The Effect of Abstinence from Smoking on Urinary Excretion of Hydroxyproline

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The effect of abstinence from smoking on the urinary hydroxyproline to creatinine ratio (HOP ratio) was assessed in 49 smokers who participated in an anti-smoking course. Urine samples were collected at the beginning of the course and weekly during the subsequent 14 weeks in 1985. The subjects were divided into five groups depending on the number of cigarettes smoked daily before abstinence: 1–10, 11–20, 21–30, 31–40 and >40 cigarettes. The urinary HOP ratio immediately after abstinence from smoking was proportional to the mean daily number of cigarettes smoked in the past. All groups showed decreasing HOP ratios with a longer period of abstinence. Half of the total decrease observed in the HOP ratios after 14 weeks was reached within 5 or 6 weeks. When using the Brinkman index to adjust for the number of smoking years, half of the maximum decrease in all groups was reached within 4 weeks. In an exponential decay model fitted to the data, the half-life time taken to reach the non-smokers' level was 9–10 weeks for all groups. The HOP level of smokers who smoked less than 41 cigarettes before cessation approached the HOP level of the control group after 61–74 weeks.

The results suggested that the urinary HOP ratio was useful as a biochemical marker for short-term breakdown of lung collagen and lung elastin.

(Key Words: Anti-smoking course, Abstinence from smoking, Decay model, Urinary hydroxyproline)

INTRODUCTION

Abstinence from smoking has been shown to have a positive effect on the mortality rate, the prevalence of lung cancer and myocardial infarction, and also on pulmonary function (1, 4, 8, 19). However, these prospective studies required a long period after smoking was given up before the effects could be assessed. Recently, Higashi (9) evaluated the effect of abstinence using alpha-1-antitrypsin (alpha-1-AT) and the trypsin inhibitory capacity (TIC) as indicators, and he reported that the effect of abstinence appeared within one to four weeks.

A number of reports show that urinary hydroxyproline excretion can reflect the collagen and elastin turnover because hydroxyproline is specifically contained in collagen and elastin (5, 20). In addition, the hydroxyproline to creatinine ratio (HOP ratio) in random urine samples is considered to be representative of the amount of urinary hydroxyproline excreted within 24 hours (12).

We previously reported that the urinary HOP ratio increased with active and passive smoking (15, 17, 18). These studies supported the hypothesis that smoking may cause a breakdown of lung collagen and elastin, thus increasing the excretion of these hydroxyproline containing peptides in the urine. The prevalence of respiratory symptoms and lung function tests were used as health markers of the effect of smoking in most of the previous studies. However, these markers are not suita-
ble for the assessment of health effects prior to the manifestation of clinical symptoms. We used the urinary HOP ratio to observe changes in collagen metabolism in smokers after they stopped smoking.

Factors, other than smoking, affecting the urinary excretion of hydroxyproline include physiological factors such as growth (13, 14) and pregnancy, and pathological factors such as abnormal secretion of various hormones, bone diseases, bone metastasis of malignant neoplasms, collagen diseases and Marfan's syndrome (20). An increase in hydroxyproline excretion may also be caused by external environmental factors such as nitrogen dioxide in the air, especially indoor air pollution by NO₂ in winter (23). In order to eliminate these factors, we conducted this study in summer (free from seasonal effects of urinary hydroxyproline excretion) using adults with no disorder other than respiratory symptoms which could be ascribed to smoking.

MATERIALS AND METHODS

A five-day anti-smoking course (Monday through Friday) was held by the Japan Health Temperance Association and the Kanagawa Health Service Association in May 1985. The participants were diagnosed to be health after periodic health examinations. Initially, there were 60 participants, 54 males and six females, who were interviewed about their smoking history, motivations for giving up smoking and health status, including the presence of respiratory symptoms.

After the end of the course, two follow-up meetings were held at intervals of two months, and on both occasions, the participants were interviewed about their non-smoking status and their health conditions in general. All the subjects of the present study smoked for at least five years, and one of them was a heavy smoker who smoked more than 100 cigarettes a day. Nevertheless, all of them were diagnosed as being healthy except for symptoms of cough, sputum and shortness of breath on periodic physical examinations held in their respective work places.

Urine samples were collected from all subjects on the opening day of the course and during 14 consecutive weeks after completion of the course. Urine samples from a control group consisting of six adult non-smokers living in the same area were collected on the same days. Since there was possibility that the amounts of urinary hydroxyproline excreted in the urine were affected by the gelatin content of food, it was desirable to use fasting urine samples (10). Practical fasting urine samples were obtained as follows: after an overnight fast, the first urine in the morning was discarded and then the fasting urine was collected 2–3 hours later.

Urine hydroxyproline was analyzed by the Parekh and Jung method modified by Matsuki et al. (16). Jaffe's test was used for the analysis of creatinine (21).

