Acute and Short-term Effects of Secondhand Smoke on Lung Function and Cytokine Production

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Rationale: The acute effect of secondhand smoke (SHS) on lung function and the duration of system disruption remain unknown.

Objectives: To assess the SHS effects and their duration on lung function and inflammatory markers.

Methods: In a randomized single-blind crossover experiment data were obtained from 16 (8 women) nonsmoking adults at baseline and at 0, 1, and 3 hours after a 1-hour SHS exposure set at bar/restaurant SHS levels.

Measurements and Main Results: Serum and urine cotinine, lung function, and cytokines IL-4, IL-5, IL-6, tumor necrosis factor (TNF)-α, and IFN-γ. At 0 hours most lung function parameters were significantly reduced (indicative: FEV_1, 4.3 ± 0.4 vs. 3.8 ± 0.3 L; FEV_1/FVC, 0.9 ± 0.1 vs. 0.8 ± 0.1; $P < 0.05$) but at 3 hours they were at baseline levels. In contrast, cotinine (serum, 8.9 ± 3.2 vs. 35.5 ± 10.2 ng·ml^{-1}), IL-4 (41.3 ± 5.8 vs. 44.2 ± 4.5 pg·ml^{-1}), IL-5 (36.1 ± 3.2 vs. 60.1 ± 7.0 pg·ml^{-1}), IL-6 (2.5 ± 0.3 vs. 7.6 ± 1.4 pg·ml^{-1}) and IFN-γ (0.3 ± 0.2 vs. 0.6 ± 0.2 IU·ml^{-1}) at 3 hours were higher than at baseline ($P < 0.05$). IL-4 and TNF-α increased only in men, whereas IL-5, IL-6, and IFN-γ were different between sexes after exposure ($P < 0.05$). Regression analyses revealed inverse associations of FEV_1 and FEV_1/FVC ratio with IL-5 ($P < 0.05$) in men and with IL-5 ($P = 0.01$), IL-6 ($P < 0.001$), IFN-γ ($P = 0.034$) and serum cotinine ($P < 0.001$) in women.

Conclusions: We conclude that 1 hour of SHS exposure at bar/restaurant levels is accompanied by significant decrements on lung function and marked increases in inflammatory cytokines, particularly in men. More importantly, whereas most smoke-induced effects on lung function appear to recede within 60 minutes, inflammatory cytokines remain elevated for at least 3 hours after exposure to SHS.

Keywords: passive smoking; cotinine; respiration; inflammatory markers

> *In the 10 minutes you will spend reading this article, 111 people will die somewhere in the world from tobacco-induced illnesses.* Fourteen of them have never smoked.†*

An overwhelming amount of evidence has emerged over the past decades on the adverse health effects of secondhand smoke

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Epidemiological studies have identified the long-term detrimental consequences of secondhand smoke (SHS), but the acute SHS effects and their duration, as well as the underlying physiological mechanisms, remain unexplored.

What This Study Adds to the Field

A 1-hour moderate SHS exposure is accompanied by significantly deteriorated lung function and marked increases in inflammatory cytokines, particularly in men. Although most SHS-induced effects on lung function appear to recede within 60 minutes, inflammatory cytokines remain elevated for at least 3 hours following SHS exposure.

(SHS) (1–5). Nevertheless, more than 126 million American and 130 million Chinese adult nonsmokers suffer daily SHS exposure, whereas global estimates include 700 million children and 50 million pregnant women (1). Latest reports show that, despite current measures, the prevalence rates of smoking are increasing (2, 3), while the tobacco industry predicts a global expansion of the tobacco epidemic in the near future (3). Moreover, arguments are being expressed that only chronic exposures to SHS represent a health risk and that there is no scientific basis for claims that brief, acute, transient SHS exposures represent a significant acute health hazard in nonsmokers (6). This is, in part, because our knowledge on the effects of SHS is based predominantly on longitudinal epidemiological studies, whereas experimental studies assessing the acute and short-term effects of SHS are scarce. Yet, the latter are essential and, comparatively, more important in elucidating the underlying physiological mechanisms involved in SHS-induced system disruption (7, 8).

