Assessment of salivary cotinine concentration among general non-smokers population: before and after Spanish smoking legislations

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Abstract word count: 208

Manuscript word count: 2,040

Highlights word count: 77

ABSTRACT

Background: In Spain, two smoke-free laws have been passed after the approval of the FCTC ((Law 28/2005 and Law 42/2010).This study evaluates the association between Spanish smoking legislations and the second-hand smoke (SHS) exposure in an adult non-smoking population cohort in Barcelona (Spain).

Methods This is a longitudinal study, before and after the implementation of two national smoking bans, in a representative sample of adults (≥ 16 years old) from Barcelona (Spain) surveyed in 2004-2005 and followed-up in 2013-2014 (n=736). We only analyzed non-smokers (n=397). We conducted a survey about the self-reported exposure to SHS and obtained 9 ml of saliva sample for cotinine analysis following the same protocol. We used cotinine as a biomarker of previous (1-2 days) tobacco exposure. We calculate geometric means of salivary cotinine concentration and their geometric standard deviation. We use linear mixed effect models with individuals as random effects to model the percentage change in salivary cotinine concentrations and their 95% confidence intervals.

Results: The geometric mean of salivary cotinine concentration significantly decreased 88% (from 0.98 ng/mL to 0.12 ng/mL, p<0.001) and salivary cotinine concentration was significantly higher only among those declaring exposure to second-hand smoke at home (exposed=0.33 ng/mL vs non-exposed=0.11 ng/mL, p<0.001); after the implementation of the two Spanish smoke-free legislations.

Conclusion: There was a large reduction in the salivary cotinine concentration among adult non-smokers and higher cotinine concentrations among those declaring exposure to SHS at home after both legislations.

Keywords: Smoke-free legislation; *Secondhand smoke; Cotinine; Biomarker*

1. INTRODUCTION

The effects of secondhand tobacco smoke (SHS) on the health of non-smokers are well-known. SHS exposure has been associated with many adverse health effects [1] and it is classified as a Group I carcinogen in humans [2] by the International Agency for Research on Cancer (IARC). In this sense, the Institute for Health Metrics estimated that about 18% of worldwide population smoked in 2012 causing 11.5% of the global deaths in 2015 attributable to active smoking and 1.6% to passive smoking[3]. In Spain, the latest data reported showed that 23.6% of Spanish population were tobacco users (2012)[4], causing 60,456 deaths attributable to active smoking[4].

Consequently, several countries have implemented tobacco control legislations, as suggested by the World Health Organization Framework Convention on Tobacco Control (WHO FCTC) [5]. Regarding Spain, two smoke-free laws have been passed after the approval of the WHO FCTC. On the 1st of January 2006, it came into effect a smoke-free legislation (Law 28/2005). This law was a great step forward for public health in Spain, but it was incomplete in terms of protection to SHS exposure because it allowed smoking in hospitality sectors depending on the size of venues [6]. Because of this exception this Spanish smoking law was known as the "Spanish model" [7]. The scientific evaluation of this law showed the need to promote a total ban [8-10] and motivated the modification of the law accordingly. Hence, a new law (Law 42/2010) came into effect on the 2nd of January 2011, applying the smoke-free regulation to all hospitality venues [11] without exception, and extending the prohibition to some outdoor areas, including hospital premises, educational campuses, and playgrounds.

In order to asses SHS exposure, it is desirable to use biomarkers as they allow to objectively quantifying SHS exposure. In this regard, cotinine, the main nicotine metabolite, has been widely used as a biomarker of tobacco exposure [12]. Cotinine concentration in biological fluids (blood, urine or oral fluid, widely referred to as saliva) [13] indicate tobacco exposure over the previous 1-2 days [14].

Currently, there are scarce studies that evaluate the impact of both Spanish smoking legislations using a general population cohort which increases the internal validity of the results [15]. Moreover, few studies assessed the legislations impact among general population using a specific biomarker of SHS exposure such as cotinine concentration, and only one in Spain using a repeated cross-sectional study [16]. In a previous Spanish cohort of hospitality workers, salivary cotinine concentration decreased significantly (by 56.6%) among hospitality workers at venues where smoking was totally banned after the Spanish partial ban (law 28/2005) took effect [17]. Therefore, the objective of this study is to evaluate the association between both Spanish smoking legislations and the SHS exposure in an adult non-smoking population cohort in Barcelona (Spain) using salivary cotinine concentrations and information on self-reported exposure.

