CLIN. CHEM. 39/1, 87–92 (1993)

Breath Acetone Analyzer: Diagnostic Tool to Monitor Dietary Fat Loss

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Acetone, a metabolite of fat catabolism, is produced in excessive amounts in subjects on restricted-calorie weight-loss programs. Breath acetone measurements are useful as a motivational tool during dieting and for monitoring the effectiveness of weight-loss programs. We have developed a simple, easy-to-read method that quantifies the amount of acetone in a defined volume of exhaled breath after trapping the sample in a gas-analyzer column. The concentration of acetone, as measured by the length of a blue color zone in the analyzer column, correlates with results obtained by gas chromatography. Using the breath acetone analyzer to quantify breath acetone concentrations of dieting subjects, we established a correlation between breath acetone concentration and rate of fat loss (slope 52.2 nmol/L per gram per day, intercept 15.3 nmol/L, n = 78, r = 0.81). We also discussed the possibility of using breath acetone in diabetes management.

Additional Keyphrases: *obesity · breath analysis*

Measurement of the rate of fat catabolism is a particular problem for many individuals on weight-loss diets, who are unable to determine their rate of fat loss because of daily variation in their body fluid content. Several methods can be used to measure body fat content at a particular time, but none of them reveal the rate of fat loss. In an indirect method to measure daily fat loss (*1*), daily fluid and protein mass changes are subtracted from daily weight changes. The percentage of body fat is generally calculated by measuring the total body water by isotopic dilution methods (*2*) and using the formula:

$$\% \text{ body fat} = 100 - \%\text{TBW}/0.732$$

where the factor 0.732 represents total body water (TBW) per unit of fat-free mass. Hydrostatic weighing is also used to measure total body fat, but has limitations relating to the density of nonfat mass, lung capacity, unknown body water content, and mineral concentrations (*3*). Skin calipers, sometimes used to calculate body fat, are inaccurate (*4*). The use of bioelectrical impedance is becoming popular in view of its claimed equivalency to isotopic dilution and hydrostatic weighing methods for estimating body fat (*5*). However, accurate measurement of body weight and variation in the body fluid content, particularly with women during menstruation cycles, limits its use in short-term weight-loss programs.

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Received April 30, 1990; accepted August 20, 1992.

Subjects who are dieting, or who are metabolizing more calories than they are consuming, utilize stored body fat to make up the dietary calorie deficit (*6*). This results in the breakdown of triglycerides in adipose tissue, liberating increased amounts of ketone bodies: 3-hydroxybutyric acid, acetoacetic acid, and acetone (*7*). The volatile acetone then diffuses through the lungs into the exhaled breath (*8*). Several studies show that breath acetone is an indicator for estimating the rate at which body fat is catabolized during a net negative calorie balance (*7–10*).

Current methods for measuring breath acetone concentrations include the use of gas chromatography and (or) mass spectrometry, either by blowing directly into the inlet tube or by collection in a suitable breath-collection device (*10–12*). These methods are feasible only in research laboratories. Colorimetric methods, which were used two decades ago, include 2,4-phenylhydrazine (*13*), salicylaldehyde (*14*), and furfural reagents (*15*). These methods are complex, require high concentrations of acid or alkali, and necessitate the use of a spectrophotometer; they are generally no longer used. A patent (*16*) was awarded to a method for measuring acetone vapor by reacting the acetone with hydroxylamine hydrochloride. This liberates hydrochloric acid, which then reacts with a pH indicator to cause a visual color change. However, the method seems to be insensitive, and utility with pure breath samples was not demonstrated.

We describe here a novel instrument-free colorimetric assay method for quantifying breath acetone content. Results of the breath acetone analyzer correlate with those of a gas-chromatographic method for measuring breath acetone in normal and dieting subjects. Moreover, the breath acetone analyzer (*17*) has been effectively utilized to monitor the rate of fat loss in restricted-calorie programs.

Materials and Methods

Analysis for Breath Acetone

By gas chromatography. A Shimadzu (Kyoto, Japan) gas chromatograph, Model GC-2A, equipped with a heated gas sampler (HGS-2) and a flame-ionization detector, was used as described previously (*17*). Acetone calibration standards ranging from 4 to 1000 nmol/L were used to determine the relationship between the height of the acetone peak and the concentration of acetone in a sample. A Shimadzu C3RA integrator was used for the calibration. Known concentrations of acetone vapor standard samples were prepared by using a gas blender (Linde Div., Union Carbide, Danbury, CT) and were used to calibrate the instrument daily.

