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A BRIEF ORIGINAL CONTRIBUTION

Collecting Saliva Samples by Mail

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Collecting saliva samples by mail can serve numerous purposes in epidemiologic research. The objectives of this study were to assess what proportion of participants in a mail survey would provide a saliva sample and whether incentives could improve participation. In 1995, 2,994 students, faculty, and staff members of Geneva University, Geneva, Switzerland, were randomized to receive, together with a mailed questionnaire about smoking, a saliva vial, a ballpoint pen, the offer of a lottery, or any combination of these. After one mailing and a reminder letter, response rates were 52% among those who had been requested to provide saliva and 63% among controls ($p < 0.001$). In the former group, most respondents (98%) provided a saliva sample. Incentives improved participation only among those who were asked to provide saliva (lottery: +11% response, $p = 0.003$; pen: +6% response, $p = 0.1$). The final participation, after up to three reminders, was 76% overall. The authors conclude that while the collection of saliva samples by mail is feasible it tends to decrease response rates. *Am J Epidemiol* 1998;147:141–6.

cotinine; data collection; incentives; postal service; randomized controlled trials; saliva; smoking; specimen handling

Collecting saliva samples by mail can serve numerous purposes in epidemiologic research, including measurement of cotinine, a metabolite of nicotine (1–3). Cotinine is stable in unrefrigerated saliva (4), even when samples are collected by mail and remain unfrozen for 12 days (5, 6). Collection of saliva samples by mail costs four times less than collection at the participant's workplace (6). Other substances that are stable in unrefrigerated saliva and could be collected by mail include thiocyanate (7, 8), genetic materials (9), testosterone (10), and human immunodeficiency virus (HIV) antibodies (11, 12).

Despite the potential benefits of collecting saliva samples by mail, to our knowledge, only studies of technical feasibility have so far been conducted (7–11). A possible concern is that requesting saliva samples may reduce response rates, but no evidence exists regarding this issue (13). We aimed to establish what proportion of potential respondents to a mailed survey about smoking would agree to provide a saliva sample, and whether requesting saliva affects the response rate.

Various incentives may increase response to mailed surveys (14), such as offering a pen (15–17) or participation in a lottery (18–22). Our second aim was to assess whether response rates could be increased by offering participation in a lottery and/or a ballpoint pen.

Finally, asking participants to provide saliva or offering incentives could bias results, either by modifying response rates in population subgroups (selection bias) or by altering respondents' answers (information bias). Our third objective was to establish whether such procedure-related bias occurred.

MATERIALS AND METHODS

Study setting and population

The study population ($n = 9,686$) included students (82 percent), faculty (12 percent), and administrative and technical staff (6 percent) of the University of Geneva, Geneva, Switzerland. A simple random sample of 3,000 persons was drawn from the university files. This file indicated the address, birth date, and sex of each participant. The study was approved by the review board of the Institute of Social and Preventive Medicine of the University of Geneva.

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Abbreviation: HIV, human immunodeficiency virus.

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Study design

Potential participants were randomized in a three-way factorial trial of the following interventions: 1) request to provide a saliva sample, 2) inclusion of a ballpoint pen (value <$1), and 3) offer to participate in a lottery if the questionnaire was returned. Lottery prizes were a weekend in Paris for two persons (valued at $540) and nine vouchers for books (valued at $40 each). Randomization resulted in groups of similar age (mean age, 29 years) and sex (men, 42 percent). After deletion of six names that appeared twice, the survey package was mailed to 2,994 persons in November 1995.

Data collection

The initial mailing contained the questionnaire, a cover letter explaining the purpose of the study, and a business reply envelope. Questions covered smoking history, exposure to environmental tobacco smoke, health status (SF-36 "general health" scale (23, 24)), stages of change of smoking (25) and, for smokers only, the pros and cons of smoking (26), level of nicotine dependence (27), and smoking self-efficacy (28). The date when the questionnaire was mailed back was assessed by the post office date of cancellation on the envelope.

Saliva was collected in a plastic vial containing a sterile dental cotton roll (Salivette, Sarstedt, Nümbrecht, Germany, article no. 511534). The cover letter indicated that nonsmokers too should provide saliva, because exposure to environmental tobacco smoke was of interest. We planned to collect saliva only in the initial mailing, because our resources allowed laboratory analysis only of a limited number of samples.

A reminder postcard was sent 2 days after the first mailing (29), and a reminder letter was sent to all nonrespondents after 15 days, without the questionnaire or saliva vial. The randomized intervention was interrupted on day 28 of data collection. To maximize the final response rate, all reminder packages sent to nonrespondents after day 28 included both incentives. Data collection ended 121 days after the initial mailing, when up to four survey packages had been sent to nonrespondents.