Chronic lung disease is generally the result of long-term processes, yet, even brief SHS exposures appear to initiate mechanisms that contribute to its development. For instance, shortly after the beginning of smoke exposure there is a rapid and marked up-regulation of growth factor production as well as production of type 1 procollagen in the small airways (9). Furthermore, spontaneous inhalation of cigarette smoke elicits acute pulmonary chemoreflexes, characterized by apnea, bradycardia, and hypotension through activation of pulmonary C fibers (10). These findings are in line with experimental data from our (11–14) and other (15–17) laboratories showing that even brief SHS exposures generate unfavorable changes in various cardiovascular, endocrine, and immune mechanisms. Additionally, human (12) and animal (18) models suggest an
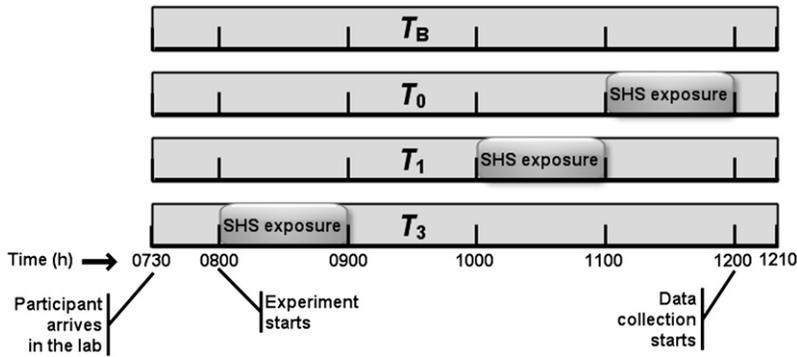
Figure 1. Overview of the experimental design: In the baseline trial (T_B) participants' data were collected at 1200 hours without any secondhand smoke (SHS) exposure. In the remaining trials, participants were exposed to SHS for 1 hour either at 0800 hours (T_3), 1000 hours (T_1) or at 1100 hours (T_0), while data were collected at 1200 hours. SHS = secondhand smoke.

association between SHS exposure and elevated proinflammatory cytokines, which may be linked to the development and/or exacerbation of chronic lung disease. However, the precise effects of SHS on the respiratory system and cytokine production remain elusive. Given the role of inflammation in the development of impaired respiratory function, understanding the acute SHS-induced respiratory and immune responses is crucial (19) because SHS is associated with an increased prevalence of chronic lung diseases and allergies (20). Notably, the duration of system disruption after brief SHS exposures has yet to be elucidated. We conducted a randomized single-blind crossover experiment to assess lung function and cytokine responses before as well as 0, 1, and 3 hours after a 1-hour moderate exposure to SHS.

METHODOLOGY

Participants and Procedures

The experimental protocol was approved by the ethical review board at the University of Thessaly. Sixteen healthy adults (8 men, 8 women, aged 27.2 ± 4.3 yr; body mass index, 22.0 ± 1.8) volunteered. Exclusion criteria included smoking, pregnancy, evidence of cardiac or pulmonary disease, and previous disease and medications known to affect lung function. All women participants were premenopausal with regular menstruation and were tested during the late luteal phase of their menstrual cycle.

Experimental Design

Participants visited the laboratory at 0730 hours for four trials conducted in a random order and separated by 7 days. In the baseline trial (T_B) participants were assessed for cotinine, lung function, and cytokine levels without any SHS exposure precisely at 1200 hours. In the remaining trials, participants were exposed to SHS for 1 hour either at 0800 hours (T_3), 1000 hours (T_1), or at 1100 hours (T_0), while cotinine, lung function, and cytokine levels were assessed at 1200 hours as illustrated in Figure 1. *See* the online supplement for further details.

All testing was conducted by the same trained investigators, who were unaware of the specific trial each participant was undergoing. To ensure that the investigators would not be able to differentiate between trials, participants were given a shirt and athletic pants to wear upon exiting the chamber after all trials. These clothes had been previously exposed to SHS inside the chamber and emitted a strong tobacco scent.

The present is a new experiment, yet methods regarding the SHS exposure as well as measurements for serum and urine cotinine and cytokine production have been used previously in our laboratory and are described in detail elsewhere (11, 12). Further detail on the method for making these measurements is provided in the online supplement.