2. METHODS

This is a longitudinal study from a representative sample of the adult population (≥16 years) of the city of Barcelona (Catalonia, Spain). The baseline study was carried out during the years

2004-2005 [18,19] (n = 1,245) and follow-up took place in 2013-1014, after both Spanish smoking legislations (n=736).

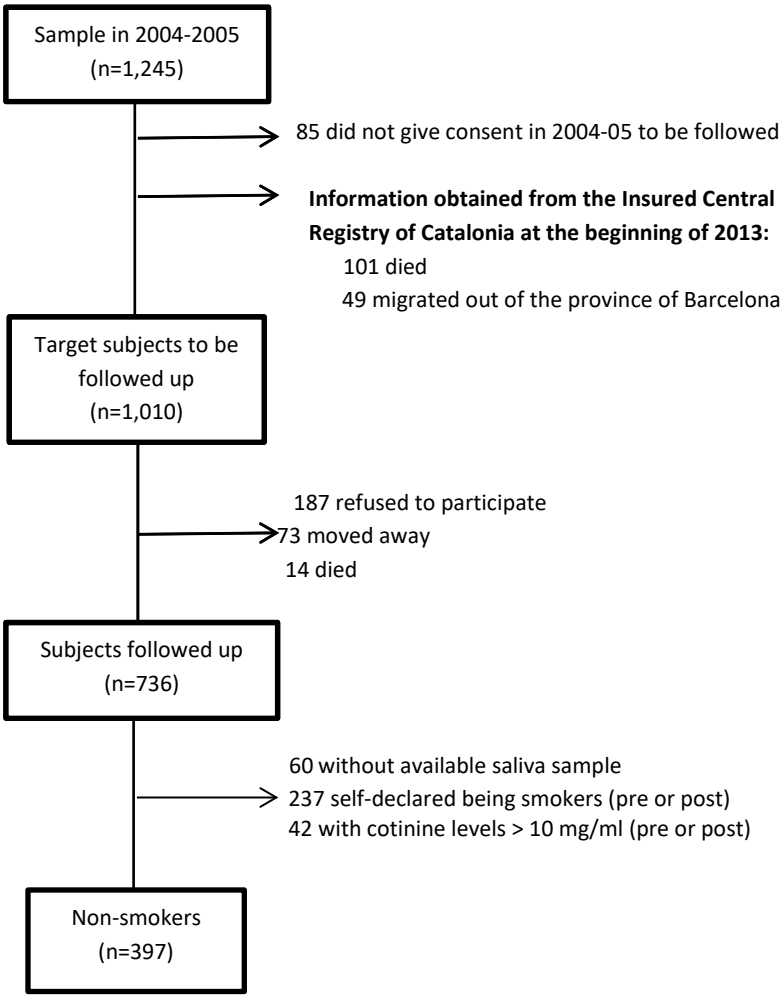
From the baseline sample, we excluded 235 subjects, 150 after checking their data in the Insured Central Registry of Catalonia (101 died and 49 migrated out of the province of Barcelona) and another 85 without consent to be followed up or being minor (<18 years old) in 2004-2005 whose parents did not provide informed consent to be re-contacted. Follow-up was conducted between May 2013 and February 2014. In total, 72.9% of the eligible sample agreed to participate, 18.5% refused to participate, 7.2% had moved elsewhere and 1.3% had died. The final sample included 736 individuals (Figure 1).

The final sample was skewed as slightly older in comparison with the general population of Barcelona. For this reason, we weight our data according to age distribution of the city of Barcelona to maintain its representativeness.

We administered the same questionnaire that gathered information on the smoking status, tobacco consumption and exposure to second-hand smoke before and after Spanish smoking legislations. Specifically, we obtained information about the self-reported exposure to SHS at home, work, public transport and leisure time (dichotomous variable of exposure to SHS in each setting). Exposure to SHS at home was determined through two questions: ‘‘Currently, how many individuals usually smoke inside your home per day?’’ and ‘‘During the last week, how many cigarettes per day have been smoked in your presence inside your home?’’ Answers were gathered for typical working and non-working days. These questions were only provided

for non-smokers (former and never smokers). Based on these two questions, we derived a dichotomous variable: non-exposed at all (responses=0 to both questions) and exposed (responses ≥1 to any of the questions).

