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A comparison of exposure to carcinogens among roll-your-own and factory-made cigarette smokers

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ABSTRACT

Consumption of roll-your-own (RYO) tobacco is rising but little is known about its in vivo delivery of toxins relative to factory made (FM) cigarettes. To start to address this issue, this study compared the concentrations of metabolites of recognised human carcinogens in smokers of RYO tobacco and FM cigarettes. We opportunistically recruited 127 FM and 28 RYO smokers in central London and collected saliva and urine samples. Saliva samples were assayed for cotinine and urinary samples for 1-HOP and Total NNAL, metabolic markers of polycyclic aromatic hydrocarbons and tobacco-specific N-nitrosamines, respectively. Data on socio-demographic, anthropometric and puffing characteristics were also obtained. Both univariate and multivariate analyses (controlling for age, sex, body mass index, puff flow, puff duration and cotinine) showed no difference in metabolic markers between RYO and FM cigarette smokers. However, significant main effects for cotinine levels and sex were observed in multivariate analyses. Greater levels of cotinine were associated with a greater concentration of both 1-HOP ($B=0.002$, $p=0.037$) and NNAL ($B=0.002$, $p<0.001$). In addition, women had significantly greater concentrations of urinary 1-HOP ($B=0.679$, $p=0.004$) and Total NNAL metabolites ($B=0.117$, $p=0.024$) than men, irrespective of the type of cigarettes smoked. More research is now needed to confirm these findings and gender-specific effects in a larger, representative sample. However, results do not support the common belief that roll-your-own cigarettes are less harmful than manufactured cigarettes.

Key words: Total NNAL; 1-HOP; tobacco carcinogens; sex-difference; roll-your-own cigarettes; factory-made cigarettes

INTRODUCTION

Consumption of roll-your-own (RYO) tobacco is on the increase in many countries including the US, UK, Australia, France and Norway (Connolly and Alpert, 2008; Oddoux and Melihan-Cheinin, 2001; Scollo and Borland, 2004; Kraft et al., 1998). This appears to be a response to tax increases of factory-made (FM) cigarettes as smokers switch to cheaper (often smuggled) products such as RYO tobacco (Gunby, 1994), especially among the most economically disadvantaged smokers (Young et al., 2006; Young et al. 2008). Yet, despite this increase in the prevalence of RYO use, relatively little is known about the health impact of RYO cigarettes.

The question naturally arises as to whether smoking RYO tobacco is less harmful, as harmful as, or more harmful than smoking FM cigarettes. Some studies suggest that compared with FM cigarettes, RYO cigarettes yield similar or even higher levels of toxins in smoke (Appel et al., 1990; Kaiserman and Rickert, 1992a; Kaiserman and Rickert, 1992b; Darrall and Figgins, 1998). Moreover, smokers of RYO and FM cigarettes suffer from the same smoking-related diseases (De et al., 1992) and RYO smokers may be at increased risk of particular cancers (e.g. lung cancer) compared with FM cigarette smokers (Engeland et al., 1996; Tuyns and Esteve, 1983).

To our knowledge, however, no studies thus far have examined *in vivo* exposure to recognised human carcinogens in smokers of RYO tobacco compared with FM cigarettes. There are many challenges to doing this in a way that would be widely generalisable because of potential confounders. This study is a first attempt to obtain a broad indication of the relative exposure of smokers of RYO tobacco to carcinogens compared with smokers of FM cigarettes. We chose to characterise the concentrations of two metabolites of known tobacco carcinogens (1-hydroxypyrene [1-HOP] and Total

4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol [NNAL]) in an opportunistic sample of FM and RYO smokers and to examine their socio-demographic correlates. We chose 1-HOP and Total NNAL as they are established metabolic markers of polycyclic aromatic hydrocarbons and tobacco specific *N*-nitrosamines (Hecht, 2002), which represent two groups of human carcinogens that are thought to play a major role in tobacco-related carcinogenesis (Hoffmann et al., 1997).

