Studies on the Regulation of the Branched Chain α-Keto Acid Dehydrogenase in the Perfused Rat Liver

(Received for publication, December 9, 1980, and in revised form, March 30, 1981)

Tarun B. Patel$, Michael S. DeBuysere, Lynn L. Barron, and Merle S. Olson

From the Department of Biochemistry, The University of Texas Health Science Center, San Antonio, Texas 78284

The regulation of the branched chain α-keto acid dehydrogenase multienzyme complex was investigated in the isolated, perfused rat liver. The metabolic flux through the branched chain α-keto acid dehydrogenase was monitored by measuring the production of \(^{14}\)CO\(_2\) from infused \(^{1-14}\)C-labeled branched chain α-keto acid substrates. The rate of decarboxylation of α-keto\(^{1-14}\)Cisocaproate exceeded that of α-keto\(^{1-14}\)Cisovalerate at all concentrations of the substrates infused. Coinfusion of either α-ketoisovalerate or α-keto-β-methylvalerate inhibited the rate of α-keto\(^{1-14}\)Cisocaproate decarboxylation. The rate of α-keto\(^{1-14}\)Cisovalerate decarboxylation was enhanced during coinfusion of \(\alpha\)-carnitine, while α-keto\(^{1-14}\)Cisocaproate decarboxylation was unaffected. The presence of pyruvate in the perfusion medium resulted in an inhibition of the flux through the branched chain complex with either α-ketoisocaproate or α-ketoisovalerate as the substrate. \(\alpha\)-β-hydroxybutyrate infusion inhibited α-keto\(^{1-14}\)Cisocaproate decarboxylation by 18% but resulted in nearly a 100% stimulation of α-keto\(^{1-14}\)Cisovalerate decarboxylation.

The evidence presented indicates that (a) the metabolic flux through the branched chain α-keto acid dehydrogenase complex can be monitored effectively in a continuous fashion in the perfused liver by following the release of \(^{14}\)CO\(_2\) from infused \(^{1-14}\)C-labeled substrates and (b) the changes observed in the metabolic flux through the branched chain complex during coinfusion of alternative substrates and other compounds may be entirely different depending upon which branched chain α-keto acid substrate is utilized to monitor this reaction.

It has been established that during starvation the circulating blood levels of the branched chain amino acids, leucine, isoleucine, and valine increase transiently both in humans and in experimental animals (1, 2). These amino acids represent a carbon source for the generation of energy, carbohydrate (e.g. valine and isoleucine), and ketone bodies (leucine) in various tissues (3). The branched chain amino acids are transaminated with α-ketoglutarate to form the corresponding α-keto acids which subsequently undergo oxidative decarboxylation in the mitochondrial compartment by the branched chain α-keto acid dehydrogenase multienzyme complex which is an analogous enzyme complex to the pyruvate and α-ketoglutarate dehydrogenase complexes (4). The three branched chain α-

* This research was supported by National Institutes of Health Grants HL-24654 and AM-19473 and Grant AQ-728 from the Robert A. Welch Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Received a travel grant from the Wellcome Trust.

keto acids, α-ketoisocaproate, α-ketoisovalerate, and α-keto-β-methylvalerate, are oxidized by a single enzyme complex which requires CoASH, NAD\(^+\), and thiamin pyrophosphate (1, 4–6). The various enzyme activities which are involved in branched chain amino acid metabolism are found in varying amounts in different tissues.

The demonstration that the transaminase activity is high in skeletal muscle and low in liver (7–9) while the branched chain α-keto acid dehydrogenase activity is high in liver and low in muscle (4) has been used as evidence that the primary role of muscle in the metabolism of branched chain amino acids is in transamination, while the liver is the primary site for oxidation of the branched chain α-keto acids (4, 8). This suggestion accounts for the observed elevation of the circulating α-keto acid concentration during starvation (12). Hence an investigation of the regulation of the branched chain α-keto acid dehydrogenase in the liver is of special interest.

Studies on the regulation of the branched chain α-keto acid dehydrogenase in rat liver have been performed using homog enates, isolated mitochondria, and hepatocytes. Few studies of this enzyme complex have been performed using the isolated perfused rat liver. Walser et al. (13) studied the disappearance of the three branched chain α-keto acids in th perfused rat liver. However, the measurements in this study were conducted in the presence of 2 mM oleate which greatly suppressed the uptake of the α-keto acids. The present study was performed in order to determine whether the metabolic flux through the branched chain α-keto acid dehydrogenase reaction could be determined continuously by measuring the \(^{13}\)CO\(_2\) production from infused \(^{1-13}\)C-labeled α-keto acids in a manner analogous to studies of the pyruvate dehydrogenase complex in the perfused liver and heart (14, 15). Previously we demonstrated that the flux through the branched chain dehydrogenase reaction could be monitored using this experimental approach in the perfused rat heart (16).

