Impact of the Spanish smoking legislation among smokers: A longitudinal study with biomarkers in Barcelona (Spain)

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Word count: 2,539

ABSTRACT

Background: The aim of this study is to assess the impact of the Spanish tobacco control legislation on smoking behavior and salivary cotinine concentration among smokers.

Methods: We used data from a longitudinal study, before (2004-2005) and after (2013-2014) the implementation of the two national smoking bans (in 2006 and 2011), in a representative sample of adults (≥16 years old) from Barcelona (Spain). We only analyzed a subsample of continuing smokers (n=116). We conducted a survey on smoking behavior and obtained saliva sample for cotinine analyses. We calculated geometric means (GM).

Results: The salivary cotinine concentration significantly increased 28.7% (GM from 91.7 ng/ml to 117.3 ng/ml, p=0.015) after the implementation of the two Spanish smoke-free bans. Nonetheless, no pattern of change was observed in the self-reported number of cigarettes smoked daily.

Conclusions: Our study shows a significant increase in the salivary cotinine concentration among adult continuing smokers after both Spanish legislations. This increase could be due to differences in smoking topography (increase in the depth of inhalation when smoking) along with changes in the type of tobacco smoked (increase in smoking roll your own cigarettes or mixed used). Our results suggest the need to extend tobacco control policies, focusing on reduction of use of any kind of tobacco product and implementing better treatment policies to help smokers stop smoking.

Keywords: *Smoke-free legislation*; *Smoking; Cotinine; Biomarker; Tobacco consumption; FTCD;*

1. INTRODUCTION

Tobacco is the first single leading cause of preventable death in the world (1), being related to more than 25 diseases and being responsible for 30% of all cancers, respiratory diseases and cardiovascular diseases (2). In 2015, WHO estimated that about 15% (1.1 billion people) of worldwide population smoked (3) and that tobacco use is responsible for about six million deaths across the world each year, including about 600,000 deaths from the effects of second-hand smoke (SHS) (4). In Spain, the latest data reported showed that 28% of men and 19% of women were tobacco users in 2014 (5) and that more than 60,000 deaths per year were attributable to active smoking (6).

Consequently, two smoke-free laws have been passed in Spain after the approval of the World Health Organization Framework Convention on Tobacco Control (WHO FCTC) (7). On the 1st of January, 2006, a smoke-free legislation came into effect (Law 28/2005). The law was a compendium of public health measures against smoking and included regulations on publicity, sale, supply, and consumption of tobacco products (8). Smoking was banned in all indoor workplaces, public places, public transport facilities including enclosed stations, hospitals and other health care facilities, schools and universities as well as in retail stores and shopping centres. However, hospitality venues were subject to only a partial ban. In bars and restaurants smaller than 100 m2, the proprietor could choose between permitting or prohibiting smoking. Those larger than 100 m2 were defined as smoke-free, but the law allowed the proprietor to provide a physically separated and independently ventilated smoking area comprising less than 30% of the total floor area. Moreover, several tax reforms followed the implementation of the partial ban (9). This law was a great advance for public health in Spain; however, it was not complete in terms of health protection from SHS exposure. The scientific evaluation of this law showed the need to promote a total ban (10-12). On the 2nd of January, 2011, a new law came into force (Law 42/2010), including several tax reforms and

extending the smoke-free regulation to all hospitality venues without exception (9) and to some outdoor areas, including hospital premises, educational campuses, and playgrounds. However, designated smoking rooms are still permitted in psychiatric services, nursing homes, prisons and up to 30% of hotel rooms.

The principal aim of both Spanish tobacco control legislations was to protect non-smoking population from tobacco smoke exposure. Therefore, their scientific evaluation focused on their effect on SHS reduction, often neglecting research about the effect on active smokers. In this sense, a systematic review (13) has concluded that the implementation of smoke-free policies and restrictions in public spaces, workplaces or residences lead to a decrease in smoking prevalence and cigarette consumption. Moreover, previous studies (14,15) suggest that active smokers could be changing their smoking pattern to consuming cheaper tobacco products, such as Roll Your Own (RYO) cigarettes, which are subject of little taxation in Spain (16). In addition, RYO are usually not filtered (17) and as a result more nicotine (as well as other toxicants) is delivered with smoke. There are few studies that assess the impact of smoking legislation among the smoking population using a specific biomarker of tobacco smoke absorption such as cotinine, which is the major proximate metabolite of nicotine (18). In Spain, a previous study assessed the impact of the first legislation among hospitality workers who smoke, using self-reported data and information regarding salivary cotinine (19). Cotinine concentration in biological fluids (blood, urine or oral fluid, widely referred to as saliva) (20) indicates tobacco exposure over the previous 1-2 days (21) and is strongly correlated with the number of cigarettes smoked daily (22). Moreover, the cotinine concentration could vary within each stage of change (defined in the methods section), according to the number of cigarettes smoked, time to first cigarette of the day, and nicotine dependence (23).

