Cotinine as a marker of exposure to lifestyle risk factors other than smoking

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[2] The abbreviations used are:

ETS,	Environmental tobacco smoke
GLC,	Gas-liquid chromatography
HALFFS,	HALS food frequency score
HALS1,	Health and Lifestyle Survey
HALS2,	Health and Lifestyle Survey Follow-up
HSE93,	Health Survey for England 1993
HSEFFS,	HSE93 food frequency score
PU,	Polyunsaturated

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Abstract:

We demonstrated previously that smokers and passive smokers have increased exposure to many lifestyle risk factors. Using two representative UK surveys which determined cotinine in serum (HSE93) or saliva (HALS2), we related 32 risk factors to cotinine level in never and current smokers. Using the same 4813 HSE93 and 3668 HALS2 subjects, we compared the associations with cotinine with those with the indices "living with a smoker" and "number of cigarettes smoked per day"

In never smokers, cotinine was strongly positively associated with numerous risk factors, including low social class, lack of education, low income, having a "risky" occupation, high alcohol and fried food consumption, high body mass index, low control at work and high extroversion. Associations were generally stronger with cotinine than with living with a smoker.

In current cigarette smokers, cotinine was strongly positively associated with lack of education, low social class, long time before first meal, high fried food, tea and coffee consumption, low fruit and salad consumption, using sugar in tea and coffee and low control at work. Number of cigarettes smoked marked some associations better than cotinine, but others worse. High alcohol consumption and body mass index were weakly positively associated with number smoked but were clearly *negatively* related with cotinine, which may indicate an effect of these risk factors on inhalation or metabolism of nicotine.

Cotinine is a marker of exposure to many risk factors other than tobacco smoke. These correlations could confound studies of the health effects of smoking, especially passive smoking.

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Introduction

Cotinine, whether measured in serum, saliva, urine or other tissues or body fluids, has been widely used as a marker of recent exposure to tobacco smoke constituents. Numerous large studies show that average cotinine levels differ markedly between current smokers and nonsmokers (1-6), that cotinine levels in current smokers are correlated with self-reported number of cigarettes smoked per day (2-9) and that cotinine levels in nonsmokers are correlated with various questionnaire indices of ETS exposure (3-7,10-15). As a measure of exposure to tobacco smoke, cotinine has the important benefit of being objective, but the disadvantage of measuring only recent exposure when past or total lifetime exposure may be necessary to assess health effects.

Our group previously (16) described results of analyses based on the Health and Lifestyle Survey (HALS1) comparing the prevalence of 33 lifestyle factors generally considered associated with adverse health between current smokers, ex-smokers, never-smokers living with a smoker ("passive smokers") and other never smokers. Of the 33 risk factors, 27 showed a significantly higher prevalence in heavy smokers than in never smokers and only two showed a lower prevalence. For many risk factors, prevalence increased with amount smoked, decreased with time of smoking cessation and was increased in passive smokers. The magnitude of bias from confounding by the risk factors was estimated and it was concluded that confounding by multiple risk factors may be an important issue in smoking studies where weak associations are observed.

Although a number of other studies have reported associations between self-reported smoking habits or ETS exposure and increased exposure to other lifestyle risk factors (17-23), little work has been conducted to investigate the relationship of cotinine to such risk factors. Although it was not determined in HALS1, salivary cotinine was determined in a follow-up survey of the same population (HALS2). Serum cotinine has also been determined in an additional large representative survey, the Health Survey for England 1993 (HSE93). Both HALS2 and HSE93 recorded extensive data on

lifestyle risk factors, recorded detailed smoking data and asked questions regarding living with a smoker.

We present results of analyses of both these surveys aimed at describing associations, in never smokers and in current smokers, between cotinine levels and a list of risk factors similar to that we considered earlier (16). The strengths of these associations are also compared with the those of the corresponding associations with living with a smoker and with number of cigarettes currently smoked.

Methods

HSE93

The survey, described in detail elsewhere (5), involved 17,687 people. The sample selection process was designed to yield a representative sample of adults aged 16 years and over living in private households in England. The survey consisted of two stages. At the first stage, interviews were conducted at home, 16,569 with the subject and 1,118 with a proxy. The completed questionnaire included details of the household, self-reported health, exercise, dietary habits, smoking and drinking habits, and work, and measurements of height and weight were taken. At the second stage, further information was gathered shortly after by a nurse during a follow-up visit. Where possible, the nurse took a sample of blood from the subject which was analysed *inter alia* for serum cotinine. Since the method used for cotinine in the first half of the survey was too insensitive to detect the increases in cotinine associated with passive smoking, a different laboratory was used for the second half, which used gas-liquid chromatography (GLC) to detect very low concentrations of serum cotinine. For the purposes of the main statistical analyses, attention is restricted to the subjects with valid GLC serum cotinine who provided information by self-, not proxy-, report.

HALS2

The initial Health and Lifestyle Survey, HALS1, conducted in 1984-85, described in detail elsewhere (24), involved 9003 people and was designed to be a representative sample of the population, aged 18+, of England, Scotland and Wales. HALS2 was a follow-up survey of 5352 subjects conducted in 1991-92. Both surveys consisted of three stages. The first stage was a questionnaire completed during an at-home interview, with information collected on many factors including self-reported health, health attitudes and beliefs, dietary habits, leisure, work, exercise, smoking and drinking habits, home and family circumstances, education and income. The second stage was a follow-up home visit by a nurse during which measurements were made, including height and weight, and tests carried out. The third stage was a self-completion questionnaire to assess personality and psychiatric status. While HALS1 and HALS2 were very similar in many respects in the questions asked and measurements taken, a notable difference was that HALS2 included saliva samples taken by a visiting nurse for cotinine estimation by GLC.

Smoking and passive smoking. For both HSE93 and HALS2, subjects were divided into current smokers, ex-smokers and never smokers. The never smokers were further sub-divided into those exposed to the cigarette smoke of others within their household (referred to as "Passive smokers" in the tables below) and those not so exposed ("Unexposed"). These classifications were based on the answers the subjects gave to questions about their own smoking habits, past and present, and the current smoking habits of those in their household and so are subjective measures. The definition of passive smoker did not take into account any cigarette smoke exposure at work or at leisure outside the home. Neither survey collected data on these other types of exposure.

20 ng/ml cotinine in serum and 30 ng/ml cotinine in saliva are both similar to levels that have been used in other surveys to distinguish true nonsmokers from misclassified smokers (25). In order to try to avoid contamination by smokers, analyses relating cotinine levels in never smokers to prevalence

of risk factors have excluded subjects with cotinine levels above these "cut-points".

Selection of risk factors. In our analyses based on HALS1 (16), we selected 33 risk factors for analysis based broadly on five criteria: (I) inclusion of variables commonly considered as risk factors in smoking-related diseases; (ii) inclusion of variables commonly considered to be part of a healthy lifestyle; (iii) avoidance of variables not generally considered to be risk factors for smoking-related diseases; (iv) avoidance of variables which are indices of morbidity (and which might have been the consequences of smoking and therefore not have true confounding effects); and (v) combination of related variables into single indices, provided that initial analysis had shown similar patterns of relationship to smoking. The selection of variables tended to include variables showing a stronger relationship with smoking than those showing little or no relationship.

Of the 33 risk factors used in HALS1, analyses for 27 are presented here for HALS2. "Never tried to lose weight" and "not cut down on fatty foods" were omitted because relevant questions were not asked in HALS2; "household size", "do not get enough exercise" and "Type A personality" as they showed little relationship with smoking in our previous study (16); and "had depression/nervous illness" as it may be considered an index of morbidity and not a confounding variable. The risk factor "underweight" must be treated with caution as it may also reflect morbidity, but it was decided to include this risk factor.

