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Nicotine dependence and urinary nicotine, cotinine and hydroxycotinine levels in daily smokers

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Abstract

Introduction: Nicotine dependence and smoking frequency are critical factors for smoking cessation. The aims of this study are 1) to determine if nicotine dependence FTND scores are associated with urinary levels of nicotine metabolites, 2) to assess the relationship of hydroxycotinine/cotinine ratio with FTND score and cigarettes smoked per day (CPD) and 3) to identify significant predictors of cigarettes per day among biomarker concentrations and individual FTND items**.**

Methods: Urine samples and questionnaire data of 239 daily smokers were obtained. Nicotine, cotinine and hydroxycotinine urinary levels were determined by UPLC MS/MS.

Multiple linear regression models were developed to explore the relationship between nicotine, cotinine , hydroxycotinine levels and separate FTND scores (for all 6 items).

Results: We found significant correlations between the different urinary biomarker concentrations, and the FTND score. The time before the first cigarette after waking (TTFC) was significantly associated with the nicotine, cotinine and hydroxycotinine concentrations. No association was found between the ratio of hydroxycotinine to cotinine and either the FTND or the CPD. A model including four FTND questions, sex, age and the cotinine concentration, accounted for 45 % of the variance of CPD.

C**onclusions**: There are significant relationships between urinary levels of nicotine, cotinine and hydroxycotinine and the FTND score. Especially the FTND question about TTFC is relevant for explaining the biomarker concentrations. CPD (below 15) was significantly explained by four FTND dependence items and urinary cotinine levels in a regression model.

Implications

We investigated associations between urinary levels of nicotine, cotinine and hydroxycotinine in daily smokers and the FTND scores for nicotine dependence. We did not find association between the hydroxycotinine/cotinine ratio and CPD. We developed a model that explains the cigarettes smoked daily (CPD) in a group of light smokers by combining FTND items, urinary cotinine levels, sex and age. Our results might be of importance for clinical use or future studies on larger smoking populations.

Introduction

Nicotine dependence is important to study with regards to smoking cessation strategies. Nicotine dependence can be measured by the Fagerström Test for Nicotine Dependence (FTND) which is a 6 item questionnaire.[1] One of the questions of the FTND is the assessment of the daily cigarette consumption (CPD). The result of the FTND is expressed as a total score made up by summing up the scores obtained for the individual questions. Otherwise, biomarker levels measured in human samples indicate objectively the degree of nicotine intake. Determinations of biomarkers for tobacco use are possible in several types of human samples including plasma, urine and saliva .[2-4] Cotinine is widely used as a biomarker for exposure to nicotine. The metabolism of nicotine to cotinine is mediated by the CYP2A6 enzyme. The major metabolite of cotinine in urine *is trans*-3'-hydroxycotinine which is also formed by mediation of CYP2A6. The ratio of *trans*-3'-hydroxycotinine to cotinine is a marker of CYP2A6 activity and the rate of nicotine metabolism. [5-6] Interestingly, some studies indicated that the ratio of hydroxycotinine to cotinine (as a (phenotypic) marker for the rate of nicotine metabolism) might be useful for selecting the optimal smoking cessation medication .[7-10] Previous studies already focused on the link between nicotine dependence and biomarkers of exposure to tobacco. Limited data are available for the ratio of hydroxycotinine over cotinine determined in urine and its relation to CPD and to nicotine dependence. The current study

investigates the relationship between urinary levels of nicotine, cotinine and hydroxycotinine and the six questions of the Fagerström Test for Nicotine Dependence (FTND) in a group of daily smokers. The specific objectives of this study are: 1) to investigate the relationship between nicotine dependence as measured by the FTND and urinary levels of nicotine and metabolites (cotinine and hydroxycotinine) of daily smokers, 2) to assess the relationship of hydroxycotinine/cotinine ratio with FTND score and cigarettes smoked per day (CPD) and 3) to identify significant predictors for cigarettes per day among biomarker concentrations and individual FTND items.

