Recovery of labeled CO2 from acetate in severely burned children

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Fram RY, Cree MG, Chinkes DL, Herndon DN, Wolfe RR. Recovery of labeled CO2 from acetate in severely burned children. Am J Physiol Endocrinol Metab 293: E1726–E1729, 2007. First published September 25, 2007; doi:10.1152/ajpendo.00388.2007.—The purpose of this study was to determine the fractional recovery rate of labeled CO2 in the breath of severely burned children. This information is needed to perform tracer studies of substrate oxidation using carbon-labeled fatty acids. Nine children, ages 4–14 yr with massive burns participated in the study. All experiments were performed 7 days post burn after an overnight fast. A primed (60 μmol/kg), constant (2.0 μmol/kg-min) infusion of [1,2-13C]acetate was given during a 4-h basal period and during a 4-h hyperinsulenic euglycemic clamp. A priming dose (150 μmol/kg) of NaH13CO3 was given at the beginning of the study. Breath samples were collected every 10 min during the last 40 min of each period. Indirect calorimetry was performed during the last 30 min of each period. The isotopic enrichment of 13CO2 was determined by isotope ratio-mass spectrometry, and total CO2 excretion was measured by indirect calorimetry. The fractional recovery of acetate label was 0.89 ± 0.05 and 0.88 ± 0.04 during the basal state and clamp, respectively. We conclude that the fractional recovery of labeled acetate in severely burned children is approximately three times the recovery of a nonburned adult and similar to the value in exercising adults. The high recovery rate reflects the rapid turnover of the TCA cycle in burned children relative to the rate of exchange reactions. Minimal correction of expired CO2 data is needed in this circumstance to quantify fatty acid oxidation using 13C-labeled fatty acids.

Acetate correction factor; fatty acid oxidation

Carbon-labeled tracers have been used extensively for calculating fatty acid oxidation. Inherent in this measurement is the notion that the rate of appearance of labeled carbon dioxide in the breath after infusion of a labeled tracer directly reflects the rate of oxidation of the labeled substrate of interest. For this assumption to be true, the rate that labeled carbon enters the tricarboxylic acid cycle (TCA) through acetyl-CoA molecules should equal the rate of loss of labeled CO2 found in the breath. However, previous studies in normal volunteers have shown that only a fraction of the labeled CO2 entering the TCA cycle in acetyl-CoA is recovered in breath (12, 16, 17).

The recovery of only a fraction of the labeled CO2 in breath can be explained by exchange reactions that occur during the TCA cycle. When labeled acetyl-CoA enters the TCA cycle, no label is lost to CO2 in the first spin of the cycle through its breakdown. However, during this spin, label can leave the cycle due to exchange reactions. Label can be transferred to glutamate and glutamine via exchange with α-ketoglutarate.

Furthermore, label that enters the oxaloacetate (OAA) pool from the first spin can enter the gluconeogenic pathway and be lost from the TCA cycle while not appearing in breath CO2. In the second spin, one-half of the label in the [1-C] position of acetyl-CoA is lost to CO2, and the remainder is lost to CO2 in the next spin of the cycle. In contrast, the loss of label from the [2-C] position of acetyl-CoA is much slower. No label is lost from the second position of acetyl-CoA until the third spin, and the loss of label from the second position is incomplete even after 10 spins (23). Therefore, 13C recovery in breath CO2 is normally lower when [2,13C]acetate is given as opposed to [1-13C]acetate (28).

Hyperglycemia, insulin resistance, and elevations of free fatty acids are common responses to injury (4, 10, 24). Investigating these responses to find new methods of treatment is important for future outcomes of the critically ill. Measuring fatty acid oxidation rates accurately by indirect calorimetry is difficult in the severely burned child. Often, children with severe burn injury are on ventilatory support or are receiving supplemental O2. Hence, accurate measurements of both carbon dioxide production (VCO2) and especially oxygen consumption (VO2) are complicated by challenges in collecting breath completely and accurately measuring inhaled O2 concentration. Since the calculation of fat oxidation from indirect calorimetry data is based on the respiratory quotient (VCO2/VO2), difficulty in measuring either or both units will translate to an inaccurate calculation of fatty acid oxidation. Thus, there is a theoretical advantage of measuring fatty acid oxidation using a 13C-labeled fatty acid.