Overall, 18 risk factors were considered for HSE93. Questions asked in HSE93 did not cover all the areas considered by HALS2, but where they did, an attempt was made to construct comparable risk factors. Some other risk factors, based on topics not studied in HALS2, were also considered.

Risk factors were of two types, "presence/absence" and "graded". For graded risk factors, significance testing always used the full data by level but frequencies are expressed in the tables as

above a critical "cut-point", usually chosen to subdivide the total distribution into two approximately equal parts. Presence, and high scores of graded variables, were chosen to indicate levels of variables generally associated with an **increased** risk of disease. The risk factors considered are listed in Appendix A, together with explanatory notes describing their levels and cut-points (26, 27).

Statistical methods. When comparing the prevalence of a risk factor in different smoking, passive smoking or cotinine groups (as in Tables 3, 4, 7 and 8), percentages and coded probability values are presented. Percentages are sex and age adjusted (see table footnotes for groupings used) by direct standardisation to the overall distribution of the population considered in the table. The probability values are also sex and age adjusted. For presence/absence risk factors, stratified chi-squared statistics (28) are used to test for trend over the smoking or cotinine groups. For graded risk factors, where percentages presented are for high or low categories as defined in Appendix A, the trend test is based on the overall distribution of the risk factor, using the Fry-Lee test (29), an extension of Kruskal-Wallis one-way analysis of variance by ranks which allows for stratification and testing for dose-related trend. Linear discriminant analysis was also used to study the relationship of cotinine levels to the risk factors considered simultaneously. Probability values for all analyses are presented as:-

+++, - - -	$p<0.001$	(+), (-)	$p<0.1$ or
++, - -	$p<0.01$	N.S.	$p \geq 0.1$ (not significant)
+, -	$p<0.05$		

with plus signs indicating a positive association between the risk factor and smoking, passive smoking or cotinine groups, and minus signs a negative association.

RESULTS

Subjects - HSE93 Of the 17,687 subjects in the survey, 16,569 were interviewed directly. Of these, 12,055 provided a blood sample. Of the subjects giving blood, valid cotinine values were available for only 5012 due to the inadequacy of the test method used for the first half of the study. To ensure

that the same population was studied in all analyses, we further restricted the sample to exclude those with social class not classified as I to V, those who could not be classified as never, former or current smokers, and those never smokers with no data on passive smoking in the household. This left 4813 subjects, 2336 males and 2477 females.

Subjects - HALS2 Of the 9003 subjects interviewed for HALS1, 5352 were re-interviewed for HALS2. Of these 3900 subjects had a valid cotinine value. As with HSE93 we further restricted the sample to exclude those with social class not classified as I to V, those who could not be classified as never, former or current smokers, and those never smokers with no data on passive smoking in the household. This left 3668 subjects, 1681 males and 1987 females.

[TABLE 1 ABOUT HERE]

Frequency of risk factors studied

Table 1 shows the crude frequency of the various risk factors in the two surveys, showing prevalence values both for the subset of subjects studied (those having a cotinine value and other critical values described above) and for the total non-proxy survey population. The subsets studied are shown to be largely representative of the total survey population. To a great extent any differences reflect the characteristics of the population which agreed to give a blood sample (and hence have a cotinine value). The most marked effect is seen in relation to employment, with many of those not in paid work refusing to give a blood sample.

For many of the risk factors the frequencies are quite similar in the two surveys. However this is not always so. The frequencies clearly differ between the surveys for no use of low fat/PU spread and low vegetable and salad consumption while smaller differences are seen for no educational qualifications, not in paid employment, low social class, high alcohol and bread consumption and low fruit

consumption. With the exception of not in paid employment, discussed above, these differences exist in the original surveys and not only within the subsets studied.

Cotinine as a marker of smoking

Median cotinine levels (ng/ml) in serum (HSE93) and in saliva (HALS2) were much higher in current smokers (HSE93 216.2, HALS2 324.2) than in never smokers (HSE93 0.6, HALS2 0.8) or in ex-smokers (HSE93 0.7, HALS2 1.1). Within never smokers, levels were significantly higher in those living with a smoker than in those not living with a smoker (HSE93 1.5 vs 0.5, $p<0.001$; HALS2 2.05 vs 0.6, $p<0.001$). Within current cigarette smokers, levels rose significantly ($p<0.001$) by amount smoked (HSE93 1-9/day 80.9, 10-19/day 242.1, 20+/day 315.4; HALS2 1-9/day 158.1, 10-19/day 324.1, 20+/day 389.4).

[TABLE 2 ABOUT HERE]

Table 2 shows the distribution of the 4813 HSE93 and 3668 HALS2 subjects by smoking group and cotinine level. In HSE93, the percentage of subjects with serum cotinine levels above 20 ng/ml was 1.1% (15/1402) for never smokers not living with a smoker, 2.9% (9/307) for never smokers living with a smoker, 5.6% (92/1643) for ex-smokers, and 87.1% (1272/1461) for current smokers. The corresponding figures for HALS2, based on saliva cotinine levels above 30 ng/ml were 0.1% (1/1049), 0.7% (2/298), 4.4% (49/1124) and 93.9% (1124/1197). Using the levels of 20 ng/ml cotinine in serum and 30 ng/ml cotinine in saliva as the maximum levels compatible with non-smoking status meant that 24 people from HSE93 and 3 people from HALS2 were excluded from analyses of never smokers.

[TABLES 3, 4 ABOUT HERE]

Cotinine level in never smokers related to risk factor prevalence

Table 3 shows the prevalence of the risk factors in HSE93 by cotinine level for those never smokers who have a level consistent with not smoking. Table 4 shows the corresponding figures for HALS2.

The tables show a good degree of agreement between the two surveys. Both surveys show strong positive trends for no educational qualifications and low social class and the surveys are similar in showing significant ($p<0.05$) positive trends for mother dead, high alcohol consumption, low fruit consumption and high body mass index. Both show a negative trend for high sweet food consumption. Both surveys found no significant trend for father dead, not in paid employment and underweight. However, for separated, divorced or widowed, high bread consumption and no use of low fat/PU spread HALS2 shows a significant ($p<0.05$) positive trend while HSE93 does not.

Of the variables available in only one of the surveys, a significant positive trend was shown for low veg/salad consumption, high salt consumption in food and low control at work (HSE93) and for "risky" occupation, low income, high fried food consumption, low breakfast cereal consumption and high extroversion (HALS2).

Comparison of cotinine level and "living with a smoker" as markers of exposure to lifestyle risk factors in never smokers

Analyses for never smokers comparable to those in Table 3 and Table 4, which use cotinine level as the index of exposure, were also run using the alternative index "living with a smoker," this being the only subjective measure of ETS exposure available in either survey. Table 5 allows comparison of age and sex adjusted trend chisquared values from both sets of analyses, attention being restricted to risk factors showing a significant ($p<0.05$) trend in at least one set.

In general, risk factors were clearly more strongly associated with cotinine level than with "living with a smoker." Thus, the only risk factors significantly ($p<0.05$) associated with living with a smoker but not with cotinine level were high pace index (HSE93 only), and high coffee consumption and high neuroticism (HALS2 only). In contrast, there were numerous risk factors where a significant association was seen only with cotinine. Furthermore, where both indices showed a significant relationship, the trend statistic was nearly always higher for cotinine, the only exceptions being no use of low fat/PU spread (HALS2 only) where the trends were quite similar, and separated, divorced or widowed (HALS2), where the directions of the associations differed. The separated, divorced or widowed are clearly more likely to live on their own and hence be less likely to live with a smoker. Their higher cotinine values may reflect increased exposure to nicotine outside the home.

