Human breath isoprene and its relation to blood cholesterol levels: new measurements and modeling

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Karl, Thomas, Peter Prazeller, Dagmar Mayr, Alfonso Jordan, Josef Rieder, Ray Fall, and Werner Lindinger. Human breath isoprene and its relation to blood cholesterol levels: new measurements and modeling. J Appl Physiol 91: 762–770, 2001.—Numerous publications have described measurements of breath isoprene in humans, and there has been a hope that breath isoprene analyses could be a noninvasive diagnostic tool to assess blood cholesterol levels or cholesterol synthesis rate. However, significant analytic problems in breath isoprene analysis and variability in isoprene levels with age, exercise, diet, etc., have limited the usefulness of these measurements. Here, we have applied proton transfer reaction-mass spectrometry to this problem, allowing on-line detection of breath isoprene. We show that breath isoprene concentration increases within a few seconds after exercise is started as a result of a rapid increase in heart rate and then reaches a lower steady state when breath rate stabilizes. Additional experiments demonstrated that increases in heart rate associated with standing after reclining or sleeping are associated with increased breath isoprene concentrations. An isoprene gas-exchange model was developed and shows excellent fit to breath isoprene levels measured during exercise. In a preliminary experiment, we demonstrated that atorvastatin therapy leads to a decrease in serum cholesterol and low-density-lipoprotein levels and a parallel decrease in breath isoprene levels. This work suggests that there is constant endogenous production of isoprene during the day and night and also reaffirms the possibility that breath isoprene can be a noninvasive marker of cholesterol-enrichment if care is taken to measure breath isoprene under standard conditions at constant heart rate.

proton transfer reaction-mass spectrometry; pulmonary gas-exchange model

HUMAN BREATH CONTAINS A VARIETY of endogenous volatile organic compounds (VOCs), the most abundant ones being acetone (~1 part per million by volume (ppmv)), methanol, ethanol, propanols, and isoprene (~few hundred parts per billion by volume (ppbv)) (reviewed in Refs. 6, 14). In healthy persons preprandial, the breath concentrations of acetone and simple alcohols typically each lie within a range of a factor of 2–3 (14), but the breath concentrations of isoprene vary by more than a factor of 50 (23). For example, we found that children aged 4–6 yr have an average breath isoprene concentration of ~100 ppbv with single cases of concentrations <20 ppbv, whereas that of adults is a factor of ~2.4 higher, with values ranging from ~50 up to >1,000 ppbv (23). However, within the group of adults, in agreement with Mendis et al. (16), we found no evidence for an age dependence of the isoprene concentration.

The possibility that breath isoprene may be linked to cholesterologenesis was suggested by experiments with liver extracts by Deneris et al. (5) and more recently in subjects treated with a cholesterol-lowering drug (22). These findings raised the possibility that measurements of breath isoprene might represent a noninvasive means of assessing body cholesterol status (23). However, given problems in 1) resolving isoprene from other breath VOCs, and 2) the variability in breath isoprene between measurements, even in the same individual, it has not yet been possible to make this connection. Here we address the question: How can we obtain reliable data to determine whether there is a direct connection between isoprene concentration in breath and isoprene production within the body? We will show below that accurate breath isoprene analysis is not trivial and that misinterpretations have been made by us and by others in earlier publications.

Measurements of the abundance of isoprene in the breath have been complicated by the difficulty in separating it cleanly during gas chromatography (GC) from such compounds as pentane and acetone, which are also commonly present in expired air (13, 16). In addition, GC or GC-mass spectrometry (MS) methods do not allow on-line analysis of breath VOCs. For the present work, we used proton transfer reaction-MS (PTR-MS), which addresses both of these analytic problems (9, 14). The PTR-MS method allows analysis of breath VOCs with no preconcentration or chromatography, and, because isoprene produces a distinctive positive ion [mass-to-charge ratio (m/z) 69], there is no
interference from detected acetone (m/z 59) or pentane, which, like other alkanes, is not detected by PTR-MS. As PTR-MS is normally operated on-line, it is possible to obtain breath isoprene analyses every few seconds (23), which has allowed us to make new observations presented here.

