ± 0.64	255.2 ± 90.8
45 – 54	162 (37.3)	1.57 ± 1.24	268.1 ± 85.0
55 – 64	79 (18.2)	1.39 ± 0.61	277.6 ± 71.8
≥ 65	25 (5.8)	1.60 ± 1.11	281.3 ± 94.9
Education			
Low (≤ 9 years)	229 (53.8)	1.45 ± 0.61	268.3 ± 81.1
Moderate (10 – 11 years)	106 (24.9)	1.44 ± 0.71	257.4 ± 94.4
High (≥ 12 years)	91 (21.4)	1.73 ± 1.57	271.9 ± 88.5
Unknown	8		
BMI			
25	192 (46.2)	1.57 ± 1.18	253.4 ± 83.1
> 25 – < 30	152 (36.5)	1.44 ± 0.66	270.7 ± 87.2
≥ 30	72 (17.3)	1.59 ± 0.67	279.2 ± 86.7
History of diseases			
Coronary heart disease	33 (8.1)	1.41 ± 0.55	292.8 ± 96.7
Type 2 diabetes mellitus	46 (11.2)	1.48 ± 0.67	290.5 ± 84.5
Cancer	16 (3.9)	1.39 ± 0.43	230.2 ± 41.4
Cigarette consumption			
10 – 19	122 (28.7)	1.40 ± 0.62	262.4 ± 95.4
20 – 29	170 (40.0)	1.54 ± 0.75	265.7 ± 84.4
≥ 30	133 (31.3)	1.59 ± 1.31	269.4 ± 80.0
Unknown	9		
Self-reported smoking status at follow-up			
Continuing smoker	370 (85.3)		
Quitter	64 (14.7)		
Cotinine-validated smoking status at follow-up			
Continuing smoker	379 (87.3)		
Abstinent quitter	55 (12.7)		

Table 2

Cross-sectional differences in plasma total glutathione and total cysteine according to level of cigarette consumption

	1 – 9 cigarettes/day	10 – 19 cigarettes/day	20 – 29 cigarettes/day	≥ 30 cigarettes/day	Non-smoking (quitter)	
Baseline						
tGSH [μM] Mean ± SD		1.40 ± 0.62	1.54 ± 0.75	1.59 ± 1.31		P^a=0.25
tCys [μM] Mean ± SD		262.4 ± 95.4	265.7 ± 84.4	269.4 ± 80.0		P^a=0.82
Follow-up						
tGSH [μM] Mean ± SD	1.47 ± 0.62	1.52 ± 0.82	1.44 ± 0.70	1.34 ± 0.42	1.91 ± 2.21	P^a=0.04
tCys [μM] Mean ± SD	217.0 ± 78.4	207.4 ± 71.9	215.9 ± 75.9	224.9 ± 66.4	221.9 ± 79.6	P^a=0.61

[a] *P*-value refers to differences between categories according to ANOVA

Table 3

Bivariate Analyses of smoking cessation and changes in plasma total glutathione and total cysteine

tGSH [μM]					
Mean ± SD					
	Baseline		**Follow-up**		***P*-value of change over follow-up**
Overall (*N*=418)	1.51 ± 0.93		1.53 ± 1.03		P^b=0.84
Baseline smokers by follow-up smoking status					
Continuing smokers (*N*=367)					
Quitters (*N*=51)	1.51 ± 0.96	P^a=0.90	1.47 ± 0.73	P^a=0.004	P^b=0.57
	1.53 ± 0.67		1.91 ± 2.21		P^b=0.22
tCys [μM]					
Mean ± SD					
	Baseline		**Follow-up**		***P*-value of change over follow-up**
Overall (*N*=419)	265.6 ± 85.9		214.2 ± 73.8		P^b<0.001
Baseline smokers by follow-up smoking status					
Continuing smokers (*N*=368)					
Quitters (*N*=51)	262.6 ± 83.8	P^a=0.06	213.1 ± 73.0	P^a=0.42	P^b<0.001
	286.8 ± 97.6		221.9 ± 79.6		P^b<0.001

[a] *P*-value refers to differences between categories according to ANOVA

[b] *P*-value refers to differences within categories over time according to paired t-test

Table 4

Multivariate Analyses (linear mixed models) of smoking cessation and changes in plasma total glutathione and total cysteine (all baseline smokers)

	tGSH [μM]		tCys [μM]	
	β	**95% CL, *P*-value**	***β***	**95% CL, *P*-value**
	Model 1			
Smoking status				
Continuing smoker	Ref.		Ref.	
Quitter	0.45	(0.14; 0.76), 0.004	7.4	(−14.7; 29.6), 0.51
N		411		412
	Model 2			
Smoking status				
Continuing smoker	Ref.		Ref.	
Quitter	0.58	(0.23; 0.93), 0.001	9.2	(−16.5; 34.8), 0.48
N		347		349

Model 1: Adjusted for age, sex, education, baseline-level of respective parameter (=correction for autocorrelation), and clustering of patients by general practitioner (random effect)

Model 2: like Model 1, but additionally adjusted for baseline level of and change in BMI, and baseline history of diseases (coronary heart disease, diabetes, hypertension, cancer)

Table 5

Multivariate Analyses (linear mixed models) of change in smoking intensity (by difference in cigarette consumption) and changes in plasma total glutathione and total cysteine (all baseline smokers)

	tGSH [µM]		tCys [µM]	
	β	**95% CL, *P*-value**	***β***	**95% CL, *P*-value**
	Model 1			
Smoking status				
Continuing smoker and…				
Increase in cigarette consumption	−0.18	(−0.63; 0.27); 0.44	−19.9	(−51.1; 11.3); 0.21
Stable cigarette consumption	Ref.		Ref.	
Decrease by 1 to 9 cigarettes	−0.07	(−0.37; 0.24); 0.67	−15.8	(−36.9; 5.26); 0.14
Decrease by 10 to 19 cigarettes	−0.04	(−0.38; 0.30); 0.82	−0.69	(−24.4; 23.0); 0.95
Decrease by ≥ 20 cigarettes	−0.33	(−0.83; 0.181); 0.20	−1.58	(−37.0; 33.8); 0.93
Quitter	0.41	(0.03; 0.77), 0.03	−0.19	(−26.2; 25.8); 0.99
N		378		379
	Model 2			
Smoking status				
Continuing smoker and…				
Increase in cigarette consumption	−0.2	(−0.72; 0.31); 0.44	−21.8	(−58.1; 14.6); 0.24
Stable cigarette consumption	Ref.		Ref.	
Decrease by 1 to 9 cigarettes	−0.11	(−0.43; 0.22); 0.53	−13.2	(−36.7; 10.3); 0.27
Decrease by 10 to 19 cigarettes	0.05	(−0.33; 0.42); 0.80	−2.3	(−29.1; 24.5); 0.87
Decrease by ≥ 20 cigarettes	−0.32	(−0.84; 0.21); 0.24	−1.3	(−39.1; 36.5); 0.95
Quitter	0.53	(0.11; 0.95), 0.01	2.1	(−28.2; 32.4); 0.89
N		318		319

Model 1: Adjusted for age, sex, education, baseline cigarette consumption, baseline-level of respective parameter (=correction for autocorrelation), and clustering of patients by general practitioner (random effect)

Model 2: like Model 1, but additionally adjusted for baseline level of and change in BMI, and baseline history of diseases (coronary heart disease, diabetes, hypertension, cancer)