[TABLE 5 ABOUT HERE]

Table 5 also shows the effect on the trend chisquared values of adjusting the association with living with a smoker for cotinine, and of adjusting that with cotinine for living with a smoker (these being additional to the normal adjustments for age and sex). The results confirm the superiority of cotinine as a marker of exposure to other risk factors. Thus, while 26 of the 31 associations with cotinine adjusted for living with a smoker were significant ($p<0.05$), only 8 of the associations with living with a smoker were significant when adjusted for cotinine. The difference is even more marked for highly significant associations ($p<0.001$, 11 vs 1), the only such adjusted association with living with a smoker being the essentially artefactual association with being separated, divorced or widowed.

Independence of the associations with cotinine level in never smokers

Analyses (results not shown in detail) were also carried out of the relationship of cotinine to the risk factors after adjustment for age, sex and social class (in six groups, I, II, III non-manual, III manual, IV and V). With the exception of high alcohol consumption, where social class adjustment somewhat

strengthened the association in both surveys, social class adjustment tended to weaken the association. However, 19 of the 31 associations with cotinine remained statistically significant at $p<0.05$ and 7 remained significant at $p<0.001$. Social class adjustment had the most marked effect for no educational qualifications (both surveys), low control at work (HSE93 only) and "risky occupation" and low income (HALS2).

[TABLE 6 ABOUT HERE]

Table 6 shows the results of linear discriminant modelling on data for never smokers, using log(cotinine+0.05) as the dependent variable and including all the risk factors and an age-sex variable as explanatory variables. (The trend codings given in Tables 3 and 4 are repeated for ease of comparison.) This shows that many of the associations between cotinine and the risk factors are independent. Of the 10 risk factors showing an association significant at $p<0.1$ in Table 3 (HSE93), only 3 (mother dead, low fruit consumption and high salt consumption) fail to do so when considered simultaneously in the model. Also marital status and high pace index are significant in the model although they did not reach significance in Table 3. Of the 19 separate risk factors showing a significant association in Table 4 (HALS2), 10 make a significant independent contribution to the model.

[TABLES 7 AND 8 ABOUT HERE]

Cotinine level in current cigarette smokers related to risk factor prevalence

Table 7 (HSE93) and Table 8 (HALS2) compare age and sex adjusted risk factor prevalence in never smokers who have a cotinine level consistent with not smoking and in current cigarette smokers grouped by cotinine level. The tables also show the significance of trends calculated including never smokers (trend 1) and excluding never smokers (trend 2). Apart from the exclusions noted earlier,

current smokers who smoked pipes and/or cigars only and current cigarette smokers who did not report the number they smoked are also excluded from Tables 7 and 8.

Considering first trends including never smokers, it can be seen that the results in Tables 7 and 8 show quite close agreement. Both surveys find a highly significant ($p<0.001$) positive trend for the risk factors no educational qualifications, separated, divorced or widowed, not in paid employment, low social class, high alcohol consumption, low fruit consumption, no use of low fat/PU spread and underweight. They both also show a highly significant negative trend for high sweet food consumption and high body mass index. There are differences between the surveys in the trends found for father dead, mother dead and, most markedly, for high bread consumption. For those risk factors available in one survey only, a highly significant positive association was found with low activity, low veg/salad consumption and high salt consumption (HSE93) and with "risky" occupation, low income, do nothing to keep healthy, little sleep, long time before first meal, high fried food consumption, low breakfast cereal consumption, low salad consumption, sugar in tea or coffee, high tea consumption, high coffee consumption, high neuroticism and high extroversion (HALS2).

Turning now to trends excluding never smokers, it can also be seen that the results in Tables 7 and 8 show some agreement. As would be expected, the trends are less marked. The only risk factor for which both surveys found a highly significant positive trend was low fruit consumption although significant ($p<0.05$) positive trends were found in both surveys for no educational qualifications, low social class and no use of low fat/PU spread. Both surveys also found a significant *negative* trend among current smokers for high alcohol consumption. Both surveys found no significant ($p<0.05$) trend for mother dead, high bread consumption, high sweet food consumption, separated, divorced or widowed and underweight. The most notable difference between the surveys is for high body mass index, for which HSE93 shows a highly significant negative trend among current smokers while HALS2 shows no significant trend. Father dead and not in paid employment showed a significant

positive trend in HSE93 but no significant trend in HALS2.

[TABLE 9 ABOUT HERE]

Comparison of cotinine level and number of cigarettes smoked as markers of exposure to lifestyle risk factors in current cigarette smokers

Analyses comparable to those in Tables 7 and 8, which use cotinine level as the index of exposure, were also run using the alternative subjective index "number of cigarettes smoked." Table 9 allows comparison of age and sex adjusted chisquared values for trend within current cigarette smokers for both indices. It also shows trends for each index further adjusted for the alternative index. Results in each survey are shown for all risk factors which showed a significant ($p<0.05$) age and sex adjusted trend for at least one index, and also for high body mass index in HALS2, significant only after further adjustment for the alternative index.

Whether one compares the magnitude of the trend statistic adjusted for age and sex only or the magnitude of the trend adjusted for age, sex and the alternative index, number of cigarettes smoked was a better marker than cotinine of the association with the risk factor for low fruit, vegetable and salad consumption (both surveys), not in paid employment, low activity and high salt consumption in food (HSE93 only), and do nothing to keep healthy, low breakfast cereal and sweet food consumption and high fried food, tea and coffee consumption (HALS2 only). In contrast, cotinine was a better marker for no educational qualifications and low social class (both surveys), father dead (HSE93 only), "risky" occupation, long time before first meal and sugar in tea or coffee (HALS2 only). The evidence in relation to no use of low fat/PU spread and low control at work was less clear. High alcohol consumption and high body mass index were of interest in that both studies showed a positive relationship with number of cigarettes per day adjusted for cotinine, but a negative relationship with cotinine adjusted for number of cigarettes per day.

The analyses in Tables 7 to 9 included subjects who smoked pipes and cigars as well as cigarettes. In additional analyses restricted to current smokers of cigarettes only, very similar conclusions were reached (results not shown).

[TABLES 10 AND 11 ABOUT HERE]

Independence of the associations with cotinine level in current smokers

Tables 10 and 11 show the results of linear discriminant modelling on data for never and current smokers together and for current smokers only, using log(cotinine+0.05) as the dependent variable and including all the risk factors and an age-sex variable as explanatory variables. (The trend codings given in Tables 8 and 9 are repeated for ease of comparison.) For both HSE93 and HALS2 many of the risk factors which are associated with cotinine level also make independent significant ($p<0.1$) contributions to the linear models. The results from the modelling, not surprisingly, reflect a lack of independence within certain groups of risk factors such as:-

- low fruit, vegetable and salad consumption
- no educational qualifications, not in paid employment and low social class and
- high body mass index and underweight.

However, the majority of those risk factors found to be significantly associated with cotinine level also made an independent significant contribution to the linear models.

Discussion

Our findings show clearly that, in both serum (HSE93) and saliva (HALS2), cotinine levels are much higher in current smokers than in never or ex-smokers. They also show that in current smokers cotinine level increases with amount smoked and that in never smokers it is higher in those living with a smoker. These findings are consistent with reports from other large studies (1-15), and demonstrate that cotinine, a major metabolite of nicotine, is strongly associated with recent exposure to tobacco smoke.

In our previous study (16) we showed that current smokers had an increased exposure to a whole range of risk factors. Our present analyses confirm and extend this finding. Of the risk factors considered, high sweet food consumption and high body mass index are the only two where smokers clearly have a lower prevalence. In contrast, smokers have a significantly higher prevalence for the great majority, including having no educational qualifications, being separated, divorced or widowed, not being in paid employment, being of low social class, working in a "risky" occupation, having a low income, having a high alcohol, tea, coffee and fried food consumption, doing nothing to keep healthy, taking little sleep, taking a long time before the first meal of the day, having a low fruit and salad consumption, using sugar in tea or coffee, not using low fat/PU spread, being underweight, having low control at work, and having high neuroticism and extroversion scores.