Each breath sample was collected into a breath sam-

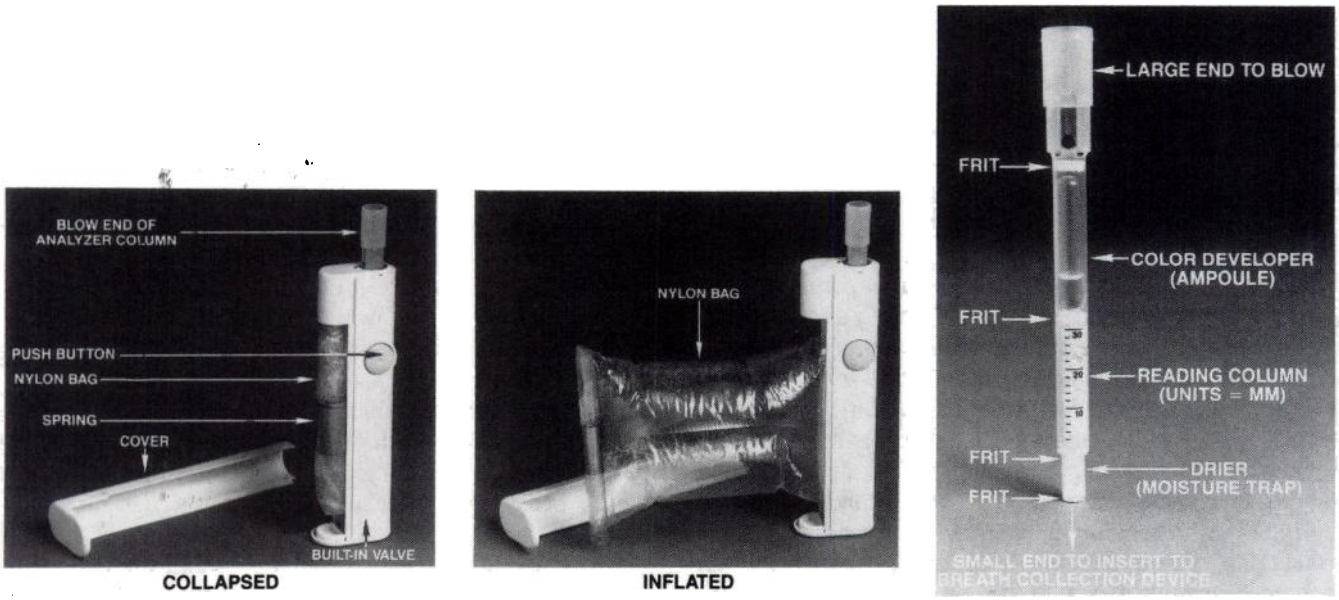

Fig. 1. Breath sampler in collapsed and inflated positions and a close-up of the analyzer column

The push button is an aid to break the glass ampul in the analyzer column. The reading column is marked in millimeters. The small end of the column goes into the breath sampler and the large end is used for blowing into

pler made of 1-mil (25.4 μm)-thick nylon film (capacity 380 mL) to which a constant-pressure spring and a valve were attached. Subjects were instructed to take a deep breath, hold it for at least 5 s, and inflate the breath sampler until it was completely full. For analysis, the 1-mL gas-loop was always purged with a breath sample or a collected acetone vapor sample for 20 s at a constant flow rate of 40 mL/min with use of a gas-flow meter.

By breath acetone analyzer. A detailed description of breath acetone analysis by breath acetone analyzer is described in a recent patent (*17*). In brief, the analyzer consists of a reusable breath sampler and a disposable analyzer column (Figure 1). Breath samples were collected as described above. The collected breath sample then passes through the analyzer column automatically until the sampler is completely collapsed. This deflation takes ~10 min. The analyzer column contains a calcium chloride trap that removes the moisture in the breath sample before it enters the reaction zone (reading column zone, Figure 1). Removal of moisture from the breath sample is essential to prevent deactivation of the reaction zone and to avoid delayed color development. The reaction zone comprises a mixture of nitroprusside (nitroferricyanide)-diethylaminoethyl silica and aminopropyl silica particles (*17*). Acetone present in the breath sample gets trapped in the reaction zone. The length of the zone to which the acetone is adsorbed depends on the concentration of acetone in the sample. The location of the adsorbed acetone color bar height in the analyzer column is made visible by breaking the ampul—which contains a mixture of dimethyl sulfoxide, methanol, and a catalytic amount of diethanolamine (*17*)—with a push button (Figure 1). The ampul can be broken immediately after the sampler collapses or within 1 h, to avoid excessive moisture accumulation in the calcium chloride trap. A blue color bar develops within 5 min. The color is stable for 30 min, so the results should be read between 5 and 30 min after breaking the ampul.