Analysis

Separate analyses were conducted for the "randomized phase" (days 1–28), the "most effective intervention phase" (days 29–121), and overall (days 1–121). At the end of the "randomized phase," we assessed selection bias by comparing response rates by age and sex across randomized groups; interactions between demographic variables and incentives were tested in logistic regression models with interaction terms (30). We also examined procedure-related bias by comparing respondent characteristics across randomized groups. We also compared participants who responded during the "randomized phase" and the "most effective intervention phase." Finally, we compared delays between the initial mailing and the return of the questionnaire among subgroups of respondents. Participation was described using Kaplan-Meier curves (30).

TABLE 1. Response rates to a mailed questionnaire in a three-way factorial randomized trial of requesting a saliva sample, offering a lottery and a ballpoint pen, Geneva, Switzerland, 1995

Incentives	Randomized intervention phase (days 1–28)				No request for saliva; lottery and pen included for all (days 29–121)				Overall (days 1–121)		
	Total	No. who responded	%	*p* value	Total	No. who responded	%	*p* value	No. who responded	%	*p* value
All participants	2,994	1,733	57.9	–	1,173	523	44.6	–	2,272	75.9	–
Saliva vial											
Yes	1,496	785	52.5	<0.001	661	262	39.6	<0.001	1,055	70.5	<0.001
No	1,498	948	63.3		512	261	51.0		1,217	81.2	
Ballpoint pen											
Yes	1,497	889	59.4	0.10	567	239	42.2	0.10	1,134	75.8	0.87
No	1,497	844	56.4		606	284	46.9		1,138	76.0	
Lottery											
Yes	1,497	902	60.3	0.009	559	242	43.3	0.39	1,152	76.9	0.17
No	1,497	831	55.5		614	281	45.8		1,120	74.8	
Incentive group											
1. Saliva vial + lottery + pen	365	209	57.3	0.001*	148	52	35.1	0.05*	261	71.5	0.53*
2. Saliva vial + pen	361	185	51.2	0.11*	161	58	36.0	0.06*	246	68.1	0.71*
3. Saliva vial + lottery	391	219	56.0	0.003*	162	65	40.1	0.28*	285	72.9	0.28*
4. Saliva vial	379	172	45.4	<0.001†	190	87	45.8	0.14†	263	69.4	<0.001†
5. Lottery + pen	374	242	64.7	0.35†	122	62	50.8	0.58†	307	82.1	0.66†
6. Pen	397	253	63.7	0.51†	136	67	49.3	0.41†	320	80.6	0.94†
7. Lottery	367	232	63.2	0.61†	127	63	49.6	0.45†	299	81.5	0.83†
8. None	360	221	61.4	–	127	69	54.3	–	291	80.8	–

* Difference with group 4 (saliva vial only).
† Difference with group 8 (no incentive or saliva vial).