We also showed previously (16) that living with a smoker was associated with an increased prevalence of many of these risk factors. In the analyses reported here, use of cotinine as a marker of exposure has tended to bring out these relationships more clearly for many of the risk factors. As shown in Table 5, adjustment for cotinine essentially eliminates the association between risk factors and living with a smoker in many cases, but the reverse is generally not true. Even after adjustment for living with a smoker, highly significant associations were still evident for having no educational qualifications, being separated, divorced or widowed, being of low social class, having low income,

having high alcohol and fried food consumption, having low veg/salad consumption and having high extroversion scores. While the association between living with a smoker and some risk factors may reflect the fact that nonsmokers living with smokers share to some extent the characteristics (including diet) of their smoking cohabitants, the associations with cotinine level in never smokers may also reflect the fact that cotinine is a marker of ETS exposure outside, as well as inside the home. It is notable that extroversion and alcohol consumption are strongly correlated with cotinine, but are virtually uncorrelated with living with a smoker. This suggests that cotinine is not only a measure of ETS exposure, and of lifestyle shared with cohabitants who smoke, but is also a measure of increased socialization outside the home (e.g. going to public houses), and all the factors with which this is correlated.

The magnitude of potential bias that may arise because of uncontrolled confounding has been discussed in detail in our previous paper (16). It is clear from the results in that paper and from the results shown in Tables 3 and 4 that some of the differences in risk factor prevalence have the potential to cause moderate confounding when attempting to assess the relationship between ETS and disease using cotinine as a marker of ETS exposure. To take an illustrative example, consider the prevalences of high alcohol consumption for HSE93 in Table 3 and assume that high alcohol consumption increases by a factor of 2 the risk of some disease which is actually unaffected by ETS exposure. One can readily calculate that one would expect to see relative risks of 1.00, 1.02, 1.10, 1.10 and 1.14 in relation to the five ascending serum cotinine level categories shown in that table. Although this effect is modest one must consider not only the strength of some of the associations in Tables 3 and 4 but also the possibility of confounding by multiple, and not just single risk factors, many of which have been shown to be independently associated with cotinine (Table 6). Although cotinine is a useful marker of ETS exposure in never smokers which may assist in investigating the relationships between ETS and disease, it is important to realise that its use in no way precludes the necessity to take careful account of potential confounding factors in analysis.

While cotinine is a better marker than is living with a smoker of the association between risk factor prevalence and ETS exposure for the great majority of the risk factors studied, the relative value of cotinine and number of cigarettes smoked as markers of the association between risk factor prevalence and exposure to active smoking is less clear. While the results in Table 9 suggest cotinine is a better marker for some risk factors, they also show that number of cigarettes smoked is a better marker for others. Differences may reflect the fact that, in smokers, cotinine is an indicator not only of how many cigarettes are smoked, but also of how much cotinine is obtained per cigarette. People not only vary in the depth to which they inhale cigarettes, but they may also vary in how they metabolize nicotine to cotinine (30). Metabolism might in theory depend on a range of factors, both genetic and environmental.

It is interesting to note that, in current cigarette smokers, the direction of the association with some risk factors differs depending on whether number of cigarettes smoked or cotinine is being used as the marker. This is most clearly evident for high alcohol consumption. It is clear that current smokers drink more alcohol than never smokers. However, within current smokers, though there is little association between number of cigarettes smoked and high alcohol consumption, frequency of high alcohol consumption *decreased* markedly with increasing cotinine level (Tables 7 and 8). In other words, increasing alcohol consumption is associated with decreased cotinine per cigarette smoked. This effect is evident in both surveys. It may be due to heavy drinking being associated with reduced inhalation, but other possible explanations include alcohol having an effect on the method used to determine cotinine or on the metabolism of nicotine. An effect of alcohol on nicotine metabolism is supported by the results of a pharmacokinetic study in rats pretreated with ethanol (31). It is interesting to note that if alcohol affects nicotine metabolism in humans, adjustment for alcohol consumption may be necessary in studies using cotinine as an index of exposure to active or passive smoking.

Body mass index is another risk factor that we found to be positively related to number of cigarettes smoked and negatively related to cotinine. Reporting a similar finding previously, Istvan et al (32) considered nicotine metabolism, energy intake, and measurement issues as possible explanations.

Whether the associations reported in Table 9 between cotinine level in cigarette smokers and various risk factors are due to associations of the risk factor with amount smoked, inhalation, metabolism of nicotine, detection of cotinine or are due to confounding by other risk factors, it is clear that many of them (e.g. with no educational qualifications, high alcohol consumption, low fruit consumption, long time before first meal and high tea consumption) are quite strong and independent (see Table 11). As with the results relating cotinine to risk factor prevalence in never smokers, they serve to underline the point that cotinine is a marker of exposure not just to smoke constituents, but to quite a wide range of other risk factors.

As others have emphasised before (33, 34), the use of biomarkers rather than subjective self-reports does not necessarily guarantee more valid research, a point which may be particularly pertinent for studies of possible effects from passive smoke exposure.

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Table 1. Frequency of risk factors analysed

Risk factor	HSE93				HALS2			
	N[a]	n[b]	%[c]	%[d] (all)	N[a]	n[b]	%[c]	%[d] (all)
Father dead	4417	2646	59.9	60.7	3604	2392	66.4	67.8
Mother dead	4362	2075	47.6	48.4	3640	1859	51.1	52.7
No educational qualifications	4776	1642	34.4	37.6	3663	1562	42.6	44.6
Separated, divorced or widowed	4813	703	14.6	15.4	3650	620	17.0	18.8
Not in paid employment	4813	1621	33.7	44.2	3668	1480	40.3	43.6
Low social class	4813	2070	43.0	44.6	3668	1993	54.3	54.7
High alcohol consumption	4808	1997	41.5	39.0	3651	1178	32.3	30.3
High bread consumption	4802	1616	33.7	34.2	3620	1838	50.8	49.7
Low fruit consumption	4803	2422	50.4	51.5	3668	1410	38.4	38.0
Low veg/salad consumption	4797	1551	32.3	33.5	*[e]	*	*	*
Low vegetable consumption	*	*	*	*	3664	1845	50.4	50.6
Low salad consumption	*	*	*	*	3668	2008	54.7	54.7
High sweet food consumption	4796	2158	45.0	46.1	3654	1779	48.7	49.3
No use of low fat/PU spread	4692	2368	50.5	53.0	3164	928	29.3	29.6
High body mass index	4633	2719	58.7	58.5	3641	2026	55.6	56.4
Underweight	4633	130	2.8	3.3	3641	99	2.7	2.9
"Risky" occupation	*	*	*	*	2741	892	32.5	32.1
Low income	*	*	*	*	3073	1625	52.9	54.6
Do nothing to keep healthy	*	*	*	*	3668	1234	33.6	35.0
Little sleep	*	*	*	*	3657	1568	42.9	43.0
Long time before first meal	*	*	*	*	3668	890	24.3	23.0
High fried food consumption	*	*	*	*	3660	948	25.9	25.3
Low breakfast cereal consumption	*	*	*	*	3665	1328	36.2	36.1
Sugar in tea or coffee	*	*	*	*	3668	1635	44.6	44.0
High tea consumption	*	*	*	*	3176	850	26.8	22.6
High coffee consumption	*	*	*	*	2577	305	11.8	7.8
High neuroticism	*	*	*	*	3160	1451	45.9	46.7
High extroversion	*	*	*	*	3142	1439	45.8	45.3
Low activity	4813	766	15.9	17.7	*	*	*	*
High salt consumption in food	4761	2288	48.1	47.9	*	*	*	*
Low control at work[f]	2745	626	22.8	24.2	*	*	*	*
High pace index[f]	2730	934	34.2	32.6	*	*	*	*

[a] Number of subjects with valid cotinine, smoking, passive smoking and social class data (the subset studied) who provided information on the risk factor.
[b] Number with risk factor present.
[c] Prevalence in the subset studied
[d] Prevalence in the whole study (excluding proxy replies for HSE93)
[e] Asterisks indicate data on the risk factor are not available from the survey.
[f] Only those aged 16-59 who are in paid employment or are self-employed.