Therefore, the objective of this study is to assess the impact of the Spanish tobacco control legislations (laws 28/2005 and 42/2010) on smoking behavior and salivary cotinine

concentration among smokers by using a cohort study of adult smokers in Barcelona (Spain) along with biomarker information (salivary cotinine concentration).

2. METHODS

This is a longitudinal study (cohort study) from a representative sample of the adult population (≥16 years at baseline, ≥25 at follow-up) of the city of Barcelona (Catalonia, Spain). The baseline study was carried out during the years 2004-2005 (24,25) (n = 1,245) and the follow-up took place in 2013-2014, after the implementation of both Spanish tobacco control legislations.

To obtain the baseline sample, a representative random sample by age, sex, and district was drawn from the official 2001 population census of Barcelona, a reliable source of population-based information explained elsewhere (24,25). Briefly, the procedure was as follows: a personal letter was sent to eligible participants, and trained interviewers contacted the subjects at home and informed them about the study. When the index person was not contacted (after several attempts following a strict protocol that included visits on weekends and during non-working hours) or refused to participate, we randomly selected a substitute in the same sex-, age-, and district-group. We asked participants to answer a face-to-face questionnaire and to sign a consent form in order to be contacted in the follow-up. From the baseline sample, we excluded 235 subjects: 150 after checking their data in the Insured Central Registry of Catalonia (101 died and 49 migrated out of the province of Barcelona) and 85 subjects who did not give consent to be followed up or were minors (<18 years old) in 2004-2005 and we did not ask consent to re-contact. The percentage of follow-up in this first stage was 81.1%. The follow-up was conducted in 2013-2014. In total, 72.9% of the eligible sample agreed to participate (736 out of 1010 traced, second stage of follow-up), 18.5% refused to participate, 7.2% had moved elsewhere and 1.4% had died. The final sample included 736 individuals (Figure 1) and was skewed as slightly older in comparison with the general

population of Barcelona. For this reason, we weighted our data according to the age distribution of the city of Barcelona to maintain its representativeness. The percentage of participation in both stages was 51.9% (736 out 1245). We did not find statistically significant differences between the followed-up sample (n=736) and the participants lost in the second stage (n=274) according to age, sex, educational level (categorized as low: unschooled, elementary school completed or uncompleted and special education; intermediate: high school and training cycles; and high: university education) and smoking status. However, we described statistically significant differences according to age, level of education and smoking status between the follow-up sample (n=736) and the participants lost in both stages of the follow-up (n=509) (Table 1).

We asked the participants to provide a 9 ml sample of saliva for cotinine analysis, using the same protocol before and after the Spanish tobacco control legislations. Participants were asked to rinse their mouths and then suck a lemon candy (Smint) to stimulate saliva production. Saliva samples were frozen and sent to the IMIM-Hospital del Mar Medical Research Institute in Barcelona. Saliva samples were analyzed using liquid chromatography-tandem mass spectrometry (LC/MS/MS) with multiple reaction monitoring. The limit of quantification was 0.4 ng/ml (quantification error was <15%) (26).

We used the same core questionnaire in both surveys to gather relevant information on smoking characteristics, along with the information on their cotinine concentration. Smoking status, was obtained from the question: “Which of the following statements better describes your smoking status?” with the possible answers: ‘Nowadays I smoke everyday (at least one cigarette per day)’, these are current daily smokers; ‘Nowadays I smoke occasionally (less than one cigarette per day)’, these are current occasionally smokers; ‘I don’t smoke now, but I smoked before every day’, these are former daily smokers; ‘I don’t smoke now, but I smoked before occasionally’, these are former occasionally smokers; and ‘I have never smoked’, these

are never smokers. We also aggregated these categories when appropriate as 'smokers' (current daily and occasionally smokers) and 'non-smokers' (former daily and occasionally smokers plus never-smokers). The percentage of self-reported smokers with salivary cotinine inconsistent with active smoking (≤ 35 ng/ml per cigarette smoked daily (27)) was 3.4% before the legislations and 12.1% after the legislations, accounting for 13.8% of the total sample of continuing smokers (n=116). Moreover, when using the self-reported number of cigarettes smoked in the last 24 hours and in the last 48 hours, the percentage of inconsistent smokers in the total sample of continuing smokers decreased to 6.0% and 0.8% respectively. However, for this analysis, we used self-reported smoking status for the identification of current smokers in order to maximize the available sample size. The final sample for this analysis consists of 116 continuing smokers, that is to say, those who self-reported active smoking before and after the legislation (Figure 1).