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 180-200 g were used in these studies. Animals were given a standard laboratory chow and water ad libitum. A nonrecirculating liver perfusion technique using \(^{2+}\)hemoglobin-free perfusion medium was employed and has been described elsewhere (17). The perfusion medium was Krebs-Henseleit bicarbonate buffer (18), pH 7.4, saturated with an oxygen/carbonate mixture (95%/5%) and maintained at 37 °C. The various \(^{13}\)C-labeled as well as the unlabeled substrates were infused into the perfusion circuit immediately prior to the liver. Oxygen consumption by the liver was monitored using a Clark type oxygen electrode placed in the perfusion circuit immediately following the liver. Effluent perfusate samples were collected at 30-s intervals. Aliquots (2.5 ml of the perfusate were placed in 25-mL Erlenmeyer flasks fitted with rubber serum stoppers equipped with plastic center wells containing 0.3 ml of phenelzine. Labeled carbon dioxide in the perfusate which was produced from the infused \(^{13}\)C-labeled substrates was released by injecting 0.5 ml of 1 N HCl through the serum stopper into the flasks. The flasks were agitated for 1 h and the center well was transferred to scintillation vials containing 10 ml of aquasol.
(New England Nuclear Corp.) and counted. Knowing the correction for quenching and the specific radioactivity of the infused \(^{14}\)C-labeled substrates, a continuous measurement of the metabolic flux through the branched chain \(\alpha\)-keto acid dehydrogenase complex was obtained.

Ketone bodies, \(\beta\)-hydroxybutyrate and acetoacetate, were measured in samples of the effluent perfusate using procedures described by Williamson and Corkey (19) and Mellanby and Williamson (20), respectively. \([1-{^{14}\text{C}}]\)Leucine, \([1-{^{14}\text{C}}]\)valine, aquasol, and phenethylamine were purchased from New England Nuclear (Boston, MA). 1-\(^{14}\)C-labeled \(\alpha\)-ketoisocaproate and \(\alpha\)-ketoisovalerate were prepared from the corresponding \(l\)-'\(^{14}\)C-labeled amino acids (leucine and valine) using a procedure described by Rudiger et al. (21). Unlabeled \(\alpha\)-ketoisocaproate, \(\alpha\)-ketoisovalerate, \(\alpha\)-ketone-\(\beta\)-methylvalerate, and \(l\)-leucine were purchased from Sigma (St. Louis, MO). P-hydroxybutyrate dehydrogenase were obtained from Boehringer Mannheim (Indianapolis, IN). \(\beta\)-Hydroxybutyrate was purchased from Calbiochem (La Jolla, CA). \((--\))Carnitine chloride was the generous gift of the Otsuka Pharmaceutical Factory (Osaka, Japan). All other reagents and materials were of the highest purity available from commercial sources.