It is the aim of this work to investigate whether other compounds in human breath react with H$_2$O$^+$ to produce ions at m/z 69. It is known that some alcohols, like methylbutenol, partly break up in the reaction with H$_2$O$^+$ under our conditions to form products at m/z 69. To check on the possible presence of compounds more polar than isoprene at m/z 69, we occasionally passed the breath gas through distilled water before introducing it into the PTR-MS system, measured the signal at m/z 69, and compared it with the one obtained with a direct inlet. Isoprene has an extremely low Henry’s law constant and thus is not readily dissolved in water (14), whereas practically all other VOCs under consideration are highly soluble in water. Under normal conditions, no such differences were observed with human breath samples, indicating that the measured signal correlates to isoprene only.

Two modes of measurement were used. First, on-line measurements were performed during performance of exercise of two test persons on a stationary bicycle. They breathed in through the nose but exhaled through a tube in the mouth. This tube led into a larger tube of ~1.6-cm diameter and 100-cm length, heated to a temperature of ~40°C to avoid condensation. At the end of this tube, a hose of ~1.6-cm diameter and 100-cm length allowed the exhaled air to finally escape into the air inside the laboratory where the experiment took place. From the tube (at middle length), a 1/8-in. Teflon tube led into the PTR-MS system for continuous sampling and on-line measurement of the ion count rates at several preselected masses. Among the masses monitored were the ones representing acetone, methanol, isoprene, acetaldehyde, and ethanol, and taking one such set of data took ~25 s. The only VOC that showed significant changes during the exercise of the test persons was isoprene; therefore, in the RESULTS section we will only address breath isoprene dynamics. During the exercise, heart rate was measured continuously with an electronic pulse meter, and breath rate was measured intermittently.

A second mode of measurement was applied during the investigation of a test person with hyperlipidemia who was undergoing lipid-lowering therapy under a physician’s care. The drug atorvastatin was administered (20 mg/day), and breath isoprene measurements were extended over a period of 15 days. Once or twice a day the test person inflated a Tedlar bag with breath air under “standard conditions” as follows. The person only moved slowly during ~1 h and then sat for 5 min on a chair, thus allowing the pulse rate to stabilize to somewhere between 60 and 70 beats/min before the breath sample was taken. After a modestly deep breath was taken, ~0.5 liter of air was exhaled into the open air, and the rest was released into the Tedlar bag, which then contained between 0.5 and 1 liter of breath gas. Typically, within 1 or 2 days the Tedlar bag was attached to the PTR-MS inlet, which was situated within an oven (temperature 40°C), and the analysis of the breath gas was done in the usual way. Separate, long-term measurements were done showing that isoprene concentrations in the air within a Tedlar bag do not decline >2%/day, as has been verified in separate test series extending over 33 days, and respective corrections were performed but were mostly insignificant.

Three times during the first 14 days of the lipid-lowering therapy, measurements of the serum cholesterol and low-density-lipoprotein (LDL) levels of the blood of the test person were performed (at the beginning of atorvastatin therapy, and 4 and 11 days later) in a routine fashion at the medical clinic of the University of Innsbruck.

