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In: Jan Chleboun and Petr Přikryl and Karel Segeth (eds.): Programs and Algorithms of Numerical Mathematics, Proceedings of Seminar. Dolní Maxov, June 6-11, 2004. Institute of Mathematics AS CR, Prague, 2004. pp. 82–87.

Persistent URL: http://dml.cz/dmlcz/702779

ON CUMULATIVE ^{13}C BREATH TESTS: AN EFFORT TO IMPROVE THE ACCURACY OF TEST RESULTS REVEALED A SUBSTANTIAL UNCERTAINTY IN INPUT DATA[*]

Jan Chleboun, Petr Kocna[†]

Abstract

A cumulative ^{13}C breath test can be used to detect a pancreatic disorder, for example. The test is burdened by uncertain input data. Among them, the estimation of CO_2 production rate is a key issue. An established estimate based on the body surface area could be replaced by an estimate using the basal metabolic rate. Although the latter estimate might be considered more appropriate than the former, the differences between them are large in some sex and age groups. Such disagreements pose a danger to the reliability of the cumulative breath tests and ask for further research.

1. Introduction

In medicine, breath tests are used to evaluate the intensity of metabolic processes and, consequently, to diagnose a disorder. Take, for instance, pancreas-oriented breath tests.

The pancreas secretes (besides hormones) digestive enzymes. If a pancreas disorder occurs, nutrients are not sufficiently extracted from food. Therefore, the human body lacks nutrients and starts to extract them from body tissues. Thus, the state of health deteriorates.

Since pancreas disorder symptoms are not much distinctive and since fairly large groups of individuals are at risk (as diabetics and alcoholics, for example), a reliable diagnostic method is an issue for medicine.

Two diagnostic approaches have established themselves in this field:

1) Imaging methods, as computer tomography, nuclear magnetic resonance, endoscopic retrograde cholangiopancreatography, or ultrasound scanning, evaluate the size, structure, or texture of the pancreas, but do not evaluate its function. They cannot recognize disorders that have not demonstrated themselves visually.

2) Pancreas function tests focus on pancreatic disorders, but their execution is laborious, clumsy, or expensive and, moreover, often asks for well-trained laboratory technicians. Although dozens of pancreas function tests have been developed, including breath tests, none is widely (internationally) accepted and used in practice.

[†]Institute of Clinical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine, Charles University, Prague.

Breath tests [5] are non-invasive indirect methods to investigate patients by observing the outflow of a stable isotope marker; their use is not limited to the examination of the pancreas.

The goal of cumulative ^{13}C breath tests is to measure the patient's ability to hydrolyze an administered ^{13}C-enriched substrate, i.e., to evaluate the activity of pancreas enzymes through the proportional cumulated dose recovery (PCDR) defined as the ratio $C(t)/D$, where D is the ^{13}C dose that the patient receives at time 0, and $C(t)$ stands for the cumulative amount (in moles) of ^{13}C that the patient exhales *above* his/her normal ^{13}C level during the time interval [0,t], i.e., above the natural (background) ^{13}C content observed in the patient's breath if no dose of ^{13}C is administered.

Basically, two methods are used to determine the amount of ^{13}C in the patient's breath, namely isotope ratio mass spectrometry (IRMS) and isotope selective nondispersive infrared spectrometry (NDIRS). The former method directly delivers the abundance of ^{13}C in breath samples, whereas the latter method reports only changes in ^{13}C with respect to a reference sample. If the ^{13}C-abundance in the reference sample is known, then the ^{13}C-abundance in the measured sample can be calculated from the NDIRS readings. In practice, the reference ^{13}C-abundance is not known in NDIRS, but can be estimated. The estimates are fairly narrow and credible; see [1]. Thus, the unknown reference ^{13}C-abundance does not undermine the usability of the NDIRS measuring instruments, which are cheaper than their IRMS counterparts.

However, both methods suffer from the uncertainty in CO_2 production rate. This has turned out to be a rather severe difficulty.

2. Mathematical model

Let R_0 denotes the $^{13}CO_2/^{12}CO_2$ ratio in the reference sample comprising the normal (^{13}C-unenriched) breath air, and let $R(t)$ be the $^{13}CO_2/^{12}CO_2$ ratio in the measured sample at time $t \geq 0$. Then

$$R(t) = \left(1 + \frac{\delta(t)}{1000}\right) R_0, \tag{1}$$

where $\delta(t)$ is the NDIRS reading in per mille. Let us note that the $^{13}CO_2/^{12}CO_2$ ratio coincides with the $^{13}C/^{12}C$ ratio.

It is assumed that the patient's CO_2 production rate P is constant during the test.

The key quantity that we need to evaluate is denoted by $C(P, R_0, \delta; T)$ and stands for the total substrate-origin-$^{13}CO_2$ breath volume produced from time $t = 0$ till time $t = T$ (T equals six hours in practice),

$$C(P, R_0, \delta; T) = P \int_0^T A(R_0, \delta; t) \, dt, \tag{2}$$

where

$$A(R_0, \delta; t) = \delta(t) R_0 / \left\{1000 \left[(\delta(t)/1000 + 1) R_0 + 1\right] (R_0 + 1)\right\}, \tag{3}$$

as can be inferred from (1) by simple algebraic manipulations; see [1].