Methods

Participants

A group of daily smokers (n = 239) was recruited among employees of the university hospitals of Leuven (n = 95) and Hasselt (n =124) and among smokers visiting the smoking cessation counsellor in the general hospital in Oudenaarde (n = 20). Study participation by smokers occurred only on a voluntary basis. Informed consent was obtained from each participant and the study was approved by the ethics committees of UZ Gasthuisberg Leuven, Jessa hospital Hasselt and general hospital Oudenaarde.

Samples and data collection

Urine sample collection was performed in collaboration with occupational medicine physicians (for Leuven and Hasselt and with the smoking cessation counsellor (Oudenaarde). Urine samples were collected during the day (between 8 am and 5pm) and all participants reported to be active smokers when urine was collected. Not all participating smokers collected their urine sample on the same time of the day because the participation occurred on a voluntary basis when there was contact with

the occupational physician or the stop smoking counsellor. A question was asked about the amount of time passed since smoking the last cigarette before the urine sampling. Urine samples were stored at -20°C before analysis.

Questionnaires providing information on sex, age, height, weight, number of years of smoking, number of cigarettes smoked per day (CPD) and the Fagerström Test for Nicotine Dependence (FTND) were completed by each participant.

Smokers within a smoking cessation attempt and who were using smoking cessation pharmacotherapy were not included.

Analysis of urine samples

The simultaneous determination of the urinary concentrations of nicotine, cotinine and hydroxycotinine was performed by online solid phase extraction-ultra performance liquid chromatography tandem mass spectrometry (SPE-UPLC MS/MS) as described previously.[11] The limits of detection (LOD) are 0.2 and 1.8 µg/L (or ng/ml) for cotinine and hydroxycotinine respectively and the limits of quantification (LOQ) are 1, 1 and 5 µg/L (or ng/ml) for cotinine, nicotine and hydroxycotinine respectively.

Briefly, urine samples were defrosted, homogenized, centrifuged for 10 min at 3000 rpm and 100 µL supernatant was diluted 100 times in 0.1% ammonium hydroxide solution after addition of a mixture of ^{13}C- labelled cotinine, nicotine and *trans*-3'-hydroxycotinine. Samples were injected in an online-SPE coupled to a UPLC (Xevo, Waters) and analysed by tandem mass spectrometry (triple quad MS, Waters) using isotope dilution. In each run, blank samples as well as quality control samples at three different concentration levels were analysed.

Data analysis/Statistical analysis

We calculated mean and standard deviations of CPD, smoking years and FTND scores and geometric means and geometric standard deviations of nicotine, hydroxycotinine and cotinine concentrations by individual characteristics, sex, age and BMI, taken from the questionnaires. We also calculated the hydroxycotinine over the cotinine concentration ratio. We tested for significant differences between groups based on individual characteristics by use of Wilcoxon test and Kruskall-Wallis analysis. Potential correlation between the CPD, smoking years, FTND scores and biomarker concentrations were explored with the Spearman correlation coefficient and scatter plots.

We developed several multiple linear regression models to explain biomarker concentrations. To investigate CPD, a Poisson Generalized Regression Model was used and partial R –squared values of each of the individual predictors were calculated. In each of these regression models other described variables are used as independent variables. For the regression models we considered as the independent variables the separate FTND question scores, sex, age, and BMI.

The distributions of nicotine, hydroxycotinine and cotinine concentrations are non-normally distributed. In order to fulfil the normality requirement of linear regression models and to stabilize the variance, a log-transformation was performed on these variables. The second type of model is a Poisson regression model where the dependent variable is CPD and where the independent variables are the scores for the separate FTND questions (excluding the question on cigarettes smoked per day), a biomarker concentration, sex, BMI and smoking years or age. The biomarker concentration is log-transformed.

All analyses were performed using the statistical programming environment R. [12]

Results

The main results of all participants, the chemical analyses and information from the questionnaires are summarised in Table 1.

There were 239 participants, having a mean FTND score of 3.4. The geometric mean (GM) nicotine, hydroxycotinine and cotinine concentrations were 593, 4069 and 1015 ng/mL respectively.