METHODS

For the present measurements, we used a PTR-MS instrument, which has been developed in our laboratory and described in detail elsewhere (9, 14). It allows for fast, simultaneous monitoring of the concentrations of many VOCs that are present in trace quantities, even <1 ppbv, in moist air like human breath. Briefly, the basic principle of PTR-MS involves the mixing of a flowing air sample in a drift tube equipped with a source of H$_3$O$^+$. H$_2$O$^+$ does not react with any of the main components of air (i.e., N$_2$, O$_2$, CO$_2$), as they all have lower proton affinities than H$_2$O$_2$, but H$_3$O$_2$ performs proton transfer to most breath VOCs in nondissociative reactions

$$H_3O^+ + R \rightarrow RH^+ + H_2O$$  (1)

where $R$ is the gas constant and $k$ is the proton transfer rate constant. The proton transfer rate constants $k$ are large, corresponding to the collisional limiting values ($\sim 10^{-9} \text{ cm}^2/\text{s}$) (15). The value for the ratio of electric field strength to buffer gas density in the drift tube is kept at ~140 Townsend, high enough to avoid strong clustering of H$_3$O$^+$ with water and other molecules in the breath gas to be investigated. The concentration of a particular VOC ($[R]$) is calculated from the count rates (cycles per second (cps)/RH$^+$) and cps(H$_3$O$^+$) obtained in the downstream ion detection system using the relation

$$\text{cps(RH}^+) = \text{cps(H}_3\text{O}^+) \cdot (1 - e^{-k[R]}) \equiv \text{cps(H}_3\text{O}^+) \cdot [R] \cdot k \cdot t$$  (2)

where $t$ is the transit time of the H$_3$O$^+$ through the drift tube. The value of $t$ is calculated from known mobility values of H$_3$O$^+$ in air (15), and the reaction rate coefficient $k$, related to the components studied in the present investigation, lies in the range $1.7 \times 10^{-9} \text{ cm}^2/\text{s} < k < 4 \times 10^{-9} \text{ cm}^2/\text{s}$. Thus Eq. 2 can be used to directly convert ion counts for detected VOCs into their respective concentrations. Isoprene, the compound of main interest in the present study, performs nondissociative proton transfer with a rate constant of $k = 1.9 \times 10^{-9} \text{ cm}^2/\text{s}$ at our operating condition of the PTR-MS system.
RESULTS

Breath isoprene during exercise. Figure 1A shows measured and calculated breath isoprene concentrations of a male adult before, during, and after he performed exercise on a stationary bicycle. The test person started pedaling after sitting still for ~5 min on the bicycle. During the exercise, the test person breathed continuously into the breath-sampling system attached to the PTR-MS apparatus, which performed on-line measurements. The heartbeat frequency (also shown in Fig. 1A) was continuously measured and was registered together with the PTR-MS data. The velocity of pedaling was increased in time intervals of ~5 min in such a way that the heartbeat frequency increased by ~20 beats/min each time until it reached 175 beats/min, where it was kept for ~7 min; then pedaling was reduced in steps so that the heartbeat frequency also decreased in steps, as shown in Fig. 1A. The breath rate was not measured continuously; however, a relative breathing rate was determined several times during the exercise (Fig. 1A). The experimental data show a rapid increase in the breath isoprene concentration right after the start of the physical exercise. Within ~90 s, isoprene increased from ~120 to 250 parts per billion (ppb), followed by a decline to minimum values of ~50 ppb 20 min later when the maximum heartbeat rate was reached in five steps of increasing exercise effort. Thereafter, with declining exercise effort, breath isoprene increased steadily to a concentration at the end of the exercise similar to that of the starting level. Notably, the marked increase in breath isoprene seen at the begin-

Fig. 1. Analyses of breath isoprene in male adults during exercise. A: 5-step increase in exercise heart rate in subject WS. B: 2-step increase in exercise heart rate in subject AW. During these experiments, breath isoprene was continuously monitored by proton transfer reaction-mass spectrometry before and during exercise on a stationary bicycle. Throughout the experiment, heart rate was measured with an electronic pulse meter, and relative breath rate was estimated intermittently and plotted on a relative scale. Breath isoprene (solid line) and arterial blood isoprene concentration, calculated from a gas-exchange model, are also plotted, the latter on a relative scale. ppb, Parts per billion.
ning of the exercise was not correlated with a large change in breath rate; as seen in Fig. 1A, the relative breath rate peaked only after \( \sim 20 \) min of exercise.

