

Carbon Monoxide Concentration in the Breath Does Not Change with Ordinary Meals: Study Based on Diurnal Variations

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We examined whether or not changes occur in the quantity of carbon monoxide (CO) production as a consequence of the intake of ordinary meals. The study was performed by following diurnal changes in the concentration of CO in the breath over approximately 12 hr after each meal on 17 healthy subjects. CO is a product of the reaction of the enzyme heme oxygenase-1 (HO-1) induced through oxidative stress. The average value for diurnal variations in the concentration of CO in the breath was 1.9 ± 0.5 (0.8 to 3.3) ppm, and the maximum range of variation in individual cases came within the narrow range of 0.4 to 1.3 ppm. There were no significant differences in average values before and after meals. The value for the total quantity of CO in the breath 12 hr sought on the basis of the area under the diurnal variation curve was 22.8 ppm (0.39 mmol). This figure was very close to the heme breakdown quantity of 0.35 mmol (theoretical value, corresponding to the CO production quantity) every 12 hr.

Key words —— carbon monoxide concentration, diurnal variations, meal influences, heme oxygenase, total carbon monoxide excretion

INTRODUCTION

Production of reactive oxygen species (ROS) and the reaction that scavenges it are maintained inside the body in a perfect balance, and thus are only rarely exposed to excess oxidative stress under normal conditions. When exposure to oxidation stress occurs, a protective reaction occurs in the form of activation of various genes,¹⁾ and attention is being directed in this regard especially to heme oxygenase-1 (HO-1), an isoform of the heme breakdown enzyme HO, which is induced due to reaction with oxidative stress.²⁾ It is generally known that carbon monoxide (CO) and biliverdin (bilirubin) generated by HO reaction have anti-inflammatory, anti-apoptotic or anti-oxidation effect.³⁾ In addition, nutrients contained in meals such glutathione, Vitamin C, Vitamin E, retinol, beta-carotene, zinc and selenium are connected to antioxidation reactions involved in the elimination of ROS.

In today's stress-filled society, how to control oxidative stress through the intake of antioxidant foods has become an important topic of research in the field nutritional science.⁴⁾ A recent report indicates that when food deficient in choline and folic acid was fed to a rat, there was a decrease in the amount of Vitamin C and Vitamin E in the blood, a decrease in hepatic retinol, and an increase in the expression of hepatic HO-1 mRNA.⁵⁾ Furthermore, it has become clear that the anti-oxidation substance resveratrol contained in grapes and red wine induces the human culture cell HO-1,⁶⁾ and that application of glutamine, which is able to control oxidative stress, to a septicemic rat is able to raise the survival rate through induction of intestinal mucosa HO-1.⁷⁾

On the other hand, the amount of CO produced inside the body is dependent mainly upon the enzyme reaction of HO-1, and it is clear this is reflected in the concentration (excretion quantity) of CO in the breath.⁸⁾ A device of analyzing breath gas that facilitates measurements of the concentration of CO in the breath has been developed recently, and it has become possible to use the concentration of CO in the breath as a low-invasive biomarker capable of indirectly expressing the amount of HO-1 expressing in tissue. Since it seems likely that the concentration of CO in the breath will change through the ingestion of foods that are likely to contain antioxidants and pro-oxidants, in the present report we have striven to clarify the influence of meals by following diurnal variations in the concentration of CO in

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the breath.

MATERIALS AND METHODS

Subjects — Seventeen healthy subjects (4 men and 13 women aged between 19 and 67, average age 25.6 years) who did not smoke and were not exposed to passive smoking were used as subjects of the present study. The purpose of the study, its methods and any foreseeable problems were explained in full to the subjects, and they were finally selected as subjects only when their understanding and agreement had been obtained. This research has been approved by the Ethics Committee of our university under the Approval Number 018.

Study Design — Diurnal variations in the concentration of CO in the breath were measured six times a day (before breakfast, one to two hours after breakfast, before lunch, one to two hours after lunch, before dinner and one to two hours after dinner). Breath samples were taken after getting the subject to breathe in lightly, hold the breath for about 15 sec and then breathe out; the final breath (terminal exhalation) of between 100 and 200 ml was blown into an exhalation bag through a mouthpiece attached to the tip of the bag.⁹⁾ Each subject was then handed a "Meals and Daily Life Survey Form" and was asked to write down diachronically the names of the foods they had ingested as meals or as snacks, the approximate quantities thereof, variations in physical condition, and if and when they went out, engaged in physical activities, went to the toilet, slept, experienced stress or fatigue, and took dietary supplements.

