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Direct measurement of oxidative metabolism in the living brain by microdialysis - Review

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Abstract

This review summarizes microdialysis studies that address the question of which compounds serve as energy sources in the brain. Microdialysis was used to introduce ^{14}C-labeled glucose, lactate, pyruvate, glutamate, glutamine and acetate into the interstitial fluid of the brain to observe their metabolism to $^{14}CO_2$. Although glucose uptake from the systemic system supplies the carbon source for these compounds, compounds synthesized from glucose by the brain are subject to recycling, including complete metabolism to CO_2. Therefore, the brain utilizes multiple compounds in its domain to provide the energy needed to fulfill its function. The physiological conditions controlling metabolism and the contribution of compartmentation into different brain regions, cell types and subcellular spaces are still unresolved. The aconitase inhibitor fluorocitrate, with a lower inhibition threshold in glial cells, was used to identify the proportion of lactate and glucose that was oxidized in glial cells versus neurons. The fluorocitrate data suggest that glial and neuronal cells are capable of utilizing both lactate and glucose for energy metabolism.

Keywords

energy metabolism; microdialysis; fluorocitrate; compartmentation; oxidation

Oxidative metabolism of ^{14}C-labeled compounds with the formation of $^{14}CO_2$ by brain tissue and cell cultures or extracts have indicated the wide range of substrates (glucose, lactate, pyruvate, glutamate, glutamine, acetate) that these preparations are able to metabolize for energy production (Bouzier-Sore *et al.* 2003; Cremer 1981; Dienel and Cruz, 2006; Edmond *et al.* 1987; Itoh *et al.* 2003; Larrabee 1995; McKenna *et al.* 1994; Peng *et al.* 1994; Poitry-Yamate *et al.* 1995; Roeder *et al.* 1984; Tildon *et al.* 1983; Zielke *et al.* 1996). These results may seem to be in contrast to the direct correlation of glucose and oxygen utilization by the intact brain that support the conclusion that glucose taken up by the brain from the blood can account for all the energy needs of the brain (Sokoloff 1992). One conclusion that might be drawn from these results is that the oxidation seen *in vitro* does not reflect the *in vivo* situation. A more nuanced conclusion is that brain energy metabolism is complex and that a wide range of compounds are formed in the brain from glucose as the carbon source. These compounds form the basis for the structural and physiological function of the brain. When these compounds are not needed to perform their basic function they are metabolized for reformation of brain constituents, released from the brain as by-products or oxidized for energy production.

Microdialysis has been adapted to both infuse radioactive substrates into the interstitial fluid of the rat brain and to sample the same fluid for the formation of $^{14}CO_2$ (Huang *et al.* 1993).

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The methodology allows study of a large number of available ^{14}C-labeled compounds by infusion of trace amounts into the interstitial space without drastically altering the physiological levels in the interstitial space.

Oxidation of compounds following infusion into the brain by microdialysis

^{14}C-Labeled glucose, lactate, pyruvate, glutamate, glutamine, and acetate were tested as potential oxidative substrates and each was shown to be oxidized to $^{14}CO_2$ (Table 1). The absolute oxidation rates for these substrates can not be calculated due to the limitations listed above. However, the data indicate that all listed compounds are actively oxidized and that the closer metabolically the compound is to the TCA cycle, the higher the rate of $^{14}CO_2$ recovery. These results are consistent with oxidative capacity reported with *in vitro* cultured brain cells. Although readily oxidized following perfusion into the interstitial fluid, glutamate and pyruvate have a very low interstitial concentration. Consequently, the amounts present in the interstitial fluid can not serve as a significant reserve as energy sources, although the intracellular concentration of glutamate is high. In contrast glutamine and lactate are present in the interstitial fluid at concentrations ranging from 0.2 to 1 mmol/L (Kanamori and Ross 2004;Kuhr and Korf 1988). Consequently, they have the potential of serving as a mobile energy source. Since neither of these compounds is taken up from the periphery in adults, they are synthesized in the brain from glucose. Glutamine is an energy source for multiple types of intact and dissociated cells in culture (Tildon and Zielke 1988), including astrocytes (Huang and Hertz 1995) and neurons (Peng *et al.* 1994;Peng *et al.* 2007; Yu *et al.* 1995). The report of pyruvate recycling in cultured neurons is supportive of neuronal metabolism of glutamine to CO_2 (Olstad *et al.* 2007).