However, there has never been an assessment of the recovery rate of labeled 13CO2 from labeled acetate in severely burned children. We thought that this was necessary, because resting energy expenditure (REE) is elevated after burn injury and persists 12 mo after injury (8), and in other circumstances in which VO2 and VCO2 are elevated, recovery rates of labeled CO2 from labeled acetate infusion are affected (15). Therefore, we infused [1,2-13C]acetate in severely burned children to determine the fractional recovery rate of [13C]acetate in the breath during the fasted basal state and during a hyperinsulinemic clamp. With these data, it is possible to determine a correction factor for estimation of fatty acid oxidation in severely burned children using [U-13C]pyruvate.

Materials and Methods

Patients and Clinical Care

Nine children who underwent acute treatment for severe burns at Shriners Hospital for Children Galveston from May 2005 to August 2006 were included in this study. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
were enrolled. Children between the ages of 4 and 18 yr with total body surface area burn ≥40% who arrived within 96 h after injury, were eligible for enrollment. This study was performed under a University of Texas Medical Branch Institutional Review Board-approved protocol. Informed written consent was obtained from each patient’s guardian with assent of patients ages ≥7 yr before enrollment into the study. All subjects admitted to the Shriners Hospital for Children Galveston were treated in an identical surgical manner by the same team of burn surgeons. Standard treatment included early excision of the burn wound, systemic antibiotic therapy, and continuous enteral feeding at a rate of 1.4× REE (14).

Experimental Protocol

An 8-h isotopic tracer study was performed ~4 days after the child’s first excision and grafting procedure. This allowed for the metabolic changes that occur with surgery to return to baseline, as well as for the use of existing catheters. All metabolic studies occurred at approximately hospital day 4 and within 7 days of initial injury. Each study was performed in the morning after a 4-h fast with intravenous fluids of 0.9 normal saline and 8 h without blood, blood products, or albumin transfusions. There were two phases to this protocol. The first 4 h of the study was used to determine acetate recovery in the fasted, resting state. The second 4 h of the study was used to determine acetate recovery during acute hyperinsulinemia-euglycemia.

Basal determination. A primed (60 μmol/kg), continuous (2 μmol/kg·min⁻¹) infusion of [1,2-13C]acetate (Cambridge Isotope Laboratories, Andover, MA) was started and maintained for 4 h. At the beginning of the experiment, a 150 μmol/kg NaH13CO3 bolus was given to prime the bicarbonate pool. Breath samples were taken at 192, 215, 226, and 235 min after the beginning of the infusion to determine 13CO2 enrichment. Expired air was collected into a 1-liter collection bag. Twenty milliliters of the expired air was injected into evacuated tubes. VO₂ and VCO₂ were measured during the last 30 min of the experiment using a Sensor-Medics Vmax 29 metabolic cart (Yorba Linda, CA). Subjects were tested in a supine position while under a large, clear, ventilated hood at ambient temperatures of 30°C, which is the standard environmental setting for all patient rooms in our acute burn intensive care unit.

Hyperinsulinemic clamp. After the basal period, the infusion of [1,2-13C]acetate was continued at the same rate. A primed (1.5 μmol/kg), constant (1.5 μmol/kg·min⁻¹) infusion of regular human insulin (Humulin; Eli Lilly, Indianapolis, IN) was started and maintained for 4 h. Plasma glucose concentrations were maintained between 80 and 90 mg/dl by simultaneously infusing 20% dextrose at variable rates. Blood glucose levels were checked every 10 min with an Accuchek Advantage (Roche Diagnostics, Mannheim, Germany) blood glucose monitor. Breath and blood samples were collected at 453, 465, 476, and 485 min after the beginning of the acetate infusion, and indirect calorimetry was performed as described during the basal determination.

Sample Analysis

The tracer-to-tracee ratio of 13CO2 to 12CO2 was determined by an isotope ratio-mass spectrometer (IRMS, SIRA II; VC Isotech, Cheshire, UK) as previously described (18).

Calculations

The values for breath CO2 enrichment measured at 192, 215, 226, and 235 min after the beginning of the labeled acetate infusion were averaged during the basal and clamp periods for the calculation of acetate kinetics. The following equation was used for whole body acetate carbon recovery: fractional recovery of label in breath CO2 = ECO2 × VCO2/F, where ECO2 is the isotopic enrichment of breath CO2, and F is the tracer infusion rate.