MATERIAL AND METHODS

Participants

The study methodology has been detailed elsewhere (Shahab et al., 2008). Briefly, FM and RYO cigarette smokers were opportunistically recruited through advertisements in local newspapers, flyers, emails, or posters on public bulletin boards at or around University College London. Participants had to be regular smokers ($5 \geq$ cigarettes/day) of a particular brand of manufactured cigarettes or rolling tobacco for the last 3 months; be between 18 and 60 years of age and not be pregnant or have heart or lung disease. A power calculation (Faul and Erdfelder, 1992) indicated that a sample of 130 FM cigarette smokers and 42 RYO smokers would be required to detect a medium effect size in two-tailed analysis. Due to time limitations and difficulty in recruiting RYO cigarette smokers and the exclusion of five participants because of violation of the study protocol and malfunctioning of machinery, the final sample was comprised of 127 FM and 28 RYO cigarette smokers, which reduced the power to detect only medium-to-large (Cohen's $d=0.6$) effects. Participant characteristics are provided in Table 1. There were no differences in any of the baseline variables between included and excluded participants.

Procedure

Smokers visited the laboratory on two occasions, 24 hours apart. At the first visit, participants' consent was obtained as well as a saliva sample and a baseline questionnaire assessing socio-demographic, anthropometric (height and weight) and smoking characteristics. Participants were asked to smoke cigarettes over the 24 hour period between visits with a topography device measuring puffing behaviour (complete results reported elsewhere, see Shahab et al., 2008). At the second visit, participants provided saliva and urine samples. The study received approval from University College

London Ethics Committee (Ref 0474/001) to confirm adherence to EU ethical research standards.

Measures

Biomarkers

Saliva samples were collected with a dental roll that participants were asked to keep in their mouth until saturated. Saliva was assayed for cotinine, a measure of nicotine intake, using a validated method involving capillary column gas-liquid chromatography (Feyerabend and Russell, 1990). Urine was collected by participants on site in a sterilised sealable cup and assayed for the presence of metabolites of two common tobacco carcinogens, 1-HOP and Total NNAL, to determine the uptake of such carcinogens in smokers. 1-HOP is a metabolite of pyrene, which is always present in mixtures of polycyclic aromatic hydrocarbons (PAH), and therefore an accepted biomarker of carcinogenic hydrocarbons such as benzo(a)pyrene (Jongeneelen, 2001). NNAL and its glucuronides are metabolites of the known lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific *N* nitrosamine (Hecht, 2002). Samples were tested for 1-HOP and Total NNAL (NNAL plus NNAL-gluc) concentrations using high-performance liquid chromatography with fluorescence detection (see Carmella et al., 2004) and gas chromatography with chemiluminescence detection (see Hecht et al., 2006), respectively. In addition, urine was assayed for creatinine (using Vitros CREA slides) to correct for variable urine flow rates in metabolite analysis.

Puffing behaviour

Puffing behaviour was measured using the CReSSmicro[®] machine, a validated battery-operated, hand-held portable device that provides data on puffing intensity including puff

duration and puff flow (Lee et al., 2003; Shahab et al., 2008). It uses an orifice flow meter mouthpiece that produces a pressure drop related to the flow rate of smoke through the mouthpiece. Smokers insert a cigarette in the device, smoke the cigarette as normal and, when finished, withdraw the cigarette butt from the device. The CReSSmicro[®] stores smoking topography data that are later downloaded for analysis. Recorded data were checked for consistency, invalid data removed and average values of puff duration and puff flow across all cigarettes smoked over 24 hours computed.

Demographic and smoking characteristics

The baseline questionnaire assessed participants' cigarette consumption, smoking history and quit plans, as well as general demographic information. Smokers of RYO tobacco were also asked to indicate whether they used filters when rolling cigarettes. Deprivation level was determined using the Index of Multiple Deprivation (IMD), a reliable measure of relative poverty based on post codes (Jordan et al., 2004) and participants' self-reported height and weight were used to calculate the body mass index (BMI in kg/m²).

Analysis

Data were analysed with SPSS 16.0. Group differences in baseline variables were assessed with the chi-square test for dichotomous data, and t-test for continuous variables. As the distribution of metabolite concentrations was positively skewed and showed significant heteroscedasticity between FM and RYO cigarette smokers, a general linear model (GLM) with a gamma distribution and robust estimates was fitted to determine group differences in Total NNAL and 1-HOP. In order to control for possible confounders, socio-demographic variables, puffing behaviour and cotinine as well as interaction terms were entered as covariates in the GLM.