Clinical Study

Subjects. The study was limited to 58 normal men and women, who were between 10% and 30% above their ideal body weight and otherwise apparently healthy. All participants underwent complete physical examinations (including blood and urine analysis) before, midway through, and at the end of the study. The study period was 30 consecutive days. Twenty volunteers included in a separate nondieting control group were studied for 19 days. The clinical study was approved by the Abbott Laboratories Institution Review Board, and all volunteers were Abbott employees.

Diet and physical activity. Two diets, 1000 and 1200 calories daily, were used in this study. The 1000-calorie plan included 60–80 g of protein, 90–130 g of carbohydrate, and 22–40 g of fat per day. The 1200-calorie diet included 80–110 g of protein, 113–147 g of carbohydrate, and 25–47 g of fat per day. The selection of the appropriate diet was made according to the basal energy expenditure requirements of the participants. The volunteers refrained from any strenuous physical activity during the entire study period.

Experimental methods. Breath samples were collected each day immediately upon awakening. The samples were assayed simultaneously with breath acetone analyzers and by gas chromatography. Whole-body weights were measured daily immediately upon awakening, after defecation and urination. Total body water and body fat were determined for each volunteer in the morning before breakfast, with a bioelectrical impedance analyzer (Model BIA-101; RJL Systems, Detroit, MI).

Results

Breath Acetone Analyzer

Correlation with gas chromatography. A standard correlation curve between the color bar heights (mm, y) obtained by the breath acetone analyzer and the acetone vapor concentrations (nmol/L, x) analyzed by gas chromatography, was developed. The standard curve gave a linear-regression equation of $y = 14.905 \log x - 14.57$ ($r = 0.96$). We used this equation to calculate the acetone concentrations of standard acetone gas mixtures analyzed by the breath acetone analyzer. To test the correlation, we assayed by gas chromatography and the breath acetone analyzer standard acetone gas mixtures of 0, 10, 40, 80, 100, 200, and 250 nmol/L (Figure 2*A*). Figure 2*B* shows the correlation for individual breath acetone concentrations from dieting and nondieting volunteers determined by both methods.

Assay precision. The assay precision of the breath acetone analyzer was evaluated at three different acetone vapor concentrations (40, 80, and 200 nmol/L), prepared by using a gas blender and verified by gas chromatography. Each concentration was measured by the breath acetone analyzer in replicates of five for 5 days. As summarized in Table 1, the coefficient of variation (CV) of the breath acetone analyzer within the same day was <9% and between days was <3.5%.

Assay sensitivity and range. The detection limit of the breath acetone analyzer was evaluated by direct measurement of standard acetone–air mixtures. Concentrations of 0, 4, 9, 15, and 20 nmol/L acetone–air mixtures (as measured by gas chromatography) in replicates of five samples were used in this study. At 0 nmol/L acetone concentration, no color was visible in the analyzer column, whereas at 4, 9, 15, and 20 nmol/L, the color bar heights were 3, 4, 5, and 7 mm, respectively (average SD 0.5 mm). Because of the exponential relationship between the concentration of breath acetone and the height of the color bar, the customary definition of detection limit, the lowest nonzero concentration detected with 95% confidence, cannot be used. Instead, we estimated sensitivity from the standard error of the intercept (*18*), 0.57 mm. The concentrations of acetone–air mixtures determined by gas chromatography were assumed to be exact, and a least-squares linear regression of the color bar heights read and the logarithm of the acetone concentrations was calculated. Inserting twice the standard error of the height estimate into the linear-regression equation for height vs log acetone concentration (see above) yielded a detection limit for the assay of 1.7 nmol/L.