Compartmentation of oxidative metabolism of glutamate and glutamine

Metabolic compartmentation in the brain was first established with the observation that radiolabeled acetate labeled glutamine more extensively than glutamate, its immediate precursor (Berl and Clarke 1969). Subsequent studies have contributed to the understanding of glutamate and glutamine metabolic compartmentation by establishing that astrocytes are the site of the enzyme glutamine synthetase (Norenberg and Martinez-Hernandez 1979) and that neurons have a much greater amount of phosphate activated glutaminase than astrocytes (Hogstad *et al.* 1988).

To ascertain if glutamine or glutamate is extensively oxidized in brain and in which compartment or cell type, it was necessary to determine their specific activity in the interstitial fluid during the oxidation study. Initial microdialysis studies (Huang *et al.* 1993; Zielke *et al.* 1997) indicated that the production of $^{14}CO_2$ was linear with time for more than 6 hours once equilibrium had been established between the perfusate and the interstitial fluid. These data suggested that a steady state specific activity had been established 1 hr after the ^{14}C-labeled compounds was perfused into the interstitial fluid. Once the steady state was achieved, the radioactive amino acid in the perfusate was replaced with artificial CSF and eluates from the microdialysis probe were collected every 2 minutes for HPLC analysis followed by determination of radioactivity in glutamate or glutamine. The specific activity dropped rapidly with time. Extrapolation of the specific activity back to the end of the oxidation study provided an estimate of the interstitial specific activity during the oxidation study (Zielke *et al.* 1998). Based on the extrapolated extracellular specific activities it was calculated that glutamine was oxidized at 5 times the rate of glutamate (Table S1). Since glutamine is metabolized via glutamate, the data support the conclusion that glutamine and glutamate are oxidized in separate compartments. Based on the primary localization of glutaminase in neurons, it suggests that glutamine is a significant oxidative substrate in neurons. *In vivo* NMR studies by Pascual et al. (1998) indicated that both

glutamate and glutamine were oxidative substrate under normoxia and that their oxidative metabolism increased dramatically during ischemic conditions. These authors calculated oxidative rates, based in part on extracellular concentration that favored glutamate oxidation over glutamine. Differences between these two studies may be related to assumption of different interstitial glutamine concentrations.

Effect of potential competitive or stimulatory metabolites

The addition of a non-radioactive substrate to oxidation experiments along with a ^{14}C-labeled substrate provides information about whether two compounds share the same uptake system, if they are intermediates in a common metabolic pathway, or if they serve as alternate energy sources. Addition of non-radioactive lactate to ^{14}C-pyruvate significantly reduced the recovery of $^{14}CO_2$ from the dialysate during *in vivo* oxidative studies and the reciprocal seemed also to be the case (Fig. S1). A minimal conclusion is that they are oxidized in the same compartment(s). The reciprocal inhibitory effects may also suggest competitive uptake by the same carrier. This is supported by the observation that α-cyano-4-hydroxycinnamate, an inhibitor of the mitochondrial monocarboxylate transporter (Halestrap and Denton, 1974) inhibited the oxidation of both ^{14}C-pyruvate and ^{14}C-lactate (Zielke *et al.* 2007a).

Addition of non-labeled pyruvate had a small but not significant effect on the amount of $^{14}CO_2$ recovery from studies with ^{14}C-glucose (Fig. S1) that was similar to a large and highly significant observation in cultured astrocytes (Hertz, 2004). Lactate had no effect in similar experiments (Zielke *et al.* 2007a). An additional study is consistent with glucose and lactate oxidation by both neurons and astrocytes (Zielke *et al.* 2007b).

Co-infusion of acetate, and to a lesser degree pyruvate, stimulated ^{14}C-glutamate and ^{14}C-glutamine oxidation, but had no effect on ^{14}C-glucose, ^{14}C-pyruvate or ^{14}C-lactate oxidation (Fig. 1). Acetate is preferentially transported into astrocytes (Waniewski and Martin, 1998) and converted to acetyl-CoA, suggesting that this effect occurs primarily in this cell type. An increase of acetyl-CoA may potentially enhance TCA cycle activity. Pyruvate could function in the same manner, but a buildup of acetyl-CoA is expected to decrease pyruvate dehydrogenase activity (Sugden and Holness, 2003), resulting in a self-limiting stimulatory effect. Pyruvate may also enhance oxidation of glutamate and glutamine by serving as an amino group acceptor (Hodgkins *et al.* 1999). Inclusion of another amino group acceptor, α-ketoisocaproate, increased two fold the rate of ^{14}C-glutamate oxidation (Zielke *et al.*, 1997). In addition, carboxylation of pyruvate to oxalacetate may dilute the specific activity of TCA cycle intermediates, decreasing the apparent stimulatory effect of pyruvate compared to acetate.