RESULTS

Smokers of hand-rolling tobacco were more likely to be male ($\chi^2(1)=6.6$, $p=.01$), consumed more cigarettes ($t(153)=2.3$, $p=0.022$) and, while not significant, had somewhat higher baseline cotinine levels. There were no other differences in terms of socio-demographic or smoking characteristics between RYO and FM cigarette smokers (Table 1).

Table 1 about here

Table 2 provides the concentrations of the creatinine-adjusted carcinogen metabolites for smokers of hand-rolling tobacco and manufactured cigarettes. Whilst 1-HOP levels were somewhat higher in RYO than FM cigarette smokers, no significant differences in 1-HOP or Total NNAL concentrations were detected between smokers of either type of cigarette.

Table 2 about here

Nearly two thirds of RYO smokers ($N=18$) used a filter for rolling cigarettes while the remaining third used either a roach (piece of card board) or nothing. There were no differences in urinary metabolites among RYO smokers or between FM and RYO smokers as function of filter use. However, 1-HOP levels were somewhat elevated among RYO smokers using a filter as compared with those not using a filter (Table 2).

As this sample was not randomly sampled, we used a general linear model to control for putative behavioural and biological confounders that may influence carcinogen exposure and metabolism. The model adjusted for socio-demographic and anthropometric factors

(age, sex, body mass index) as well as a biomarker of nicotine intake (cotinine). As a previous study indicated that RYO compared with FM cigarettes smokers puff cigarettes less hard but for longer (Shahab et al, 2008), markers of puffing intensity (puff flow and puff duration) were also included in this model. Lastly, while cotinine levels significantly increased from baseline to follow-up ($t(154)=4.3$, $p<0.001$), there was no significant change in model estimates if either baseline or follow-up cotinine levels were entered and average cotinine values are therefore used in the analysis. Table 3 provides the final model.

Table 3 about here

Confirming the univariate analysis, results suggest that there was no effect of the type of cigarette smoked on creatinine-adjusted 1-HOP and Total NNAL concentrations.

However, cotinine level had a significant association with 1-HOP and Total NNAL concentrations; higher cotinine concentrations were associated with a greater urinary concentration of carcinogen metabolites. In addition, there was a main effect of sex on 1-HOP and Total NNAL levels. As men were more likely than women to smoke RYO cigarettes, we included a cigarette type by sex interaction term in the GLM to explore this further. No significant interaction was observed for either 1-HOP or Total NNAL levels.

As shown in Figures 1 and 2, for both men and women there was a tendency towards higher 1-HOP concentrations among smokers of RYO tobacco than FM cigarettes but virtually identical levels of Total NNAL. Moreover, irrespective of the cigarette type smoked and the metabolite that was assessed, concentrations, and thus likely carcinogenic exposure, were greater among women than men.

Figures 1 and 2 about here

DISCUSSION

This is the first study comparing carcinogen metabolites in vivo in smokers of RYO and FM cigarettes. Our data suggest a similar intake of the carcinogens concerned. The relatively greater level of 1-HOP but not Total NNAL in RYO smokers may be due to a combination of behavioural factors and product design differences; smokers of hand-rolling tobacco are more likely to relight their cigarette (thus increasing the level of combustion-related carcinogens such as benzo(a)pyrene) and RYO cigarettes tend to have inferior filtering to FM cigarettes. Indeed, the use of filters by RYO smokers if anything appeared to increase 1-HOP levels compared with the levels in smokers of unfiltered RYO cigarettes. Moreover, as RYO smokers in contrast to smokers of manufactured cigarettes can manipulate the physical characteristics of their cigarettes, they are potentially exposed to a wider range of carcinogen yields than FM cigarette smokers (Kaiserman and Rickert, 1992b).

There was a marked sex difference in carcinogen metabolites; women had higher concentrations of 1-HOP and Total NNAL than men irrespective of the cigarette type smoked. This result is consistent with studies reporting that women may be at greater risk to develop lung cancer than men when controlling for consumption (Zang and Wynder, 1996; Kreuzer et al., 2000; Henschke and Miettinen, 2004; but see Bain et al., 2004). Moreover, in agreement with the results reported here, it has been suggested that women have a greater expression of particular polymorphisms in genes that are likely to be involved in metabolising tobacco-related carcinogens (CYP1A1 and GSTM1) leading to increased lung cancer susceptibility (e.g. Dresler et al., 2000; Mollerup et al., 2006).