Table 1. Assay Precision of Breath Acetone Analyzer

	Within-day (n = 25)			Between days (n = 25)		
	Acetone, nmol/L			Acetone, nmol/L		
Acetone, nmol/L	Mean	SD	CV, %	Mean	SD	CV, %
40	35	3.0	8.6	35	0.8	2.3
80	65	4.2	6.5	65	2	3.1
200	200	2.4	1.2	200	3	1.5

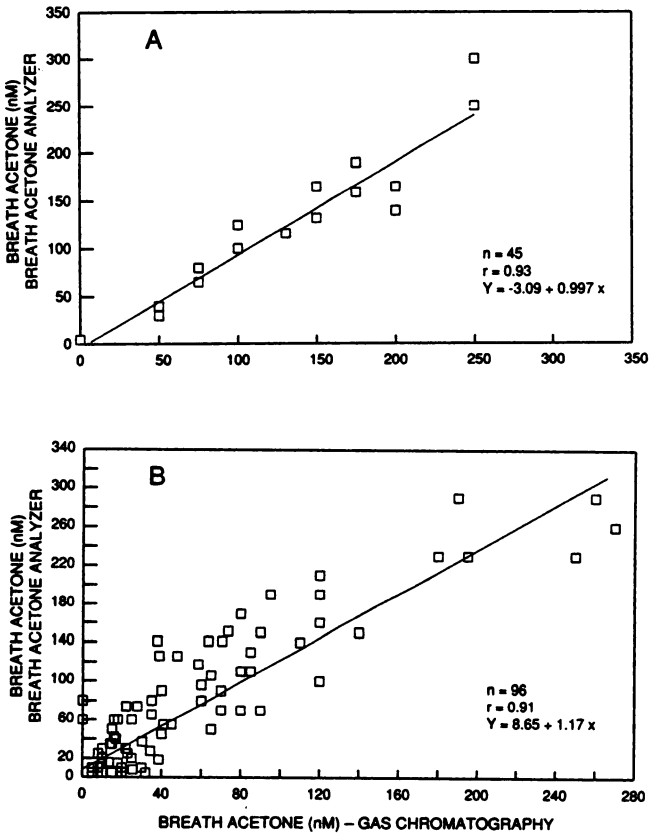

Fig. 2. Correlation of breath acetone analyzer and gas-chromatographic measurements of acetone concentration: (*A*) standard acetone vapor samples; (*B*) breath samples from dieting and nondieting (control) subjects

We used two lots of breath acetone analyzer columns, one covering the assay range 0 to 300 nmol/L and the other 0 to 450 nmol/L. For each lot of columns, we calculated the measured acetone concentrations by using the standard correlation curve equation between breath acetone analyzer and gas chromatography for that specific lot. The calculated acetone concentrations as calibrated for each lot of analyzer columns against the color bar heights were incorporated in a chart so that the breath acetone concentrations could be obtained directly from the observed color bar height readings. All dieting and nondieting volunteers who participated in this study recorded their own daily breath acetone concentration by using this chart.

Assay specificity. In normal breath samples, acetone is the major compound containing a carbonyl moiety (91% of the total carbonyl compounds); the other carbonyl compounds constitute a small fraction (*11*). In addition, ethanol, methanol, and isopropanol are present in normal breath samples as minor components, each <100 nmol/L. We tested the breath acetone analyzer to evaluate the percentage of change in an observed acetone concentration at 80 nmol/L acetone after a known concentration of possible interferents was added to the sample. To avoid contamination, we used separate breath samplers in this study. In no case was color

Table 2. Specificity of Breath Acetone Analyzer

	Interferent conc, nmol/L		Acetone observed, nmol/L[a]				
			0 std.		80 std.		
	Normal physiological	Used in the study	Mean	SD	Mean	SD	% change
Control (no component added)[b]			0	0	65	0	—
2,2-Dimethyl-3-hexanone	0.006	0.031	0	0	68	6	5
Methyl-*tert*-butyl ketone	0.01	0.05	0	0	68	6	5
2-Methyl-3-heptanone	0.006	0.016	0	0	65	0	0
2,4-Dimethyl-3-hexanone	0.0016	0.031	0	0	65	0	0
5-Methyl-3-heptanone	0.002	0.016	0	0	65	0	0
Ethanol	10	10.8×10^3	0.4	0.05	80	4	23
Methanol	9.37	625	0.8	0.05	68	6	5
Isopropanol	8.6	500	0	0	65	0	0
Propiophenone	0.004	0.002	0	0	68	6	5
N-Butyl acetoacetate	0.003	0.016	0	0	65	0	0
2-Methyl-3-pentanone	0.004	0.02	0	0	68	6	5
p-Tolualdehyde	0.08	0.42	0	0	68	6	5
Acetaldehyde	0.016	0.80	0	0	68	6	5