Lactate Metabolism

A current issue in brain metabolism is the role of lactate in brain, and specifically in neuronal metabolism. The astrocyte-neuron shuttle hypothesis (ANLS) was proposed in 1994 to interrelate neurotransmitter glutamate uptake by astrocytes, enhanced glycolysis and lactate release by astrocytes (Pellerin and Magistretti 1994). In the extreme, proponents proposed that neurons did not utilize glucose, but rather relied solely on lactate. Multiple studies and reviews with different conclusions have resulted (Ames 2000; Chih *et al.* 2001; Dienel and Cruz 2004; Dienel and Hertz 2001; Hertz 2004; Pellerin 2003; Aubert *et al.* 2005). Because the methodology is not available to measure oxidative metabolism during a single synaptic event, we addressed the issue of glucose and lactate oxidation over a longer time frame in the intact *in vivo* brain using microdialysis and inhibition of TCA cycle activity in glial cells using the aconitase inhibitor fluorocitrate (Paulsen *et al.* 1987; Clarke 1991).

Fluorocitrate has been utilized *in vitro* at concentrations of 5–100 μmol/L (Hassel *et al.* 1995) to inhibit glial but not neuronal aconitase activity. Recent studies with hippocampal slices utilized 10 and 20 mmol/L fluoroacetate to demonstrate that synaptic transmission was inhibited, but electrogenic membrane function was unaffected (Canals *et al.* 2008). The lactate dehydrogenase activity in the medium remained unaffected after a 24 h exposure to fluorocitrate indicating that cell disruption had not occurred even at this elevated level of inhibitor. *In vivo* inhibition of glial aconitase activity was achieved by the injection of 1 nmol into the brain (Paulsen *et al.* 1987). The range of fluorocitrate concentrations that inhibited glial aconitase activity and not neuronal activity was quite narrow. If the quantity injected was increased to 2 nmol, neuronal damage was also observed. A one time injection approach was not suitable for the desired oxidation studies needed to address questions about cellular metabolic activity because the requirement of pre-implantation of the guide cannula and the desire to assure inhibition throughout the oxidation study. Using studies with *in vitro* concentrations of 5–100 μmol/L as a guide (Hassel *et al.* 1995), the studies by Zielke et al. (2007b) were performed at 5, 20 and 100 μmol/L fluorocitrate in the microdialysis perfusate. Based on an estimated equilibration of 15% of the fluorocitrate across the dialysis membrane, Zielke et al. (2007b) proposed that the brain would be exposed to fluorocitrate concentrations that had been shown *in vitro* to only affect astrocytes. The interstitial level of two astrocytic products, glutamine and lactate, was used as determinant of astrocyte TCA cycle inhibition. Their concentrations in the interstitial fluid were a function of increasing fluorocitrate levels in the perfusate.

Studies were performed to ascertain if 100 μmol/L fluorocitrate caused cellular damage. Rats were perfused by microdialysis for 6 hours with 100 μmol/L fluorocitrate and the animals were euthanized 24 hours later. Nissl stained brain sections showed no evidence of neuronal damage (Fig. 2). The results were interpreted as indicating that minimal blockage of the TCA cycle had occurred in neurons. The behavior of rats perfused with 100 μM fluorocitrate ranged from sleep to being awake. When rats were perfused with 300 μmol/L fluorocitrate, the animals were hyper active and had bursts of rapid activity, circling rapidly in their enclosure.

Potential alterations of glial and neuronal metabolism due to fluorocitrate were further assessed by measuring the effect on oxidation of compounds that enter the TCA cycle after the aconitase reaction. Oxidation studies were performed using [^{14}C]glutamate and [^{14}C]glutamine which enter the TCA cycle via α-ketoglutarate. Oxidation of neither of these compounds was affected by 100 μmol/L fluorocitrate (Fig. S2) indicating that cellular metabolism was not grossly affected by microdialysis perfusion at this fluorocitrate concentration. We concluded that 100 μmol/L fluorocitrate should be considered the upper level that primarily affects the glial compartment and that the optimum concentration is probably 20–50 μmol/L.