Virtually identical results were seen in a second individual, as shown in Fig. 1B. In this case, the exercise effort was increased in two steps. Again, breath isoprene increased within a few seconds of the start of exercise, by a factor of \( \sim 4 \) in this case, and then followed the same pattern seen in Fig. 1A. A total of eight individuals were tested in this type of experiment. As summarized in Table 1, for all eight individuals and one individual tested twice, breath isoprene rapidly increased at the start of the exercise to a maximum that was \( \sim 1.6–4.4 \)-fold higher than before the exercise. In each case, the resting heart rate increased 1.3–1.8-fold during this same period (3–5 min). In these test subjects, the maximal increase in breath isoprene was positively correlated with the maximal increase in heart rate (\( R^2 = 0.68 \)); the lack of a stronger correlation may be due to differences in lung capacity or blood flow characteristics among individuals. Nevertheless, these results confirm the importance of heart rate during breath isoprene concentration measurements.

**Breath isoprene during sleep.** A related issue concerns the reported diurnal cycle of breath isoprene that is maximal during sleep (4, 22, 23). As it seems likely from the above results that breath isoprene concentrations are very sensitive to changes in heart rate, we tested whether an individual, either reclining and then standing or sleeping and awakened by an alarm, experiences parallel changes in heart rate and breath isoprene concentrations. As shown in Table 2, breath isoprene in each case increased significantly in parallel with the increase in heart rate associated with reclining and then standing (daytime measurements). These increases are similar to those seen during exercise. In addition, when the same individual was awakened during the night by an alarm and filled a gas-sampling bag, the heart rate increased from 51 to 80 beats/min during the sampling; the resulting isoprene concentration (398 ppbv) was higher than any of the daytime samples. Then, \( \sim 3 \) min after awakening, the individual stood up and obtained another breath sample; in this case, the heart rate rose to 114 beats/min, and the isoprene concentration was found to be 540 ppbv. These results show how rapidly breath isoprene can rise in a sleeping individual as a result of increased heart rate; they also illustrate the elevated nighttime breath isoprene levels reported previously (and discussed below).

**Modeling isoprene exhalation.** Can we model human breath isoprene dynamics in the resting and exercising states? This is important because, as described in the Introduction, previous measurements of breath isoprene have shown large variability. As a result, it has not been possible to clearly link breath isoprene measurements to physiological processes. The calculation of breath isoprene concentration as dependent on time was done on the assumptions of a constant endogenous isoprene production during the course of the experiment and that the loss of isoprene occurs only by exhalation. A physiological two-compartment model similar to the one of Filser et al. (7) was applied but with some modification. As shown in Fig. 2, it contains two circuits: the blood-lung circuit and the breath air-lung circuit. The source of isoprene is situated within the first circuit, and, although the tissue source of isoprene is not known (10), its exact location is not essential for this model. Venous blood with enhanced isoprene concentration enters the lungs, where part of the isoprene diffuses into breath air, so that arterial blood with lower isoprene concentration leaves the lungs. Within the lungs, there is a gradient of blood isoprene concentration, and, independent of the actual geometry of the lung, we may assume that the lung compartment consists of adjacent segments of lung volumina, with the blood isoprene concentration declining from the venous to the arterial side (Fig. 2). Because of the large alveolar surface with respect to alveolar volume, in each of the partial lung volumina,

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate during rest, beats/min</td>
<td>71</td>
<td>70</td>
<td>55</td>
<td>79</td>
<td>61</td>
<td>85</td>
<td>70</td>
<td>82</td>
</tr>
<tr>
<td>Heart rate during bicycle exercise, beats/min</td>
<td>115</td>
<td>95</td>
<td>95</td>
<td>105</td>
<td>99</td>
<td>111</td>
<td>91</td>
<td>150</td>
</tr>
<tr>
<td>Breath isoprene during rest, ppbv</td>
<td>82</td>
<td>250</td>
<td>90</td>
<td>160</td>
<td>240</td>
<td>110</td>
<td>200</td>
<td>120</td>
</tr>
<tr>
<td>Breath isoprene during bicycle exercise, ppbv</td>
<td>244</td>
<td>520</td>
<td>270</td>
<td>380</td>
<td>527</td>
<td>175</td>
<td>441</td>
<td>522</td>
</tr>
</tbody>
</table>

