NMR IN BIOMEDICINE
NMR Biomed. 2002;**15**:45–51
DOI:10.1002/nbm.745

Hepatic gluconeogenesis and Krebs cycle fluxes in a CCl_4 model of acute liver failure

Rui A. Carvalho,[1,2]* John G. Jones,[3] Chris McGuirk,[3] A. Dean Sherry[2,3] and Craig R. Malloy[3,4]

[1]Department of Biochemistry and Center of Neurosciences, University of Coimbra, Portugal
[2]Department of Chemistry, University of Texas at Dallas, Dallas, Texas, USA
[3]The Mary Nell and Ralph B. Rogers Magnetic Resonance Center, Department of Radiology, University of Texas Southwestern Medical Center, Dallas, Texas, USA
[4]Department of Internal Medicine, University of Texas Southwestern Medical Center and Department of Veterans Affairs Medical Center, Dallas, Texas, USA

Received 1 August 2001; Accepted 24 September 2001

ABSTRACT: Acute liver failure was induced in rats by CCl_4 administration and its effects on the hepatic Krebs cycle and gluconeogenic fluxes were evaluated *in situ* by ^{13}C NMR isotopomer analysis of hepatic glucose following infusion of [U-^{13}C]propionate. In fed animals, CCl_4 injury caused a significant increase in relative gluconeogenic flux from 0.80 ± 0.10 to 1.34 ± 0.24 times the flux through citrate synthase ($p < 0.01$). In 24-h fasted animals, CCl_4-injury also significantly increased relative gluconeogenic flux from 1.36 ± 0.16 to 1.80 ± 0.22 times the flux through citrate synthase ($p < 0.01$). Recycling of PEP via pyruvate and oxaloacetate was extensive under all conditions and was not significantly altered by CCl_4 injury. CCl_4 injury significantly reduced hepatic glucose output by 26% (42.8 ± 7.3 vs 58.1 ± 2.4 μmol/kg/min, $p = 0.005$), which was attributed to a 26% decrease in absolute gluconeogenic flux from PEP (85.6 ± 14.6 vs 116 ± 4.8 μmol/kg/min, $p < 0.01$). These changes were accompanied by a 47% reduction in absolute citrate synthase flux (90.6 ± 8.0 to 47.6 ± 8.0 μmol/kg/min, $p < 0.005$), indicating that oxidative Krebs cycle flux was more susceptible to CCl_4 injury. The reduction in absolute fluxes indicate a significant loss of hepatic metabolic capacity, while the significant increases in relative gluconeogenic fluxes suggest a reorganization of metabolic activity towards preserving hepatic glucose output. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: glucose homeostasis; hepatic glucose output; intermediary metabolism; Krebs cycle flux

INTRODUCTION

Hepatic glucose metabolism is disrupted in the setting of cirrhosis.[1–5] In the absorptive state it is characterized by an inability to extract glucose from the portal vein circulation leading to a reduction in hepatic glycogen deposition.[3,4,6] Consequently, in the postabsorptive state, hepatic glucose production from glycogenolysis is reduced. Given that fasting hypoglycemia is typically rare in cirrhosis, hepatic glucose output is believed to be maintained by a compensatory increase in gluconeogenic flux.[5,6] Since gluconeogenesis is largely dependent on a functional Krebs cycle for energy and gluconeogenic precursors, it is likely that such alterations reflect a reorganization of metabolic fluxes at the Krebs cycle level. These could include changes in the relative rates of acetyl-CoA oxidation and conversion of anaplerotic substrates (such as pyruvate) to glucose. Additionally, loss of functional hepatic mass and metabolic capacity may impose limits on absolute metabolic fluxes through these pathways.