Oxidative studies were performed on the effect of fluorocitrate on lactate and glucose oxidation. The 5 μmol/L fluorocitrate had no effect on either lactate or glucose oxidation while 20 and 100 μmol/L fluorocitrate reduced production of $^{14}CO_2$ from ^{14}C-lactate by 55% and 68%, respectively (Fig 3). These results support the conclusion that glial cells are capable of oxidizing lactate as well as producing lactate. As a corollary, Zielke *et al.* (2007b) proposed that the portion of lactate oxidation that was not inhibited by fluorocitrate occurred in the less fluorocitrate sensitive compartment, neurons. In comparable experiments, ^{14}C-glucose oxidation was reduced in a non-dose dependent manner, 50% and 24%, respectively (Fig. 4). Using analogous reasoning, a significant portion of glucose is oxidized in both the glial and neuronal cells. These indicated that a threshold level of inhibitor had been reached at near 20 μmol/L fluorocitrate.

Addition of non-radioactive lactate in addition to ^{14}C-glucose and 20 or 100 μmol/L fluorocitrate further decreased the rate of glucose oxidation by 31% and 38%, respectively (Fig. 4). These data further support the conclusion that neurons oxidize lactate. Based on these findings we propose that under normal physiological circumstances, both neurons and astrocytes utilize glucose and lactate for energy. The quantitative values from these studies differ in magnitude with *in vitro* studies that indicated a almost 4-fold higher contribution from lactate metabolism than glucose metabolism to oxidative metabolism in cultured cortical neurons (Bouzier-Sore *et al.* 2004). This may reflect the differences between pure *in vitro* cultures versus an intact brain with a mixed population of cells.

Concluding remarks

The use of microdialysis for oxidative studies shares a common advantage with all other types of microdialysis studies. Namely, that one obtains *in vivo* results from a non-anaesthetized animal that more closely reflects the functioning of a normal brain. However, not all of metabolic interactions are revealed since the data reflects the results in the interstitial fluid rather than in the individual cells. Glucose, lactate, pyruvate, acetate, glutamate and glutamine are some of the multiple compounds that were oxidized to $^{14}CO_2$ by brain cells when perfused into the interstitial fluid. It is further concluded that equal amounts of lactate are oxidized in neurons and astrocytes, whereas oxidative metabolism of glucose accounts for 2/3 of total glucose metabolism in neurons and 1/3 in astrocytes (Fig S3). The data support the conclusion that both astrocytes and neurons utilize the enzymatic mechanisms present in their cells to obtain energy. Because of the difference in time frame, the current studies do not directly address the energetics during synaptic transmission.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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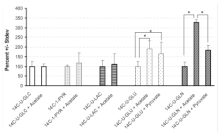

Fig. 1.

Effect of acetate and pyruvate on in vivo oxidation. The mean ± stdev.is expressed as percent, normalized to an infusion rate of 1 μCi/hr. The 100% values for each compound, expressed as dpm/1μCi/hr, are: ^{14}C-U-glucose 391 ± 96, n=5; ^{14}C-1-pyruvate, 3,349 ± 198, n=5; ^{14}C-U-lactate, 713 ± 220, n=7; ^{14}C-U-glutamate, 1,775 ± 438, n=5; and ^{14}C-U-glutamine, 573 ± 126, n=4 (*P<0.05). From Zielke *et al.* 2007a with permission.

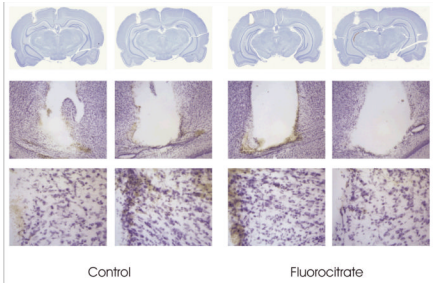

Fig. 2.

Nissl stain of rat brain 24 hrs after perfusion with 100 μM fluorocitrate for 6 hrs. The brains were perfused *in situ* with 4% paraformaldehyde and processed for Nissl staining (FD Neurotechnologies, Inc, Ellicott City, MD).