RESULTS

In order to assess the validity of measuring \(^{14}\)CO\(_2\) production from \(1-{^{14}\text{C}}\)-labeled \(\alpha\)-ketoisocaproate and \(\alpha\)-ketoisovalerate as a monitor of the flux through the branched chain \(\alpha\)-keto acid dehydrogenase reaction in the perfused rat liver, the experiment illustrated in Fig. 1A was performed. The liver from a fed rat was perfused in the presence of \(1 \text{ mM}\) unlabeled \(\alpha\)-ketoisocaproate for a period of \(15 \text{ min}\). At \(15 \text{ min}\), a tracer amount of \(\alpha\)-keto\([1-{^{14}\text{C}}]\)isocaproate was infused for \(10 \text{ min}\) and samples were collected at \(30 \text{ s}\) intervals to monitor \(^{14}\)CO\(_2\) production. Following the termination of the infusion of \(\alpha\)-keto\([1-{^{14}\text{C}}]\)isocaproate, samples were collected every \(12 \text{ s}\) for \(3 \text{ min}\) and then every \(30 \text{ s}\). The "off" or "washout" kinetics of \(^{14}\)CO\(_2\) production shown in Fig. 1A indicates two distinct components with approximate half-times of \(27 \text{ s}\) and \(4.8 \text{ min}\), respectively. The rapid kinetic component with a \(t_{1/2}\) of \(27 \text{ s}\) accounted for \(94\%\) of the \(^{14}\)CO\(_2\) "washed out" of the liver within the initial \(2\frac{1}{2} \text{ min}\) following the termination of the infusion of the labeled branched chain \(\alpha\)-keto acid. This rapid kinetic component is very similar to the rapid component observed in the perfused rat heart with \(\alpha\)-ketoisocaproate (18). In view of the fact that there are few metabolic fates for \(\alpha\)-ketoisocaproate that would result in the rapid decarboxylation of \(\alpha\)-ketoisocaproate, it was inferred that the rapid kinetic component observed in Fig. 1A was due to the branched chain \(\alpha\)-keto acid dehydrogenase reaction. The slower kinetic component (i.e. \(t_{1/2} = 4.8 \text{ min}\)) observed in the experiment shown in Fig. 1A may be the result of a variety of factors, among which are: (a) if the \(\alpha\)-keto\([1-{^{14}\text{C}}]\)isocaproate was not entirely labeled in the 1 position, \(^{14}\)C derived from the other positions would be released at a slower rate; or (b) if the infused \(\alpha\)-keto\([1-{^{14}\text{C}}]\)isocaproate was transaminated to \([1-{^{14}\text{C}}]\)leucine using glutamate as the amino group donor, the \(^{14}\)C-labeled leucine presumably present in an intracellular pool might be expected to be metabolized at a slower rate than the infused branched chain \(\alpha\)-keto acid. In order to examine the second of these suggestions the experiment shown in Fig. 1B was performed. A liver was perfused for \(15 \text{ min}\) with \(1 \text{ mM}\) \(l\)-leucine following which a tracer amount of \([1-{^{14}\text{C}}]\)leucine was infused during the \(10 \text{ min}\) interval indicated. Under these conditions, the metabolic flux through the branched chain \(\alpha\)-keto acid dehydrogenase reaction was calculated as \(1.2 \mu\text{mol of } l\text{-leucine decarboxylated/g of liver/h.}\) The decline in the production of \(^{14}\)CO\(_2\) upon withdrawal of the \([1-{^{14}\text{C}}]\)leucine exhibited one major kinetic component which accounted for \(87\%\) of the \(^{14}\)CO\(_2\) "washed out" of the liver in the initial \(3\frac{1}{2} \text{ min}\) following the cessation of the \([1-{^{14}\text{C}}]\)leucine infusion. The \(t_{1/2}\) of this kinetic component was approximately \(1.3 \text{ min}\) (Fig. 1B). Each time \([1-{^{14}\text{C}}]\)leucine was infused, there occurred a sharp transient peak in the rate of \(^{14}\)CO\(_2\) production immediately after the infusion of the \(1-{^{14}\text{C}}\)-labeled substrate was initiated. Repeating this type of experiment using \(\alpha\)-keto\([1-{^{14}\text{C}}]\)isovalerate as the substrate provided results nearly identical with those with \(\alpha\)-keto\([1-{^{14}\text{C}}]\)isocaproate shown in Fig. 1A (results not shown).

The effect of increasing the concentration of the infused \(\alpha\)-ketoisocaproate on the flux through the branched chain dehydrogenase reaction in the perfused liver is illustrated in Fig. 2. Increasing the concentration of \(\alpha\)-keto\([1-{^{14}\text{C}}]\)isocaproate in the perfusate increased the rates of oxygen consumption, ketogenesis, and \(^{14}\)CO\(_2\) production until the branched chain \(\alpha\)-keto acid concentration exceeded \(1 \text{ mM}\). The metabolic flux through the branched chain \(\alpha\)-keto acid dehydrogenase plotted as a function of infused \(\alpha\)-keto acid concentration exhibited saturation kinetics in this type of experiment (see inset of Fig. 2). The \(\beta\)-hydroxybutyrate/acetoacetate ratio in the effluent perfusate increased from values of \(0.11 \times 0.05 \text{ mm of } \alpha\)-ketoisovalerate to \(0.19 \times 0.5 \text{ mm of } \alpha\)-ketoisocaproate and finally to \(0.22 \times 5 \text{ mm of } \alpha\)-ketoisocaproate. The overall rate of ketogenesis (i.e. \(\beta\)-hydroxybutyrate plus acetoacetate) upon infusion of the ketogenic branched chain \(\alpha\)-keto acid, \(\alpha\)-ketoisocaproate

![Fig. 1. Kinetics of \(^{14}\)CO\(_2\) production from \(\alpha\)-keto\([1-{^{14}\text{C}}]\)isocaproate (A) and \([1-{^{14}\text{C}}]\)leucine (B) in the perfused rat liver. Livers were perfused for 15 min with the unlabeled substrate before infusing the \(1-{^{14}\text{C}}\)-labeled substrate for 10 min. Samples were collected and analyzed for \(^{14}\)CO\(_2\) content as described under "Materials and Methods."](image-url)
Branched Chain α-Keto Acid Metabolism

FIG. 2. Effect of varying the α-keto-[1-14C]isocaproate concentration on the rates of α-ketoisocaproate decarboxylation and ketogenesis in a perfused rat liver. The infused α-ketoisocaproate concentration was increased in a stepwise manner as indicated by the horizontal bars. 14CO2 production, oxygen consumption, and ketone bodies, β-hydroxybutyrate □ and acetoacetate □, were measured as described under “Materials and Methods.” The inset at the top of the figure demonstrates the relationship between α-ketoisocaproate concentration in the perfusion medium and the rate of decarboxylation of this substrate.