[a] Mean and SD of two measurements each of 0 and 80 nmol/L standards in the presence of the potential interferent.
[b] Normal breath acetone concentration, 15 nmol/L.

observed with any of the interferents. Moreover, the percent of difference in observed acetone concentration due to the possible breath interferents (Table 2) was not significant ($0.4 > P > 0.3$). Interference from ethanol (up to 6.5 μmol/L) was also not significant. However, ethanol concentrations above the legal limit, 10.3 μmol/L (*19*), caused an increase in apparent acetone concentration of 23% (Table 2), which is also not significant ($0.25 > P > 0.1$).

Clinical Study to Monitor Fat Loss

During the course of this study, the breath acetone concentrations of all dieting subjects increased during the first few days of the diet, reached a plateau after ~7 days, and remained increased during the course of the 30-day study. Figure 3 (left) shows the average breath acetone concentration and the daily rate of fat loss during the study for the 58 dieting volunteers and the 20 nondieting (control) volunteers. The rate of fat loss of the dieters was negative before day 3 of the diet, but on day 4 the rate of fat loss increased sharply and finally stabilized from day 8 onwards. The possible reasons for such erratic results in the rate of fat loss as measured by the bioelectrical impedance analyzer in the first few days of the diet were due to the fluctuation in the body water content and the stored glycogen reserve. However, from day 8 to day 30, the daily rate of fat loss remained almost parallel to the daily breath acetone concentration of the dieters and averaged 114 g/day (SD 17.4, range 100–152 g/day). The daily average acetone concentration of the dieters during this period was 290 nmol/L (SD 8.1, range 280–300 nmol/L). The control subjects showed a daily average breath acetone concentration of 15 nmol/L (SD 11 nmol/L) during the entire study period. The daily rate of fat loss of the control group was almost negligible and remained parallel to the daily average breath acetone concentration.

Each dieter showed differences in the breath acetone concentration as well as in the rate of fat loss, as illustrated in the individual profiles of two dieters (Figure 3, right). The breath acetone concentration of dieter no. 1 increased progressively from day 1 and reached a

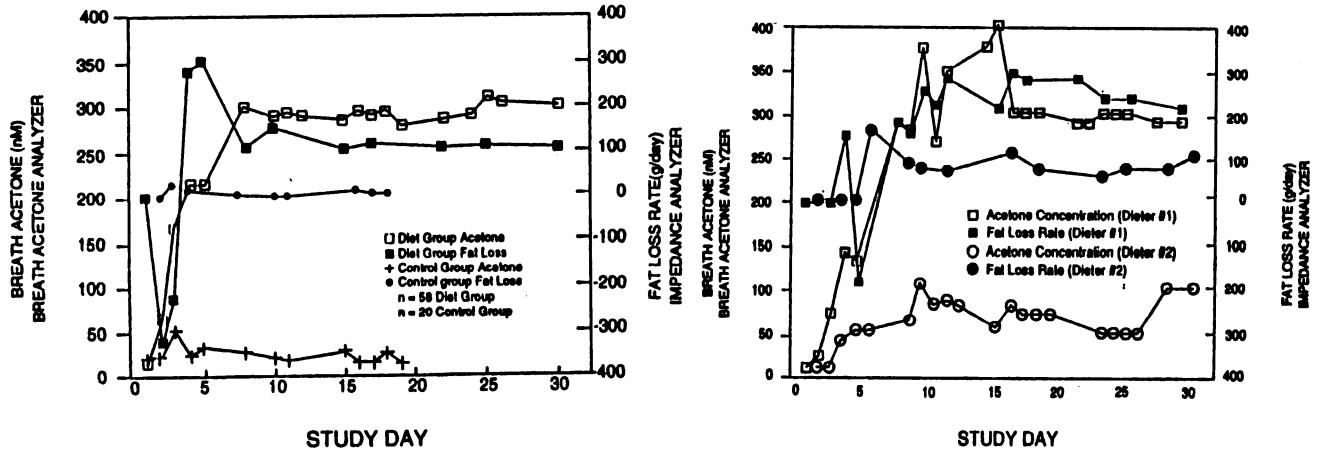

Fig. 3. Daily changes in fat loss rate and daily average breath acetone concentration of dieting and nondieting (control) groups (*left*) and in two individual dieters (*right*)