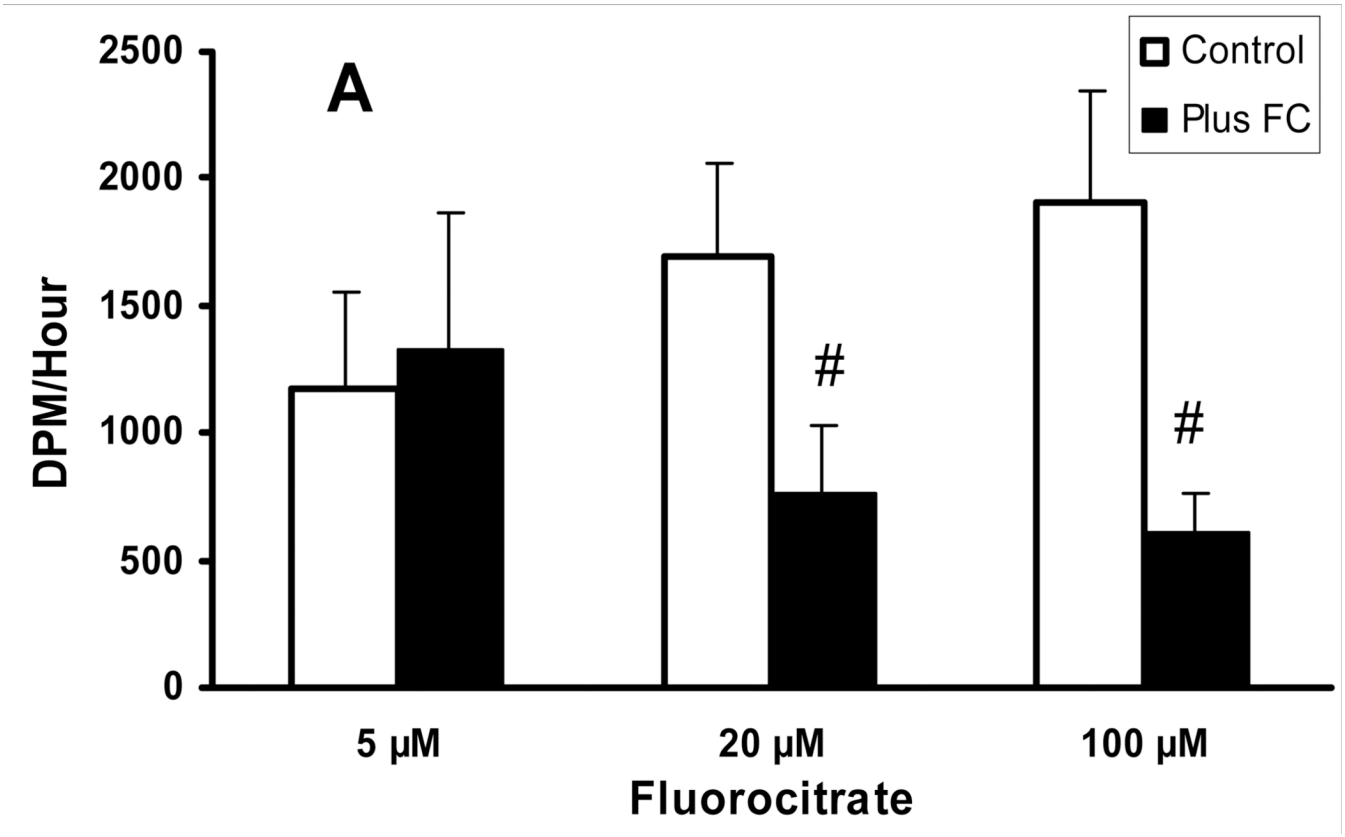

Fig. 3.
Concentration effect of fluorocitrate on $^{14}CO_2$ recovery following perfusion with [U-^{14}C]lactate. Mean ± stdev. (0 µmol/L, n= 13; 5 µmol/L, n=4; 20 µmol/L, n=5; 100 µmol/L, n=4; #p<0.001). From Zielke *et al.* 2007b with permission.