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



Exposure to SHS at work, only provided for non-smokers, was determined through two questions: ''Does anybody smoke in close proximity to you at work?'' and ''How many hours per day do you think you are exposed to tobacco smoke at work?". We also derived a dichotomous variable of exposure to SHS at the workplace: non-exposed at all (responses=0 to

both questions) and exposed (responses ≥1 to any of the questions). Exposure to SHS at public transport, only provided for non-smokers, was obtained through the questions: ''During the last week, were you in a public transport vehicle while someone was smoking?'' We defined a dichotomous variable of exposure to SHS in public transport: non-exposed at all (responses=0 to both questions) and exposed (responses ≥1 to any of the questions). Exposure to SHS at leisure time, only provided for non-smokers, was determined through the question ''How much time have you spent in any place with tobacco smoke that was not at home or at work?". The answers were gathered for typical working and non-working days. We derived a dichotomous variable of exposure to SHS during leisure time: non-exposed at all (responses=0 to both questions) and exposed (responses ≥1 to any of the questions). Exposure to SHS in any setting was defined as exposure in at least one of the above mentioned settings. We also obtained 9 ml of saliva sample (i.e. oral fluid) for cotinine analysis before and after the Spanish smoking legislations, following the same protocol before and after smoking 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 'Hospital del Mar' Medical Research Institute (IMIM) in Barcelona. Salivary samples from baseline survey (2004-2005) were analyzed with gas chromatography followed by mass spectrometry detection (GC/MS). The limit of quantification was 1 ng/mL and the limit of detection was 0.3 ng/mL. Salivary samples from the follow-up survey (2013-14) were analyzed with liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) [20] with multiple reaction monitoring. The limit of quantification was 0.1 ng/mL and the limit of detection was

0.03 ng/mL (quantification error <15%). Because the latter method was more sensitive and had a lower limit of quantification than the former method, all available saliva samples from the baseline survey (2004-2005) with cotinine concentrations below 1 ng/mL (n= 245) were reanalyzed with the LC/MS/MS method. For cotinine concentrations below the limit of quantification a value of half the level of quantification (0.05 ng/mL) was assigned.

The same definition of smoking status was used in both studies. We considered as non-smoker the person who declares to have never smoked or to have formerly smoked, and has a salivary cotinine concentration compatible with non-smoking (≤10 ng/ml) [21]. The final sample for this study consists of 397 non-smokers before and after the Spanish smoking legislations (Figure 1). Given the skewed distribution of cotinine concentration, we calculated geometric means (GM) and their geometric standard deviation (GSD). We used linear mixed effect models with individuals as random effects adjusted for basal sex, age and educational level to model the change percentage in salivary cotinine concentrations (after log 10 transformation) and their 95% confidence intervals. We obtained the p-value for the median difference through Wilcoxon test for paired samples and Mann Whitney test for independent samples. Moreover, the results were stratified by sex, age and educational level. The statistical program used was R-3.0.2 and Stata v14.

3. RESULTS

There were no statistically significant differences between the followed-up sample (n=736) and the participants lost in the second stage (n=274) according to age, sex, level of education and

smoking status. However, there were statistically significant differences according to age, level of education and smoking status between the follow-up sample (n=736) and the participant lost in both stages of the follow-up (n=509) (Table 1).