Breath Analysis — One ml of breath was taken from the exhalation bag using a special syringe and injected into a TRIlyzer mBA-3000 device for the simultaneous measurement of breath gas (CO, CH₄, H₂) manufactured by Taiyo Co., Ltd. of Osaka. Three gas concentrations were consecutively measured simultaneously at a speed of 4 minutes 20 sec in the case of each sample. We decided to conduct the measurements in the course of the day, but in no case later than one to three days after the breath sample was taken. The measurement principle employed by this device is gas chromatography using a high-sensitivity semiconductor gas detector. The lower limit for detection of the gas constituent of the three types was 0.1 ppm, with reproducibility of $\pm 2\%$ and linearity of up to 100 ppm. Ultra-high purity synthetic air (less than 0.1 ppm of impurities) was used as the carrier gas, and high-concentration

(50 ppm with all three gases) and low-concentration (5 ppm) mixed gas was used for calibration (100 samples were measured at one time). The respective concentrations of CO, CH₄ and H₂ in the air in the vicinity of the laboratory were approximately 0.2 to 0.4 ppm, 1.5 ppm and 2.0 ppm.

Statistical Analysis — The results obtained are expressed in terms of the mean value \pm the standard deviation, and the significance is verified by means of Student *t* test. We estimated the significant difference when *p* is less than 0.05.

RESULTS

Life Profile of the Subjects

The everyday life profiles of the 17 subjects used on this study were analyzed with reference to the completed "Meals and Daily Life Survey Form." We looked into the content of the food ingested, and we found that there were considerable differences between one subject and another when it came to an approximate calculation of ingested quantities of the three main nutrients, anti-oxidants and pro-oxidants, vitamins, glutamine and other amino acids, and zinc and other minerals. The day on which diurnal variations were examined was Sunday in the greatest numbers of cases (ten), and examinations were carried out on Saturday and Tuesday in two cases each. The times at which meals were taken varied within a maximum range of three hours, but breath sampling after meals was carried out within one to two hours of the meal in every case (in most cases, diurnal variations over around 12 hr were observed).

Diurnal Variations in Concentration of CO in the Breath

The average value for CO concentration in diurnal variations in the 17 cases examined was 1.9 ± 0.5 (0.8 to 3.3) ppm (Fig. 1). The maximum extent of variation in the six values measured in the course of a single day in a single subject was 0.4 to 1.3 ppm, and only 5 (29.4%) of the 17 subjects showed variations of 1.0 ppm or more. In the case of the subject who showed the higher values, we looked to see if there was a large difference (Δ ppm) between the maximum value and the minimum value, and correlation between the two was observed (*p* = 0.04). We examined whether there was any difference in CO concentration before and after each meal, but no significant differences were noted. The values in question were 2.0 ± 0.5 ppm and 2.1 ± 0.5 ppm be-

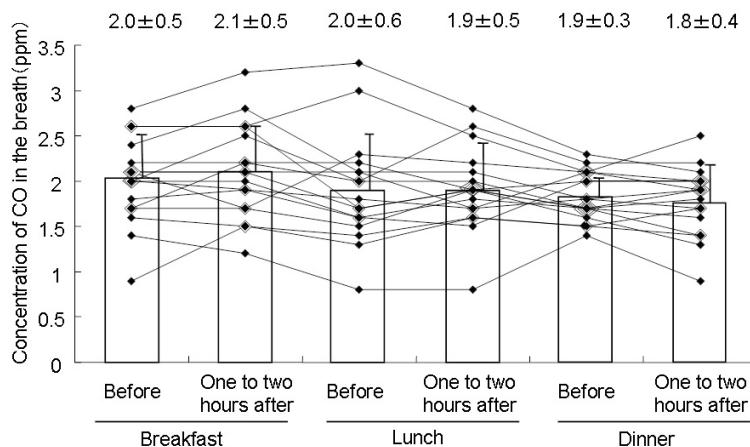


Fig. 1. Diurnal Variation Patterns for CO Concentration in the Breath of 17 Subjects

Mean values \pm standard deviation (Mean \pm S.D.) before breakfast and between one to two hours after, before lunch and between one to two hours after, and before dinner and between one to two hours after. No significant differences in concentration at each point in time were observed. [◆] shows two cases and [◇] shows three cases. The results for the 17 cases are shown at each point in time.

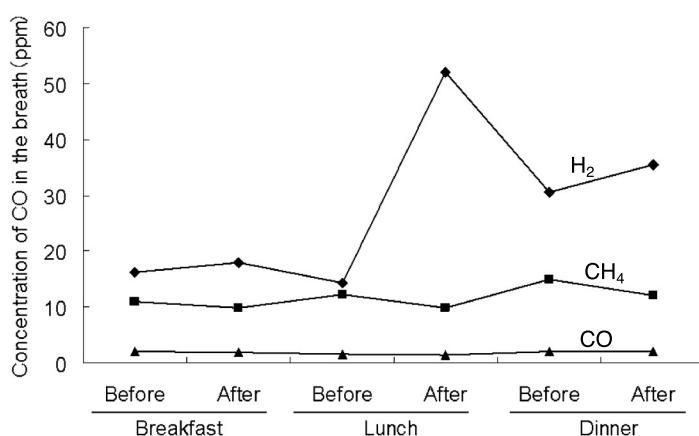


Fig. 2. An Example of Diurnal Variations in the Concentration of CO in the Breath (K. M., Woman Aged 20)

There is almost no change in CO concentration in comparison with the concentration of H₂ and CH₄ in the breath measured at the same time.

fore and after breakfast; 2.0 \pm 0.6 ppm and 1.9 \pm 0.5 ppm before and after lunch; and 1.9 \pm 0.3 ppm and 1.8 \pm 0.4 ppm before and after dinner.

