On the basis of observations made in the present study, we recommend that direct wiping be avoided, preferring application of an established volume of saliva that could allow delivery of a sufficient quantity to the test pad, which can be easily done “on site” by collecting saliva and applying it to the test pad. In the future, manufacturers of Drugwipe may want to standardize batch-to-batch devices to preselected concentrations for amphetamine-related drugs, taking into account the differences in doses and routes of administration currently in use as well as the time window to be covered by the analytic device in relation to peak effects of the drug. In fact, although a limited number of individuals participated in this study, the present results show that the Drugwipe in combination with the Drugread adequately detected MDMA in saliva in the first 6 h after administration. On the other hand, the analytic device gives a negative response in a range of salivary concentrations down to ~450 μg/L (0.9 ng of MDMA in 2 μL of saliva applied to the test pad). These concentrations can be found in individuals ~6–10 h after the administration of 100 mg of MDMA, corresponding to a mean range of 80–120 μg/L in plasma and 3–12 mg/L in urine (10, 11). Conversely, the 0–6 h time window is the period of maximal pharmacologic effects of MDMA. In this time interval, an individual is at highest risk of psychomotor impairment that may have consequences in some demanding tasks, such as driving. Six hours after MDMA ingestion, although the drug is still present in several biologic fluids, most subjective and physiologic effects (i.e., cardiovascular function) return to basal conditions (10). Hence, if the objective of on-site saliva testing is not only to detect the consumption of a given drug but also to determine whether an individual is under the effects of the drug, on-site saliva testing with the Drugwipe coupled with the Drugread fits that purpose. Ultimately, appropriate confirmation with a reference chromatographic method for saliva samples should be performed.

This investigation was supported by the Department of Social Affairs (Italy), FIS 97/1198 and 98/0181, CIRIT 99-SGR-242, and PNSD (Spain). We thank Esther Menoyo and Isabel Sanchez for assistance in the experimental sessions and laboratory tests, Dr. Marta Pulido for editing the manuscript, and Securetec (Ottobrunn, Germany) for technical support.

References

Addition of Quantitative 3-Hydroxy-Octadecanoic Acid to the Stable Isotope Gas Chromatography—Mass Spectrometry Method for Measuring 3-Hydroxy Fatty Acids, Patricia M. Jones,1* Susan Tjoa,2 Paul V. Fennessey,2 Stephen L. Goodman,2 and Michael J. Bennett1 (1 University of Texas Southwestern Medical Center, Department of Pathology, and Children’s Medical Center of Dallas, Dallas, TX 75235; 2 University of Colorado Health Sciences Center, Department of Pediatrics, Denver, CO 80262; * address correspondence to this author at: Children’s Medical Center, Department of Pathology, 1935 Motor St., Dallas, TX 75235; fax 214-456-6199, e-mail Patricia.Jones@email.swmed.edu or pjones@childmed.dallas.tx.us).

Mitochondrial fatty acid oxidation (FAO) is a catabolic pathway that supplies energy for the normal physiologic functioning of many tissues when glucose is unavailable, and it also supplies energy for some tissues even when glucose is available (1, 2). The FAO pathway is complex and not fully understood. Quantitative measurement of the concentrations of 3-hydroxy-fatty acids (3-OHFA}s) in plasma or serum samples from individuals who are suspected of having a deficiency in FAO, especially in the enzyme step involving the 3-hydroxyacyl-CoA-dehydrogenases, is a useful tool to aid in diagnosis (3, 4). This study adds the quantitative measurement of 3-hydroxy-octadecanoic acid (3-OH-C18) to the previously reported assay (4) that measures the six shorter chain-length FAO intermediates, 3-hydroxy-hexanoic acid (3-OH-C6), 3-hydroxy-octanoic acid (3-OH-C8), 3-hydroxy-decanoic acid (3-OH-C10), 3-hydroxy-dodecanoic acid (3-OH-C12), 3-hydroxy-tetradecanoic acid (3-OH-C14), and 3-hydroxy-hexadecanoic acid (3-OH-C16).