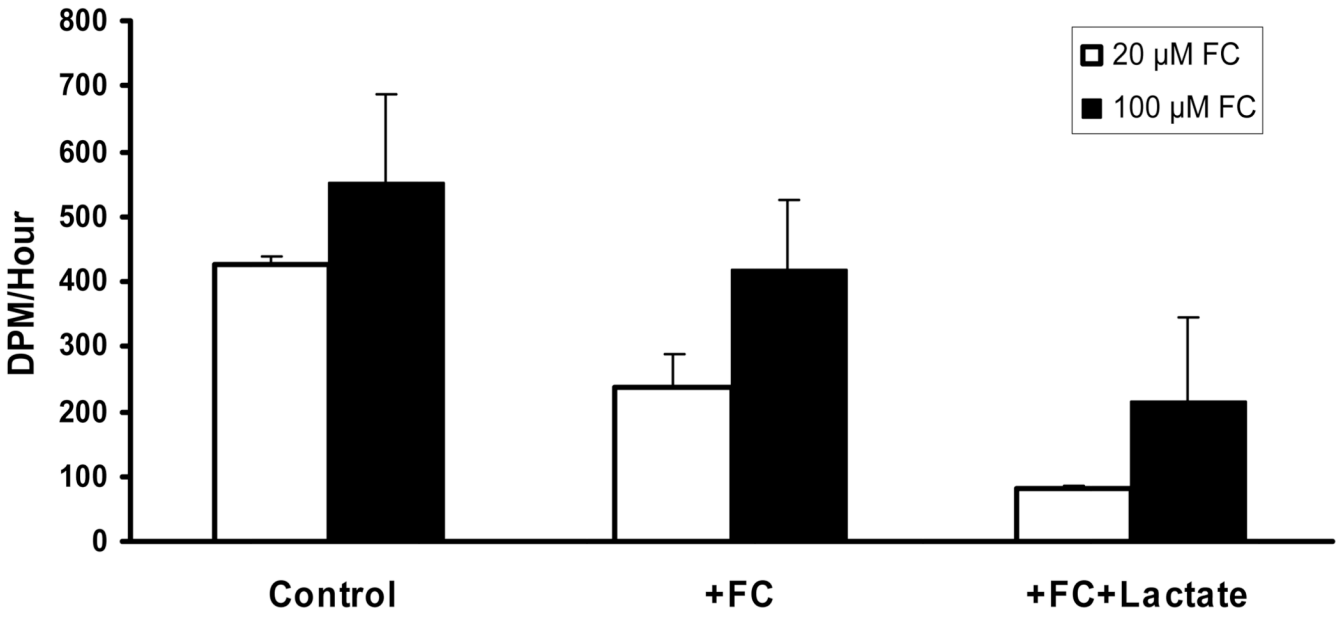

Fig. 4.

Effect of fluorocitrate and lactate on recovery of $^{14}CO_2$ following perfusion of [U-^{14}C]glucose into the interstitial space by microdialysis. Three one hour dialysate fractions were collected as described in Material and Methods to establish the baseline (Control) rate of $^{14}CO_2$ recovery from [U-^{14}C]glucose. Perfusion was continued with [U-^{14}C]glucose plus 20 or 100 µmol/L fluorocitrate (+FC). The perfusate was then changed to [U-^{14}C]glucose plus fluorocitrate plus 50 mmol/L lactate (+FC+Lactate). In studies with 20 µmol/L fluorocitrate each set of samples was significantly different from each other. In studies with 100 µmol/L fluorocitrate, control values were significantly different only from (+FC+Lactate) samples. (Analysis of variance and Tukey's test; $p<0.05$; n=4). From Zielke *et al.* 2007b with permission.

Table 1

Oxidation rate of substrate perfused into the hippocampus of the rat

Substrate	μCi perfused/hr	Ave ± Stdev dpm/1μCi/hr	n
[U-^{14}C]Glucose	3.55	194 ± 149	33
[U-^{14}C]Lactate	1.48	539 ± 177	39
[1-^{14}C]Pyruvate	2.05	2312 ± 1118	20
[U-^{14}C]Glutamate	1.30	1015 ± 335	12
[U-^{14}C]Glutamine	1.21	456 ± 179	18
[U-^{14}C]Acetate	0.95	1204 ± 540	5

The recovery of $^{14}CO_2$ is expressed as “Ave ± Stdev dpm/1μCi/hr.” The data were normalized to a perfusion rate of 1μCi/hr. No corrections were made for interstitial concentration, rate of cellular uptake or intracellular pool size. Reproduced with permission (Zielke *et al.* 2007a).