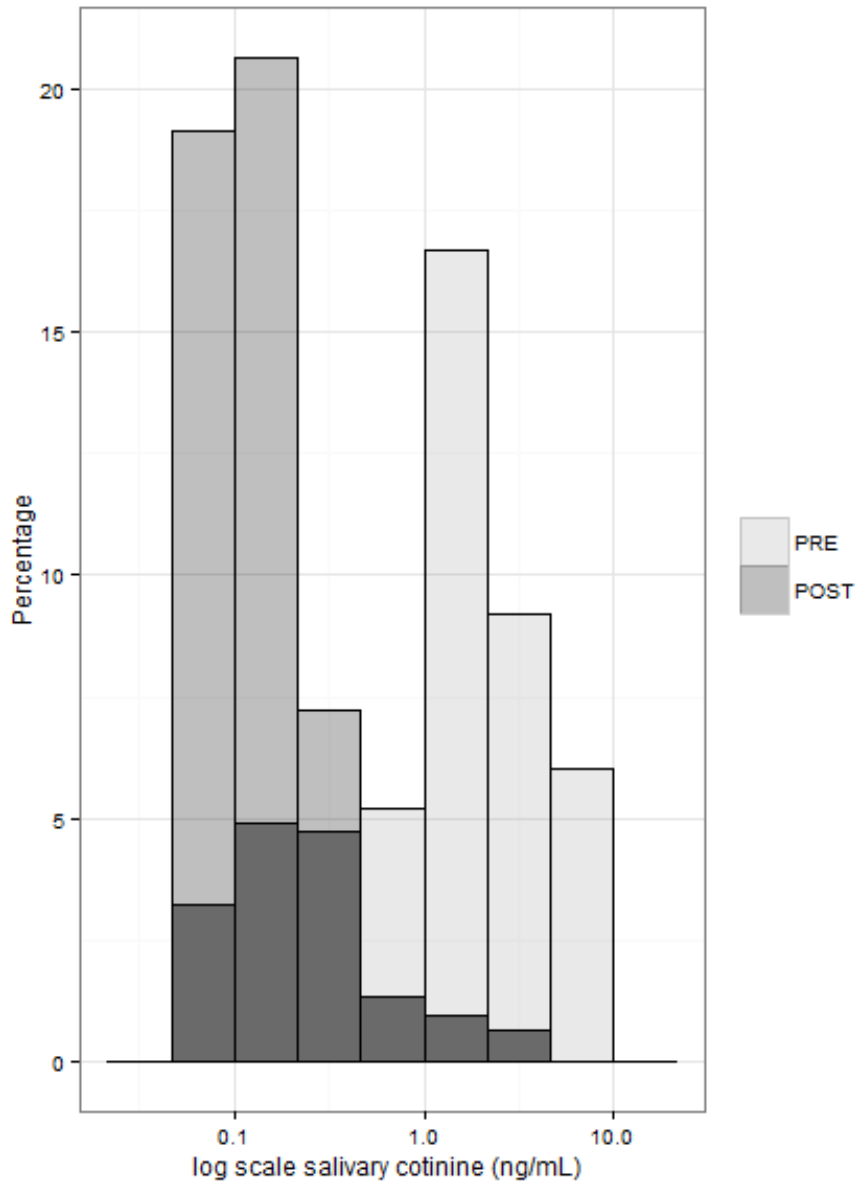
Table 1: Differences (prevalence for qualitative variables and mean (standard deviation) for quantitative variable) between follow-up sample and lost in both stages of the follow-up.

	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	
Studies			
Primary	53%	38.9%	<0.001[a]
Secondary	20.5%	24.2%	
Superior	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

The percentage of participants with saliva samples with measurable concentrations of cotinine fell from 92.4% to 64.2% after both Spanish smoking legislations. We also found a leftward shift in the salivary cotinine distribution after both Spanish smoking legislations (Figure 2), i.e. it is more frequent to obtain lower values in the salivary cotinine concentration after the legislations.

Figure 2. Distribution (histogram) of cotinine in saliva (ng/mL) before and after the implementation of both Spanish smoking bans.



Pre: before Spanish smoke-free bans (2004-2005).
Post: after Spanish smoke-free bans (2013-2014).
The darker color in the histogram indicates the overlap of the two distributions.

The overall GM of salivary cotinine concentration significantly decreased 87.9% (from 0.98 ng/mL to 0.12 ng/mL, $p<0.001$) after the implementation of the two Spanish smoke-free bans (Table 2). We found a statistically significant decrease in the GM of salivary cotinine concentration independently of the sociodemographic variables (sex, age, and level of

education) (Table 2). Before both legislations, there was higher salivary cotinine concentrations among young (GM 26-44 years=1.12 ng/mL, GM 45-64 years=0.80 ng/mL, GM 65-98 years=0.87 ng/mL), men (GM men=1.22 ng/mL vs GM women=0.84 ng/mL), and with intermediate educational level (GM low level=0.78 ng/mL, GM intermediate level=1.11 ng/mL, GM high level=1.07 ng/mL) (Table 2). Nevertheless, after both legislations, the salivary cotinine concentration was similar according to sociodemographic variables (p-value sex: 0.635, p-value age: 0.210, p-value educational level: 0.163) (data not shown).