3-OH-C18 was synthesized by the method of Jones et al. (4), with the following changes. The precursor for 3-OH-C18 was not commercially available; thus the 3-OH-C18 precursor, hexadecanal, was synthesized first by the method of Landini et al. (5). A saturated solution of potassium chromate (0.55 mol/L) in 300 mL/L aqueous sulfuric acid was reacted with 0.01 mol of 1-hexadecanol dissolved in 60 mL of methylene chloride in the presence of 0.001 mol of tetrabutylammonium hydrogen sulfate as
a catalyst (ratio of 1-hexadecanol to catalyst, 10:1). The unlabeled and [1,2]-13C2-labeled 3-OH-C18 were then synthesized from the hexadecanal as described previously (4). The methylene chloride, 1-hexadecanol, and tetrabutyldiammonium hydrogen sulfate were obtained from Aldrich Chemical Co. Analysis of the naturally occurring and stable-isotope 3-OH-C18s after synthesis was also performed as described previously (4). This analysis demonstrated that the naturally occurring 3-OH-C18 was 89% pure with the expected composition, and the stable-isotope 3-OH-C18 was 95% pure with the expected composition. Mass spectra revealed patterns that reflected the structure of the authentic compounds and demonstrated that the impurities would not affect the ions used for quantification of 3-OH-C18. Because of the synthetic process, very little of the naturally occurring compound was expected in the isotope-labeled 3-OH-C18, and the mass spectra confirmed this by showing 0.06%.

The 3-OH-C18 stable-isotope-labeled calibrator used in the assay and the natural compound used for linearity and precision studies were made by weighing the 3-OH-C18 species and dissolving each to a concentration of 500 μmol/L in dichloromethane.

Control samples were serum, heparinized plasma, or EDTA-plasma, left over from previous studies, from apparently healthy individuals. The control samples included those from nonfasting individuals (n = 35) who were not on any special diet. The control individuals ranged in age from 2 days to 53 years (15 females and 20 males).

In addition to the control group, we assayed samples from 10 ketotic patients who had increased concentrations of the short-chain 3-OHFA s. These patients showed no increases in their 3-OH-C18 concentrations above the control group. Samples were also obtained from two ketotic patients diagnosed with deficiency in long-chain l-3-hydroxyacyl-CoA dehydrogenase (LCHAD). Both of these patients were being managed with medium-chain triglyceride supplementation and carnitine. Nonfasting samples were collected at routine clinical follow-up visits.

Samples were extracted, derivatized, and assayed as described previously (4), except that 10 μL of 500 μmol/L 3-OH-C18 calibrator was added to the sample separately, and six other 3-OHFA stable-isotope calibrators were added. Gas chromatographic–mass spectrometric analysis was carried out on an Agilent Technologies 6890 gas chromatograph with a 5973 Network Series quadrupole mass spectrometer as described previously (4).

Calibration curves were constructed for 3-OH-C18 to determine the linearity, precision, and accuracy of the assay. These curves were constructed by adding 0.01–25 μmol/L of the naturally occurring 3-OH-C18 into an essentially fatty-acid-free albumin at physiologic protein concentrations and then adding 5 nmol of the isotope-labeled calibrator to each sample, as described above. The signal-response ratio of the naturally occurring compound to isotope-labeled calibrator was then plotted against the known analyte concentration. We subjected these calibration curves to linear regression analysis using the signal-response ratio as the dependent variable. To evaluate the method, we repeated calibration curves (n = 6) and used the signal-response ratios to back-calculate concentrations from the derived regression equations.

Quantification of the naturally occurring 3-OH-C18 in patient samples was accomplished as described previously (4) for the other six 3-OH species.

The [M-CH$_3$]$_2$ fragment ions used to identify and quantify 3-OH-C18 were m/z 429 for the naturally occurring compound and m/z 431 for the isotope-labeled calibrator. 3-OH-C18 also had ions resulting from the 3-OH fragments, which were m/z 233 for the naturally occurring compound and m/z 235 for the isotope-labeled calibrators. These fragments were used to help identify the correct compounds, but could also be used to quantify the naturally occurring compound.

The linear regression analysis of the calibration curves led to a linear regression equation of $y = 0.0849x + 0.0163$ ($r^2 = 0.9992$) for the m/z 429/431 pair. For the m/z 233/235 pair, the equation was $y = 0.0792x + 0.0168$ ($r^2 = 0.9988$). For both ion pairs, the 3-OH-C18 was linear in the concentration range of 0.2–10 μmol/L. The lower limit of detection, defined as 3 SD above the mean value for the blank, was 0.12 μmol/L. Imprecision (CV) at 8, 1.5, and 0.45 μmol/L was 5.3%, 6.8%, and 13%, respectively, for the m/z 429/431 ion pair, and 5.0%, 6.5%, and 6.8%, respectively, for the m/z 233/235 ion pair. The differences from the target concentrations at the concentrations given above were 2.5%, –4.7%, and 10% for the m/z 429/431 ion pair and –6.2%, –3.3%, and –4.0% for the m/z 233/235 ion pair.