There were 68 male participants (28.5%) and 171 female participants (71.5%). The male participants had a mean FTND score of 3.7 and GM nicotine, hydroxycotinine and cotinine concentrations of 681, 3950 and 1100 ng/mL respectively, while the female participants had a mean FTND score of 3.3, and GM nicotine, hydroxycotinine and cotinine concentrations of 562, 4117 and 983 ng/mL respectively. Male and female participants had significantly different FTND scores, nicotine, hydroxycotinine and cotinine concentrations. The males had higher FTND scores and nicotine and cotinine concentrations while females had higher hydroxycotinine levels than males.

In terms of age, we defined three age-groups: younger than 35, from 35 to 50, and older than 50. Each of these age groups contained approximately one third of the study population. Only one participant was older than 65. Via Kruskall-Wallis analysis, the differences between these age groups were tested. Only the cotinine concentration differed significantly at the 5% level. Participants in the age group of 17-34 years had a GM cotinine concentration of 788 ng/mL, the age group of 35-50 years had a GM cotinine concentration of 1060 ng/mL and participants older than 50 years had GM cotinine concentration of 1230 ng/mL.

As a last individual characteristic, we looked at Body Mass Index (BMI), where we had three categories. The first category was defined as normal and had a BMI of less than 25, the second group was overweight with a BMI between 25 and 30, and the last group was obese with a BMI of over 30. A large majority (61.90%) of the participants fell in the normal BMI group. In terms of these BMI groups we did not find significant differences for any of the dependent variables.

Next to the absolute biomarker concentrations we also calculated the ratio of the hydroxycotinine over the cotinine concentration. The rationale behind this is that this ratio serves as a proxy of the rate of nicotine metabolism. For the total population the mean hydroxycotinine over cotinine ratio is 5.2. Following the above method, we tested this ratio for differences following individual characteristics, and we present these results in Table 1. The difference in hydroxycotinine over cotinine ratio between women and men proved to be significant ($p<0.001$), where women had a mean ratio of 5.5 and men a mean ratio of 4.3 . The analysis focussing on CPD and smoking years yielded very similar results. The overall mean CPD for the whole study population was 12.1, while the mean number of smoking years was 21.5. Men and women had significantly different results for CPD and self-reported number of years of actual smoking. Women generally smoked slightly less than men (11.4 vs 13.6 CPD), but for a little longer duration (21.7 years vs. 21.1 years). The different age groups also had a significantly different CPD and smoking years variable which is not unexpected. In terms of BMI we did not find any significant differences.

Table 2 summarises the correlation coefficients between the FTND, the measured biomarkers in urine, the ratio of hydroxycotinine/cotinine, CPD and years smoking. Significant correlations were observed between the FTND score and the nicotine, hydroxycotinine and cotinine concentrations. The levels of the 3 biomarkers were also significantly correlated as well as CPD and the 3 biomarker levels (Table 2). There was a significant inverse correlation between hydroxycotinine/cotinine ratio and smoking years but no correlations between this ratio and other variables were found. These correlations point out the lack of association of the hydroxycotinine/ cotinine ratio with FTND score, and CPD.

In Table 3, an overview of three multiple linear regression models is shown, each having one biomarker concentration as dependent variable. The model explaining cotinine concentrations performed by far the best with an adjusted R-squared measure of 0.24. In this model the significant independent variables are the time to first cigarette score and age of the smoker. Shorter time to

first cigarette and increased age predicted cotinine level. The models explaining nicotine and hydroxycotinine concentrations performed far worse with an adjusted R-squared measure of 0.10 and 0.14, respectively.

Table 4 shows the results of the Poisson regression model explaining CPD by means of the other FTND question scores, the log-transformed cotinine concentration, sex and age. The model at hand had an adjusted R-squared measure of 0.45. In order to explain CPD, four FTND items (the time to the first cigarette, the difficulty to not smoke, the type of cigarette not willing to give up, smoking when sick), the urinary cotinine concentration, sex and age were found to be significant variables.

All previously described models were tested for error specification, normality of errors, homoscedasticity, multicollinearity and self-correlation, where the diagnostics showed that the model requirements were fulfilled.