The study has a number of limitations. The sample was self-selected and relatively small, particularly the pool of RYO smokers. However, the socio-demographic profile of

RYO smokers was relatively similar to that of RYO smokers in the population as shown by a greater number of men than women smoking RYO tobacco (Goddard, 2006), 78.6% of RYO smokers in our sample were male. We did not measure *in vivo* tobacco carcinogen levels directly but rather assessed metabolites of carcinogens. Yet, these metabolites are generally considered to be reliable biomarkers of carcinogen exposure and are therefore unlikely to have biased results (Hecht, 2002). Lastly, considering that tobacco smoke is thought to contain around 60 human carcinogens (International Agency for Research on Cancer, 2004), this study does not allow us to make any statements as to whether RYO and FM or male and female smokers are exposed to different levels of carcinogen *in toto*. However, we purposefully selected biomarkers of those carcinogens (PAH and TSNA) that count among the best characterized of all tobacco carcinogens and are likely to play a major role in the development of smoking-related cancers (Hecht, 1999). Moreover, the fact that observed sex-differences were found for the metabolites of both these groups of carcinogens would confer some degree of generalisability of this finding to other tobacco-related carcinogens.

While there is some variation from country to country (Young et al., 2008), many smokers tend to think that RYO cigarettes are more 'natural' and therefore may confer some health benefit over FM cigarettes (O'Connor et al., 2007; Young et al., 2006). Our results suggest that this is not the case. This finding highlights that greater health education efforts are needed to make smokers of all tobacco products, including hand-rolled cigarettes, aware that there is no completely safe alternative to smoking cessation. Very little is currently known as to why women may have a greater risk to develop smoking-related diseases, and more research is now needed to confirm findings reported here and expand our knowledge of the impact of increasingly popular RYO cigarettes on carcinogenic exposure and ultimately health.

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Tables

Table 1: Baseline socio-demographic and smoking characteristics

	All smokers (N=155)	FM (N=127)	RYO (N=28)
<i>Demographic data</i>			
Mean (SD) age	31.8 (10.9)	31.4 (10.5)	33.4 (12.3)
Percent (N) male	56.8 (88)	52.0 (66)	78.6 (22)*
Mean (SD) IMD	31.7 (13.1)	31.9 (13.4)	30.4 (11.6)
Mean (SD) BMI	24.0 (4.0)	24.1 (4.1)	23.3 (3.5)
<i>Smoking data</i>			
Mean (SD) cigarettes per day	13.9 (5.9)	13.4 (5.7)	16.1 (6.4)*
Mean (SD) length of time of smoking in years	14.5 (11.2)	14.1 (10.8)	16.2 (12.9)
Percent (N) smoking marijuana	36.8 (57)	36.2 (46)	39.3 (11)
Percent (N) Want to quit next month	10.3 (16)	11.0 (14)	7.1 (2)
Mean (SD) Baseline Salivary Cotinine (ng/ml)	272 (154)	265 (153)	305 (158)

IMD: Index of multiple deprivation; BMI: Body-mass index; *p<0.05

Table 2: Level of carcinogen metabolites by cigarette type

	All smokers (N=155)	Factory made (N=127)	Roll-Your-Own		
			Total (N=28)	Filter (N=18)	No Filter (N=10)
<i>Carcinogen metabolites</i>					
Mean (S.D.) in pmol/mg creatinine					
1-HOP	1.58 (1.61)	1.46 (1.25)	2.10 (2.69)	2.36 (3.05)	1.62 (1.95)
Total NNAL	0.60 (0.45)	0.62 (0.47)	0.53 (0.32)	0.50 (0.36)	0.58 (0.22)

Table 3: General Linear Model for carcinogen metabolites controlling for confounders