The upper limit of the reference interval for 3-OH-C18 for both free and total species in controls (n = 35) was <0.5 μmol/L. For free 3-OH-C18, this follows the pattern seen previously (4), with concentrations decreasing as chain length increases. For total 3-OH-C18, there appears to be little to no conjugated 3-OH-C18 in plasma or serum, even under rigorous hydrolysis conditions (10 mol/L NaOH, 70 °C, 45 min). Only 4 of 35 control samples showed any increase in concentrations between the free and total 3-OH-C18 values, and these increases ranged from 20% to 25%. This is in contrast to 3-OH-C14 and 3-OH-C16, which are ~25–50% conjugated even in apparently healthy individuals (6). Representative concentrations and a representative pattern in a control sample are demonstrated in Fig. 1, which also shows the abnormal pattern and concentrations displayed by an LCHAD-deficient patient. In LCHAD deficiency, there was a marked increase of 3-OHFA s of chain lengths 3-OH-C14 and –C16 and an increase in 3-OH-C18. Although the 3-OH-C18 concentration was above the upper limit of the reference interval, it did not show the pattern of increase seen with the 3-OH-C14 and –C16 species, nor did it show any difference in free and total 3-OH-C18. For example, for the LCHAD-deficient patient depicted in Fig. 1, the 3-OH-C14 concentration was 5-fold higher (2.0 μmol/L) than the upper limit of the 3-OH-C14 reference interval.
(0.4 μmol/L), and the 3-OH-C16 concentration was 20-fold higher (10 μmol/L) than the upper limit of the 3-OH-C16 reference interval (0.5 μmol/L). The 3-OH-C18 concentration was only threefold higher (1.5 μmol/L) than the upper limit of the 3-OH-C18 reference interval (0.5 μmol/L). Another LCHAD-deficient patient, who was in better metabolic control, had a concentration that was 1.5-fold above the upper limit of the reference interval for 3-OH-C14, 3.4-fold above the upper limit of the reference interval for 3-OH-C16, and 1.2-fold above the upper limit of the reference interval for 3-OH-C18. Again, the 3-OH-C18 concentration did not follow the pattern of increasing metabolite accumulation established by 3-OH-C12 to -C16.

Mitochondrial fatty acid β-oxidation defects are often difficult to diagnose (7–9). This is attributable, in part, to the fact that routinely detectable metabolites are often absent when a patient is clinically well and, in part, to the fact that this pathway is still not fully understood. Assays that contribute to the knowledge base for this pathway are useful not only for diagnostic purposes, but also to increase understanding of the mechanisms involved. The addition of quantitative 3-OH-C18 to our original assay has the potential to be useful in these ways.

As demonstrated in Fig. 1, measurable amounts of the 3-OH-C18 can be found in serum and plasma samples; however, almost none of it is found in conjugated form. This finding is in contrast to 3-OH-C14 and 3-OH-C16, where a significant percentage of the circulating form is conjugated, much of which is probably conjugated to carnitine. In serum samples, linoleic acid (C18:2) and oleic acid (C18:1) contribute a large proportion of the total C18 fatty acid content. We have used the 3-OH-C18:0 stable isotope internal standard to attempt to approximate the amounts of 3-OH-C18:2 and -C18:1 present. We used the [M-CH3]⁺ ion pairs m/z 425/431 for 3-OH-C18:2 and m/z 427/431 for 3-OH-C18:1 and then m/z 233/235 for each to estimate these concentrations. We analyzed two serum samples from an LCHAD-deficient individual and four apparently healthy controls. For the control samples, the 3-OH-C18:1 and -C18:2 values varied from undetectable to 0.38 μmol/L and undetectable to 0.24 μmol/L, respectively. For the LCHAD-deficient individual, the sample drawn when the patient was not in metabolic control showed approximate concentrations of 3-OH-C18:1 and -C18:2 of 1.96 and 0.88 μmol/L, respectively, compared with 0.78 μmol/L for 3-OH-C18. For the sample drawn when the patient was in better metabolic control, the results of these three forms were 0.42, 0.16, and 0.45 μmol/L, respectively. The two samples differed because the unsaturated 3-OH-C18 concentration was lower than the saturated when the patient was in relative metabolic control. The opposite was true when the patient was not in control. In addition, the unsaturated forms of the 3-OH-C18 species were more conjugated, with the concentrations after hydrolysis being 1.5- to 4-fold higher than the unhydrolyzed concentrations.