We recently developed non-invasive methods for measuring hepatic gluconeogenic and Krebs cycle fluxes following administration of ^{13}C tracers.[7–9] The measurements can be performed in a clinical setting since they are based on non-radioactive tracers and the withdrawal and analysis of small amounts of blood.[9] In its simplest form, the method provides a direct measurement of relative gluconeogenic and oxidative Krebs cycle fluxes.[7,9] By incorporating an additional measurement of hepatic glucose output into the assay, absolute fluxes can be derived, allowing the total metabolic capacity for

**Correspondence to:* R. A. Carvalho, Department of Biochemistry, Apartado 3126, 3001-401 Coimbra, Portugal.
Email: carvalho@condor.ci.uc.pt
Contract/grant sponsor: National Science Foundation; *contract grant number:* NSF/AR1 DB1-9612144.
Contract/grant sponsor: National Institute of Health; *contract grant number:* NIH/SIG 1-S10-RR11488.

Abbreviations used: ATP, adenosine triphosphate; CS, citrate synthase; GC/MS, gas chromatography/mass spectrometry; GOT, glutamate–oxaloacetate transaminase; GPT, glutamate–pyruvate transaminase; OAA, oxaloacetate; PEP, phosphoenolpyruvate; PEP-ck, phosphoenolpyruvate carboxykinase; PDH, pyruvate dehydrogenase; PK, pyruvate kinase.

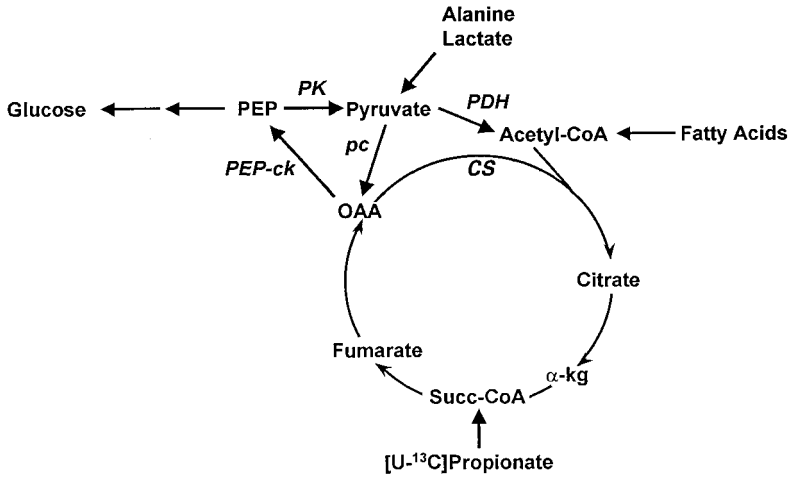

Figure 1. Simplified model of the Krebs cycle and gluconeogenic fluxes. Key enzymes are also included: CS, citrate synthase; PDH, pyruvate dehydrogenase; PEP-ck, phosphoenolpyruvate carboxykinase, and PK, pyruvate kinase

gluconeogenesis and Krebs cycle oxidation to be evaluated.[8] To determine the utility and sensitivity of these measurements for characterizing Krebs cycle and gluconeogenic fluxes, we applied them to a standard rat model of acute liver failure induced by CCl_4 ingestion. Although this acute injury does not constitute a model of cirrhosis, usually described as a chronic liver disease, it provides a good approach towards the validation of the above ^{13}C-tracer methodologies as tools for monitoring changes in glucose metabolism in the injured liver.

MATERIALS AND METHODS

Materials

Sodium [U-^{13}C]propionate and [1,6-$^{13}C_2$]glucose were obtained from Cambridge Isotope Laboratories (Cambridge, MA, USA). Other materials were analytical grade and used without further purification (Sigma, St Louis, MO, USA).

Animals

Acute liver injury was induced both in fed and fasted animals by intragastric administration of CCl_4 mixed with corn oil (1:1) 24 h prior to experimentation (1.0 ml/240 g body weight). Four groups of animals were studied: (i) fed controls; (ii) fed CCl_4 injured; (iii) fasted control; and (iv) fasted CCl_4 injured. Male Sprague–Dawley rats (190–310 g, Sasco, Houston, TX, USA) were used throughout.