Lung Function

FVC, FEV_1, FEV_1/FVC ratio, peak expiratory flow (PEF), as well as maximum expiratory flow (MEF) when 75%, 50% and 25% of FVC remains in the lungs ($MEF_{75\%}$, $MEF_{50\%}$, and $MEF_{25\%}$, respectively) were measured using a hand-held spirometer (Spiromed 180, Fukuda Sangyo, Pulmonary Products, Tokyo, Japan) calibrated before each use. The lung function assessment protocol conformed to the American Thoracic Society recommendations (21).

Statistical Analysis

Given the significant sexual dimorphism in the acute SHS effects (12), participants' data were divided by sex into groups. A two-group (men, women) by four-times (T_B, T_0, T_1 and T_3) factorial analysis of variance (ANOVA), followed by sex-specific post-hoc t tests incorporating a Bonferroni adjustment, was used to assess the effect of sex and time after SHS on all the examined variables. Given the statistically significant effect of SHS on FEV_1 and the FEV_1/FVC ratio (see RESULTS), sex-specific stepwise multiple linear regression analyses incorporating backward elimination at the $P < 0.05$ level were introduced to model the effect of cotinine and cytokine concentrations (independent variables) on these lung function parameters (dependent variables). The level of significance was set at $P < 0.05$ except for post-hoc tests in which a Bonferroni adjustment was applied. Additional detail on the statistical analyses is provided in the online supplement.
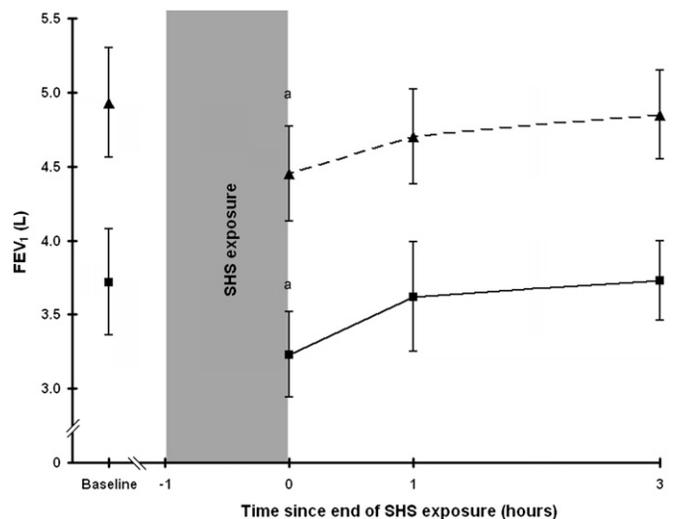

Figure 2. Mean ± SD of FEV_1 for men (*triangles*) and women (*squares*) in each trial. Values dropped 9.7% in men and 13.2% in women between T_B and T_0. a = significant ($P < 0.05$) difference from previous trial (timepoint).

TABLE 1. LUNG FUNCTION AND TIME COMPARISONS FOR MEN AND WOMEN IN EACH TRIAL

		T_B	T_0	T_1	T_3
FVC, L	M	6.0 ± 0.7	6.0 ± 0.7	6.0 ± 0.8	5.9 ± 0.6
	W	4.0 ± 0.6	4.0 ± 0.6	4.0 ± 0.5	4.1 ± 0.5
FEV_1, L	M	4.9 ± 0.4	4.5 ± 0.3*	4.7 ± 0.3	4.9 ± 0.3
	W	3.7 ± 0.4	3.2 ± 0.3*	3.6 ± 0.4	3.7 ± 0.4
FEV_1/FVC	M	0.8 ± 0.1	0.7 ± 0.1*	0.8 ± 0.1	0.8 ± 0.1
	W	0.9 ± 0.1	0.8 ± 0.1*	0.9 ± 0.1*	0.9 ± 0.1
PEF, L·sec^{-1}	M	9.8 ± 0.7	9.5 ± 0.3	9.6 ± 1.0	9.7 ± 0.9
	W	7.8 ± 1.2	7.8 ± 1.2	8.1 ± 0.9	8.1 ± 0.9
$MEF_{75\%}$, L·sec^{-1}	M	8.0 ± 0.3	6.9 ± 0.4*	6.9 ± 0.6†	7.3 ± 0.7
	W	6.6 ± 0.4	6.0 ± 0.3*	6.3 ± 0.3	6.4 ± 0.4
$MEF_{50\%}$, L·sec^{-1}	M	5.8 ± 0.5	4.7 ± 0.5*	4.7 ± 0.8†	5.2 ± 0.9
	W	4.7 ± 0.3	4.1 ± 0.1*	4.4 ± 0.3	4.6 ± 0.3
$MEF_{25\%}$, L·sec^{-1}	M	2.8 ± 0.3	2.4 ± 0.3*	2.5 ± 0.3	2.6 ± 0.3
	W	3.1 ± 0.5	2.7 ± 0.2	2.7 ± 0.3	3.0 ± 0.3