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. Before (2004-2005) and after (2013-2014) the implementation of both Spanish smoking legislations.

	n	GM (GSD) ng/mL (PRE)	GM (GSD) ng/mL (POST)	% change *	p-value
Overall	397	0.98 (0.08)	0.12 (0.05)	-87.9 (-89.8; -85.6)	<0.001
Sex					
Men	170	1.22 (0.11)	0.12 (0.08)	-89.9 (-92.3; -86.7)	<0.001
Women	227	0.84 (0.10)	0.12 (0.06)	-86.2 (-88.9; -82.8)	<0.001
Age**					
26-44	224	1.12 (0.11)	0.11 (0.07)	-90 (-92.3; -87.1)	<0.001
45-64	109	0.80 (0.11)	0.13 (0.07)	-83.8 (-87.3; -79.4)	<0.001
65-98	64	0.87 (0.16)	0.12 (0.10)	-85.6 (-90; -79.4)	<0.001
Educational level					
Low	120	0.78 (0.11)	0.14 (0.08)	-82.6 (-86.6; -77.5)	<0.001
Intermediate	100	1.11 (0.18)	0.12 (0.12)	-89.5 (-93.1; -84.1)	<0.001
High	177	1.07 (0.11)	0.11 (0.06)	-89.7 (-91.9; -86.9)	<0.001

*: adjusted by basal sex, age and educational level.
**: age measured after smoke-free legislations (2013-2014).
Pre: before Spanish smoke-free bans (2004-2005).
Post: after Spanish smoke-free bans (2013-2014).
P-value: Wilcoxon test for paired samples.

Before both legislations, salivary cotinine concentration was significantly higher among those declaring to be exposed to SHS at any setting (GM exposed=1.07 vs GM non-exposed=0.67) and at home (GM exposed=1.75 vs GM non-exposed=0.85) (data not shown). Although the higher prevalence of self-reported SHS exposure post-legislations was found in leisure time (50.8%), salivary cotinine concentration was significantly higher only among those declaring to be exposed to SHS at home (GM exposed=0.33 vs GM non-exposed=0.11) (Table 3).

Table 3. Prevalence of self-declared exposure to SHS at all the analyzed settings, geometric mean (GM) and geometric standard deviation (GSD), of salivary cotinine concentration (ng/mL) according to self-declared exposure to SHS after the implementation of both Spanish smoking legislations (2013-14).

	n[a]	% POST	Geom. Mean (POST)	p-value
SHS exposure (Any setting)				
Yes	225	56.7	0.13 (0.07)	0.080
No	172	43.3	0.11 (0.7)	
SHS exposure (Home)				
Yes	38	9.5	0.33 (0.18)	<0.001
No	359	90.5	0.11 (0.05)	
SHS exposure (Work)				
Yes	67	29.4	0.12 (0.13)	0.876
No	161	70.6	0.11 (0.08)	
SHS exposure (Public transport)				
Yes	15	4.7	0.09 (0.18)	0.542
No	291	95.3	0.12 (0.06)	
SHS exposure (Leisure time)				
Yes	201	50.8	0.13 (0.07)	0.077
No	194	49.2	0.11 (0.06)	

n[a]: The sum does not up the total for some variables because of some missing values.
Post: after Spanish smoke-free bans (2013-2014).
P-value: Mann-Whitney test for independent samples (Mann Whitney)

4. DISCUSSION

Our study shows an important reduction of salivary cotinine concentration (around 88%) among non-smokers after both Spanish smoking legislations, this reduction keep according to sex, age, and educational level (between 82% to 90%).