Rats were anesthetized by intramuscular injection of a mixture of ketamine (85 mg/ml) and xylazine (15 mg/ml). For measurement of relative Krebs cycle and gluconeogenic fluxes an aqueous solution of sodium [U-^{13}C]propionate was infused by a catheter inserted into the jugular vein at a rate of 25.0 μmol/kg/min over 1 h.[7] For measurement of hepatic glucose output and absolute metabolic fluxes in 24 h-fasted rats, sodium [U-^{13}C]propionate infusion (25.0 μmol/kg/min) was combined with a primed infusion of [1,6-$^{13}C_2$]glucose (25.00 μmol prime, 2.75 μmol/kg/min) over 2 h.[8]

For all measurements, blood was collected from the carotid artery and the liver was removed and freeze-clamped. Blood and tissue samples were extracted with cold perchloric acid, neutralized with KOH and freeze-dried. Glucose and carboxylic acids in the liver extract were separated from amino acids by cation exchange chromatography. The glucose/carboxylic acid fraction was treated with 100 units of glucose oxidase (6–12 h at 25 °C, Type VII-S, Sigma) and bubbled with air (about 10–20 ml/min) to quantitatively oxidize all glucose to gluconate.[10] The reaction was terminated by lowering the pH to ~1 with concentrated perchloric acid. Plasma samples were treated with perchloric acid and prepared for NMR analysis as previously described.[8] In separate experiments, levels of serum transaminases (GPT and GOT) were measured in a separate blood sample (16 rats, four in each group) to monitor the extent of hepatic injury induced by CCl_4.

NMR methods

Proton-decoupled ^{13}C NMR spectra of liver extracts were obtained by using a 5 mm broadband probe on a 9.4 T GE-Bruker Omega spectrometer. Proton decoupled ^{13}C NMR spectra of serum extracts were acquired by using a 5 mm broadband probe on a 14.1 T Unity INOVA (Varian Instruments, Palo Alto, CA). ^1H NMR spectra

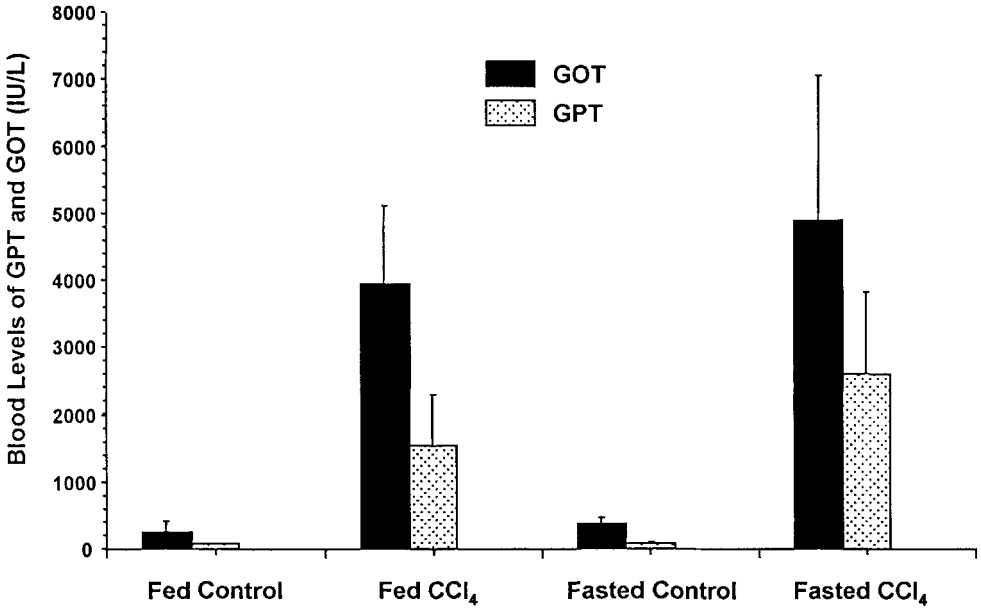

Figure 2. Histogram of serum transaminase GPT (IU/l) and GOT (IU/l) for the four groups of rats studied