Table 2. Number of subjects by survey, sex, cotinine level and smoking group

Survey	Sex	Cotinine[a]	Unexposed[b]	Passive smokers[c]	Ex-smokers	Current smokers
HSE93	Male	0.0-1.0	374	32	572	44
		1.1-2.0	92	21	158	21
		2.1-20.0	49	52	119	64
		20.1-100.0	5	2	21	96
		100.1-350.0	4	2	23	397
		>350.0	0	0	7	181
		Total	524	109	900	803
	Female	0.0-1.0	731	91	513	15
		1.1-2.0	105	45	111	14
		2.1-20.0	36	57	78	31
		20.1-100.0	3	4	19	110
		100.1-350.0	3	0	15	387
		>350.0	0	1	7	101
		Total	878	198	743	658
HALS2	Male	0.0-1.0	219	16	289	3
		1.1-5.0	144	50	237	7
		5.1-30.0	15	24	76	22
		30.1-100.0	0	0	8	44
		100.1-350.0	0	1	11	229
		>350.0	0	0	2	284
		Total	378	91	623	589
	Female	0.0-1.0	483	71	251	3
		1.1-5.0	164	104	202	16
		5.1-30.0	23	31	20	22
		30.1-100.0	0	0	7	40
		100.1-350.0	1	0	14	277
		>350.0	0	1	7	250
		Total	671	207	501	608

[a] ng/ml in serum (HSE93) and in saliva (HALS2).
[b] Never smokers not living with a smoker.
[c] Never smokers living with a smoker

Table 3. Relationship of risk factor prevalence to serum cotinine in never smokers[a] (HSE93)

	Serum cotinine level (ng/ml)					
Risk factor	0.0-0.2 %[b]	0.3-0.5 %[b]	0.6-1.0 %[b]	1.1-2.0 %[b]	2.1-20.0 %[b]	Trend p[c]
Father dead	55.0	52.8	55.2	52.6	54.1	NS
Mother dead	39.0	41.4	43.5	48.9	43.5	+
No educational qualifications	22.0	26.9	24.0	33.8	37.2	+++
Separated, divorced or widowed	10.8	14.4	11.7	13.4	17.0	NS
Not in paid employment	34.1	27.7	28.1	32.0	29.8	NS
Low social class	27.5	31.1	39.1	44.6	53.8	+++
High alcohol consumption	23.0	24.9	35.0	34.7	40.6	+++
High bread consumption	33.9	30.2	28.9	32.7	32.8	NS
Low fruit consumption	45.3	40.9	42.7	44.0	60.5	+++
Low veg/salad consumption	27.6	27.1	27.8	32.9	39.7	+++
High sweet foods consumption	53.1	49.9	47.1	50.2	43.3	-
No use of low fat/PU spread	47.7	49.7	47.4	46.3	45.5	NS
High body mass index	55.2	55.4	59.5	62.7	65.4	++
Underweight	2.4	2.0	3.2	2.0	2.2	NS
Low activity	13.1	15.8	14.6	12.9	15.3	NS
High salt consumption in food	37.4	39.0	40.3	38.3	47.4	++
Low control at work	17.7	20.4	24.1	28.8	32.2	+++
High pace index	31.6	39.6	37.1	33.7	33.8	NS
Number of subjects	353	485	390	263	194	

[a] Excluding never smokers with serum cotinine above 20 ng/ml, or with no data on social class or living with a smoker.
[b] Percentages are adjusted for sex and age (<30, 30-39, 40-49, 50-59, 60-69, 70+) to the overall population of never smokers considered.
[c] Significance based on chisquared test or Fry-Lee stratified rank test (see methods); +++ $p<0.001$, ++ $p<0.01$, + $p<0.05$, - $p<0.05$ decrease, NS not significant ($p \geq 0.1$).

Table 4. Relationship of risk factor prevalence to saliva cotinine in never smokers[a] (HALS2)

Risk factor	Saliva cotinine level (ng/ml) 0.0-0.5 %[b]	0.6-1.0 %[b]	1.1-2.0 %[b]	2.1-5.0 %[b]	5.1-30.0 %[b]	Trend p[c]
Father dead	61.1	59.8	64.6	61.4	67.7	NS
Mother dead	44.6	41.5	45.7	46.7	59.5	+
No educational qualifications	28.2	30.1	39.0	38.7	56.7	+++
Separated, divorced or widowed	12.5	15.0	18.4	18.5	17.5	+
Not in paid employment	38.2	32.9	34.3	34.1	40.2	NS
Low social class	37.3	41.6	53.3	54.1	63.1	+++
High alcohol consumption	18.2	23.5	23.2	35.7	29.3	++
High bread consumption	45.5	42.0	47.0	49.6	52.2	+
Low fruit consumption	26.3	22.8	31.8	30.2	39.5	++
Low vegetable consumption	50.1	48.1	51.0	49.4	56.6	NS
Low salad consumption	49.3	46.0	46.6	49.2	52.5	NS
High sweet food consumption	59.8	58.9	55.0	49.7	48.1	- -
No use of low fat/PU spread	21.7	20.5	20.1	27.7	38.6	++
High body mass index	51.1	55.9	58.1	62.7	64.6	+++
Underweight	2.3	1.0	1.1	1.3	2.8	NS
"Risky" occupation	21.9	21.2	26.5	33.2	44.3	+++
Low income	45.2	44.0	49.2	53.8	57.6	+++
Do nothing to keep healthy	28.3	29.6	28.6	35.8	43.8	++
Little sleep	37.7	38.5	36.5	46.5	42.9	NS
Long time before first meal	14.7	17.4	19.0	21.1	15.9	++
High fried food consumption	15.3	20.1	20.5	24.6	29.8	+++
Low breakfast cereal consumption	25.1	28.4	30.3	29.1	39.0	+++
Sugar in tea or coffee	35.6	35.5	39.8	36.8	51.7	+
High tea consumption	18.5	21.0	17.8	20.0	22.2	NS
High coffee consumption	7.2	5.3	8.5	6.7	7.1	NS
High neuroticism	39.8	44.6	54.0	48.2	42.2	(+)
High extroversion	32.0	42.5	43.1	49.2	56.1	+++
Number of subjects	511	278	241	221	93	

[a] Excluding never smokers with saliva cotinine above 30 ng/ml, or with no data on social class or living with a smoker.
[b] Percentages are adjusted for sex and age (<30, 30-39, 40-49, 50-59, 60-69, 70+) to the overall population of never smokers considered.
[c] Significance based on chisquared test or Fry-Lee stratified rank test (see methods); +++ $p<0.001$, ++ $p<0.01$, + $p<0.05$, - $p<0.05$ decrease, (+) $p<0.1$, NS not significant ($p \geq 0.1$).