	1-HOP			Total NNAL		
	B	95%CI	p	B	95%CI	p
Cigarette type*	0.530	-0.739 – 1.800	0.413	-0.008	-0.120 – 0.104	0.892
Sex [^]	0.679	0.214 – 1.145	0.004	0.117	0.015 – 0.219	0.024
BMI	-0.030	-0.085 – 0.026	0.294	-0.001	-0.011 – 0.009	0.807
Age	0.000	-0.019 – 0.019	0.986	0.005	-0.002 – 0.011	0.162
Puff flow	-0.030	-0.067 – 0.007	0.107	-0.003	-0.010 – 0.004	0.445
Puff duration	0.000	-0.001 – 0.000	0.148	0.000	0.000 – 0.000	0.579
Cotinine	0.002	0.000 – 0.003	0.037	0.002	0.001 – 0.002	<0.001

*Reference category: factory-made cigarettes; [^]Reference category: men; BMI: Body-mass index

Figure Legend

Figure 1: ^Estimated marginal means adjusted for covariates (age, body mass index, puff flow, puff duration, cotinine); 1-HOP – 1-hydroxypyrene; FM – Factory-made; RYO – Roll-your-own; Error bars indicate 95% confidence interval of the mean

Figure 2: ^Estimated marginal means adjusted for covariates (age, body mass index, puff flow, puff duration, cotinine); Total NNAL – 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; FM – Factory-made; RYO – Roll-your-own; Error bars indicate 95% confidence interval of the mean

Figure 1: 1-HOP levels in men and women by cigarette type

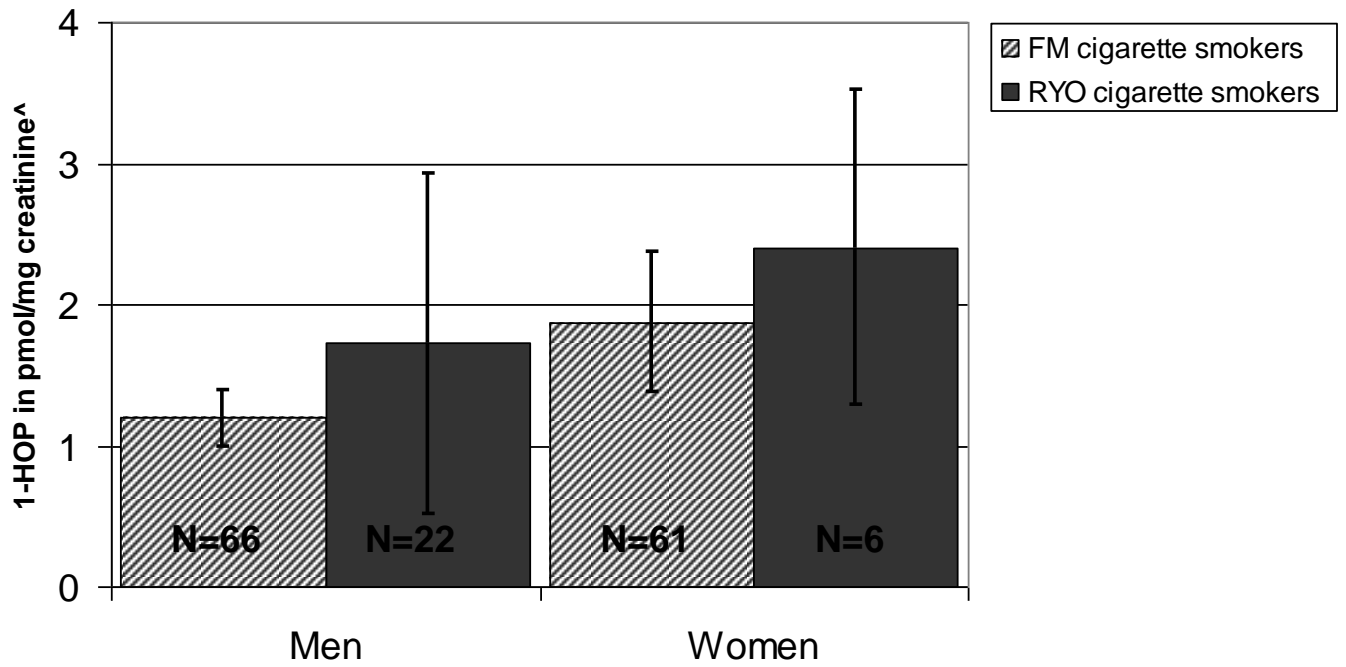


Figure 2: Total NNAL levels in men and women by cigarette type