were acquired using a 5 mm indirect detection probe and the 14.1 T system. ^{13}C broadband decoupling covering the C2–C5 region of glucose (70–76 ppm) was applied during ^{1}H acquisition to eliminate all ^{1}H-^{13}C long-range couplings in the glucose H1 resonance.[8] Free induction decays were zero-filled and multiplied by a 0.25 Hz exponential function prior to Fourier transformation. Line fitting and spectral simulations were performed using the PC-based NMR software, NUTS™ (Accorn NMR, Freemont) or the standard Varian NMR software.

Measurement of hepatic fluxes using [U-^{13}C]propionate and ^{13}C NMR

We have shown that [U-^{13}C]propionate is an effective tracer of gluconeogenesis and Krebs cycle activity in both rats and humans.[8,9] [U-^{13}C]propionate is metabolized via activation to propionyl-CoA, carboxylation to methylmalonyl-CoA, and entry into the Krebs cycle as succinyl-CoA. Figure 1 shows a standard model of the Krebs cycle featuring anaplerotic and gluconeogenic pathways.[8,9,11,12] Plasma glucose becomes enriched with ^{13}C following incorporation of [U-^{13}C]propionate into the Krebs cycle as a consequence of gluconeogenic outflow via phosphoenolpyruvate (PEP). PEP can also be recycled via pyruvate and oxaloacetate (OAA) by a so-called futile cycle.[13,15] The major inflow of gluconeogenic carbons for PEP synthesis occurs via carboxylation of pyruvate. While these carbons can also be oxidized to acetyl-CoA via pyruvate dehydrogenase, this flux is typically very low compared to pyruvate carboxylase flux.[7,9,16–19]

The labeling distribution in plasma glucose reflects the integrated activities of these pathways and given certain assumptions. Key assumptions are: (1) isotopic steady-state conditions; (2) negligible labeling of acetyl-CoA; and (3) complete randomization of OAA carbons via fumarate. Simple mathematical equations relate the glucose ^{13}C isotopomers (as reported by the glucose C2 ^{13}C-NMR signal) to relative fluxes for OAA $\rightarrow$ PEP, PEP $\rightarrow$ pyruvate, PEP $\rightarrow$ glucose and OAA $\rightarrow$ citrate.[7,9] These can be converted to absolute values when integrated with an independent measurement of absolute flux through any one of the pathways.[8]

RESULTS

Serum transaminase levels

Conventional blood chemistry was performed on samples collected from 16 animals (four in each group). Plasma levels of glutamate-pyruvate (GPT) and glutamate-oxaloacetate (GOT) transaminases are reported in Fig. 2 for the four groups: fed control, fed CCl_4 injured, fasted control and fasted CCl_4 injured. As anticipated for animals with damaged livers, serum GPT and GOT were significantly elevated in both CCl_4 injured groups compared to controls.