Table 5. Effects of adjustment on trend chisquared values in never smokers[a] associated with cotinine level and with living with a smoker

		Trend chisquared values[b]			
		Living with a smoker adjusted for:-		Cotinine adjusted for:-	
Survey	Risk factor	Age[c], sex only	Age[c], sex cotinine	Age[c], sex only	Age[c], sex, Living with a smoker
HSE93	Mother dead	0.0	0.0	6.0	6.2
	No educational qualifications	10.3	3.7	19.4	13.4
	Low social class	19.6	4.0	46.4	32.5
	High alcohol consumption	0.8	0.6	27.9	27.7
	Low fruit consumption	9.5	1.5	17.0	9.3
	Low veg/salad consumption	0.7	0.7	20.2	19.3
	High sweet food consumption	0.0	0.3	(4.6)	(4.9)
	High body mass index	1.2	0.0	10.0	9.5
	High salt consumption in food	2.3	0.2	7.0	5.0
	Low control at work	10.6	4.0	16.5	9.0
	High pace index	(4.8)	(3.9)	0.0	0.4
HALS2	Mother dead	0.5	0.0	6.1	5.0
	No educational qualifications	7.3	0.0	35.9	27.1
	Separated, divorced or widowed	(13.6)	(27.1)	6.2	18.5
	Low social class	24.9	5.7	43.4	23.0
	High alcohol consumption	2.0	0.4	10.3	6.4
	High bread consumption	1.5	0.0	4.1	1.9
	Low fruit consumption	4.7	1.6	9.4	4.4
	High sweet food consumption	2.9	0.2	(7.7)	(5.2)
	No use of low fat/PU spread	7.8	2.8	6.7	2.7
	High body mass index	11.5	4.5	11.8	5.9
	"Risky" occupation	10.5	2.6	20.0	12.2
	Low income	0.3	1.9	18.0	15.9
	Do nothing to keep healthy	7.7	1.3	9.3	4.3
	Long time before first meal	3.1	0.3	8.6	5.9
	High fried food consumption	5.3	1.0	17.8	11.7
	Low breakfast cereal consumption	9.5	2.6	11.1	4.4
	Sugar in tea or coffee	1.2	0.0	6.0	3.9
	High coffee consumption	6.1	5.7	0.3	0.0
	High neuroticism	5.2	3.9	2.9	0.3
	High extroversion	3.2	0.2	35.7	28.7

[a] Excluding never smokers with serum cotinine above 20 ng/ml (HSE93) or saliva cotinine above 30 ng/ml (HALS2) or with no data on social class or living with a smoker.

[b] Critical values of the trend chisquared are 10.83 $p<0.001$, 6.63 $p<0.01$ and 3.84 $p<0.05$.

[c] Adjusted for age in 10 year groups (<30, 30-39, 40-49, 50-59, 60-69, 70+).

Bracketed chisquared values indicate significant negative trends.

Table 6. Results of linear discriminant modelling of log(cotinine+0.05) in never smokers

Risk factor	HSE93		HALS2	
	Trend p [a]	Significance in model	Trend p[b]	Significance in model
Father dead	NS	NS	NS	NS
Mother dead	+	NS	+	NS
No educational qualifications	+++	(+)	+++	NS
Separated, divorced or widowed	NS	+	+	(+)
Not in paid employment	NS	NS	NS	NS
Low social class	+++	+++	+++	NS
High alcohol consumption	+++	+++	++	++
High bread consumption	NS	NS	+	NS
Low fruit consumption	+++	NS	++	NS
Low veg/salad consumption	+++	(+)		
Low vegetable consumption			NS	NS
Low salad consumption			NS	NS
High sweet food consumption	-	-	- -	-
No use of low fat/PU spread	NS	NS	++	(+)
High body mass index	++	(+)	+++	+
Underweight	NS	NS	NS	NS
"Risky" occupation			+++	+
Low income			+++	NS
Do nothing to keep healthy			++	+
Little sleep			NS	NS
Long time before first meal			++	NS
High fried food consumption			+++	+
Low breakfast cereal consumption			+++	NS
Sugar in tea or coffee			+	NS
High tea consumption			NS	NS
High coffee consumption			NS	NS
High neuroticism			(+)	(+)
High extroversion			+++	+++
Low activity	NS	NS		
High salt consumption in food	++	NS		
Low control at work[c]	+++	+		
High pace index[c]	NS	(+)		

[a] Trend p repeated from Table 3
[b] Trend p repeated from Table 4
[c] Only those aged 16-59 who are in paid employment or are self-employed.

Table 7. Risk factor prevalence in never smokers and, by serum cotinine level, in current cigarette smokers[a] (HSE93)

	Serum cotinine level (ng/ml) in current cigarette smokers						
Risk factor	Never smoked %[b]	<100 %[b]	100.1-250 %[b]	250.1-350 %[b]	>350 %[b]	Trend1[c] p[e]	Trend2[d] p[e]
Father dead	52.6	50.4	55.1	55.5	58.9	+	+
Mother dead	40.9	40.9	42.9	46.0	41.7	NS	NS
No educational qualifications	25.4	30.1	40.1	49.7	55.0	+++	+++
Separated, divorced or widowed	11.7	14.3	19.6	14.3	19.5	+++	NS
Not in paid employment	27.6	29.0	32.2	40.2	35.8	+++	+
Low social class	37.0	37.7	50.8	56.5	58.0	+++	+++
High alcohol consumption	32.0	57.4	47.2	46.9	39.4	+++	- - -
High bread consumption	31.9	30.6	35.0	30.3	29.7	NS	NS
Low fruit consumption	45.0	54.0	66.3	72.6	72.2	+++	+++
Low veg/salad consumption	30.5	39.4	39.1	41.2	43.4	+++	+
High sweet food consumption	49.1	34.0	39.2	38.9	32.7	- - -	NS
No use of low fat/PU spread	47.9	51.8	55.5	59.6	58.2	+++	+
High body mass index	58.1	55.0	56.8	44.4	45.1	- - -	- - -
Underweight	2.6	3.9	3.4	3.4	7.5	+++	(+)
Low activity	13.8	10.9	20.3	15.0	11.0	+++	NS
High salt consumption in food	40.1	55.0	51.4	61.0	56.0	+++	+
Low control at work	23.0	14.3	28.5	29.4	23.5	++	++
High pace index	35.6	39.6	29.3	36.2	33.3	NS	NS
Number of subjects	1685	278	414	317	262		

[a] Excluding never smokers with serum cotinine above 20 ng/ml, and subjects with no data on social class or number of cigarettes smoked per day

[b] Percentages are adjusted for sex and age (<30, 30-39, 40-49, 50-59, 60-69, 70+) to the overall population considered.

[c] Trend including never smokers.

[d] Trend excluding never smokers.

[e] Significance based on chisquared test or Fry-Lee stratified rank test (see methods); +++ $p<0.001$, - - - $p<0.001$ decrease, ++ $p<0.01$, + $p<0.05$, (+) $p<0.1$, NS not significant ($p \geq 0.1$).

Table 8. Risk factor prevalence in never smokers and, by saliva cotinine level, in current cigarette smokers[a] (HALS2)