Table 1. Summary of breath isoprene vs. heart rate in 8 test subjects during rest and bicycle exercise

Table 2. Evidence for a rapid increase in breath isoprene when a reclining or sleeping individual assumes a standing position

<table>
<thead>
<tr>
<th>Isoprene Concentration, ppbv</th>
<th>Heartbeat, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reclining</td>
<td>193</td>
</tr>
<tr>
<td>Standing position</td>
<td>333</td>
</tr>
<tr>
<td>Reclining</td>
<td>194</td>
</tr>
<tr>
<td>Standing position</td>
<td>276</td>
</tr>
<tr>
<td>Reclining</td>
<td>209</td>
</tr>
<tr>
<td>Standing position</td>
<td>327</td>
</tr>
<tr>
<td>Night: 4:19 AM lying</td>
<td>398</td>
</tr>
<tr>
<td>Night: 4:22 AM standing position</td>
<td>540</td>
</tr>
</tbody>
</table>

Values are from 1 subject.
there exists near equilibrium between the blood iso-
prene concentration and the breath isoprene concen-
tration. This is due to Henry’s law partitioning of blood
with alveolar air. An average blood isoprene
concentration thus is correlated to an average breath
isoprene concentration over the whole lung volume.
With this model, it becomes obvious that, with a given
isoprene production rate, the average breath isoprene
concentration is governed by the rates at which blood is
pumped through the lungs (heartbeat rate) and breath
air is exchanged (breath rate).

In a typical situation of an adult person being at slow
movement close to rest, the breath rate volume (Vbr) is
~7.5 l/min, and the heart pump volume is ~5 l/min. As
the Henry’s law constant for isoprene is extremely low
at 0.029 M/atm (water-air at 310K; Ref. 14), only a very
small amount of isoprene is dissolved in the human
body and thus also in the blood (~2 × 10^{-4} mg/l at an
average concentration of 100 ppb). When isoprene-free
air is breathed into the lungs, it becomes enriched with
isoprene dissolved in the blood, causing a marked re-
duction in the blood isoprene concentration within the
lungs and, therefore, causing a substantial drop in the
isoprene concentration between the venous and arte-
rial blood of ~85%. This drop is calculated in the
following way.

Under stationary conditions, the venous blood enter-
ing the lungs has an isoprene concentration CV0, and
the arterial blood leaving the lungs has a concentration
CA0. Within the lungs, we can connect to each differ-
ential volume (using the compartment model) a con-
centration CD, and, assuming an exponential drop of
the isoprene concentration along the axis of the differ-
ential volumes, we obtain

\[ C_D(V) = C_{V0} \cdot \exp \left( - \frac{Vbr \cdot V}{H \cdot R \cdot T \cdot V_0 \cdot HBV} \right) \]  

(3)

where V is volume, H is Henry’s law constant, T is
temperature, V0 is the total volume of the lungs, and
HBV is the heartbeat volume. The concentration CD is
changing from one differential volume of the lung to
the other.

Before entering the lungs at V = 0, the isoprene
concentration in the venous blood is CV0 = CD(0), and,
at the exit, at V0, the arterial blood has the concen-
tration CD(V0) = CA0, with both being connected by the
relation

\[ C_{A0} = C_{V0} \cdot \exp \left( - \frac{1}{HRT \cdot HBV} \cdot Vbr \right) \]  

(4)

Using the above-mentioned values, Vbr = 7.5 l/min
and HBV = 5 l/min for a person under normal condi-
tions, as well as the Henry’s law constant for isoprene
(0.029 M/atm), Eq. 4 yields a difference between the
concentrations of isoprene in the arterial and venous
blood of ~85%.