From the same questionnaire before and after the two laws, we also obtained information about the type of tobacco smoked, obtained through the question: "What kind of tobacco product do you habitually consume?" with the possible answers: "cigarettes", "RYO cigarettes", "cigars", "little cigars", "pipes", "hookah" and "e-cigarettes". The answers to this question were dichotomized as 'CC-CC' indicating those who only smoked conventional cigarettes (CC) before and after the two laws, 'CC - RYO', indicating those who switched from conventional to RYO cigarettes, 'CC – MU', indicating those who switched from conventional to mixed use (CC and RYO) and 'Other', indicating other possible options. Moreover, we collected self-reported information about the smokers' stage of change (28): precontemplation, when smokers were not seriously considering quitting within the next 6 months; contemplation, when smokers were seriously considering quitting within the next 6 months but not within the next 30 days; and preparation, when smokers were planning to quit within the next 30 days and had attempted to quit for at least 24 hours in the past year. The information to obtain

smoker's stage of change was obtained through two different questions. 1) "Are you seriously considering the possibility of…?" with the possible answers: 'quit smoking during the following week', 'quit smoking during the following month', 'quit smoking during the following three months', quit smoking during the following six months', 'quit smoking during the following twelve months', 'quit smoking but not during the following twelve months', and 'no quit smoking'. 2) "In the last year, have you been at least one day without smoking?" (not taking into account days in which you have been ill), with the possible answers: 'yes', 'no', and 'doesn't know or doesn't answer'. We also gathered information about the number of cigarettes smoked daily (either conventional or RYO cigarettes) and the Fagerström Test for Cigarette Dependence (FTCD) score (28) categorized as 'low' (≤4), 'medium' (5) and 'high' (≥6). FTCD score includes six items: time to first cigarette (0 – 3 points), difficulty to refrain (0 – 1 points), hardest cigarette to give up (0 – 1 points), cigarettes per day (0 – 3 points), smoking more in the morning than in the rest of the day (0 – 1 points), and smoking while ill (0 – 1 points). FTCD score ranges from 0 to 10 and assesses distinguishable self-reportable pharmacological dimensions of nicotine addiction (29). Finally, we gathered self-reported information about several smoking characteristics, such as use of regular or non-regular cigarettes (light, ultralight, etc.), type of tobacco smoked (blond or black), use of cigarettes with or without filter, length of cigarettes left after smoking (in centimeters), depth (superficial, intermediate, deep) and frequency of inhalation (continuous, regular, scarce).

For statistical analysis we calculated geometric means (GM) and their geometric standard deviation (GSD), given the skewed distribution of cotinine concentration. We used linear mixed effect models with individuals as random effects to model the change in cotinine concentration (after log10 transformation), their 95% confidence intervals and the p-value, adjusted for sex, age, and educational level. The results were stratified by sex, age, educational level, kind of tobacco smoked, FTCD score and stages of change. We also used generalized

linear mixed models with individuals as random effects to calculate the prevalence ratio of the change in smoking characteristics. The statistical programs used were R-3.0.2 and Stata v14.

3. RESULTS

When analysing self-reported data from the whole sample (n=736), 166 participants (22.6%, 95% CI: 19.7–25.8) were continuing smokers and 456 participants (62.0%, 95% CI: 58.4–65.5) were continuing non-smokers (never and former smokers). In other words, they showed the same smoking status at baseline and at follow-up. Moreover, 88 participants (11.9%) quit at follow-up and 26 participants (3.5%) initiated or relapsed tobacco use at the follow up (2 participants, 0.2%, were never smokers at baseline and 24 participants, 3.3%, were former smokers).

According to cotinine levels from the whole sample (n=503 due to missing data), 115 participants (22.9%, 95% CI: 19.3-26.8) were continuing smokers and 360 participants (71.6%, 95% CI: 67.4-75.4) were continuing non-smokers (never and former smokers). Moreover, 20 participants (4.0%) quit at follow-up, 5 participants (1.0%) initiated or relapsed tobacco use at the follow up, and 3 participants (0.6%) were not properly classified.