Discussion

We found that in this study population of daily smokers the FTND score differed significantly between men and women, with men having a higher nicotine dependence than women. The hydroxycotinine/cotinine ratio for females was significantly higher than for males. This confirms that females have a higher nicotine metabolism rate than males as demonstrated before.[2] We found correlations between the three biomarkers determined in this study. CPD are correlated with cotinine levels (r = 0.48) as observed in other studies reporting correlation coefficients between 0.28 and 0.55.[13-14] The CPD and smoking years also differed significantly between males and females in our study. Males have a higher mean CPD value than females while females had slightly higher smoking years which is in accordance with data on smoking behaviour of the Belgian general population although the general male population starts smoking at younger age than the females.[15]

Our investigation of the relationship between the measured nicotine biomarker levels and the FTND items revealed that time to the first cigarette after waking was most associated with the nicotine, cotinine and hydroxycotinine levels in urine. Our results may be compared with only a few studies with urine samples that focused mainly on the association between nicotine dependence and cotinine levels. More studies focused on the relationship between the hydroxycotinine/cotinine ratio in blood, plasma or saliva than in urine.[9] In the current study, we investigated three multiple linear regression models, each having one biomarker concentration as dependent variable and the separate FTND scores, sex , age and BMI as independent variables. The model examining the cotinine levels performed best. The main results of our study are in agreement with the conclusions of other studies. One study found a relationship between the time to the first cigarette after waking (TTFC) and cotinine levels determined in plasma and urine of daily smokers where the lowest cotinine levels were found in smokers with the largest TTFC.[13] In another reported approach the urinary cotinine levels were used to predict the nicotine dependence level.[14]

Our study included levels of nicotine and hydroxycotinine in addition to cotinine. It is well known that nicotine levels depend highly on the time of the last smoked cigarette due to the short half-life of nicotine (1-2h) in comparison with that of cotinine and hydroxycotinine (6-22h and 4.6-8.3h, respectively).[2,16-17] This inverse relation between the time since the last cigarette and the nicotine level was also observed in the current study (results not shown). This fact might also explain the lower R-squared value for the model wherein nicotine levels are explained by single FTND items. In addition to using multiple nicotine biomarkers, we used the information from all FTND items.

Our urinary nicotine biomarker results can be compared with results obtained from saliva samples. An inverse association between the time to the first cigarette after waking up and salivary cotinine levels has been described where salivary cotinine levels decreased as TTFC increased .[18] As in the current study, the relationship between salivary cotinine levels and nicotine dependence measured by the FTND has been investigated.[19] As in our study, it was found that the FTND of men was significantly higher than for women. Unlike in our study no significant age effect for the cotinine

levels was found while we found that older people have significantly higher cotinine levels. Unlike for results for salivary cotinine, we did not find that the FTND question about the CPD was significant for explaining the variance of urinary cotinine levels. Previous results indicated that the Heaviness of Smoking Index (HSI) is the best predictor of biochemical measures such as salivary cotinine and CO.[1,20] TTFC and CPD are combined when the Heaviness of Smoking Index (HSI) is used and these have been identified as important predictors of quitting behaviour.[21] Another study confirmed that the time to the first cigarette had the most impact on nicotine biomarkers of exposure. The latter study investigated nicotine equivalents in urine, serum cotinine and blood carboxyhemoglobin.[22]

Another objective of our study was to explore the ratio of hydroxycotinine to cotinine. The mean value of 5.2 for this ratio is similar to that observed in other studies using urine. [23,24] It must be noticed that the ratio hydroxycotinine/cotinine determined in urine is higher than values for blood [25] or saliva.[26] Our results reveal a significant negative association between the hydroxycotinine /cotinine ratio and smoking years. This finding is supported by previous research indicating that nicotine metabolism rate decreases with age.[2] As smoking years increase, exposure to nicotine increases and it has been shown that nicotine metabolism is slower in smokers and that nicotine exposure may reduce its own metabolism.[5] In accordance with this, our results indicated a weak but insignificant negative correlation between hydroxycotinine/cotinine ratio and CPD.