fairly steady state from day 8 to day 30. The breath acetone concentration of this dieter remained >280 nmol/L, with a daily average concentration of 311 nmol/L (SD 38.4, range 280–400 nmol/L) during this period. The daily rate of fat loss of this dieter fluctuated widely until day 7 and then remained fairly steady onwards, almost paralleling the daily breath acetone concentration from day 8 to day 30: the daily average rate of fat loss from day 8 to day 30 was 238 g/day (SD 47, range 183–300 g/day).

The breath acetone concentration of dieter no. 2 increased at a slow rate from day 2 and remained low (range 43–100 nmol/L) during the entire study period. The breath acetone concentration reached almost a steady state from day 8 to day 30, with a daily average of 71 (SD 20.3) nmol/L. This dieter did not show any fat loss until day 4, but on day 5 had an unusual increase in the daily fat loss rate. However, from day 8 to day 30, the daily rate of fat loss remained steady and paralleled the daily breath acetone concentration. The daily average rate of fat loss of this dieter from day 8 to day 30 was 78 g/day (SD 17.4, range 50–100 g/day).

To establish a correlation between the breath acetone concentration and the daily rate of fat loss, we calculated the breath acetone concentration and the daily rate of fat loss during day 8 and day 30 of the diet study (steady phase) for each dieter. For each control subject, we used the breath acetone concentration and the rate of fat loss during their entire study period of 19 days. The breath acetone concentrations were determined directly from the breath acetone analyzer column readings and were averaged for the dieters and control subjects for the days stated above. Similarly, we determined the fat loss rates with the bioelectrical analyzer and averaged the daily rate of fat loss as above. The results (Figure 4) demonstrate that breath acetone concentration as measured by breath acetone analyzer correlated with the rate of fat loss during the diet program (r = 0.81).

Discussion

The present study was designed to validate the use of a simple and easy-to-read breath acetone analyzer we developed (*17*). The device is intended to be used to monitor the ketotic state of an individual for estimating fat catabolism in weight-loss programs and to monitor patients with diabetic ketosis.

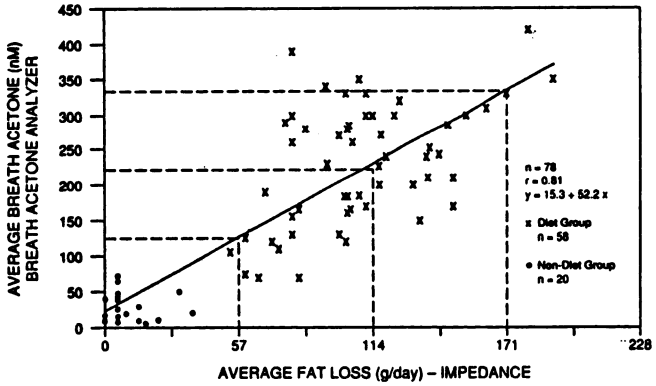

Fig. 4. Correlation of breath acetone concentrations determined by breath acetone analyzer to rate of fat loss determined by bioelectrical impedance