(1 mM), was 59.5 μmol·g⁻¹·h⁻¹ which is approximately 75% of the rate observed in the perfused liver from a fed rat with octanoate (0.5 mM) as the ketogenic substrate.

Infusing varying concentrations of α-keto-[1-14C]isovalerate into a perfused liver from a fed rat resulted in sequential increases in the flux through the branched chain α-keto acid dehydrogenase reaction (Fig. 3) and the rate of oxygen consumption by the liver. Again the flux through the enzyme complex saturated above 1 mM α-ketoisovalerate. It is evident that the flux through the branched chain complex was greater with α-keto[1-14C]isocaproate as the infused substrate than with α-keto[1-14C]isovalerate by nearly a factor of 2 (e.g. 30 compared to 54 μmol·g⁻¹·h⁻¹). This observation stands in contrast to the situation in the perfused rat heart (16) and in rat liver mitochondria (22) where α-ketoisovalerate was a better substrate than α-ketoisocaproate at the same concentration.

In a liver perfused with α-keto[1-14C]isocaproate (1 mM), co-infusion of either unlabeled α-ketoisovalerate (1 mM) or DL-α-keto-β-methylvalerate (2 mM) resulted in a 54% and 67% decrease in the rate of 14CO2 production from the infused α-keto[1-14C]isocaproate, respectively (see Fig. 4). The rate of oxygen consumption by the liver was elevated by 10% upon infusion of either of the alternative branched chain substrates.

FIG. 3. Effect of varying the α-keto[1-14C]isovalerate concentration on the rate of α-ketoisovalerate decarboxylation in the isolated perfused rat liver. For experimental details, see legend to Fig. 1 and “Materials and Methods.” The inset at the top of the figure represents the relationship between α-keto[1-14C]isovalerate concentration in the perfusion medium and the rate of α-ketoisovalerate decarboxylation.

FIG. 4. Effect of α-ketoisovalerate and DL-α-keto-β-methylvalerate co-infusion on the rate of α-keto[1-14C]isocaproate decarboxylation in a perfused rat liver. The various branched chain α-keto acids were infused into the liver as indicated by the horizontal bars. 14CO2 production and oxygen consumption by the liver were monitored as described under “Materials and Methods.”
Conflicting evidence has been published concerning the effects of L(-)-carnitine on the decarboxylation of branched chain α-keto acids. Although carnitine has been reported not to affect the rate of α-ketoisocaproate oxidation in liver (23) and muscle mitochondria (24), information from other laboratories indicates that L(-)-carnitine significantly stimulates oxidation of leucine, valine, and α-ketoisocaproate in rat skeletal muscle mitochondria (25) and the rate of α-ketoisocaproate decarboxylation in rat liver homogenates and mitochondria (26, 27). The effect of L(-)-carnitine on the decarboxylation of 1-14C-labeled α-ketoisocaproate and α-ketoisovalerate in the perfused liver is illustrated in Fig. 5, A and B, respectively. With α-keto[1-14C]isocaproate as the substrate, L(-)-carnitine infusion resulted in a 13% increase in oxygen consumption by the liver with no change in the rate of α-ketoisocaproate decarboxylation. On the other hand, oxygen consumption by the liver and the decarboxylation of α-keto[1-14C]isovalerate were stimulated by approximately 11% and 60%, respectively, during the 8 min of L(-)-carnitine infusion.

We have demonstrated that pyruvate infusion into the perfused rat heart resulted in up to a 90% inhibition of the metabolic flux through the branched chain dehydrogenase reaction (16). While the exact mechanism of this pyruvate-mediated inhibition of the branched chain complex is not known, it was shown that pyruvate infusion resulted in a decrease in the extractable branched chain α-keto acid dehydrogenase activity in the perfused heart (28). The experiments depicted in Fig. 6, A and B, demonstrate the effect of pyruvate infusion (10 mM) on the flux through the branched chain dehydrogenase reaction in the perfused liver. Oxygen consumption increased by approximately 15% upon co-infusion of pyruvate and either branched chain substrate. However, pyruvate infusion inhibited the decarboxylation of α-keto[1-14C]isocaproate (1 mM) by 18% (Fig. 6A), while the decarboxylation of α-keto[1-14C]isovalerate was inhibited by 12% (Fig. 6B). In the rat heart, pyruvate infusion inhibited α-ketoisocaproate decarboxylation to a considerably greater extent than α-ketoisovalerate decarboxylation (16).