We did not find a significant correlation for the ratio of hydroxycotinine to cotinine neither with CPD nor with the FTND score. It has been previously reported that there is little evidence that the ratio of *trans*-3'-hydroxycotinine and cotinine is related to questionnaire measures of dependence.[9]

Nicotine metabolism rate or nicotine metabolite ratio (NMR) data has been associated with nicotine dependence. In a study on nicotine metabolism rate in adolescents it was concluded that slower metabolizers showed greater dependence .[27]For the NMR determined in blood by LC-Mass Spectrometry it was observed that there was variation in the relationship between nicotine metabolism and nicotine dependence across different measures of dependence and sex and race

and an association between NMR and CPD was also found .[25] Lower plasma NMR has been linked to lower FTND score. [28] Our results for the NMR ratio determined in urine are not in accordance with previous results demonstrating a positive association between the hydroxycotinine to cotinine ratio determined in urine and CPD[23] but are in agreement with another study that did not find an association between the ratio in urine and CPD.[24] It must be noted that the positive association between the ratio and CPD was found for the ratio of total cotinine (free plus glucuronidated form) over total hydroxycotinine (free plus glucuronidated form) while we measured free cotinine and free hydroxycotinine as in the study not finding an association. [24] These mixed results for the urinary NMR ratio might be due to the higher variability and less suitability of the urinary ratio in comparison to the ratio determined in blood or saliva, although it has been reported that the nicotine metabolite ratio (NMR) is stable in blood, plasma and saliva and that urine NMR is a good proxy for these NMR measures.[29] Important to note is also that the glucuronidation might impact the ratio. A recent study showed that variation in the glucuronidation of hydroxycotinine did not alter the nicotine metabolite ratio in urine or plasma [30] while another study showed that the nicotine metabolite ratio in urine was influenced by UDP-glucuronyltransferase phenotype for cotinine in Caucasians. [31]

Based on the correlation results obtained in this study, we conclude that the hydroxycotinine over cotinine or NMR ratio does not have a great predictive value for nicotine dependence and CPD.

In the present study, we further developed a regression model by including the FTND items (excluding CPD), the cotinine concentration, sex, age and BMI, that explained 45 % of variance of CPD. In order to best explain CPD four FTND questions, the cotinine concentration, sex and age were found to be significant variables. We could not find any other study that combined data of nicotine dependence, cotinine levels and cigarettes per day in one model. Only one study reported a linear regression model that was used to predict the CPD by using the urinary cotinine concentration.[32] In that study, urinary and plasma levels of nicotine, cotinine and hydroxycotinine from 91 male

Japanese smokers were determined by LC MS. The mean urinary levels for nicotine, cotinine and hydroxycotinine measured were lower than our mean levels which could be related to the fact that our population mainly consisted of females (having higher hydroxycotinine levels) and because our study participants smoked on average 21 years compared to 16 years in the Japanese group. In our study, data were obtained from a larger group of smokers (n=239), smoking on average 12 cigarettes per day while the Japanese study population smoked on average 23 cigarettes per day. It must be noted that other studies found a non-linear effect between CPD above 20 and cotinine.[5,13] As the smokers included in our study were on average smoking 12 CPD our model might be valid for this population but not necessarily for smokers with higher CPD.

Limitations of our study

The results obtained for the urinary biomarkers are not corrected for creatinine. Urinary cotinine levels adjusted for creatinine are more associated to plasma cotinine at low level exposure.[3] The inverse conclusion that creatinine correction did not improve correlation with plasma levels was also reported.[33] For the hydroxycotinine/cotinine ratio this is irrelevant. But it is possible that cotinine adjustment for creatinine might affect the outcome analysis of our study.

We have examined a daily smokers' population consisting mainly of female participants, participants that were included on a voluntary basis. Apparently, women were more willing to participate and to provide a urine sample. We are aware that this group of smokers may be less representative for the general smokers' population in Belgium. The most recent data obtained by the Belgian health interview Survey indicate that 18.9% of the population are daily smokers with 16.4% among females and 21.6% among males .[15]

Strengths of this study

The applied method of simultaneously determining nicotine, cotinine and hydroxycotinine in a minimal invasive (urinary) sample that can be easily obtained from the participant is useful for large population studies. The developed model that can explain CPD might be useful to obtain highly objective data about CPD which may differ from self-reported data about cigarette consumption.