In this line, a previous repeated cross-sectional study showed that GM of the salivary cotinine concentration, among all adult non-smokers in Barcelona (Spain), significantly decreased, from 0.93 ng/mL before the legislations, to 0.12 ng/mL after the legislations [16]. Moreover, the change percentage in cotinine concentration obtained in this same study, after adjusting for sex, age, and educational level, was 87.9% [16]. Similarly, another Spanish study evaluating the impact of both Spanish smoke-free laws with urinary sample in 2010 and 2011, showed a significant decrease in median urinary cotinine concentrations between 2010 (0.8 ng/mL) and 2011 (0.7 ng/mL) among passive smokers [22].

Other Spanish studies showed significant decrease in the environmental nicotine in hospitals after both Spanish legislations [23] and in hospitality venues between 2010 and 2011 [24]. Moreover, another longitudinal study, showed a significant decrease in the prevalence of self-declared SHS exposure at workplaces, during leisure time, at home, and public transport after the application of the two Spanish smoking legislations [25]. Other studies, carried out in Spain using questionnaire, showed a decrease in self-declared SHS exposure in all the studied settings after the application of the smoking legislations [16,26,27]. Similar results can be found in

Ireland [28], Scotland [29], Italy [30], and USA [31]. In Europe, a secondary analysis showed that the enforcement of smoke-free legislation is inversely associated with SHS exposure [32].

The higher prevalence of self-reported SHS exposure post-legislations was found in leisure time (50.8%) however the exposure in leisure time, particularly in hospitality venues, is thought to be shifted to the entrances of venues [24] and therefore the exposure during leisure time is expected to occur mainly outdoor. In addition, our results show higher cotinine concentrations among those declaring exposure to SHS at home. This could be because the shift on exposure in leisure time to outdoors reduces the time and intensity of exposure. Moreover, in a regular day people usually spend more time at home than at leisure time, therefore, being exposed at home could be harder in terms of time and intensity. However, only 9.5% declared to be exposed at home. In this sense, an increase in the prevalence of smoke-free homes was observed after the implementation of the two Spanish smoke-free bans in Spain [27] and more than half (57.4%) of the population of Barcelona (Spain) had complete indoor smoke-free rules at home in 2013-2014 [33]. Moreover, in 2011, 6.7% of non-smokers reported SHS exposure at home indoors, 18.8% at home outdoors, 1.3% at work indoors, and 15.0% at work outdoors [34]. Therefore, there is a need to implement some public health interventions to continue reducing SHS exposure at home. The interventions may focus in convincing or helping smokers to quit or in getting smokers moving their smoking away from their home, that is to say, trying to promote smoke-free homes and smoke-free multi-unit housing [35,36].

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 participant lost in both stages of the follow-up [27]. Follow-up participants overestimate the young people

and smokers in comparison with lost participants. Previous cross-sectional studies conducted in Spain[19,25,37] found that you people are more exposed to SHS exposure than older one. This aspect could affect in the reduction of SHS exposure observed in our study. On the other hand, our final sample 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 [18,19] and the longitudinal design of our work maximizes the internal validity of the study. Other potential limitations are those related to survey based studies, as the use of a questionnaire to collect self-reported information, and bias due to non-response. However, we used salivary cotinine as a specific biomarker of SHS exposure, we use the same definition of smoking status in both studies, we remove individuals with a salivary cotinine concentration incompatible with non-smoking (>10 ng/ml) and we used a face-to-face questionnaire with trained interviewers potentially increasing the internal validity of our results.

5. CONCLUSION

In conclusion, this study shows a large reduction in the salivary cotinine concentration among adult non-smokers after both Spanish legislations independently of sociodemographic variables. However, our results show higher cotinine concentrations among those declaring exposure to SHS at home after both legislations, revealing the need to implement some public health interventions to continuing reducing SHS exposure in homes.

6. COMPLIANCE WITH ETHICAL STANDARDS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

7. FUNDING

This project was co-funded by the Instituto de Salud Carlos III, Subdirección General de Evaluación, Government of Spain (RTICC RD12/0036/0053, PI12/01114 and PI12/01119), and the Ministry of Universities and Research, Government of Catalonia (grant 2014SGR999 and 2014SGR00680) from the Government of Catalonia, and co-funded by ISCIII-Subdirección General de Evaluación and by FEDER funds/ European Regional Development Fund (ERDF) –a way to build Europe-. This work was also partially funded by the European Union's Horizon 2020 Research and Innovation Programme (The TackSHS Project; grant agreement: 681040).

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