In addition, when comparing self-reported smoking status with cotinine classification at baseline (n=566 due to missing data), 144 out of 207 (69.6%) self-reported smokers were correctly classified as such according to cotinine levels, and 100% of self-reported non-smokers (359) were also correctly classified. Similarly, when comparing at the follow-up (n=582 due to missing data), 107 out of 140 (76.4%) self-reported smokers were correctly classified as such according to cotinine levels, and 428 out of 442 (96.8%) of self-reported non-smokers were also correctly classified.

Among self-reported continuing smokers, we observed a general increase in salivary cotinine concentration after the implementation of the two Spanish smoke-free laws (Figure 2, panel B). Salivary cotinine concentration significantly increased by 28.7% (GM from 91.1 ng/ml to 117.3 ng/ml, p=0.015) after the implementation of the two Spanish smoke-free laws (Table 2). The increase in the salivary concentration was statistically significant among young people, highly educated and among those who switched from conventional to RYO (Table 2). In addition, a statistically significant decrease was found in the proportion of individuals classified in the medium category of FTCD score (from 23.3% to 9.1%, p=0.017) when comparing pre and post legislations (Figure 2, panel A). Nonetheless, no clear pattern of change was observed in the number of cigarettes smoked daily (Figure 2, panel C). Even though, a non-significant increase in the number of cigarettes smoked daily can be observed among those who switched from conventional cigarettes only to mixed use (from 16.6 cigarettes/day to 24.3 cigarettes/day, p=0.270) when differentiating between type of tobacco smoked (Figure 2, panel C). In this regard, 8.3% of smokers switched from conventional to mixed use, 13.0% switched from conventional to RYO, and 59.1% smoked only conventional cigarettes before and after the two laws.

Regarding other smoking characteristics (Table 3), we observed an increase in the use of regular cigarettes (from 66.6% to 76.3%, p=0.019), in the length of cigarettes left after smoking (from 1.13 cm to 2.26 cm, p=0.023) and in the depth of inhalations when smoking at the expense of the reduction of intermediate depth of inhalation (deep inhalation from 42.4% to 57.5%, p=0.001) along with a reduction in low frequency of inhalation (from 28.2% to 10.1%, p=0.036) (Table 3).

4. DISCUSSION

Among continuing smokers we found an increase in salivary cotinine concentration of around 29% after both Spanish tobacco control legislations, particularly among younger and highly educated smokers. We also observed a switch in the type of tobacco used, from conventional to RYO cigarettes or to mixed use in 21.3% of smokers. In addition, we found a statistically significant decrease in the proportion of individuals classified as medium in the FTCD score, which represents those individuals with a FTCD score of 5 out of 10, that is to say, medium dependence to nicotine.

The hardening hypothesis (30) suggests that smokers who quit over the time are those who are less dependent, and the remaining smokers are more dependent. However, previous studies have counteracted this hypothesis (31-33) using self-reported questionnaires to measure the tobacco or nicotine dependence. In this sense, we did not find differences in salivary cotinine concentration, nor in FTCD scores, among those who quit smoking after Spanish tobacco control legislations and those who continue smoking (data not shown). On the other hand, we found an increase in salivary cotinine among continuing smokers. In this sense, when considering biomarkers as a proxy of tobacco dependence (34), our results could indicate that continuing smokers became more dependent after Spanish tobacco control legislations. However, our study did not show any significant increase in the FTCD score among smokers who continue smoking; this could be due to the fact that FTCD only measures the dependence of conventional manufactured cigarettes (29). A previous study showed that the FTCD has some limitations, such as low internal consistency, floor effects, and that it does not address important aspects of addiction to cigarettes (35). Although there is a positive relation between the FTCD score, tobacco consumption and salivary cotinine (34,35), other factors might also have an effect on cotinine concentration, such as diet and meals, age, sex, race, pregnancy, kidney disease, use of estrogen-containing hormone preparations and other medications (36). In our work, the effect of age may play a prominent role given the

longitudinal structure of our data, that is to say, given that the cohort has aged by ten years between baseline and follow-up. In this sense, a previous review showed that total clearance of nicotine lowered by 23% in the elderly (age >65) compared to adults (37). If nicotine is cleared at a slower rate, it will lead to a prolonged exposure (i.e. it would take more time to remove cotinine from the system), and it could also yield to obtain higher levels of cotinine under the same smoking consumption patterns. Therefore, the observed increase in salivary cotinine could be related to the aged of our cohort. However, the increase in the older group of age at follow-up was only of 11%, which represents 13 individuals out of the 116 continuing smokers. Our models were adjusted by sex, age and educational level, obtaining similar results in the adjusted and unadjusted models. Moreover, smoking topography (24) could also affect cotinine concentration.