The effects of fatty acids and ketone bodies on the decarboxylation of the branched chain amino acids and α-keto acids have been investigated in various tissues with somewhat conflicting results. While Odyssey and Goldberg (10) observed an inhibition of [1-14C]leucine decarboxylation in rat diaphragm, Buse et al. (11) demonstrated that octanoate caused a stimulation in the rate of decarboxylation of branched chain amino acids in the diaphragm and a recirculating heart perfusion system. Additionally, stimulation of valine oxidation by octanoate and palmitate in the perfused rat hindquarter has been reported (29). Our data in the nonrecirculating heart perfusion indicate that octanoate, β-hydroxybutyrate, and acetocetate all inhibit strongly branched chain α-keto acid decarboxylation (16). Crabb and Harris (30) also demonstrated a decreased rate of [1-14C]leucine decarboxylation in hepatocytes upon addition of either oleate or β-hydroxybutyrate.

The effects of infused β-hydroxybutyrate on the metabolic flux through the branched chain α-keto acid dehydrogenase reaction in the perfused liver with α-keto[1-14C]isocaproate or α-keto[1-14C]isovalerate are depicted in Fig. 7, A and B, respectively. Co-infusion of β-hydroxybutyrate and either α-ketoisocaproate or α-ketoisovalerate resulted in an 18% increase in oxygen consumption over the rates observed with the branched chain substrates alone. However, while β-hydroxybutyrate infusion resulted in a 12% inhibition of the decarboxylation of α-keto[1-14C]isocaproate (Fig. 7A), the decarboxylation of α-keto[1-14C]isovalerate was stimulated greatly (i.e. 100%) (Fig. 7B). The stimulation of flux through the branched chain α-keto acid dehydrogenase reaction with α-ketoisovalerate as the substrate was reversed completely.
**FIG. 6.** The effect of pyruvate co-infusion on the rates of \( \alpha \)-keto[1-\( ^{14}C \)]isocaproate (A) and \( \alpha \)-keto[1-\( ^{14}C \)]isovalerate (B) decarboxylation. In each experiment, pyruvate was co-infused for a period of 8 min as indicated by the horizontal bars. \( ^{14}CO_2 \) production and oxygen consumption by the livers were measured as described under “Materials and Methods.”

**FIG. 7.** Effect of (DL)-\( \beta \)-hydroxybutyrate co-infusion on the rates of \( \alpha \)-keto[1-\( ^{14}C \)]isocaproate (A) and \( \alpha \)-keto[1-\( ^{14}C \)]isovalerate decarboxylation (B). (DL)-\( \beta \)-Hydroxybutyrate was co-infused into livers for periods of 8 min as indicated by the horizontal bars. For experimental details, see “Materials and Methods.”
when \( \beta \)-hydroxybutyrate was withdrawn from the perfusion system. Increasing the perfusion concentration of \( \alpha \)-ketol-\( \text{[1-}^{14}\text{C}\)isocaproate was "washed out" of the liver in the initial 2/3 min with a \( t_{1/2} \) of 27 s a following cessation of the tracer infusion. The \( t_{1/2} \) of the kinetic component due to the pyruvate dehydrogenase reaction in the perfused liver is very similar but not identical with that calculated for the branched chain \( \alpha \)-keto acids in the present study (e.g. compare \( t_{1/2} \) of 18 s for pyruvate with \( t_{1/2} \) of 27 s for \( \alpha \)-ketoisocaproate) (15). Also, \( \alpha \)-ketol-\( \text{[1-}^{14}\text{C}\)isocaproate and \( \alpha \)-ketol-\( \text{[1-}^{14}\text{C}\)isovalerate (data not presented) exhibited very similar \( t_{1/2} \) values for the decline of \( ^{14}\text{CO}_2 \) production following termination of the tracer infusion, suggesting a similar metabolic fate for the two branched chain \( \alpha \)-keto acids in the perfused liver. Performing a similar type of experiment with \( \text{L-}\text{[1-}^{14}\text{C}\)leucine indicated that the major kinetic component had a \( t_{1/2} \) of 1.3 min and the rate of \( \text{L-}\text{leucine} \text{ decarboxylation} \) was less than 4\% of the rate of \( \alpha \)-ketoisocaproate decarboxylation. This observation suggests that the transamination of branched chain amino acids in the liver is the rate-limiting step. Evidence for this suggestion has been provided by Ichihara et al. (7) and Shinnick and Harper (8).