The carbonyl function of an aldehyde or a ketone reacts with a nitroprusside (nitroferricyanide) salt in the presence of an amine to form a colored complex (*20*). The reaction of acetone with nitroprusside in aqueous solution proceeds very slowly compared with the reaction of acetoacetic acid, which is ~100–200 times faster (*17, 21*). The color complex is unstable, however, because nitroprusside decomposes rapidly in alkaline solutions. Numerous attempts have been made to stabilize the color complex by using mixtures of nitroprusside and amines or amino acids in combination with various buffers, metal salts, organic salts, organic stabilizers, and polymers. Many assay devices described in the literature utilize dry tablets or powders, and others utilize adsorbent carriers on which some or all of the reagents have been dried. Because the indicator materials are merely adsorbed onto the carriers, the strips bleed a certain amount of the color product into the aqueous environment. Because of these inherent problems associated with all previous nitroprusside-based methods, none of the methods are suitable for breath acetone measurements or for blood or serum acetone quantification. The method of utilizing hydroxylamine hydrochloride and a pH indicator dye (*16*) for measuring acetone vapor appears to be insensitive for measuring small volumes (<500 mL) of pure breath samples. The method we report utilizes a novel solid-phase chemistry based on a nitroprusside and amine reaction with the carbonyl function of a ketone or aldehyde. A mixture of nitroprusside-diethylaminoethyl-silica salt and covalently bound aminopropyl silica particles was used in this method (*17*). The use of silica serves two purposes: it traps the acetone from a breath sample to a distance dependent on the concentration of acetone; and the color that is developed after the addition of a solvent mixture remains bound to the solid phase, thus allowing quantification of the acetone concentration.

In the present study, use of the breath acetone analyzer demonstrated that the breath acetone concentration during diet was correlated to the rate of fat loss. Individuals with breath acetone concentrations <120 nmol/L lost fat at a rate of 0–57 g per day, those with acetone concentrations between 120 and 220 nmol/L lost fat at 57–114 g per day, and those with acetone concentrations <220 nmol/L had a daily fat loss rate between 114 and 171 g per day (Figure 4). Moreover, we demonstrated that we could measure the rate of fat loss during diet by quantifying breath acetone. Figure 5 shows a typical column-lot-specific "Diet Progress Chart," which can be used to monitor the daily breath acetone concentration during a weight-reduction program. The fat loss rates are derived from the correlation curve between the daily breath acetone concentration and the daily rate of fat loss as shown in Figure 4. A person who intends to undertake a weight-reduction program could be provided with a "Diet Progress Chart," use the breath analyzer each day in the morning, and record each day's column reading on the chart, drawing a line to connect

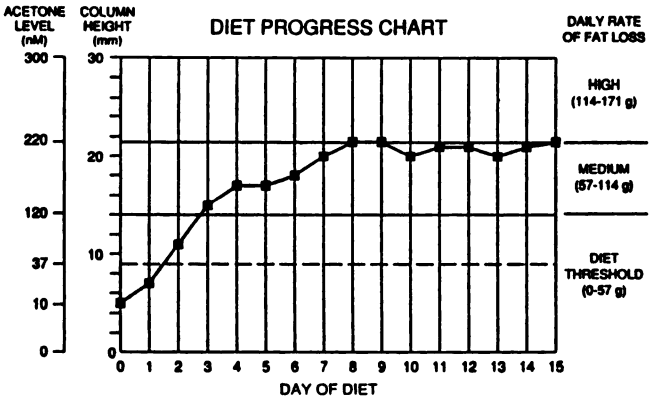

Fig. 5. Diet progress chart: breath analyzer column heights and corresponding acetone concentrations for a specific lot of analyzer columns vs the daily rate of fat loss (g/day)

The *dotted line* at 37 nmol/L represents the diet threshold, above which body fat is lost

each day's reading, as shown in Figure 5. The breath acetone concentrations for the color bar height readings are included directly on the calibrated chart, and the daily fat loss rate can be predicted from the same chart. The dotted line indicating the 37 nmol/L breath acetone concentration, the "diet threshold level," was calculated (95% confidence) from the average breath acetone concentration (15 nmol/L) of nondieting subjects (n = 78) plus 2 SD (2 × 11 nmol/L). An individual who exceeds this threshold is expected to lose fat.

Thus, the breath acetone analyzer reading allows an estimate of the rate of fat loss that is independent of the daily fluctuations in body weight. Loss in body weight is a combination of the loss in water, lean body mass, and fat. During the first several days of a diet, the loss in body weight may be mostly due to water loss. As the diet continues, it may be difficult to see small daily changes in body weight despite the fact that body fat is still being lost. The breath acetone analyzer will indicate whether the body is still catabolizing fat, even when there is a gain in body weight due to fluid retention.

Other uses of the analyzer. In insulin-dependent (type 1) diabetic patients, blood acetone concentrations correlate very well with breath acetone (*10, 12*). Urinary ketones, although measured in highly ketotic uncontrolled or poorly controlled diabetic patients, do not provide results as accurate as blood ketones do (*7, 11*). Thus the breath acetone analyzer can measure ketonemia more effectively than do available methods for quantifying urinary ketones.