According to our results, an increase in smoking regular cigarettes, in the centimeters left without smoking and in the depth of inhalation when smoking, along with a reduction in low frequency of inhalation, can be observed after the application of the Spanish tobacco laws. This could be explained because, under these smoking restriction policies, smokers have fewer opportunities to smoke in public places and less time to do so. Thus, changes in the smoking topography may be evident. In addition, the low increase in salivary cotinine found in our study in high dependent smokers at baseline could be due to a ceiling effect, since the highest value of salivary cotinine before bans was observed in this group. Furthermore, it is important to bear in mind that the information about FTCD score and the number of cigarettes smoked daily was self-reported, therefore it can be subject to potential limitations related to survey based studies. Further research is needed to analyze possible factors related to cotinine concentration, as well as to ensure cotinine concentration properly measures nicotine dependence.

The increase in the salivary concentration was statistically significant among young people, highly educated, and among those who switched from conventional cigarettes to RYO. These results are likely to be related, since previous works (38) showed a great increase in the consumption of hand-rolled tobacco and other tobacco products, especially among young people; in fact, according to those results, older people did not smoke hand-rolled tobacco, neither before nor after the implementation of the Spanish smoke-free legislations. Moreover, we found a significant association between age and education, age and type of tobacco smoked, and education and type of tobacco smoked (data not shown). Therefore, the increase in the salivary concentration among high-educated individuals might be confounded by age.

Additionally, our study showed a switch from conventional to RYO cigarettes or to mixed use in 21.3% of continuing smokers, after both Spanish tobacco control legislations. Thereby, our results could be backing the hypothesis of a switch of smokers to cheaper tobacco products, such as RYO cigarettes (14,15), because the tobacco control policies, particularly increasing of prices, are traditionally focused on conventional cigarettes. Regarding Spain, in recent years, the prices of these products have been surprisingly different, with RYO costing remarkably less than manufactured cigarettes. Moreover, the economic crisis that took place in Spain in 2008 could have affected the shift on tobacco products as has been also reported (10). Bearing this in mind, there is a need to equalize the prices of all tobacco products by applying the same taxing level as, indeed, recommended by the article 6 of the FCTC (39). Furthermore, the highest value of salivary cotinine after Spanish smoking bans, according to the kind of tobacco smoked, was observed in smokers who switched from conventional to RYO cigarettes or to mixed use, and may counteract the popular belief that RYO cigarettes are less harmful than conventional cigarettes (40). This aspect may also be involved in the observed increase in salivary cotinine after the implementation of Spanish smoking bans. Given that many smokers

switched from conventional cigarettes to other tobacco products, there is a need to implement new instruments to measure dependence not limited to a specific tobacco product.

Finally, 68% of continuing smokers were on the preparation stage, indicating that they were planning to quit within the next 30 days and that had attempted to quit for at least 24 hours in the past year; also 45.7% were classified as having low (FTCD score ≤4) nicotine dependence. However, according to the Tobacco Control Scale report of 2013 (36,37), the Spanish score for policies related to treatment to help smokers quit was low (6 out of 10 points) in comparison with other policies. This could mean that smokers may not receive the help needed to succeed when trying to stop smoking or that they are not properly identified. Therefore, there is a need to implement better treatment policies to help smokers stop smoking.

The main limitation of our study is the potential participation bias due to the attrition of the cohort of participants. In this sense, there were statistically significant differences according to age, level of education, and smoking status between the follow-up sample and the participants lost in the follow-up (41). The followed-up sample overestimated young people and smokers in comparison with lost participants. For this reason, the increase in salivary cotinine could be smaller among lost participants. On the other hand, our sample, being a cohort, overestimated the older people compared with the distribution of population in Barcelona. However, we weighted the sample to minimize these limitations and to generate estimations representative of the general population. Moreover, the baseline sample size was representative of the city of Barcelona (24,25) and the longitudinal design maximizes the internal validity of the study. Other potential limitations are those related to potential information biases derived from the use of a self-reported questionnaire to collect information, and non-response. However, trained interviewers administered a face-to-face questionnaire and used the same definition of smoking status in both studies, potentially increasing the internal validity of our results. In addition, we used salivary cotinine, a specific biomarker of nicotine.

In conclusion, this study shows a significant increase in salivary cotinine concentration among adult continuing smokers after the implementation of both Spanish legislations. Moreover, we observed a shift in the type of tobacco product used, particularly from conventional cigarettes to RYO. Our results, therefore, suggest the need to extend tobacco control policies, focusing on reduction of use of any kind of tobacco product (i.e. equalizing the prices of all tobacco products) and implementing better treatment policies to help smokers stop smoking.