	Saliva cotinine level (ng/ml) in current cigarette smokers						
Risk factor	Never smoked %[b]	<200 %[b]	200.1-350 %[b]	350.1-450 %[b]	>450 %[b]	Trend1[c] p[e]	Trend2[d] p[e]
Father dead	60.6	60.1	63.7	65.9	62.5	(+)	NS
Mother dead	43.9	41.2	48.6	49.6	48.3	++	NS
No educational qualifications	32.7	45.0	52.2	58.5	54.5	+++	+
Separated, divorced or widowed	14.1	19.7	19.7	23.9	22.2	+++	NS
Not in paid employment	34.5	41.4	41.5	40.5	43.6	+++	NS
Low social class	45.3	61.0	65.7	65.5	72.9	+++	++
High alcohol consumption	24.4	43.7	42.5	35.4	30.7	+++	- -
High bread consumption	47.6	46.9	53.5	50.6	52.1	+++	NS
Low fruit consumption	28.7	49.6	51.5	60.1	59.8	+++	+++
Low vegetable consumption	51.5	51.6	51.2	55.1	58.3	+	NS
Low salad consumption	48.9	52.6	61.4	66.0	71.5	+++	+++
High sweet food consumption	55.6	38.6	36.7	33.2	38.4	- - -	NS
No use of low fat/PU spread	22.4	34.0	32.5	35.2	47.3	+++	++
High body mass index	55.3	48.5	56.3	47.3	48.1	- - -	(-)
Underweight	1.9	2.7	5.1	4.7	5.0	+++	NS
"Risky" occupation	26.3	31.7	38.9	36.6	46.6	+++	+
Low income	46.1	61.4	58.1	60.9	68.0	+++	(+)
Do nothing to keep healthy	30.9	39.2	40.7	39.8	48.1	+++	+
Little sleep	38.6	46.6	46.4	47.6	47.7	+++	NS
Long time before first meal	17.4	23.9	40.1	48.5	52.3	+++	+++
High fried food consumption	20.1	30.5	33.6	33.2	40.4	+++	++
Low breakfast cereal consumption	28.8	43.1	50.4	54.9	54.9	+++	++
Sugar in tea or coffee	37.8	50.9	53.7	56.4	66.0	+++	+++
High tea consumption	19.2	28.7	39.6	44.4	50.5	+++	+++
High coffee consumption	6.6	17.3	20.2	27.5	27.7	+++	++
High neuroticism	43.1	55.9	53.1	47.7	49.0	+++	NS
High extroversion	40.6	56.3	53.3	56.2	53.1	+++	NS
Number of subjects	1344	229	340	235	259		

[a] Excluding never smokers with saliva cotinine above 30 ng/ml, or with no data on social class or living with a smoker.
[b] Percentages are adjusted for sex and age (<30, 30-39, 40-49, 50-59 and 60-69, 70+ for males or 60+ for females) to the overall population considered.
[c] Trend including never smokers.
[d] Trend excluding never smokers.
[e] Significance based on chisquared test or Fry-Lee stratified rank test (see methods); +++ $p<0.001$, - - - $p<0.001$ decrease, ++ $p<0.01$, + $p<0.05$, (+) $p<0.1$, NS not significant ($p\geq 0.1$).

Table 9. Effects of adjustment on trend chisquared values within current cigarette smokers[a] associated with cotinine and number of cigarettes smoked per day

		Trend chisquared values[b]			
		Number cigs. smoked/day adjusted for:-		Cotinine adjusted for:-	
Survey	Risk factor	Age[c], sex only	Age[c], sex cotinine	Age[c], sex only	Age[c], sex, number smoked
HSE93	Father dead	0.3	0.5	4.5	3.3
	No educational qualifications	17.8	1.4	38.3	19.8
	Not in paid employment	11.0	3.8	6.5	0.8
	Low social class	15.5	2.1	22.2	5.3
	High alcohol consumption	0.6	3.3	(17.3)	(14.1)
	Low fruit consumption	61.4	27.9	27.7	2.6
	Low veg/salad consumption	9.2	5.1	4.2	0.2
	No use of low fat/PU spread	1.4	0.0	4.7	3.4
	High body mass index	2.4	14.9	(19.6)	(34.3)
	Low activity	21.5	17.7	0.3	(4.9)
	High salt consumption in food	9.0	6.1	4.4	0.8
	Low control at work	8.8	0.3	10.2	1.4
HALS2	No educational qualifications	3.1	0.1	4.7	2.3
	Low social class	1.9	0.0	7.5	5.2
	High alcohol consumption	2.8	13.3	(9.9)	(13.2)
	Low fruit consumption	36.9	23.2	20.0	6.4
	Low vegetable consumption	5.4	3.7	2.3	0.4
	Low salad consumption	23.8	11.1	12.1	2.6
	High sweet food consumption	(6.2)	(6.5)	0.1	0.1
	No use of low fat/PU spread	7.2	5.8	6.8	3.9
	High body mass index	2.9	4.2	3.2	(5.4)
	"Risky" occupation	1.9	0.0	5.6	3.1
	Do nothing to keep healthy	15.3	13.0	4.2	0.7
	Long time before first meal	33.9	12.5	51.1	27.4
	High fried food consumption	20.3	13.6	7.1	0.9
	Low breakfast cereal consumption	25.3	16.7	9.5	1.5
	Sugar in tea or coffee	11.0	3.4	14.1	5.2
	High tea consumption	27.6	17.3	15.7	5.9
	High coffee consumption	20.1	13.5	9.8	1.0

[a] Excluding current smokers with no data on cotinine, social class or number of cigarettes smoked/day.

[b] Critical values of the trend chisquared are 10.83 $p<0.001$, 6.63 $p<0.01$ and 3.84 $p<0.05$. Based on data excluding never smokers.

[c] Adjusted for age in groups (<30,30-39, 40-49,50-59, and 60-69, 70+ for males or 60+ for females). Bracketed chisquared values indicate significant negative trend.

Table 10. Results of linear discriminant modelling of log(cotinine+0.05) in never plus current smokers.

Risk factor	HSE93		HALS2	
	Trend p[a]	Significance in model	Trend p[b]	Significance in model
Father dead	+	NS	(+)	NS
Mother dead	NS	NS	++	+
No educational qualifications	+++	+++	+++	+++
Separated, divorced or widowed	+++	+++	+++	+
Not in paid employment	+++	NS	+++	NS
Low social class	+++	+++	+++	+
High alcohol consumption	+++	+++	+++	+++
High bread consumption	NS	NS	+++	(+)
Low fruit consumption	+++	+++	+++	+++
Low veg/salad consumption	+++	+		
Low vegetable consumption			+	NS
Low salad consumption			+++	NS
High sweet food consumption	- - -	- - -	- - -	- - -
No use of low fat/PU spread	+++	(+)	+++	++
High body mass index	- - -	- - -	- - -	-
Underweight	+++	NS	+++	+
"Risky" occupation			+++	NS
Low income			+++	++
Do nothing to keep healthy			+++	+
Little sleep			+++	+
Long time before first meal			+++	+++
High fried food consumption			+++	+++
Low breakfast cereal consumption			+++	++
Sugar in tea or coffee			+++	+++
High tea consumption			+++	+++
High coffee consumption			+++	+++
High neuroticism			+++	(+)
High extroversion			+++	+++
Low activity	+++	++		
High salt consumption in food	+++	+++		
Low control at work[c]	++	NS		
High pace index[c]	NS	+		

[a] Trend p repeated from Table 8
[b] Trend p repeated from Table 9
[c] Only those aged 16-59 who are in paid employment or are self-employed.

Table 11. Results of linear discriminant modelling of log(cotinine+0.05) in current smokers only.

Risk factor	HSE93		HALS2	
	Trend p[a]	Significance in model	Trend p[b]	Significance in model
Father dead	+	NS	NS	NS
Mother dead	NS	NS	NS	NS
No educational qualifications	+++	+++	+	+
Separated, divorced or widowed	NS	NS	NS	NS
Not in paid employment	+	NS	NS	NS
Low social class	+++	+++	++	NS
High alcohol consumption	- - -	- - -	- -	NS
High bread consumption	NS	NS	NS	(+)
Low fruit consumption	+++	+++	+++	NS
Low veg/salad consumption	+	NS		
Low vegetable consumption			NS	NS
Low salad consumption			+++	(+)
High sweet food consumption	NS	NS	NS	NS
No use of low fat/PU spread	+	NS	++	NS
High body mass index	- - -	- - -	(-)	-
Underweight	(+)	NS	NS	NS
"Risky" occupation			+	NS
Low income			(+)	NS
Do nothing to keep healthy			+	NS
Little sleep			NS	NS
Long time before first meal			+++	+
High fried food consumption			++	NS
Low breakfast cereal consumption			++	NS
Sugar in tea or coffee			+++	(+)
High tea consumption			+++	+
High coffee consumption			++	+
High neuroticism			NS	-
High extroversion			NS	NS
Low activity	NS	NS		
High salt consumption in food	+	NS		
Low control at work[c]	++	++		
High pace index[c]	NS	+		

[a] Trend p repeated from Table 8
[b] Trend p repeated from Table 9
[c] Only those aged 16-59 who are in paid employment or are self-employed.