We conclude that our data confirm the association between FTND and urinary levels of biomarkers of tobacco exposure and a model combining four FTND items ('Morning smoking 'item from FTND was not significant in the model), urinary cotinine levels, sex and age explains CPD.

We believe that our model might be of importance for clinical use or future epidemiological studies on larger smoking populations. For clinical practice it is important to have a model for CPD as it is important to obtain objective data about cigarette beyond questionnaire data.

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Declaration of interests

None declared

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Table 1: General characteristics of participants and urinary biomarker data

		n (%)	FTND Mean (SD)	Nicotine (ng/mL) GM (SDG)	Hcot (ng/mL) GM (SDG)	Cotinine (ng/mL) GM (SDG)	Hcot/cot Mean (SD)	CPD Mean (SD)	Smoking years Mean (SD)
Total		239 (100)	3.4 (2.4)	593 (7.0)	4069 (3.2)	1015 (2.5)	5.2 (3.9)	12.1 (7.8)	21.5 (11.5)
Sex									
	Men	68 (28.5)	3.7 (2.6)**	681 (7.2)**	3950 (2.9)**	1100 (2.6)**	4.3 (2.8)**	13.6 (8.5)**	21.1 (13.0)**
	Women	171 (71.5)	3.3 (2.4)**	562 (7.0)**	4117 (3.4)**	983 (2.5)**	5.5 (4.2)**	11.4 (7.4)**	21.7 (10.9)**
Age (years)		238							
	17-34	76 (31.9)	2.9 (2.3)	454 (8.1)	3509 (3.6)	788 (2.7)*	5.8 (4.2)	9.8 (5.7)*	10.0 (5.5)**
	35-50	85 (35.7)	3.7 (2.4)	517 (7.8)	4354 (3.0)	1061 (2.5)*	5.0 (3.4)	12.4 (7.3)*	22.8 (7.7)**
	≥50	77 (32.4)	3.5 (2.5)	882 (5.0)	4343 (3.2)	1230 (2.3)*	4.7 (4.1)	14.0 (9.4)*	31.9 (8.6)**
BMI (kg/m^2)		231							
	Normal	143 (61.9)	3.2 (2.4)	597 (7.6)	4184 (3.5)	1014 (2.6)	5.4 (4.2)	11.2 (6.7)	20.6 (11.6)
	Overweight	67 (29.0)	3.5 (2.5)	602 (7.9)	3974 (2.9)	1026 (2.4)	5.0 (3.8)	13.0 (9.9)	23.6 (11.6)
	Obese	21 (9.1)	4.4 (2.4)	525 (2.9)	3618 (2.7)	963 (2.1)	4.2 (2.0)	14.3 (7.6)	20.6 (11.0)

Population percentage, FTND score, nicotine and metabolite concentrations, hydroxycotinine over cotinine concentrations ratio, cigarette consumption per day and smoking years per subgroup of sex, age and BMI. Within each subgroup the different characteristics were tested on difference at a 5%-level with the Wilcoxon test (sex) or the Kruskall-Wallis test (between the three age and BMI categories, respectively). Where the respective test yielded a significant result, this was denoted with asterisks. **:p value for difference between groups <0.001; * p value for difference between groups <0.05**.** Hcot = hydroxycotinine, cot=cotinine, CPD = cigarettes smoked per day, GM= geometric mean, SDG = GM's standard deviation

Table 2: Spearman correlation coefficients between measured biomarkers in urine, the ratio Hcot/cot and variables obtained from questionnaires