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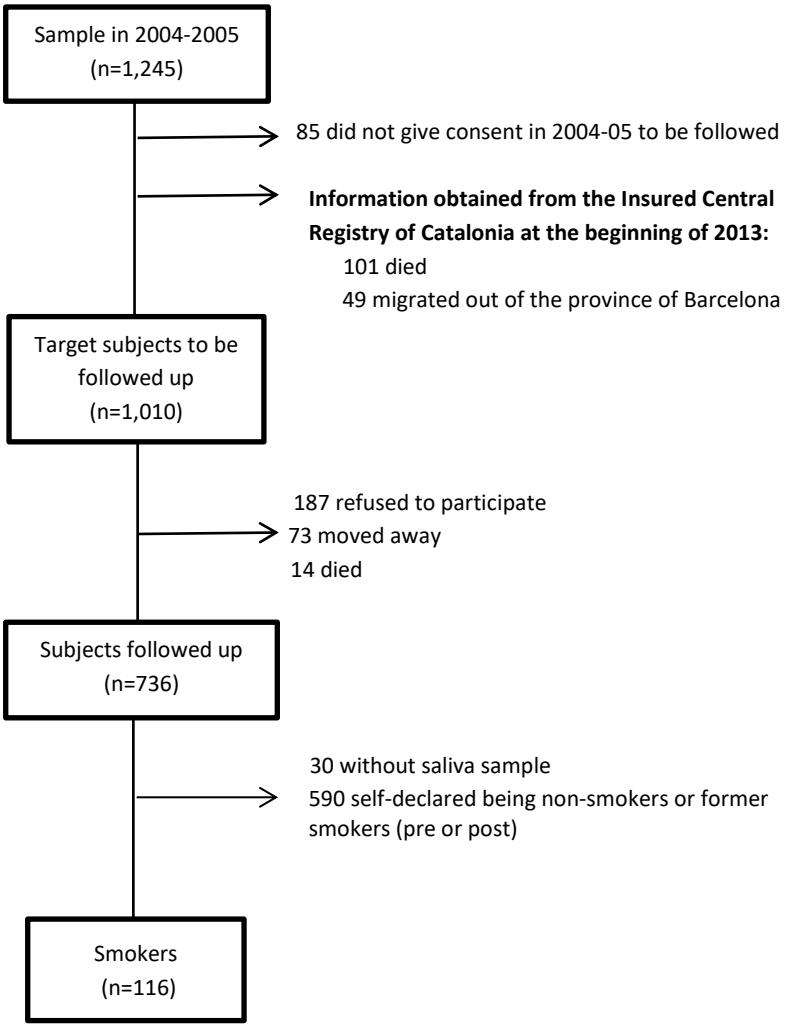
Figure1. Flow chart of sample followed-up from Barcelona, Spain, in 2013-2014.



Pre: before Spanish smoke-free bans (2004-2005).
Post: after Spanish smoke-free bans (2013-2014).