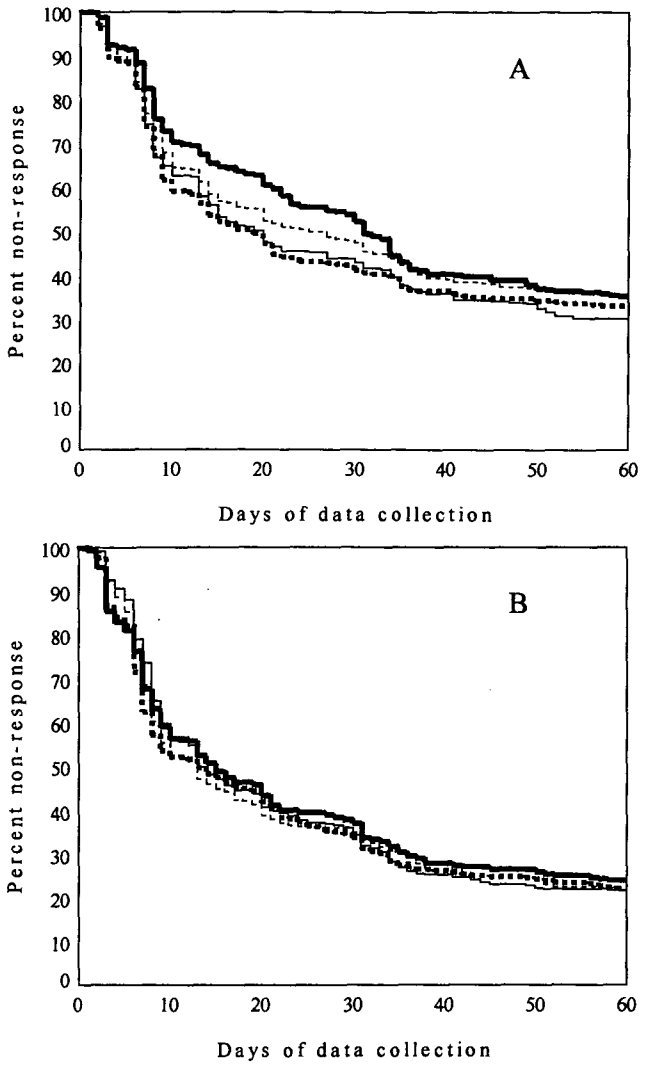

FIGURE 1. Cumulated nonresponse rate to a mail survey in eight randomized groups, Geneva, Switzerland, 1995. The individuals in the eight groups were requested to provide a saliva sample (A) or no saliva sample (B), and received the following incentives: lottery and ballpoint pen (heavy dashed line), lottery only (light solid line), pen only (light dashed line), or no incentive (heavy solid line).

RESULTS

Response rates

During the "randomized phase," including a saliva vial in the survey package decreased the response rate by 11 percent (table 1). Almost all participants in the saliva vial group (769 out of 785 (98 percent)) also returned the saliva sample.

In the saliva vial group, during the "randomized phase," both the lottery (+11 percent) and the pen (+6 percent) improved response rates, but this trend lacked statistical significance. Cumulative incentives was hardly better than the lottery alone (+1 percent). When no saliva sample was requested, incentives had no statistically significant effects on response rates (table 1).

During the "most effective intervention phase," persons who had received a saliva vial with the first survey package had persistently lower response rates, even when they were no longer reminded to provide saliva. Persons who received incentives for the first time on day 29 had higher response rates that persons who had already received these incentives with the first survey package.

Response delay among respondents

Respondents in the saliva vial group returned the questionnaire on average 2 days later than controls (19 vs. 17 days, $p = 0.009$) (figure 1). The lottery accelerated response only in the saliva vial group (−2.6 days, $p = 0.03$). Effects of the pen, age, and sex were nonsignificant.

Incentive-related selection bias. In the "randomized phase," increasing age was significantly negatively associated with response rate, regardless of incentive group (table 2). The impact on response rates of a saliva sample request was stronger in women (−13.7 percent) than in men (−6.7 percent, between-sex difference; $p = 0.04$). The response rate was similar in men and women in the saliva vial group, but it was lower in men than in women in the control group. The lottery and the pen had similar effects on response in men and women and across age groups.

Procedure-related bias. At the end of the "randomized phase," respondents in the saliva vial group were similar to controls in terms of age, sex, level of education, health status, proportion of smokers, and stages of change (table 3). Among smokers, the two groups had similar scores on the nicotine dependence test, the pros and cons of smoking, and self-efficacy. Smokers who had been asked to provide saliva reported smoking on average 1.4 fewer cigarettes per day than controls, but the difference was not statistically significant. Answers were similar in groups defined by receipt of incentives (data not shown).

Early vs. late respondents

The proportion of smokers was 6 percent lower among persons who returned the questionnaire during the "randomized phase" than among persons who returned it later (table 3). In all other respects, early and late respondents were similar.

DISCUSSION

In a mail survey of university students, faculty, and staff, half the potential participants provided a saliva sample after an initial mailing, reminder postcard, and

TABLE 2. Response rates to a mailed questionnaire after one mailing and a reminder letter ("randomized phase"), according to sex, age, and three randomly allocated incentives, Geneva, Switzerland, 1995

	Sex			Age	
	Men	Women	*p* value*	Change in response rate†	*p* value
All participants	54.2	60.6	<0.001	−4.6	<0.001
Saliva vial					
Yes	50.9	53.7	0.29	−4.7	0.02
No	57.6	67.4	<0.001	−4.4	0.01
Ballpoint pen					
Yes	55.7	62.1	0.01	−3.4	0.08
No	52.6	59.1	0.01	−5.8	0.002
Lottery					
Yes	57.6	62.2	0.07	−3.8	0.04
No	50.8	58.9	0.002	−5.6	0.006
Incentive groups					
1. Saliva vial + lottery + pen	55.4	58.5	0.55	−5.1	0.07
2. Saliva vial + pen	48.1	53.7	0.29	−1.7	0.54
3. Saliva vial + lottery	57.1	55.1	0.68	−5.3	0.04
4. Saliva vial	42.1	47.6	0.29	−6.6	0.02
5. Lottery + pen	60.0	68.2	0.10	−1.7	0.52
6. Pen	59.2	67.1	0.10	−3.4	0.20
7. Lottery	57.7	67.0	0.07	−8.0	0.003
8. None	53.0	67.3	0.006	−4.9	0.08

* Chi-square test.
† Change in response rate (percent) for a 10-year increase in age (linear regression).