Since $\delta(t)$ is measured at discrete time points t_i, the integral (2) is approximated by the trapezoidal or Simpson's rule using $A(R_0, \delta; t_i)$. Thus, the PCDR mentioned in the Introduction is calculated as $C_{\mathrm{appr}}(P, R_0, \delta; T)/D$.

3. Uncertainties

The quantities R_0, δ, and P are uncertain. The reference value R_0 can be bounded by the range of the natural $^{13}CO_2$ abundance in (^{13}C-unenriched) human breath. Although measurements have revealed diet-dependent differences, the whole range is considerably limited, and the impact of the uncertainty in R_0 is almost negligible [1].

The uncertainty in δ is caused by the variability of measurements and by the inaccuracy of the measuring instrument. A properly calibrated and maintained instrument delivers sufficiently accurate measurements [1]. The influence of uncertain δ is relatively strong when the PCDR is low, but not strong enough to question that a low PCDR is really low. The impact of uncertainty in δ diminishes if the PCDR increases [1].

Let us now focus on P. We observe that $C(P, R_0, \delta; T)$ is linear in P, so that the uncertainty in P propagates in results in a simple way. However, how much uncertainty is in P?

There are various approaches to the determination of P in breath tests. For their simplicity, estimates using body height and weight are popular. Among them, estimates based on the body surface area (BSA) are widely used. It is assumed that the CO_2 (hour) production rate P_{BSA} in the resting state is equal to the BSA multiplied by a constant.

Various BSA formulae have been derived [10]. Let us only mention the Haycock formula [2]

$$s = 0.024265 \, w^{0.5378} h^{0.3964}, \tag{4}$$

where s is in m^2, w means body weight in kg, and h stands for body height in cm. Then, under the assumption that the measured individual is in the resting state,

$$P_{\mathrm{BSA}} = 0.3 \, s, \tag{5}$$

where the multiplicative constant is given in mol/(m^2 hour); see [4].

Although (4) and the other BSA formulae show increased inaccuracy when applied to individuals from some of "extremal" groups as newborn babies, obese or gaunt humans, it has been confirmed that their inaccuracy does not exceed $\pm 10\%$ in general, and $\pm 5\%$ if they are applied to a "standard" population; see [7], for instance.

However, much less is known about the validity of the constant in (5). First of all, is it correct to assume that it is a quantity that does not depend on age and sex?

In the effort to improve the accuracy of P, a BSA-based formula can be replaced by a formula based on the basal metabolic rate (BMR). The BMR is the energy necessary for homeostasis when the body is at digestive, physical, and emotional rest [9]. Again, various formulae for the BMR and, consequently, for P_{BMR} are available. We use [6]:

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	Female			Male		
Age (years)	a	b	c	a	b	c
3 − 10	0.071	0.677	1.533	0.082	0.545	1.736
10 − 18	0.035	1.948	0.837	0.068	0.574	2.157
18 − 30	0.057	1.184	0.411	0.063	−0.042	2.953
30 − 60	0.034	0.006	3.530	0.048	−0.011	3.670

Tab. 1: *Age and sex dependent coefficients a, b, and c.*

$$e = aw + b\widehat{h} + c, \tag{6}$$

where the BMR e is given in MJ per day, w stands for body weight in kg, $\widehat{h}$ means body height in m, and the coefficients a, b, and c are specified in Table 1; see [6]. The BMR formula (6) is discontinuous with respect to time, i.e., non-negligible jumps occur at the age of 10, 18, and 30 years. It is estimated in [6] that the individual true BMR belongs to $[0.8e, 1.2e]$ with probability at least 0.95.

Since the body obtains energy through oxidation, e is related to the amount of exhaled CO_2 (again in mol/hour) [3]

$$P_{BMR} = 0.10375\, e. \tag{7}$$

The constant can be inferred from the de Weir equation [8], which links the BMR with the oxygen consumption and the CO_2 production. The constant comprises several parameters as the kcal–MJ conversion coefficient, liter–mole conversion coefficient, and a physical activity coefficient, which relates an actual metabolic rate with the BMR.

Let us depict the ratio P_{BMR}/P_{BSA}; see Figure 1, where graphs for four of the eight groups covered by Table 1 are displayed.

We can see that the ratio is greater among younger humans. This is in agreement with the fact that the BMR decreases with age, and P_{BSA} is age-independent. We can also observe that the ratio is greater for men than for women. This is explainable because, in general, men have more muscle, and P_{BSA} does not depend on sex. However, it is difficult to explain why the ratios are so high in all the groups (also in 18 − 30 years old men and women, and, especially, in 3 − 10 years old children) with the only exception of 10 − 18 years old females, where the ratio is only about 1.2 and more. A partial explanation, based on the physical activity coefficient value, is propounded below.