From Eq. 4 we also see that, for compounds like
methanol with large Henry’s law constants (i.e., >100
M/atm), the exponential factor approaches unity, indi-
cating that there is no concentration gradient within
the lungs.
Integration of Eq. 3 from 0 to \( V_0 \) yields an average isoprene concentration (\( C_M \)) in the blood of the lungs

\[
\bar{C}_M = \frac{1}{V_0} \int_0^{V_0} C_V(\nu) d\nu = C_{V_0} \cdot \frac{HRT \cdot HBV}{Vbr} \times \left[ 1 - \exp \left( - \frac{1}{HRT \cdot HBV} \right) \right]
\]

(5)

We obtain the breath isoprene concentration (\( \bar{C}_L \)) by dividing \( \bar{C}_M \) by the \( H \) of isoprene

\[
\bar{C}_L = \frac{\bar{C}_M}{H}
\]

(6)

From Eq. 5 we see that an increase in the pulse frequency (proportional to HBV) causes the breath isoprene concentration to increase, whereas an increase in the breath frequency (proportional to Vbr) reduces the isoprene concentration.

Taking into account the changes of the two flow rates HBV and Vbr as a function of time, as shown in Fig. 1, yields the calculated time dependence of the breath isoprene concentration also shown in Fig. 1. The extremely good agreement between the measured and calculated concentrations is convincing evidence that there is no change in the isoprene source strength during the time span of the experiments, despite the large variation in breath isoprene concentration by a factor of >5.

These results clearly show that changes in physical activity cause strong changes in breath isoprene concentrations, resulting from changing heartbeat rate and breath rate, even though the endogenous source for isoprene may stay constant. Therefore, if the measured breath isoprene concentration is to be indicative of endogenous isoprene production, such measurements need to be done under well-defined standard conditions.

**Breath isoprene during cholesterol-lowering therapy.** The measurements of the second step of our investigations were done under standard conditions reached in the following way. Before each measurement, the test subject was allowed to move only modestly for \( \sim 1 \) h (i.e., walking slowly and sitting), and the subject sat still for \( \sim 5 \) min before filling a Tedlar bag with breath air. In this fashion, the heartbeat rate of an adult was stabilized at typically 60–70 beats/min, and the breath volume per minute was \( \sim 5 \) liters.

Some time ago, one of us was diagnosed with a high serum cholesterol level, and, when a physician prescribed cholesterol-lowering therapy, we used the opportunity to monitor breath isoprene levels during the therapy. Breath isoprene measurements and a routine blood test for serum cholesterol and LDL levels were performed 2 days before the beginning of treatment with the cholesterol-lowering drug atorvastatin. Breath isoprene analyses were continued for an additional 14 days, and two additional blood tests were done 4 and 11 days after the start of the medication. The results are shown in Fig. 3. Both the breath isoprene concentrations (measured under standard conditions) and the serum cholesterol and LDL levels declined proportionally by \( \sim 35\% \) during the investigation. It should be noted that the breath samples taken on the mornings of July 6 and 12 showed rather high values of isoprene. These samples were considered atypical because in each case the subject underwent extreme exercise and ingested alcohol the previous afternoon and evening. Overall, these preliminary results confirm earlier findings of Stone et al. (22) and a recent, brief report (26) that cholesterol-lowering drugs of the lovastatin type also reduce the isoprene production in the body. Most importantly, they show

![Fig. 3. Breath isoprene and serum cholesterol and low-density-lipoprotein (LDL) levels in an individual undergoing treatment with atorvastatin (Lipitor). Isoprene levels in breath samples collected under standard conditions were measured intermittently by proton transfer reaction-mass spectrometry. Two samples (□) were deemed atypical because of exercise and dietary extremes on the afternoons and evenings preceding breath measurement; these were not included in the curve fit to the data. 29.6–15.7, June 29–July 15.](http://jap.physiology.org/10.1152/jappl.00856.2001)
that there is a linear relation between breath isoprene concentration (under standard conditions) and serum cholesterol and LDL levels.