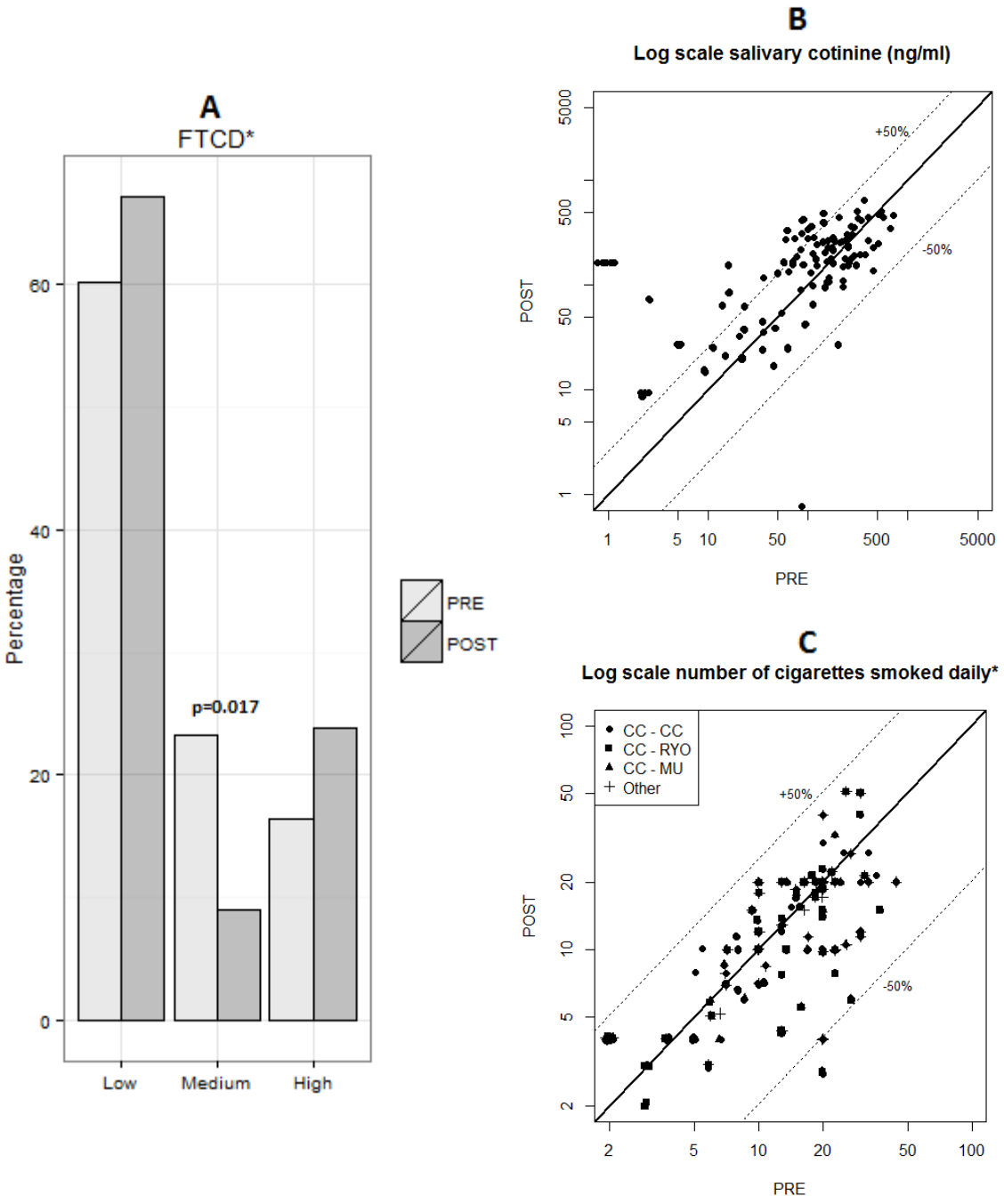
Table 1: Differences between follow-up sample and lost in both stages of the follow-up (prevalence for qualitative variables and mean (standard deviation) for quantitative variable).

	Lost (both stages) n=509	Follow-up n=736	P-value
Sex			
men	42.2%	45.7%	0.257[a]
women	57.8%	54.3%	
Age (years)	58 (39)	49 (26.25)	<0.001[b]
Age			
<45	35.2%	43.9%	<0.001[a]
45-64	22%	37.5%	
>=65	42.8%	18.65	
Educational level			
Low	53%	38.9%	<0.001[a]
Intermediate	20.5%	24.2%	
High	26.5%	36.9%	
Smoking status (follow-up)			
smoker	24.1%	31.1%	0.009[a]
no smoker	75.9%	68.9%	

a. Chi-square test

b. Mann-Whitney test

Figure 2. Changes in salivary cotinine concentration (log scale), in the number of cigarettes smoked daily (log scale), and in the Fagerström Test for Cigarette Dependence (before and after the implementation of both Spanish tobacco control legislations).



*Daily smokers

FTCD categorized as 'low' (≤4), 'medium' (5) and 'high' (≥6)

Statistically significant differences were only found in the 'medium' group when comparing FTCD categories pre and post legislation (proportion test).

Pre: before Spanish smoke-free bans (2004-2005).

Post: after Spanish smoke-free bans (2013-2014).

Table 2. Geometric mean (GM) and geometric standard deviation (GSD), adjusted linear mixed effect coefficient (% change*) and their 95% confidence interval (95%CIs) of salivary cotinine concentration (ng/mL) according to sociodemographic variables, kind of tobacco smoked, Fagerström Test for Cigarette Dependence score (FTCD) and stages of change before (2004-2005) and after (2013-2014) the implementation of both Spanish tobacco control legislations.