Studies of the concentration dependence of the rate of decarboxylation of infused branched chain \( \alpha \)-keto acids in the perfused liver indicated that the flux through the enzyme complex is saturated at substrate concentrations in excess of 1 mM. A noticeable difference emerges between the decarboxylation rates of \( \text{L-}^{14}\text{C}\)labeled \( \alpha \)-ketoisocaproate and \( \alpha \)-ketoisovalerate in the perfused liver. At each concentration examined, the rate of \( \alpha \)-ketoisocaproate decarboxylation exceeded the rate of \( \alpha \)-ketoisovalerate decarboxylation. Evidence obtained in isolated liver mitochondria (22), in the perfused rat heart (16), and in a variety of other systems (5, 34, 35) has indicated that \( \alpha \)-ketoisocaproate is decarboxylated at a more rapid rate than \( \alpha \)-ketoisovalerate by the branched chain complex. It is possible that the various short and branched chain acyl-CoA intermediates in the pathway involved in the breakdown of \( \alpha \)-ketoisocaproate and \( \alpha \)-ketoisovalerate, respectively, may affect differentially the regulation of the branched chain complex in the intact liver. In the present experiments, it is suggested that acyl-CoA intermediates produced during \( \alpha \)-ketoisocaproate oxidation may be more inhibitory to the enzyme complex than those produced during the oxidation of \( \alpha \)-ketoisovalerate. Furthermore, because the liver is capable of rapid rates of ketogenesis using \( \alpha \)-ketoisocaproate as the substrate, the potentially inhibitory intermediates in the metabolism of \( \alpha \)-ketoisocaproate may not accumulate to any appreciable extent.

The rate of ketogenesis from \( \alpha \)-ketoisocaproate (Fig. 2) exhibited saturation kinetics very similar to the kinetics of the increase in the rate of decarboxylation of \( \alpha \)-ketol-\( \text{[1-}^{14}\text{C}\)isocaproate as the concentration of the branched chain \( \alpha \)-keto acid was increased in the perfusion medium. Such a correlation suggests that the measurement of \( ^{14}\text{CO}_2 \) production from \( \alpha \)-ketol-\( \text{[1-}^{14}\text{C}\)isocaproate as a monitor of metabolic flux through the branched chain complex is valid.

Studies using either partially or highly purified preparations of the branched chain \( \alpha \)-keto acid dehydrogenase complex have demonstrated that all three branched chain \( \alpha \)-keto acids, \( \alpha \)-ketoisocaproate, \( \alpha \)-ketoisovalerate, and \( \alpha \)-keto-\( \beta \)-methylvalerate, are decarboxylated oxidatively by a single multi-enzyme complex. The experiment illustrated in Fig. 4 indicated that co-infusion of either \( \alpha \)-ketoisovalerate or \( \alpha \)-keto-\( \beta \)-methylvalerate with \( \alpha \)-ketol-\( \text{[1-}^{14}\text{C}\)isocaproate resulted in an increased hepatic oxygen consumption and an appreciable and reversible inhibition of the rate of \( ^{14}\text{CO}_2 \) from the labeled \( \alpha \)-ketoisocaproate. This experimental finding may be interpreted as a competition between the unlabeled and \( ^{14}\text{C}\)-labeled \( \alpha \)-keto acid at the level of the branched chain enzyme complex or at the level of the mitochondrial transport of the two branched chain \( \alpha \)-keto acids. We observed a differential effect of L-(\(-\))-carnitine infusion on the rate of decarboxylation of \( \alpha \)-ketoisocaproate and \( \alpha \)-ketoisovalerate. This effect (e.g. a stimulation of the rate of \( \alpha \)-ketoisovalerate decarboxylation and no appreciable change in the rate of \( \alpha \)-ketoisocaproate decarboxylation) likely is a result of the differential accumulation of acyl-CoA intermediates involved in the respective pathways for the metabolism of these two compounds in the absence and presence of L-\( (\text{--})\)-carnitine. Indeed, Solberg and Bremer (36) demonstrated in rat liver mitochondria incubated in the presence of L-\( \text{[Me-}^{3}\text{H}\)carnitine and \( \alpha \)-ketoisovalerate or \( \alpha \)-ketoisocaproate that levels of propionylcarnitine and medium chain acylcarnitine were much greater with \( \alpha \)-ketoisovalerate as the substrate than with \( \alpha \)-ketoisocaproate. Hence, L-\( (\text{--})\)-carnitine may remove propionyl-CoA and other medium (branched) chain acyl-CoAs formed from \( \alpha \)-ketoisocaproate oxidation which either may be inhibitory to the branched chain complex or may release free CoA which could accelerate the oxidation of the branched chain \( \alpha \)-keto acid. If acyl-CoA intermediates in the pathway of \( \alpha \)-ketoisocaproate oxidation do not accumulate or if the acyl-CoA species which accumulate are not as inhibitory to the branched chain complex, L-\( (\text{--})\)-carnitine infusion would not cause an observable effect. It is unlikely that L-\( (\text{--})\)-carnitine causes an appreciable diversion of intermediates toward acetyl-CoA formation during \( \alpha \)-ketoisocaproate oxidation as the ketogenic rate in the presence and absence of L-\( (\text{--})\)-carnitine was unchanged (data not shown).