Definition of abbreviations: M = men; $MEF_{75\%}$, $MEF_{50\%}$, $MEF_{25\%}$: maximum expiratory flow when 75%, 50% and 25% of FVC remains in the lungs, respectively; PEF = peak expiratory flow; TB = time at baseline; T_0, T_1, T_3 = time at 0, 1, and 3 hours, respectively; W = women.

Values are mean ± SD. All sex-comparisons were significant at $P < 0.05$ except $MEF_{50\%}$ at T_1 and T_3 and $MEF_{25\%}$ at T_B and T_1.

* Significant difference from previous trial (time-point); $P < 0.05$.

† Significant difference of T_1 and T_3 from T_B; $P < 0.05$.

TABLE 2. COTININE CONCENTRATIONS, CYTOKINE LEVELS, AND TIME COMPARISONS FOR MEN AND WOMEN IN EACH TRIAL

		T_B	T_0	T_1	T_3
Serum cotinine, ng·mL^{-1}	M	8.5 ± 2.5	27.4 ± 12.1*	39.9 ± 14.1†	38.0 ± 10.1†
	W	9.3 ± 3.6	21.8 ± 8.3*	35.6 ± 9.7*†	32.9 ± 10.3†
Urine cotinine, ng·ml^{-1}	M	6.4 ± 2.2	21.1 ± 10.6	29.6 ± 13.0†	29.4 ± 13.8†
	W	7.9 ± 3.3	22.0 ± 8.3*	30.5 ± 8.8†	28.3 ± 10.5†
IL-4, pg·ml^{-1}	M	44.9 ± 3.5‡	49.6 ± 4.3*	46.4 ± 4.5	47.0 ± 4.6†‡
	W	37.7 ± 6.4‡	43.8 ± 5.3	43.3 ± 6.1	41.4 ± 4.3‡
IL-5, pg·ml^{-1}	M	37.2 ± 6.8	69.7 ± 7.5*‡	79.7 ± 5.2*†‡	65.7 ± 5.8*†‡
	W	35.1 ± 1.4	56.0 ± 8.0*‡	66.4 ± 2.6*†‡	54.5 ± 7.6*†‡
IL-6, pg·ml^{-1}	M	2.4 ± 0.3	6.4 ± 2.5*	8.2 ± 1.3†	9.0 ± 1.5*†‡
	W	2.5 ± 0.4	5.8 ± 1.9*	7.4 ± 1.2†	6.3 ± 1.1*†‡
TNF-α, pg·ml^{-1}	M	9.3 ± 1.6‡	12.4 ± 2.4*‡	11.2 ± 2.1‡	8.4 ± 0.9*‡
	W	6.4 ± 1.5‡	8.0 ± 2.2d	7.4 ± 1.8‡	6.4 ± 1.2‡
IFN-γ, IU·ml^{-1}	M	0.3 ± 0.2	0.6 ± 0.2*‡	0.8 ± 0.2†‡	0.7 ± 0.1†‡
	W	0.3 ± 0.1	0.4 ± 0.1‡	0.6 ± 0.2†‡	0.5 ± 0.2†‡

Definition of abbreviations: M = men; TB = time at baseline; T_0, T_1, T_3 = time at 0, 1, and 3 hours, respectively; TNF-α: tumor necrosis factor-α; W = women.

Values are mean ± SD.

* Significant difference from previous trial (time-point); $P < 0.05$.

† Significant difference of T_1 and T_3 from T_B; $P < 0.05$.

‡ Significant difference between sexes for the same measurement; $P < 0.05$.