The variable differences between P_{BSA} and P_{BMR} implicate a difficulty with the interpretation of a cut-off level, i.e., a fixed reference value that determines whether a test result is considered normal, or abnormal, that is, marking a disorder. Originally and in the literature, the cut-off level for pancreas-oriented cumulative ^{13}C breath
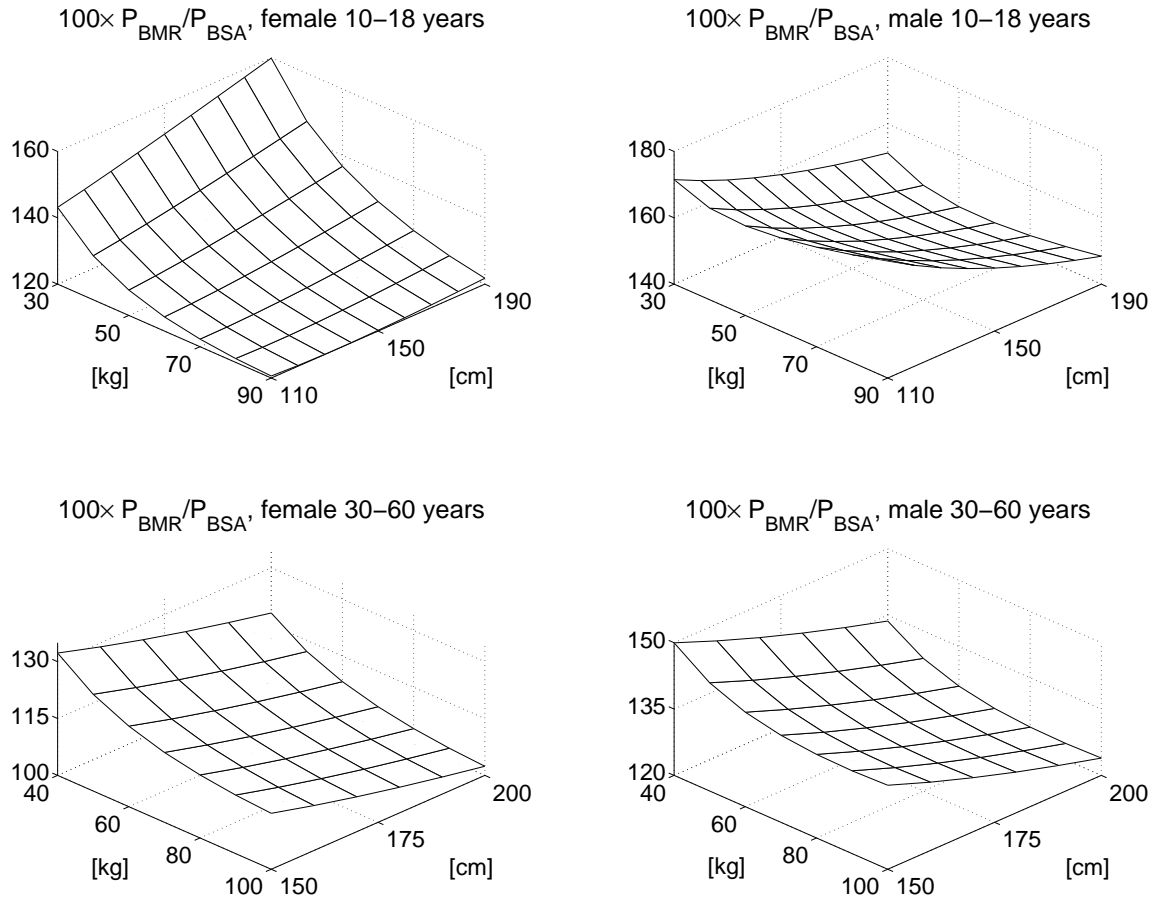
Fig. 1: P_{BMR} compared with P_{BSA}; weight in kg, height in cm.

test stemmed from the BSA-based CO_2 production rate estimates and amounted to 23%, i.e., CPDR=0.23. By (7) and by the observation (Figure 1), the BMR-based cut-off level appears greater than the BSA-based cut-off level. However, a direct conversion is hardly possible. An estimated (7)-based cut off level for male and female adults equals 29%.

The discrepancy between P_{BSA} and P_{BMR} as well as between the respective cut-off levels is strange and strong. It initiated an analysis of the procedure of deriving (7) in [3]. The analysis has led to a suspicion that (7) is valid only under special circumstances that do not apply to common ^{13}C breath tests. Although [3] lacks details on the setting of the measurements, there is an indication that P_{BMR} (7) might be large due to a large physical activity coefficient. Since ^{13}C breath tests assume a resting state, a relevant physical activity coefficient should be 1.0–1.1.

An inspection of [3] reveals a coefficient equal to 1.4 that could be interpreted as the physical activity coefficient. If a correction is made, that is, 1.1 instead of 1.4 is used in the deduction of the coefficient on the right-hand side of (7), then P_{BMR} is in a better agreement with P_{BSA}. However, to be fully justified, such a correction

has to be reinforced by further arguments and a detailed analysis. In other words, the assessment of the (in)accuracy of the CO_2 production rate estimates as well as the validation of the cut-off level ask for further investigation.

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