	FTND	NIC	Hcot	Cot	Hcot/cot	Smoking years*	CPD
FTND	1	0.31 ($p<0.05$)	0.32 ($p<0.05$)	0.42 ($p<0.05$)	-0.011 ($p = 0.86$)	0.33 ($p<0.05$)	0.77 ($p<0.05$)
NIC	0.31 ($p<0.05$)	1	0.48 ($p<0.05$)	0.63 ($p<0.05$)	0.012 ($p = 0.86$)	0.25 ($p<0.05$)	0.38 ($p<0.05$)
Hcot	0.32 ($p<0.05$)	0.48 ($p<0.05$)	1	0.68 ($p<0.05$)	0.65 ($p<0.05$)	0.15 ($p<0.05$)	0.31 ($p<0.05$)
Cot	0.42 ($p<0.05$)	0.63 ($p<0.05$)	0.68 ($p<0.05$)	1	-0.056 ($p = 0.39$)	0.33 ($p<0.05$)	0.48 ($p<0.05$)
Hcot/cot	-0.011 ($p=0.86$)	0.012 ($p = 0.86$)	0.65 ($p<0.05$)	-0.056 ($p = 0.39$)	1	-0.13 ($p<0.05$)	-0.076 ($p = 0.25$)
Smoking years*	0.33 ($p<0.05$)	0.25 ($p<0.05$)	0.15 ($p<0.05$)	0.33 ($p<0.05$)	-0.13 ($p<0.05$)	1	0.43 ($p<0.05$)
CPD	0.77 ($p<0.05$)	0.38 ($p<0.05$)	0.31 ($p<0.05$)	0.48 ($p<0.05$)	-0.076 ($p = 0.25$)	0.43 ($p<0.05$)	1

FTND: Fagerström Test for Nicotine Dependence (n = 238); NIC: nicotine (n = 239); Hcot: hydroxycotinine (n = 239); Cot: cotinine (n = 239); CPD: cigarettes per day (n = 239).

*: (n = 234 for smoking years)

Table 3: Multiple linear regression models for the cotinine, nicotine and hydroxycotinine concentrations

		Cotinine (adj. R-squared = 0.24)		Nicotine (adj. R-squared = 0.10)		Hydroxycotinine (adj. R-squared = 0.14)	
	Possible score in FTND	β	p	β	p	β	p
Time to first cigarette	1 (31-60 min); n= 51	0.417	<0.05	0.862	<0.05	0.228	0.3
	2 (5-30 min); n= 92	0.548	<0.05	1.02	<0.05	0.586	<0.05
	3 (within 5 min); n= 32	0.737	<0.05	1.253	<0.05	0.795	<0.05
Difficulty to not smoke		-0.075	0.66	0.406	0.34	0.116	0.63
Type of cigarette not willing to give up		0.099	0.42	0.142	0.65	0.092	0.6
CPD	1 (=11-20); n = 93	0.262	0.06	0.021	0.95	0.294	0.14
	2 (=21-30); n=15	0.359	0.12	0.818	0.16	0.472	0.15
	3 (>30); n=4	0.22	0.59	0.092	0.93	0.153	0.79
Morning smoking		-0.137	0.32	-0.324	0.35	-0.471	<0.05
Smoking when sick		0.168	0.15	0.38	0.2	0.189	0.26
Age		0.016	<0.05	0.025	<0.05	0.009	0.17
Sex		0.04	0.73	0.002	0.99	-0.108	0.5
BMI		-0.0215	0.08	-0.035	0.26	-0.036	<0.05

Multiple linear regression models for the cotinine, nicotine and hydroxycotinine concentrations are denoted in the columns, with the independent variables in the rows. For each independent variable we give the estimated parameter value (β) and the significance level (p).

Table 4: Poisson regression model explaining the CPD consumption

		CPD (adj. R-squared = 0.45)		Partial R - squared
	Possible score in FTND	β	p	
Time to first cigarette	1 (31-60 min)	0.412	<0.05	0.4
	2 (5-30 min)	0.561	<0.05	
	3 (within 5 min)	0.797	<0.05	
Difficulty to not smoke		0.272	<0.05	0.029
Type of cigarette not willing to give up		0.114	<0.05	0.016
Morning smoking		-0.091	0.06	
Smoking when sick		0.218	<0.05	0.014
Age		0.012	<0.05	0.049
BMI		0.006	0.23	
Sex		0.155	<0.05	0.0002
Cotinine conc.		1.20E-04	<0.05	0.022

Poisson regression model explaining the CPD consumption of the participants by means of their score on five of the FTND questions, age, BMI, sex and cotinine concentration in urine. For each independent variable the estimated parameter value (β) and the significance level (p) is given. Partial R-squared values are given for the significant variables.