TABLE 3. Impact of request to provide a saliva sample on the information obtained from a mailed questionnaire among persons who returned the questionnaire during the "randomized phase," Geneva, Switzerland, 1995

Respondent characteristics	Randomized phase			Most effective intervention phase	
	Requested to provide a saliva sample		*p* value*	All	*p* value†
	Yes	No			
No.	785	948	–	539	–
Men (%)	41.0	38.0	0.20	43.6	0.09
Mean age (years)	28.4	28.6	0.63	28.6	0.89
Education (years)	17.3	17.3	0.87	17.7	0.05
SF-36 "general health" scale‡	76.3	76.2	0.98	75.3	0.29
Stages of change			1.0		0.21
Precontemplation (%)	46.2	46.9		49.4	
Contemplation (%)	12.5	12.8		15.4	
Preparation (%)	5.0	4.9		5.7	
Action (%)	12.9	12.2		13.4	
Maintenance (%)	23.4	23.2		16.2	
Current smokers (%)	27.1	27.9	0.69	33.8	0.005
Among current smokers					
Cigarettes per day	10.7	12.2	0.07	11.4	0.84
Nicotine dependence§	1.85	2.04	0.36	1.91	0.79
Pros of smoking¶	50.0	50.7	0.48	49.1	0.14
Cons of smoking¶	49.8	50.4	0.57	49.8	0.68
Self-efficacy¶	50.8	49.4	0.08	50.7	0.26
Missing answers (%)	11.8	11.9	0.86	11.7	0.82

* *p* value on difference between saliva and no-saliva groups during randomized phase.
† *p* value on difference between persons who returned the questionnaire during the randomized phase and persons who returned it during the best intervention phase.
‡ Scaled between 0 (worst) and 100 (best) (23, 24).
§ Fagerström test, mean score between 0 (least dependent) and 10 (most dependent).
¶ Mean scores, standardized to mean = 50 and standard deviation = 10.

reminder letter. Requesting a saliva sample reduced the initial response rate to the questionnaire; because we asked for a saliva sample only in the first mailing, we do not know how much repeated requests for saliva would have affected the final response rate. When a saliva sample was requested, offering a pen or participation in a lottery increased returns of questionnaires and saliva vials.

The request for a saliva sample may reduce participation for several reasons. First, asking for a biologic sample may irretrievably antagonize some potential participants. This hypothesis is suggested by persistently lower participation rates among persons who had received a saliva vial in the first mailing, even after requests for saliva samples were discontinued. A better explanation of the purpose of the study may alleviate this problem. Second, some persons may have been concerned about confidential handling of the samples (for instance, some may have feared that drug or HIV tests would be performed). We have no evidence that this occurred, but better explanations and an anonymous data collection procedure may minimize such concerns. Third, the fact that incentives were effective only in the saliva group suggests that some people failed to return the saliva vial because they did not receive any compensation in exchange for their collaboration. Only this barrier can be overcome by appropriate incentives. However, what incentives to choose remains an open question.

Results observed among persons who were not requested to provide saliva are consistent with previous studies which showed that a lottery ticket (18) and a raffle (19) have little influence on response rates, but conflict with the findings of others (20–22). Inconsistencies between studies suggest that incentives should be tailored to particular study groups. The choice of incentives may be optimized by appropriate pretests (20, 31). One option worth exploring is whether promising to inform participants of their laboratory result would increase their willingness to provide a saliva sample.

In view of the reduced participation caused by saliva sample requests, an important result is that such a request did not cause bias, particularly regarding smoking-related information. In adolescent populations, saliva collection is sometimes used to increase the sincerity of answers (32, 33); in the adult population that we studied, this would be unnecessary. The selection bias associated with the timing of response (higher proportion of smokers among late respondents) was unrelated to saliva sample requests.

Several features of this study may limit its generalizability. First, we studied a highly educated population, and participation in mail surveys increases with education level (34, 35). Therefore, our response rates were probably higher than those that could be expected in general population surveys. Moreover, the survey was sponsored by a university, which also increases participation (36, 37). Finally, populations of other countries may react differently to request for saliva samples, for various cultural reasons. Nevertheless, the feasibility of collecting saliva samples by mail opens promising perspectives in epidemiologic research.

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