Infusion of relatively low levels of pyruvate into the perfused rat heart resulted in a 90\% inhibition of the metabolic flux through the branched chain complex using \( \alpha \)-ketoisovalerate as the substrate. A less extensive flux inhibition was noted using \( \alpha \)-ketoisocaproate as the substrate (16). Additionally, the extractable activity of the branched chain complex was diminished by 72\% upon infusion of pyruvate into a heart which had been perfused under conditions which maximally activated the branched chain complex (28). Infusion of pyruvate into the perfused liver resulted in an inhibition of \( \alpha \)-ketol-\( \text{[1-}^{14}\text{C}\)isocaproate decarboxylation to a greater degree than \( \alpha \)-ketol-\( \text{[1-}^{14}\text{C}\)isovalerate decarboxylation, just the reverse situation as was observed in the heart. Whether the flux inhibition observed in the liver was the result of an inactivation of the enzyme complex or was the result of a simple competitive inhibition of the branched chain complex by pyruvate or NADH is currently under investigation.

The decarboxylation of \( \alpha \)-ketol-\( \text{[1-}^{14}\text{C}\)isocaproate was inhibited by the co-infusion of \( \beta \)-hydroxybutyrate into the liver.
On the surface, this effect would seem to be a simple NADH-mediated inhibition of the enzyme complex since few other fates for \( \beta \)-hydroxybutyrate than oxidation to acetoacetate can occur in the liver.

However, despite the increase in the mitochondrial NADH/NAD\(^+\) ratio upon infusion of \( \beta \)-hydroxybutyrate, the rate of decarboxylation of a-keto-[\( ^{1-13}C \)]isovalerate was stimulated by 100%. Stimulation of the decarboxylation of [\( ^{1-13}C \)]pyruvate in the perfused liver upon infusion of octanoate, oleate, or \( \beta \)-hydroxybutyrate has been reported (15, 37). It was proposed that the stimulation of pyruvate decarboxylation and the activation of the pyruvate dehydrogenase complex at low (i.e., physiological) pyruvate levels were results of an accelerated acetoacetate/pyruvate exchange across the mitochondrial membrane on the monocarboxylate transporter. Under conditions of rapid ketogenesis (i.e., rapid acetoacetate efflux), the mitochondrial pyruvate level would be elevated and the pyruvate dehydrogenase kinase would be inhibited by pyruvate leading to an interconversion of the complex by the phosphoprotein phosphatase to its active form (15, 37). This mechanism by which a-keto-[\( ^{1-13}C \)]isovalerate decarboxylation was stimulated upon infusion of \( \beta \)-hydroxybutyrate may occur in a similar fashion. First, both a-ketoisocaproate and a-ketoisovalerate have been shown to be transported across the liver mitochondrial membrane on the monocarboxylate transporter (22). While a-ketoisocaproate oxidation in the liver results in the formation of acetoacetate and acetyl-CoA, a-ketoisovalerate produces only propionyl-CoA. Papa and Paradies (38) demonstrated that acetoacetate could replace OH\(^-\) ions as an effective antipot for pyruvate on the monocarboxylate transporter. Hence, it is likely that as a-ketoisocaproate is oxidized, acetoacetate is generated and an exchange of the branched chain a-keto acid substrate for the product, acetoacetate, is established. Also, this exchange phenomena could explain why a-ketoisocaproate decarboxylation exceeds a-ketoisovalerate decarboxylation in the liver. In the case of the stimulation of the decarboxylation of a-ketoisovalerate by \( \beta \)-hydroxybutyrate, there may occur a situation analogous to the case for pyruvate. As acetoacetate is produced intramitochondrially, an exchange is established between acetoacetate and a-ketoisovalerate. Thus, an acceleration of the decarboxylation of this branched chain a-keto acid occurs either by mass action (e.g., an increase in the intramitochondrial concentration of the substrate) or by virtue of the fact that the branched chain a-keto acids tend to activate or to stabilize the enzyme complex (6, 28). This scenario requires that the transport of a-ketoisovalerate is limiting the oxidative system when infused alone into the perfusion system.