RESULTS

The factorial ANOVA ($R^2 = 0.799$; $_{adj}R^2 = 0.774$) revealed that the values of all variables except FVC ($P = 0.999$), PEF ($P = 0.889$), and IL-4 ($P = 0.079$) changed depending on the time of measurement (significant main effect: $F_{(36, 141)} = 6.687$; $P < 0.001$). The same analysis also showed that the values of all variables except IL-4 ($P = 0.218$) were different between sexes (significant main effect: $F_{(12, 45)} = 35.488$, $P < 0.001$). Furthermore, the aforementioned time-induced changes in cotinine, lung function, and cytokines were not the same for men and women (significant interaction effect: $F_{(36, 141)} = 2.127$; $P = 0.001$). For instance, IL-4 and tumor necrosis factor (TNF)-α increased after the SHS exposure only in men ($P < 0.05$), whereas in women there was no change across time ($P > 0.05$).

As expected, the sex-specific post-hoc t tests (Figure 2 and Tables 1 and 2) demonstrated an effect of SHS on pulmonary function characterized by an acute response at T_0 that was attenuated with longer exposure lags (T_1 and T_3). Specifically, although at T_0 the values of FEV_1, FEV_1/FVC ratio, $MEF_{75\%}$, $MEF_{50\%}$ and $MEF_{25\%}$ were markedly reduced ($P < 0.05$), at T_3 they were at levels similar to T_B ($P > 0.05$). In contrast, mean values at T_3 of cotinine levels and all inflammatory markers except TNF-α remained higher than T_B levels ($P < 0.05$). Notably, in some cases (e.g., IL-4, IL-6, and IFN-γ) the effect of SHS increased with greater lag.

The stepwise multiple linear regression analyses for FEV_1 demonstrated an inverse association with IL-5 ($\beta = -0.007$; $P = 0.05$) in men ($R^2 = 0.339$; $F_{(1, 30)} = 3.886$; $P = 0.05$) as well as inverse associations with IL-6 ($\beta = -0.16$; $P < 0.001$) and serum cotinine ($\beta = -0.025$; $P < 0.001$) in women ($R^2 = 0.645$; $F_{(3, 28)} = 6.661$; $P = 0.002$). The same analysis for FEV_1/FVC ratio revealed an inverse association with IL-5 ($\beta = -0.002$; $P = 0.022$) in men ($R^2 = 0.41$; $F_{(2, 29)} = 2.931$; $P = 0.05$) as well as inverse associations with IL-5 ($\beta = -0.003$; $P = 0.01$) and IFN-γ ($\beta = -0.144$; $P = 0.034$) in women ($R^2 = 0.47$, $F_{(2, 29)} = 4.102$; $P = 0.027$).

DISCUSSION

To the best of our knowledge, this is the first experiment to investigate the duration of the acute SHS effects on lung function and cytokine levels. Our results demonstrated that 1 hour of moderate SHS exposure generates significant decrements on lung function and marked increases in the vast majority of the cytokines investigated. More important, whereas most SHS-induced effects on lung function appear to recede within 60 minutes, inflammatory cytokines remain elevated for at least 3 hours after exposure.

Previous epidemiological studies have shown that chronic SHS is associated with a reduction of several lung function measures, yet these findings have not been consistent, and methodological issues have constrained interpretation of the findings (22). The present data show clearly a significant effect of acute SHS on FEV_1, FEV_1/FVC ratio, $MEF_{75\%}$, $MEF_{50\%}$, and $MEF_{25\%}$. The SHS-induced changes in FEV_1 and FEV_1/FVC ratio resemble closely the airway obstruction apparent in smokers (23), although the present FEV_1/FVC ratio at T_0 was 0.74 and not 0.70. This notion is further supported by the SHS-induced changes in $MEF_{75\%}$, $MEF_{50\%}$, and $MEF_{25\%}$, which show a MEF-volume curve convex to the volume axis with an increasing curve in late expiration. These results are typical for obstructive diseases like cystic fibrosis, bronchial asthma, and wheezy bronchitis (24). Furthermore, our findings confirm a previous epidemiological study suggesting that acute SHS was associated with symptoms of chronic bronchitis (25).