**DISCUSSION**

The analysis of VOCs in breath could be an important investigative tool for assaying biological processes noninvasively, particularly if the analytic method is sufficiently sensitive that preconcentration of the sample is not required and if VOCs can be measured on-line. PTR-MS is such a method, and our laboratory has shown previously that PTR-MS can be used for analysis of a variety of human breath VOCs, including isoprene (12, 23–25). For breath VOC analysis, the PTR-MS methodology has the advantage over another on-line method using selected ion flow tube technology (20) in that it operates at high enough energy in the drift region to break up most water clusters. Thus by PTR-MS the isoprene signal is detected as a single positive ion at m/z 69. It should be noted that some other potential biogenic VOCs, such as methylbutenols or methylbutanals, also give a PTR-MS signal of m/z 69. However, there is little evidence for the release of these alcohols or aldehydes at concentrations of hundreds of parts per billion by volume in human breath (see Ref. 6), and we determined for several breath samples that the m/z 69 signal is not greatly diminished by passage through water, as expected for a hydrocarbon-like isoprene. As discussed elsewhere (23), there is compelling evidence that the great majority of the abundant m/z 69 signal is due only to breath isoprene in humans.

The measurement of breath isoprene is especially interesting, because of suggestions that it might be derived from the cholesterol biosynthetic pathway. Let us briefly summarize what is known about human isoprene and its linkage to cholesterologenesis. Isoprene is clearly endogenous in origin (reviewed in Refs. 5, 10), most likely as a by-product of the biosynthesis of isoprenoid compounds, their decomposition, or both. Deneris et al. (5) have demonstrated the in vitro synthesis of isoprene from DL-mevalonate and proposed that breath isoprene is linked to cholesterol biosynthesis, which relies on the mevalonate pathway. Administration of the drug lovastatin or other statins, competitive inhibitors of the rate-limiting step of cholesterol biosynthesis in humans, has been demonstrated to suppress isoprene in the breath (22, 26). Small amounts of breath isoprene may arise from peroxidation of the cholesterol precursor squalene (21). These results strongly support the hypothesis that breath isoprene is related to some aspect of cholesterologenesis, although the tissue or tissues responsible for isoprene formation are unknown.

Despite these links between cholesterologenesis and breath isoprene, the large variability in isoprene levels among individuals has limited breath analysis as a diagnostic tool. Why is breath isoprene so variable? At first sight one might be tempted to interpret the variation of the isoprene concentration as indicative of the variation in endogenous isoprene production in the body of the test person. As shown here, this is unlikely to be the case. Instead, we suggest a model where the breath isoprene source in an individual is relatively constant during exercise or even during sleep. Isoprene has a low water solubility and high volatility (i.e., it has a small Henry’s law constant), and, when transported via the bloodstream to the lungs, it evaporates quite efficiently. Thus the actual concentration of the isoprene in the breath is governed by the production term (which is constant) and by the velocity of the bloodstream pumped through the lungs (which is proportional to the heartbeat frequency) and the breathing rate. The validity of this model is illustrated quite well in the exercise experiments shown here, where we show the following. First, breath isoprene increases dramatically at the beginning of exercise in parallel with an increase in heart rate. During this period, blood is pumped at a higher speed through the lungs, and more isoprene evaporates through the lungs. Second, as the source of isoprene stays constant, the enhanced rate of evaporation leads to a decline in the blood isoprene concentration and thus in the evaporation rate, causing a lower isoprene concentration in the breath. Third, a few minutes after the start of the exercise, the breath rate increases, leading to an enhanced dilution of the isoprene, again resulting in a decline in the breath isoprene concentration to a new, lower steady-state level; because of the enhanced breath rate, the isoprene concentration is lowered inversely proportional to the breath rate. Fourth, these effects are reversed at the end of exercise. Thus the observed strong variations in the breath isoprene concentrations (Fig. 1) are well explained on the basis of constant endogenous isoprene production but varying heart rate and exhalation rate.