Non-insulin-dependent (type 2) diabetic patients are hyperglycemic and are distinguishable from insulin-dependent patients by having normal concentrations of breath acetone (*11*). Thus, the breath acetone analyzer can be suitably used to distinguish type 1 and type 2 diabetic patients.

The breath acetone analyzer system is not validated for patients with disorders in the digestive organs, renal insufficiency, uremia, and malignant carcinoma or other metabolic states. The test is not intended for use in patients who exhibit chronic and habitual alcohol consumption.

We thank David Milligan, William Stadtlander, and Marc Minkus for their support and encouragement; Ed Moore, Steve March, Terry Pry, and Dave Nevalainen for their valuable advice; Glen Larson, Sam Rutnarak, Robert Hammond, Lydia Milne, and the Pilot Plant Team for development of the product; Lorri Sullivan in preparation of the diet protocols and overall supervision of the clinical study; and Maryann Bliss Monsalud, Dorie D'Hane, and Sean Morin for their help in the clinical study.

References

1. Wynn V, Abraham RR, Densem JW. Method for estimating rate of fat loss during treatment of obesity by calorie restriction. Lancet 1985;i:482–6.
2. Lukaski HC, Johnson PE. A simple, inexpensive method of determining total body water using a tracer dose of D_2O and infrared absorption of biological fluids. Am J Clin Nutr 1985;41: 363–70.
3. Lohman TG. Skinfolds and body density and their relation to body fatness: a review. Hum Biol 1981;53:181–225.
4. Katch VL. Body composition of humans: standards, examples and the prediction dilemma. In: Hansen BC, ed. Controversies in obesity, endocrinology and metabolism, Vol. 5. New York: Praeger Publishers, 1983:301–17.
5. Segal KR, Gutin B, Presta E, et al. Estimation of human body composition by electrical-impedance methods: a comparative study. J Appl Physiol 1985;58:1565–71.
6. Guyton AC. Textbook of medical physiology, 6th ed. Philadelphia: WB Saunders Publishers, 1981:853.
7. Rooth G, Carlstrom S. Therapeutic fasting. Acta Med Scand 1977;88:128–39.
8. Briggs AP, Shaffer PA. The excretion of acetone from the lungs. J Biol Chem 1921;48:413–28.
9. Goschke H, Lauffenburger TH. Breath acetone and ketonemia in normal and overweight subjects during total fasting. Res Exp Med 1975;165:233–44.
10. Crofford OB, Mallard RE, Winton RE, et al. Acetone in breath and blood. Trans Am Clin Climatol Assoc 1977;88:128–39.
11. Krotoszynski B, Gabriel G, O'Neill H. Characterization of human expired air; a promising investigative and diagnostic technique. J Chromatogr Sci 1977;15:239–44.
12. Riechard GA, Haff AC, Skutches CL, et al. Plasma acetone metabolism in the fasting human. J Clin Invest 1979;63:619–26.
13. Greenberg LA, Lester D. A micromethod for the determination of acetone and ketone bodies. J Biol Chem 1944;154:177–89.
14. Peden V. Determination of individual serum ketone bodies with normal values in infants and children. J Lab Clin Med 1964;63:322–43.
15. Lyon JB, Bloom WL. The use of furfural for the determination of acetone bodies in biological fluids. Can J Biochem 1958;36:1047–56.
16. Prior JN. Breath acetone indicator media and device. Australian Patent A-77259, 1987.
17. Kundu SK, George RW, March SC, et al. Method and device for ketone measurement. Eur Patent 0279069A2, 1988.
18. Davis RB, Thompson JE, Pardue HL. Characteristics of statistical parameters used to interpret least-squares results. Clin Chem 1978;24:611–20.
19. Mason MF, Dubowski KM. Breath-alcohol analysis: uses, methods and some forensic problems—review and opinion. J Forensic Sci 1976;21:9–41.
20. Magers TA, Tabb DL. Test means and method for detecting ketone bodies. US Patent 4147514, 1979.
21. Kobayashi K, Okada M, Yusuda Y, et al. A gas chromatographic method for the determination of acetone and acetoacetic acid in urine. Clin Chim Acta 1983;133:223–6.