Interestingly, the 3-OH-C18:1 and -C18:2 values obtained from controls were comparable whether they were calculated with the [M-CH3]⁺ ion pair or the m/z 233/235 ion pair. In contrast, with the LCHAD-deficient patient, the values were quite different when calculated with the [M-CH3]⁺ vs the m/z 233/235 pair. For example, 3-OH-C18:1 was 1.96 vs 1.0 μmol/L, and 3-OH-C18:2 was 0.88 vs 0.42 μmol/L. This finding held true for the other LCHAD-deficient sample also, with a 3-OH-C18:1 concentration of 0.42 vs 0.23 μmol/L, and a 3-OH-C18:2 concentration of 0.16 vs 0.10 μmol/L. These results illustrate that without a native compound calibrator to establish a credible calibration, the use of a native compound/internal standard ratio to calculate a concentration is, at best, an approximation. However, the results for the unsaturated species could still be used to monitor the status of a previously diagnosed LCHAD-deficient patient.

The results we obtained with the saturated and unsaturated species, as well as the finding that the 3-OH-C18 concentration in LCHAD-deficient individuals is not increased as much above the upper limit of the reference interval as 3-OH-C14 and -C16, suggest that stearic acid may be handled by a different mechanism than other long-chain fatty acids. The addition of 3-OH-C18 to this assay has increased its utility as a research and a diagnostic tool.

The synthesis of compounds for this work, and also for the original assay reported by Jones et al. (4), were partially funded by the Mental Retardation Research Center Core Grant No. PO1 HD 08315.

Fig. 1. Concentrations of 3-OHFA’s of chain lengths C6–C18 in an apparently healthy individual for both free ( - ) and total (——) fatty acids and free (— ——) and total (•••••) fatty acids in an LCHAD-deficient patient.

References
Performance of Precision G Blood Glucose Analyzer with a New Test Strip G2b on Neonatal Samples, Burcu Meric,1 Nazife Kilicaslan,2 Kagan Kerman,1 Dilsat Ozkan,3 Umran Kurun,3 Nejat Aksu,3 and Mehmet Ozsoz1*

In newborns, hypoglycemia must be detected in venous specimens with a wide range of hematocrits and oxygen tensions. The need for a simple and rapid test for glucose to meet special applications such as those found in pediatric wards has been suggested frequently (1–4). Simple tests are available, but their use in neonatal units has usually not been recommended, reflecting, at least in part, the influence on results of sample hematocrit and oxygen tension (5, 6).

The Precision G System is a new instrument with a new electrode strip (G2b), which is purported to address many of the shortcomings found in previous generations of biosensors. The bioactive component of Precision G System is glucose oxidase. This is also used in many other glucose measuring systems (7). The component that makes the Precision G System unique is the signal transducer and mediator ferrocene (8).

We performed laboratory and clinical evaluations of the Precision G System with the G2b test strips to determine its suitability for use in a neonatal unit. We compared the performance of the Precision G System with a laboratory method using venous specimens with a wide range of hematocrits taken from patients in a neonatal intensive care unit.

After approval from the Institutional Review Board of the Izmir Social Security Institute (SSK) Tepecik Educational Hospital, the instrument was permitted to be used in the neonatal unit of the hospital. After informed consent was received from the parents, 3.5 μL of the 1.5-mL venous blood samples, taken for the necessary routine biochemical analysis during the treatment period of each neonate, were used for studies described in this report. The samples were kept at room temperature in heparinized tubes before use. The samples were applied to the Precision G system within 5 min after they were taken from the neonates. Venous samples from 100 patients had whole-blood glucose measured on the Precision G System and plasma glucose measured on the Hitachi 911 reference system. The hematocrit of each sample was also measured (Bayer Advia 120 Hematology System).

In the Precision G System, glucose reacts with glucose oxidase on the test strip. The chemical reaction releases electrons, which are transferred from the enzyme to the electrodes by ferricinium+, the oxidized form of the mediator ferrocene. These electrons form a small current. The electrical current, detected by the electrodes on the test strip, is proportional to the concentration of glucose in the specimen. The analysis time is 20 s.

The Precision G test strip contains three electrodes (reference, working, and background compensation electrodes). The background compensation electrode, containing no glucose oxidase, measures the nonspecific current from potentially interfering substances such as ascorbic acid and urea. This background current is subtracted from the current measured on the working electrode.

The Precision G System starts testing when it detects that a sample has been applied to the test strip. If the test fails to start because of an insufficient sample amount, the user may apply a second drop of blood to the same test strip within 30 s.

Unlike photometric (reflectance) analyzers, blood does not enter the sensor during testing with the Precision G System. Each Precision G2b is sealed in an individual foil packet, which has a barcode on the exterior. The barcode contains lot-specific information, including calibration.

Fig. 1. Linear regression of 100 blood-glucose measurements with the Hitachi 911 reference system and the Precision G System with the G2b.