Previous workers have also noted that mild exercise can decrease breath isoprene concentrations (8, 19). However, because they were not able to make on-line measurements, they did not see the rapid increase in breath isoprene noted here at the beginning of exercise and thus did not make the connection among heart rate, breath rate, and breath isoprene levels suggested here.

Similarly, the observed diurnal variations in breath isoprene concentrations with maxima during the night (4, 22, 23) can be explained as an artifact of the measurement method. During the night, the average breath rate is somewhat lowered, thus resulting in an enhanced breath isoprene concentration. Furthermore, the heartbeat rate is lowered during the night, resulting in an enhanced blood isoprene concentration. However, as soon as a test person is awakened by an alarm signal and moves to inflate a test bag, the heartbeat rate immediately increases (Table 2). This results in an overall increase in the breath isoprene concentration, which is explained in the same fashion as the initial increase observed at the beginning of the physical exercise described above (Fig. 1). We believe that the results can be explained by a model in which endogenous isoprene synthesis rate is relatively constant dur-
ing the day or night. If 1) this model is correct and 2) isoprene is a by-product of the cholesterol biosynthetic pathway, then it remains to be explained why isoprene formation does not correlate with reported diurnal periodicity in human cholesterol biosynthesis (11). From our model, it is also unclear why Stone et al. (22) observed inhibition of nighttime breath isoprene with lovastatin treatment. Cailleux and Allain (1) have already noted that the apparent diurnal variation in breath isoprene is associated with the state of sleep and wakefulness, and it is possible that administration of lovastatin alters these physiological states in a way that decreases nighttime breath isoprene. In light of the dramatic effects of heart rate on breath isoprene levels shown here, future studies of diurnal cycles of breath isoprene should take care to control for rapid changes in heart rate that occur when sleeping individuals are aroused for breath sampling.

An especially interesting development is the isoprene exhalation model presented here, which argues that breath isoprene is simply a volatile metabolic product that is efficiently (~85%) and continuously partitioned to alveolar air with each passage of venous blood through the lungs. We predict that measurements of venous and arterial blood will show this difference in isoprene concentration (see Fig. 2); blood levels of isoprene in humans have been reported only from samples of venous blood (2, 3). Such experiments are planned in the near future. It will also be of interest to obtain more extensive data on blood cholesterol and mevalonate levels (17) to help reveal suspected linkages between cholesterol biosynthesis and breath isoprene levels. It is also interesting that measurements of venous blood isoprene in patients undergoing anesthesia showed a threefold decrease during anesthesia and then an increase to normal levels during recovery (3). These results can be explained qualitatively by our gas-exchange model if one considers the enhanced ventilation rates used during anesthesia.

The results presented here may provide a new perspective on noninvasive diagnosis of cholesterol levels by measurement of breath isoprene. From the results shown in Fig. 3, it appears that changes in blood cholesterol and LDL in a patient can be monitored by breath isoprene analysis, as long as a standard breath-sampling protocol is followed. It is still an open question whether the large differences in the breath isoprene concentrations among individuals, noted in the Introduction, are primarily a result of differences in rates of cholesterologenesis or are due to other factors, such as differences in lung capacity and/or pulmonary circulation. From the results in Fig. 3, we calculate the ratio of breath isoprene concentration (under standard conditions) to serum cholesterol level to be $-6 \times 10^{-8}$ l/g. If this ratio were the same in the adult population, breath isoprene measurements, under standardized measurement conditions, could be used as a quantitative, noninvasive indicator of cholesterologenesis rates. Investigations addressing these issues are in progress.

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