**Statistical Analysis**

Data are expressed as means ± SE. Paired t-tests were performed to evaluate differences between the basal state and clamp. Significance was accepted at P < 0.05.

**RESULTS**

**Demographics**

Nine children, 5 girls and 4 boys, with an average age of 8 ± 1 yr were enrolled in our study. The average time from burn injury to admission to the acute burn unit was 3 ± 1 days. Average total body surface area (TBSA) burned was 65 ± 5% with 63 ± 5% third degree. The mean heart rate at the time of the study was 148 ± 7 beats/min. REE was normalized for body surface area and was 1,477 ± 36 kcal·m⁻²·day. This value was 136% of the value predicted by the Harris Benedict equation (7). These values indicate the children were hypermetabolic, which is common after an acute burn injury (22).

**Basal Determination**

Steady state for breath CO2 enrichment was reached within 190 min after the start of infusion of [1, 2-13C]acetate and was maintained until the end of the study period (Fig. 1). The average breath CO2 enrichment during the basal period was 0.00578 ± 0.000128 (tracer/tracer ratio). Whole body fractional acetate carbon recovery as CO2 was 0.89 ± 0.05.

**Hyperinsulinemic Clamp**

Steady state in expired CO2 enrichment was achieved within 90–120 min from the start of insulin infusion. Average breath CO2 enrichment during the clamp was 0.00516 ± 0.00036 and was significantly lower than the value during the basal period of 0.00578 ± 0.00013 (P = 0.0004). However, average VCO2 during the clamp (0.185 ± 0.019 l/min) tended to be greater than during the basal period (0.172 ± 0.015 l/min; P = 0.054). Therefore, fractional carbon recovery during the clamp was 0.88 ± 0.04 and was not statistically significant from the basal value of 0.89 ± 0.0003 (P = 0.95).

![Fig. 1](http://ajpendo.physiology.org/DownloadedFrom/10.22032.247/2017.AJP-EndocrinolMetab.VOL293.DECEMBER2007)
The purpose of the present study was to determine a new correction factor for estimation of fatty acid oxidation in severely burned children. This factor allows for the interpretation of substrate oxidation kinetics with the infusion of labeled acetate. Acetate is the tracer of choice because acetate enters the TCA cycle directly as acetyl-CoA and should therefore provide a quantitative collection of $^{13}$CO$_2$ if all acetyl-CoA molecules are oxidized. The acetate correction factor takes into account carbon fixation that occurs with isotopic exchange reactions during the TCA cycle. The acetate correction factor has been shown to differ in adult populations in different energy states (12, 16, 17). The result of the current study shows that the acetate recovery factor in hypermetabolic, severely burned children at rest is equivalent to the highest values recorded in normal adult populations.

We studied nine children with burns $\geq$40% of TBSA within 7 days of their injury. After a 3-h infusion of [1,2-$^{13}$C]acetate during the basal resting state, we found the whole body recovery of acetate to be 89%. This value is twice the amount of recovery that was calculated in previous studies of resting adult volunteers. Sidossis et al. (17) found that the recovery of labeled CO$_2$ in a resting adult was 56% after infusion with [1-$^{14}$C]acetate for 3 h. In addition, Mittendorfer et al. (12) infused [1,2-$^{13}$C]acetate for 3 h and found the recovery of acetate was 38%. The latter value is more directly comparable to our data. We found that severely burned children have a rate of recovery of labeled CO$_2$ from acetate that is in the same range as the value of an exercising adult. In Siddossi's previous study, it was found that an exercising adult had an 80% recovery of CO$_2$ in breath during the resting basal state. In addition, Sidossis et al. infused [1-$^{14}$C]acetate compared with our infusion of [1,2-$^{13}$C]acetate, and recovery should be greater when using a [1-C] position labeled acetate (20, 28). Consequently, the acetate recovery factor found in burned children would likely be higher than 90% if [1-$^{13}$C]acetate had been used.

During the second half of the study (protocol 2), the recovery of labeled acetate in breath was also measured during a hyperinsulinemic euglycemic clamp. There was no significant change in the acetate recovery factor during the clamp period compared with the basal period in severely burned children. In contrast, Wolfe et al. (26) found recovery of labeled CO$_2$ from [1,2-$^{14}$C]acetate doubled from the basal resting value when insulin was infused into normal resting adults. However, during hyperinsulinemia the recovery of labeled CO$_2$ in breath was 83% in the previous study, which was similar to our results in severely burned children.