RESULTS

Among the 60 participants, 49 cooperated by submitting urine samples, answering questionnaires and refraining from smoking for 14 weeks. These subjects were 44 males and 5 females. In 39 cases the motivation for giving up smoking was the smokers' concern for their own health. The remaining 10 cases stopped smoking on advice of family members or friends. Before stopping, 46.9% of the subjects complained of persistent cough and sputum, and 12.2% of them complained of anorexia. Two months after giving up smoking, 96.2% of the subjects reported the disappearance of these respiratory symptoms, and 61.5% of them gained 2 or more kilogramms in body weight. In the control group urine collection was impossible in the 2nd, 6th and 12th weeks due to logistic problems.

The subjects were classified into groups according to the number of cigarettes smoked daily before they gave up smoking: 1–10, 11–20, 21–30, 31–40 and over 41 cigarettes. The greater the number of cigarettes smoked daily, the higher the initial HOP ratio was. This was in agreement with the results of a similar survey undertaken in adult men in the same district in the same year (Table 1).

Figure 1 shows the arithmetic means of the HOP ratio as a function of the time for the different smoking groups. The HOP ratios of the smokers decreased gradually after abstinence from smoking, while the HOP ratios of non-smokers remained constant. The decrease with time of the HOP ratio was evaluated using an exponential decay model. The assumption of the model was that the rate of decrease
Table 1  HOP-ratio of anti-smoking course group and a group of fathers of school children in the same area.

<table>
<thead>
<tr>
<th></th>
<th>Anti-smoking course group (Just after abstinence)</th>
<th>Fathers group in the same area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>6</td>
<td>21.9</td>
</tr>
<tr>
<td>1-10 cig./day</td>
<td>4</td>
<td>31.9</td>
</tr>
<tr>
<td>11-20 cig./day</td>
<td>17</td>
<td>36.5</td>
</tr>
<tr>
<td>21-30 cig./day</td>
<td>11</td>
<td>38.3</td>
</tr>
<tr>
<td>31-40 cig./day</td>
<td>10</td>
<td>40.8</td>
</tr>
<tr>
<td>41+ cig./day</td>
<td>7</td>
<td>41.3</td>
</tr>
</tbody>
</table>

Fig. 1  Arithmetic means of HOP-ratio by amount of cigarette smoking after abstinence from smoking. Urine of non-smokers could not be collected after 2, 6 and 12 weeks.
of the HOP ratios after abstinence from smoking was proportional to the HOP levels due to smoking. Mathematically:

\[ C - C_0 = A \exp(kt) \]  

(1)

Where \( C \) is the HOP ratio at time \( t \) (week) after abstinence from smoking, \( C_0 \) stands for the average HOP ratio of non-smokers which was 21.4 in this study, \( A \) is the proportional constant and \( k \) is the rate constant. Equation (1) was converted to a natural logarithmic form (Equation (2)) and the linear result was evaluated by least square regression.

\[ \ln(C - C_0) = \ln(A) + kt \]  

(2)

The estimated theoretical curves of each group are shown in Figure 2. The estimated rate constants \( k \) and the proportional constants of each smoking group are summarized in Table 2. The half-life time taken to reach the non-smokers' level for each group was 9–10 weeks. About 60 per cent of the total observed decrease (after 14 weeks) occurred within 4 weeks. Using the calculated A and k constants, we estimated the time required for each group to approach the non-smokers' HOP levels. Since the non-smokers' HOP level is asymptomatic with respect to the smokers curves in this model, we arbitrarily chose as a criterion the point at which the HOP levels become indistinguishable when using our measurement method. The results showed that the HOP levels of smokers who smoked less than 41 cigarettes before stopping approach the HOP levels of the control group after 61–74 weeks. This period for the most heavy smokers was almost double (120 weeks).

The Brinkman index (2) was calculated for each subject on the basis of the daily number of cigarettes smoked before giving up smoking and the number of smoking years. The subjects were divided into mild smokers (Brinkman index: 1–400), moderate smokers (401–800) and heavy smokers (over 800). The HOP ratios as a function of time for these groups are shown in Figure 3. Urinary HOP ratios of subjects who smoked before abstinence were higher than the non-smokers' ratios for the whole period. Both the heavy and mild groups showed rapid decreases in HOP ratios during the first 4 weeks. In the moderate group, the decrease in the urinary HOP ratio was more gradual. However, after 14 weeks there were no marked differences between the mild and moderate groups. These results suggested that the decrease of the urinary HOP ratio depends on the number of cigarettes smoked before giving up smoking as well as the duration of the non-smoking period.

**DISCUSSION**

The efficacy of abstinence from smoking for health has been described in a number of papers (1, 4, 8, 19, 22). A study on American veterans revealed that a decrease in the mortality rates of ex-smokers was proportional to the number of non-smoking years. While the ex-smokers after 5 years of abstinence still showed similar overall mortality rates as smokers, after 15 years they exhibited a decrease in mortality rates to a level similar to that of non-smokers (4). A survey conducted in Sweden revealed that the rate of recurrence of myocardial infarction decreased by 50% after the subjects had given up smoking (22). According to investigations in England and U.S.A., quitting smoking resulted in a decreased risk of myocardial infarction to the level of non-smokers (11). In addition, 10–15 years after smokers gave up smoking, their risk for developing lung cancer approached that of non-smokers (8).