Appendix A. Description of risk factors

Risk factor	Present/Absent (P/A) or Graded	Explanatory details
Father dead	P/A	Included as an indicator of genetic risk.
Mother dead	P/A	Included as an indicator of genetic risk.
No educational qualifications	P/A	No university degree, GCE 'A' or 'O' level, CSE grade 1-5 or equivalent.
Separated, divorced or widowed	P/A	Derived from marital status variable.
Not in paid employment	P/A	'Present' includes retirees.
Low social class	Graded	The 6 Registrar General's classifications I, II, III non-manual, III manual, IV and V. "Low" = III manual, IV and V.
High alcohol consumption	Graded	Units of alcohol per week in 4 groups:- For men: none, 1-10, 11-50 and 51+. "High" = 11+. For women: none, 1-5, 6-35 and 36+. "High" = 6+.
High bread consumption	Graded	HALS2: Number of slices per day (1 roll = 1.5 slices). "High" = 4+. HSE93: HSEFFS[1]. "High" = 8.
Low fruit consumption	Graded	HALS2: Decreasing combined HALFFS[2] for fresh fruit in summer, fresh fruit in winter and fresh fruit juice. "Low" = <8. HSE93: Decreasing HSEFFS[1] for fruit. "Low" = <7.
Low veg/salad consumption	Graded	HSE93 only. Decreasing HSEFFS[1] for vegetables/salads. "Low" = <7.
Low vegetable consumption	Graded	HALS2 only. Decreasing combined HALFFS[2] for root vegetables, green vegetables and other cooked vegetables. "Low" = <8.
Low salad consumption	Graded	HALS2 only. Decreasing combined HALFFS[2] for salads (or raw vegetables) in summer and in winter. "Low" = <5.
High sweet food consumption	Graded	HALS2: Combined HALFFS[2] for tinned fruit, sweets/chocolates, biscuits, cake, sweets/puddings, jam/marmalade/syrup/honey, cream, and ice-cream/mousse/yogurt/milk puddings. "High" = >12. HSE93: Combined HSEFFS[1] for biscuits, sweets and cakes. "High" = >10.
No use of low fat/PU spread	P/A	Based on the question "What do you usually spread on your bread?".
High body mass index	Graded	Body mass index (weight in kg / (height in m)^2) in 4 groups:- For men: ≤20.0, 20.1-25.0, 25.1-29.9, 30.0+. "High" = 25.1+ For women: ≤18.6, 18.7-23.8, 23.9-28.5, 28.6+. "High" = 23.9+
Underweight	P/A	Body mass index of ≤20.0 for men and ≤18.6 for women.
"Risky" occupation	P/A	HALS2 only. Present if most recent occupation is thought to increase the risk of lung cancer, as defined by Sterling and Weinkam (26). Excludes those aged 60+.
Low household income	Graded	HALS2 only. 12 decreasing categories from £600+ per week to <£50 per week. "Low" = <£250 per week.
Do nothing to keep healthy	P/A	HALS2 only.

Appendix A. Description of risk factors continued.

Risk factor	Present/Absent (P/A) or Graded	Explanatory details
Little sleep	Graded	HALS2 only. 7 decreasing categories from 10+ to <6 hours per day. "Little" = <7 hours per day.
Long time before first meal	Graded	HALS2 only. 6 categories from <30 minutes to 4+ hours. "Long" = >2 hours.
High fried food consumption	Graded	HALS2 only. Score created by summing the scores in the HALFFS[2] for fried food (other than chips), chips, eggs and sausages.
Low breakfast cereal consumption	Graded	HALS2 only. Decreasing HALFFS[2] for breakfast cereal. "Low" = never or less than once a week.
Sugar in tea or coffee	Graded	HALS2 only. Scores of 0 for none or non-drinker, 1 for one or less teaspoons, 2 for over 1 to two teaspoons and 3 for more than two teaspoons, combined for tea and for coffee. Frequencies in tables are shown for any sugar in tea or coffee.
High tea consumption	P/A	HALS2 only. "High" = 7+ cups per day.
High coffee consumption	P/A	HALS2 only. "High" = 7+ cups per day.
High neuroticism	Graded	HALS2 only. Based on the Eysenck Personality Inventory (27). "High" = >9
High extroversion	Graded	HALS2 only. Based on the Eysenck Personality Inventory (27). "High" = >11
Low activity	Graded	HSE93 only. 4 decreasing categories of activity: vigorously active, moderately active, lightly active and inactive. "Low" = lightly active or inactive.
High salt consumption in food	Graded	HSE93 only. 7 categories which combine the scores for adding salt to cooking and for adding salt at the table. "High" = 5+.
Low control at work	Graded	HSE93 only. 3 categories: high, medium and low. "Low" = the low category.
High pace index	Graded	HSE93 only. 3 categories for low, medium and high speed/pressure at work. "High" = the high category.

[1] The HSE93 food frequency score (HSEFFS) uses the classifications:-

1 : rarely or never	4 : 1-2 days a week	7 : once every day
2 : less than once a month	5 : 3-4 days a week	8 : more than once a day.
3 : at least once a month	6 : 5-6 days a week	

[2] The HALS food frequency score (HALFFS) uses the classifications:-

0 : never	2 : once or twice a week	4 : once a day
1 : less than once a week	3 : most days	5 : more than once a day.

Table 5a. Effect of adjustment for social class on trend chisquared values in never smokers[a] associated with cotinine level

Survey	Risk factor	Trend chisquared values[b]	
		Adjusted for age[c] and sex	Adjusted for age[c], sex and social class[d]
HSE93	Mother dead	4.8	3.2
	No educational qualifications	16.6	1.9
	High alcohol consumption	28.1	36.5
	Low fruit consumption	19.8	15.4
	Low veg/salad consumption	22.6	15.2
	High sweet food consumption	(3.2)	(5.5)
	High body mass index	6.5	4.0
	High salt consumption in food	7.3	3.9
	Low control at work	19.3	4.0
HALS2	Mother dead	4.1	2.4
	No educational qualifications	31.9	13.5
	Separated, divorced or widowed	4.7	3.4
	"Risky" occupation	18.0	5.2
	Low income	15.4	5.2
	High alcohol consumption	10.8	17.2
	Do nothing to keep healthy	8.8	6.7
	Long time before first meal	9.0	5.5
	High fried food consumption	19.4	11.7
	Low breakfast cereal consumption	11.1	9.1
	High bread consumption	4.0	2.5
	Low fruit consumption	8.7	3.5
	Sugar in tea or coffee	5.9	3.2
	High sweet food consumption	(8.6)	(6.1)
	No use of low fat/PU spread	6.3	5.6
	High body mass index	10.3	7.4
	High extroversion	37.4	33.0

[a] Excluding never smokers with serum cotinine above 20 ng/ml (HSE93) or saliva cotinine above 30 ng/ml (HALS2) or with no data on social class or living with a smoker.

[b] Critical values of the trend chisquared are 10.83 $p<0.001$, 6.63 $p<0.01$ and 3.84 $p<0.05$.

[c] Adjusted for age in broad groups (<39, 40-59, 60+).

[d] Adjusted for social class in groups (I, II, III non manual, III manual, IV and V)

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The authors of this paper confirm that:

a) the material has not been published in whole or in part elsewhere;

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c) all authors have been personally and actively involved in substantive work leading to the report and will hold themselves jointly and individually responsible for its content;

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