Many reasons can be postulated as to why severely burned children have a high rate of recovery of labeled acetate in the resting state. An isotopic equilibration occurs between infused acetate and acetyl-CoA. Therefore, the fate of carbons in acetyl-CoA will be traced when labeled acetate is given. For the purpose of calculating a “correction factor” to calculate fatty acid oxidation using labeled fatty acids, it is assumed that all labeled acetate enters the TCA cycle in the form of acetyl-CoA. In this case, any labeled carbon that does not appear in breath can be considered to be lost to exchange reactions within the TCA cycle. However, if not all acetyl-CoA enters the TCA cycle, then recovery of labeled carbon would be less than 100%, and the recovery would not entirely reflect the extent of exchange reactions within the TCA cycle. The most likely fate of acetyl-CoA other than entering the TCA cycle is de novo lipogenesis. Under normal conditions, de novo lipogenesis from acetate accounts for a negligible percentage of infused acetate (9, 18). However, we (19) have previously reported that under conditions of high carbohydrate intake there may be a significant rate of de novo lipogenesis, which would cause an underestimation of the true recovery factor. In the current study, the high recovery of labeled CO$_2$ indicates that lipogenesis and other enzymatic reactions were not significant in relation to the rate of entry of acetyl-CoA into the TCA cycle.

In the case of exercise, we proposed that more rapid cycling of the TCA cycle due to increased energy expenditure explained the more complete recovery of labeled carbon from acetate. Since the REE was increased above normal in these burned children, the same explanation could apply. However, the increase in metabolic rate following burn injury is modest compared with even minimal exercise and may not be sufficient to explain the high recovery. Furthermore, gluconeogenesis, which is a potential pathway of isotopic exchange of $^{13}$C from acetate, is elevated in burn children (27). On the other hand, glutamine is a major fate of $^{13}$C from exchange reactions (23), and the muscle glutamine pool is largely depleted in burn children (1, 5, 11).

The previously reported increase in recovery in normal humans during hyperinsulinemia (26) suggests that there may be metabolic control factors governing acetate recovery as well. The increase in recovery during hyperinsulinemia in normal adults was due, at least in part, to a reduction of transfer of OAA to lactate, stemming from suppression of the phosphoenolpyruvate cycle (26). Perhaps a further increase in the fractional recovery was not observed during hyperinsulinemia in burned children because recovery was already so high it approached the maximal value of 1.0. Also, burned children are resistant to the actions of insulin in terms of both glucose uptake and production (2, 3, 25), so the lack of response to insulin in the current study may further reflect insulin resistance.

The role of mitochondrial function in the genesis of insulin resistance has recently become an area of interest. A study by Padfield et al. investigated the effects of burn injury on skeletal muscle mitochondria in mice. They found that mice, subjected to a 5% scald injury showed downregulation of the GLUT4 receptor, fatty acid oxidation, oxidative phosphorylation, mitochondrial respiration, and a reduction in ATP synthesis (13) compared with noninjured mice. In addition, Gore et al. (6) found a decrease in ATP and ADP concentrations in mice after severe burn injury. Vanhorebeek et al. (21) found that adult patients in the surgical intensive care units who had been treated with an intensive insulin therapy protocol had maintained normal hepatic mitochondrial structure compared with patients who had been treated conventionally. The conventionally treated patients had altered mitochondrial morphology and function. Mitochondrial dysfunction that occurs in burn injury could possibly play a role in limiting the exchange reactions that occur in the TCA cycle and thus increase acetate recovery during burn injury.

In conclusion, the fractional recovery of labeled carbon in breath CO$_2$ resulting from the oxidation of infused acetate in

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severely burned children was found to be approximately three times the recovery of a nonburned adult and close to the value of exercising adults. The high recovery rate reflects the rapid turnover of the TCA cycle in burned children relative to the rate of exchange reactions. The practical implications of these results is that, in contrast to the situation in normal humans, there is little need for correction of expired CO₂ data when a labeled fatty acid is used as a tracer to measure oxidation in severely burned patients. Further understanding of the mechanism for the alteration in basal acetate recovery in burned children may provide insight into the nature of alterations in the mitochondrial metabolism of substrates.

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AJP-Endocrinol Metab • VOL. 293 • DECEMBER 2007 • www.ajpendo.org