	n[a]	GM (GSD) ng/mL (PRE)	GM (GSD) ng/mL (POST)	% change* (95% CI)	p-value
Overall	116	91.1 (0.16)	117.3 (0.18)	28.7 (4.9; 58.0)	0.015
Sex					
Men	62	103.9 (0.26)	135.2 (0.28)	30.1 (-0.1; 69.4)	0.051
Women	54	78.3 (0.19)	99.6 (0.22)	27.2 (-7.8; 75.6)	0.143
Age					
26-44	62	104.3 (0.15)	174.3 (0.09)	67.2 (30.7; 113.8)	<0.001
45-64	34	132.7 (0.18)	112.9 (0.28)	-14.9 (-46.5; 35.2)	0.494
65-98	20	30.7 (0.22)	35.3 (0.34)	15.0 (-13.5; 52.9)	0.336
Educational level					
Low	46	102.1 (0.32)	112.5 (0.35)	10.2 (-11.8; 37.7)	0.392
Intermediate	33	100.9 (0.22)	142.2 (0.27)	40.9 (-21.1; 151.6)	0.246
High	37	72.4 (0.16)	104.0 (0.24)	43.8 (13.9; 81.4)	0.002
Kind of tobacco smoked[1]					
CC - CC	64	106.6 (0.18)	114.9 (0.20)	7.8 (-18.0; 41.9)	0.590
CC – RYO	14	145.6 (0.16)	243.2 (0.12)	70.0 (16.7; 138.8)	0.005
CC – MU	9	205.2 (0.16)	236.9 (0.14)	15.5 (-12.1; 51.6)	0.301
Other	21	74.3 (0.55)	80.8 (0.63)	8.8 (-15.2; 39.5)	0.509
$FTCD_d$ score (PRE)					
Low	53	115.7 (0.12)	137.4 (0.18)	18.7 (-15.3; 66.3)	0.319
Medium	20	86.6 (0.43)	103.3 (0.39)	19.3 (-0.9; 43.5)	0.062
High	14	237.6 (0.15)	272.3 (0.12)	14.6 (-12.8; 50.7)	0.329
Stages of Change (PRE)					
Precontemplation	6	186.7 (0.36)	257.1 (0.20)	37.7 (-26.0; 156.3)	0.313
Contemplation	9	136.8 (0.30)	172.5 (0.25)	26.1 (-11.5; 79.8)	0.200
Preparation	79	93.5 (0.21)	115.1 (0.23)	23.1 (-7.3; 63.6)	0.151

$FTCD_d$: FTCD daily smokers.

n[a]: The sum does not up the total for some variables because of some missing values.

Pre: Before Spanish smoke-free bans (2004-2005).

Post: After Spanish smoke-free bans (2013-2014).

% change*: Adjusted by sex, age, educational level.

P-value: Obtained through adjusted linear mixed effect coefficient.

[1]'CC-CC' only smoked conventional cigarettes before and after the two laws, 'CC - RYO' switched from conventional to RYO cigarettes, 'CC – MU' switched from conventional to mixed use (CC and RYO) and 'Other' indicates other possible options.

Table 3. Prevalence and generalized linear mixed effect coefficient (Prevalence Ratio PR), or mean (standard deviation) and mean difference, smoking characteristics before (2004-2005) and after (2013-2014) the implementation of both Spanish tobacco control legislations.

	n[a]	PRE	POST	PR (95% CI)	p-value
Type of cigarettes (%)					
Regular	66	66.6	76.3	1.15 (1.02, 1.28)	0.019[1]
Other	33	33.4	23.7	0.71 (0.50, 0.99)	0.049[1]
Type of tobacco (%)					
Blond	82	82.2	88.1	1.07 (0.99, 1.16)	0.097[1]
Black	18	17.8	11.9	0.67 (0.42, 1.06)	0.086[1]
Filter (%)					
Yes	97	98	98	1.00 (0.98, 1.02)	0.96[1]
No	2	2	2	1.04 (0.35, 3.07)	0.94[1]
Length of cigarettes left after smoking in cm (mean, SD)	107	1.13 (0.12)	2.26 (0.39)	-	0.023[2]
Frequency of inhalation (%)					
Continuous	13	11.7	16.9	1.44 (0.83, 2.52)	0.198[1]
Regular	65	60.1	73	1.21 (0.86, 1.71)	0.264[1]
Scarce	30	28.2	10.1	0.36 (0.14, 0.93)	0.036[1]
Depth of inhalation (%)					
Superficial	17	16.2	24.7	1.56 (0.73, 3.32)	0.246[1]
Intermediate	45	41.4	17.8	0.43 (0.26, 0.72)	0.001[1]
Deep	46	42.4	57.5	1.35 (1.13, 1.61)	0.001[1]

n[a]: The sum does not up the total for some variables because of some missing values.

Pre: Before Spanish smoke-free ban of 2004-2005.

Post: After Spanish smoke-free ban of 2013-2014.

PR: Prevalence ratio obtained through generalized linear mixed effect coefficient.

[1]Generalized linear mixed effect coefficient p-value.

[2]T-test for paired samples.