Diurnal variations in the concentration of breath CO in the case of one of the 17 subjects (female student aged 22) are shown in Fig. 2 as a typical case, together with the concentration of H₂ and CH₄, which were measured at the same time. This particular subject was a methane producer. Considerable variations in the concentration of H₂ in the breath that are probably ascribable to meal intake and, as if influenced by this, there was a slight variation in the concentration of CH₄ (reduction reaction by methanogenic bacteria: CO₂ + 4H₂ \rightarrow CH₄ + 2H₂O). However, the maximum difference in concentration between the highest and the lowest val-

ues for the concentration of CO in the breath was only 0.5 ppm, and there were almost no changes throughout the day, including before and after meals.

The Total Excretion of CO in the Breath 12 hr

We did not study diurnal variations over 24 hr, but a calculation of the average value for total breath CO excretion over a duration of approximately 12 hr on the basis of six values (area under the curve) produces the value 22.8 \pm 4.8 (13.2 to 33.6) ppm. Assuming a body weight of 70 kg and mean cell hemoglobin concentration (MCHC) of 30%, the average hemoglobin breakdown over 12 hr amounts to 5.7 g (0.088 mmol), meaning that breakdown of hemoglobin (*i.e.* the amount of CO production) may be estimated as 0.35 mmol. Assuming that the aver-

age quantity of breath in a day is 10^4 l, then 22.8 ppm corresponds to 0.39 mmol at a room temperature of 25°C. This means that this figure (0.39 mmol) closely resembles the theoretical value of 0.35 mmol. This close resemblance between the theoretical value for production of CO and the figures actually measured for CO excretion suggests the correctness of the method of examination of diurnal variations that we employed on the present study.

DISCUSSION

Considering that anti-oxidants and pro-oxidants contained in meals bring about changes in the state of oxidative stress inside the body and that HO-1 reactions decrease or increase (including enzyme induction) in consequence, it is inconceivable that there should be any change in the concentration of CO in the breath within the short period of one or two hours after eating. However, as shown in Fig. 2, which shows the pattern of change in the concentration of H₂ in the breath due to meals, if there is any change in the concentration of CO in the breath due to the intake of meals, it should be possible to gauge this change in terms of changes in diurnal variations as in the case of H₂ concentration. However, no significant changes in the concentration of CO in the breath influenced by the intake of ordinary meals were observed on the present study.

From among previous reports involving tests carried out on humans, there is one report describing a study that involved injecting glutamine (0.8 mmol/kg per hour; 4.2 g in the case of a person weighing 60 kg) or another amino acid mixture (control: same molar as glutamine) for six hours continuously into the duodenum of a healthy subject. The study showed the apparent expression of HO-1 in duodenal mucosa obtained by an endoscope 30 min later.¹⁰⁾ According to this particular report, injection of glutamine into the intestine brings about an increase in production of HO-1 mRNA in the case of the epithelial cells of the intestine and the tunica propria cells, and bears a clear correlation with positive cells involving immune staining. This means that, since oral intake of glutamine increased the amount of HO-1 mRNA in intestinal villi cells and the enzyme protein content in the duodenum, it seems likely that HO-1 induction occurred rapidly due to nutritive substrates coming into direct contact with the mucosa of the digestive tract. In this study, no measurements were taken of the concen-

tration of CO in the breath, and so it is unclear to what extent changes occurred in the concentration of CO in the breath.

In another report, it was found that, in the case of acute inflammation of the intestine in human beings, HO-1 was induced and that there was an increase in the concentration of CO contained in intestinal gas (sampled under an endoscope), but CO concentration normalized as the acute inflammation receded.¹¹⁾ On the other hand, it is possible that HO-1 induction is insufficient during chronic inflammation, and this may be connected with the continuation of the illness and its incurability.¹²⁾ It seems likely therefore that application of an HO-1 inducer even at times of chronic inflammation will raise CO concentration in the affected area, will have anti-oxidizing and anti-inflammatory effects, and will cure the illness.¹³⁾ In the present study, it became evident that there are no changes in the concentration of CO in the breath in the case of ordinary meals. Accordingly, if the HO-1 induction effect becomes evident through the application of specific nutrients alone, it seems likely that HO-1 may be considered as a target molecule in dietary treatment.¹⁴⁾

Another result that emerged in the course of the present study is that values extremely close to those for the quantities of CO produced inside the body (theoretical values) were obtained by following diurnal variations of breath CO concentration. This implies that, in the case of patients suffering from illnesses that involve changes in the concentration of CO in the breath, even if there is no change in the concentration of CO in the breath at a single point in time (e.g. on the starved state early in the morning), significant changes may arise if the total excreted quantities of CO in the breath are calculated within a specific duration on the basis of values (diurnal variations) measured several times at different times of the day.

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