The mechanism underlying the airflow limitation observed immediately after the SHS exposure (i.e., T_0) may be related to SHS-induced airway irritation. Previous research has shown that cigarette smoke inhalation elicits irregular breathing patterns, cough reflex, and bronchoconstriction through activation of vagal afferents (26). On the other hand, recent evidence suggests that SHS induces rapid profibrotic growth factor production in the walls of small airways through an oxidant mechanism (9). These findings suggest that the initial response to cigarette smoke may reflect direct induction of growth factors resulting in airway remodeling. Fibrosis and thickening of the airway wall, particularly in the subepithelial compartment in the small airways, are an important part of the pathogenesis of SHS-induced airflow limitation, yet the precise effect of airway remodeling type on airflow obstruction remains to be determined (9).

The observed SHS-induced increase in circulating inflammatory markers extends the findings of a small number of previous human experiments reporting SHS-induced increases of IL-1β (12), white blood cell count, C-reactive protein, homocysteine, fibrinogen (17), as well as leukocyte counts accompanied by an activation of the immune cells (27). The present results also confirm another study by our group reporting an increased SHS-induced inflammatory reaction in men compared with women (12). In the present experiment we found that IL-4 and TNF-α increased only in men, while there was significant sexual difference in IL-5, IL-6 and IFN-γ after SHS exposure.

Given that the cytokines investigated are closely associated with the chronic lung inflammation and structural changes observed in pulmonary disease patients (28), it could be suggested that chronic SHS may have clinical implications—especially in men, given their increased inflammatory response—such as increased susceptibility to infection, chronic lung inflammation, as well as pathological airway changes including chronic obstructive pulmonary diseases. The present concentrations of IL-5, IL-6, and IFN-γ after SHS exposure were linked with the marked decreases in FEV_1 and FEV_1/FVC ratio, confirming the association between circulating inflammation markers and FEV_1 (29). In turn, lower FEV_1 and FEV_1/FVC ratio are associated with a greater prospective risk of cardiovascular mortality among nonsmokers (22). The current finding that inflammatory cytokine levels remain elevated for at least 3 hours after SHS exposure alludes to chronic low-grade systemic inflammation in individuals exposed to SHS on a daily basis and/or at higher smoke concentrations. This is particularly true for IFN-γ, which is closely linked with chronic obstructive pulmonary disease and asthma (30). At present, the physiological mechanisms linking low-grade systemic inflammation and pulmonary disease is not entirely understood (31). However, a number of studies have reported higher levels of systemic fibrinogen and C-reactive protein in individuals with impaired lung function (32) and in patients suffering from chronic obstructive pulmonary disease (33).

Prosmoking groups use the reduced sales of tobacco products in selected few countries to argue that smoking bans are not necessary. This delusion is accompanied by an equally pernicious myth that there is "no scientific basis for claims that brief, acute, transient exposure to secondhand smoke...represents any other significant acute...health hazard in nonsmokers" (6). Several studies referenced in the present article reject this argument. In fact, to our knowledge, all human exposure studies investigating the acute effects of SHS have reported significant detrimental results. With regard to the present experiment, it is important to note that the observed changes in lung function and inflammatory cytokines did not arise from an extreme and/or prolonged SHS exposure. The reported cotinine levels suggest a moderate and brief SHS exposure (34) confirming a successful simulation of a bar/restaurant smoking environment. Further, it is not possible that the comparatively long half-life of cotinine, the diurnal variation in lung function and inflammatory markers, or the investigators' expectations were reflected in our results. This is because the adopted design enabled data collection without SHS exposure (T_B) as well as immediately after (T_0), 1 hour after (T_1), and 3 hours after SHS exposure (T_3), with the data being collected at the same time of the day and without the effect of previous or subsequent measurements insofar as only one assessment was conducted per trial. Yet, our lung function results are limited by the impossibility of blinding our participants to SHS exposure. However, suggestibility does not appear to underlie acute physiological responses to SHS (35). Moreover, the present SHS-induced inflammatory reaction observed at T_0 was similar to a previous study by our group that incorporated

a repeated measures–randomized block design with a sham SHS exposure (11), thus supporting our protocol. With these in mind, it is concluded that a 1-hour SHS exposure at levels similar to those of bars and restaurants is accompanied by significant decreases in lung function and marked increases in inflammatory cytokines, particularly in men. Importantly, although most SHS-induced effects on lung function appear to recede within 60 minutes, inflammatory cytokines remain elevated for at least 3 hours after SHS exposure.

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