Although abstinence from smoking improved the peripheral airways (7) and pulmonary function in terms of FEV1 (19), no consistent age-corrected changes in pulmonary function have been found in the literature.

Buckley and Balchum (3) examined the biochemical effects of exposure to NO2 and found that activity of the LDH isoenzyme showed NO2 induced changes before pulmonary lesions and clinical symptoms became manifest, suggesting that this enzyme was a sensitive biochemical marker for the health effects of NO2. We used the urinary HOP ratio as a biochemical marker to assess the effect of abstinence from smoking on the breakdown of lung collagen and lung elastin. Since cigarette smoke contains a number of oxidants, including nitrogen oxides and acrolein, urinary hydroxyproline, which is one of the end-products of collagen and elastin, is considered to be related to smoking.

Proteins in the lung structure are composed of collagen, elastin, proteoglycan and glycoprotein, and polymorphonuclear leuko-
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(1) 40-cig./day
(2) 31–40cig./day
(3) 21–30cig./day
(4) 11–20cig./day
(5) 1–10cig./day
(6) Non-smokers

Fig. 2 Theoretical curves of HOP-ratio by amount of cigarette smoking after abstinence from smoking.

Table 2 Calculated proportional and rate constants, correlation and time required to reach non-smokers HOP ratio levels for each group of ex-smokers.

<table>
<thead>
<tr>
<th>Ex-smoking (cig./day)</th>
<th>k (week)</th>
<th>( \ln(A) )</th>
<th>r</th>
<th>No.</th>
<th>time (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–10</td>
<td>-0.0751</td>
<td>2.279</td>
<td>0.451**</td>
<td>4</td>
<td>61</td>
</tr>
<tr>
<td>11–20</td>
<td>-0.0673</td>
<td>2.630</td>
<td>0.613***</td>
<td>17</td>
<td>73</td>
</tr>
<tr>
<td>21–30</td>
<td>-0.0754</td>
<td>2.821</td>
<td>0.611***</td>
<td>11</td>
<td>68</td>
</tr>
<tr>
<td>31–40</td>
<td>-0.0709</td>
<td>2.925</td>
<td>0.819***</td>
<td>10</td>
<td>74</td>
</tr>
<tr>
<td>41–</td>
<td>-0.0429</td>
<td>2.853</td>
<td>0.391**</td>
<td>7</td>
<td>120</td>
</tr>
</tbody>
</table>

**p<0.01
***p<0.001

model: \( \ln(C_t - C_0) = \ln(A) + kt + e_t \)

where \( C_t \) is the HOP ratio of \( t \)th sample,
\( t \) is the time (week) of the \( t \)th sample,
\( e_t \) is the error of the \( t \)th sample and has a normal distribution with mean 0 and a variance of \( \sigma^2 \),
\( C_0 \) is the mean HOP ratio value of the non-smokers.
cytes and alveolar macrophages recruited by cigarette smoke in the lung or airways. Cigarette smoke also enhances the dissociation of elastase and proteolytic enzyme. On the other hand, the elastase inhibitory activity of alpha-1-antitrypsin is inactivated by oxidants in cigarette smoke. Thus, cigarette smoke is considered to play a key role in the occurrence of pulmonary emphysema and chronic bronchitis, which is consistent with the "protease-antiprotease imbalance theory" advocated by Eriksson (6). In other words, due to a loss of the balance between protease and antiprotease, proteins in the lung structure were destroyed. As a result, urinary hydroxyproline excretion is increased.

Soon after the subjects stopped smoking, the urinary HOP ratio decreased relatively rapidly within the first 4 weeks, followed by a gradual decrease up to the 14th week. The smokers' group who smoked 41 or more cigarettes a day before stopping required a longer period of abstinence until the ratios decreased to the level of non-smokers, compared with groups who smoked less. It was also found that the total number of cigarettes consumed until the day of stopping was related to the degree of decrease of urinary HOP ratios.

The results of cohort studies showed that a significant difference in lung cancer mortality rate due to abstinence from smoking did not appear after less than 5 to 15 non-smoking years and that the pulmonary function, once it was reduced by smoking, required 6 to 12 years to recover. However, the results of the present study indicated that lung collagen and elastin metabolism in terms of the urinary HOP ratios recovered significantly within 61–74 weeks after smoking had been given up except for the most heavy smokers. According to the above results, collagen metabolism may be recovered by abstinence from smoking relatively quickly. It appeared that the recovery rate was faster than the clinical condition which involved respiratory symptoms such as persistent cough and sputum and high mortality rates of lung cancer. The recovery rates of urinary HOP ratios in smokers who smoked less than 41...
cigarettes a day before abstinence was faster than that of those who smoked more. From the view point of preventive medicine, smoking causes numerous diseases. It is very important that people who want to give up smoking can soon see the effects of abstinence from smoking using urinary HOP ratios.

In conclusion, it was found that biochemical markers revealed the effect of abstinence from smoking before the disappearance of certain clinical symptoms. Among these markers, hydroxyproline, an indicator of collagen and elastin breakdown in the lungs, was a relatively simple and objective parameter for assessing the positive effects on the lungs of abstinence from smoking.

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