REFERENCES
1. Adibi, S. A. (1976) Metabolism 25, 1297–1302
2. Sherwin, R. S. (1978) J. Clin. Invest. 61, 1471–1481
3. Goldberg, A. L., and Chang, T. W. (1978) Fed. Proc. 37, 2301–2307
4. Wohlhueter, R. M., and Harper, A. E. (1979) J. Biol. Chem. 254, 2391–2401
5. Parker, P. J., and Randle, P. J. (1978) Biochem. J. 171, 761–775
6. Parker, P. J., and Randle, P. J. (1979) FEBS Lett. 90, 183–186
7. Ichihara, A., Noda, C., and Ogawa, K. (1973) Adv. Enzyme Regul. 11, 155–166
8. Shinnick, F. L., and Harper, A. E. (1976) Biochim. Biophys. Acta 437, 477–486
9. Hutton, S. M., Cree, T. C., and Harper, A. E. (1978) J. Biol. Chem. 253, 8126–8133
10. Odessey, R., and Goldberg, A. L. (1972) Am. J. Physiol. 223, 1376–1385
11. Buse, M. G., Biggers, J. D., Friderici, K. H., and Buse, J. F. (1972) J. Biol. Chem. 247, 8085–8096
12. Kaser, M., Kaser, R., and Lestradt, H. (1969) Metabolism 18, 926–931
13. Walsier, M., Lunt, P., Ruderman, N. B., and Coulder, A. W. (1973) J. Clin. Invest. 52, 2965–2977
14. Olson, M. S., Dennis, S. C., DeBuysere, M. S., and Padma, A. (1978) J. Biol. Chem. 253, 7369–7375
15. Scholz, R., Olson, M. S., Schwab, A. J., Schwabe, U., Noell, C., and Braun, W. (1978) Eur. J. Biochem. 86, 519–530
16. Buffington, C. K., DeBuysere, M. S., and Olson, M. S. (1979) J. Biol. Chem. 254, 10453–10455
17. Scholz, R., Hansen, W., and Thurman, R. G. (1973) Eur. J. Biochem. 38, 64–72
18. Krebs, H. A., and Helselet, K. (1932) Z. Physiol. Chem. 210, 33–36
19. Williamson, J. R., and Corkey, B. E. (1969) Methods Enzymol. 13, 434–512
20. Mellanby, J., and Williamson, D. H. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. V., ed) Vol. 4, pp. 1840–1843, Academic Press, New York
21. Rudiger, M. W., Langenbeck, V., and Goedde, H. W. (1972) Biochem. J. 124, 445–446
22. Patel, T. B., Waymack, P. P., and Olson, M. S. (1980) Arch. Biochem. Biophys. 201, 629–635
23. Noda, C., and Ichihara, A. (1974) J. Biochem. (Tokyo) 76, 1123–1130
24. Odessey, R., and Goldberg, A. L. (1979) Biochem. J. 178, 475–479
25. Van Hinanber, V. W., Veerkamp, J. H., Engelen, P. J. M., and Ghijsen, W. J. (1978) Biochem. Med. 20, 115–124
26. Paul, H. S., and Adibi, S. A. (1978) Am. J. Physiol. 234, E494–E499
27. May, M. E., Aftrung, R. P., and Buse, M. G. (1980) J. Biol. Chem. 255, 8394–8397
28. Waymack, P. P., DeBuysere, M. S., and Olson, M. S. (1980) J. Biol. Chem. 255, 9773–9781
29. Spydevold, O. (1979) Eur. J. Biochem. 97, 389–394
30. Crabb, D. W., and Harris, R. A. (1978) J. Biol. Chem. 253, 1481–1487
31. Williamson, J. R., Wajdyys-Rode, E., and Coll, K. E. (1979) J. Biol. Chem. 254, 11511–11520
32. Wajdyys-Rode, E., Coll, K. E., and Williamson, J. R. (1979) J. Biol. Chem. 254, 11521–11529
33. Wajdyys-Rode, E., and Williamson, J. R. (1980) J. Biol. Chem. 255, 415–418
34. Pettit, F. H., Yeaman, S. J., and Reed, L. J. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 4881–4885
35. Danner, D. J., Lemmon, S. K., Beshearse, J. C., and Elssas, L. J., II (1979) J. Biol. Chem. 254, 5522–5526
36. Selberg, H. E., and Bremer, J. (1970) Biochim. Biophys. Acta 222, 372–380
37. Dennis, S. C., DeBuysere, M., Scholz, R., and Olson, M. S. (1978) J. Biol. Chem. 253, 2229–2237
38. Papa, S., and Paradies, G. (1974) Eur. J. Biochem. 49, 265–274

1 T. B. Patel, M. S. DeBuysere, R. Scholz, and M. S. Olson, manuscript in preparation.