Relative Krebs cycle and gluconeogenic flux measurements

The ^{13}C-labeling distribution of hepatic glucose follow-

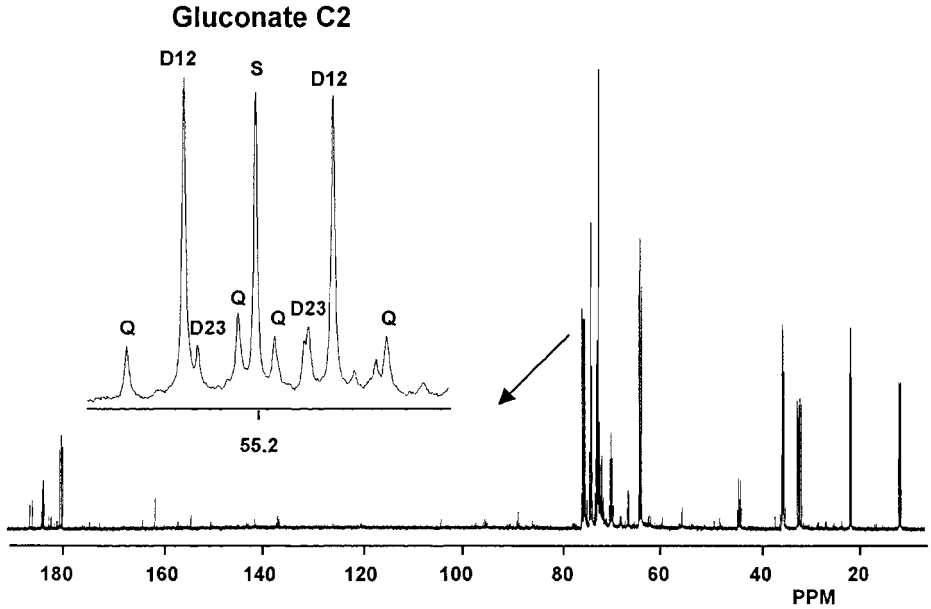

Figure 3. 9.4 T ^{13}C NMR spectra of the carboxylic acid fraction of an extract of a liver from a CCl_4 injured, fasted rat. The expansion of the gluconate C2 resonance shows the resolution of multiplet components (D12, D23 and Q) whose relative areas are used to estimate relative TCA cycle and gluconeogenic fluxes

ing infusion of [U-^{13}C]propionate was obtained from the ^{13}C NMR spectrum of hepatic glucose following derivatization to gluconate[7,9,10] and an example is shown in Fig. 3. In all cases, the gluconate C2 multiplet components were well resolved and had sufficient signal-to-noise for accurate quantitation of glucose isotopomer ratios and relative metabolic fluxes by ^{13}C NMR.

Relative flux estimates from the NMR analysis of glucose are summarized in Table 1. Analysis of 24 h-fasted rat livers showed that fluxes through PEP-ck were about four times the flux through citrate synthase. In addition, a significant portion of PEP was recycled via pyruvate and OAA, the recycling flux being about 2.6 times flux through citrate synthase. Net outflow of PEP to glucose was about 1.4 times citrate synthase flux. These observations agree with our previous baseline measurements from both intact rats and perfused livers obtained from fasted rats.[7,8] Compared to fasted rats, fed control rats showed a tendency toward lower PEP-ck flux and higher pyruvate kinase fluxes, resulting in significantly lower relative rates of gluconeogenesis. In fed animals, CCl_4 injury caused modest but significant increases in relative gluconeogenic flux from baseline values. This resulted from non-significant increases in PEP-ck flux coupled to reductions in PEP recycling via pyruvate kinase and resembles the changes observed between fed and fasted baseline measurements. In fasted animals, while CCl_4 injury also increased relative gluconeogenic flux from baseline values, this was accompanied by a tendency for increased recycling of PEP via pyruvate kinase.

Absolute gluconeogenic and Krebs cycle fluxes in 24 h fasted rats

By combining [U-^{13}C]propionate administration with a primed infusion of [1,6-$^{13}C_2$]glucose, Krebs cycle flux and hepatic glucose output measurements can be

Table 1. Relative hepatic Krebs cycle and gluconeogenic fluxes from control and CCl_4 treated rats

Group	PEP-ck flux (OAA → PEP)	PK flux (PEP → pyruvate)	Gluconeogenic flux (PEP → glucose)	CS flux (OAA → citrate)
Fed control ($n = 6$)	3.68 ± 0.42	2.87 ± 0.42	0.80 ± 0.10	1.00
Fed CCl_4 injured ($n = 7$)	4.00 ± 0.59	2.65 ± 0.42	1.34 ± 0.24*	1.00
Fasted control ($n = 6$)	3.92 ± 0.37	2.55 ± 0.52	1.28 ± 0.16	1.00
Fasted CCl_4 injured ($n = 8$)	5.03 ± 0.48	3.22 ± 0.49	1.80 ± 0.22*	1.00

* Significantly different ($p < 0.005$).

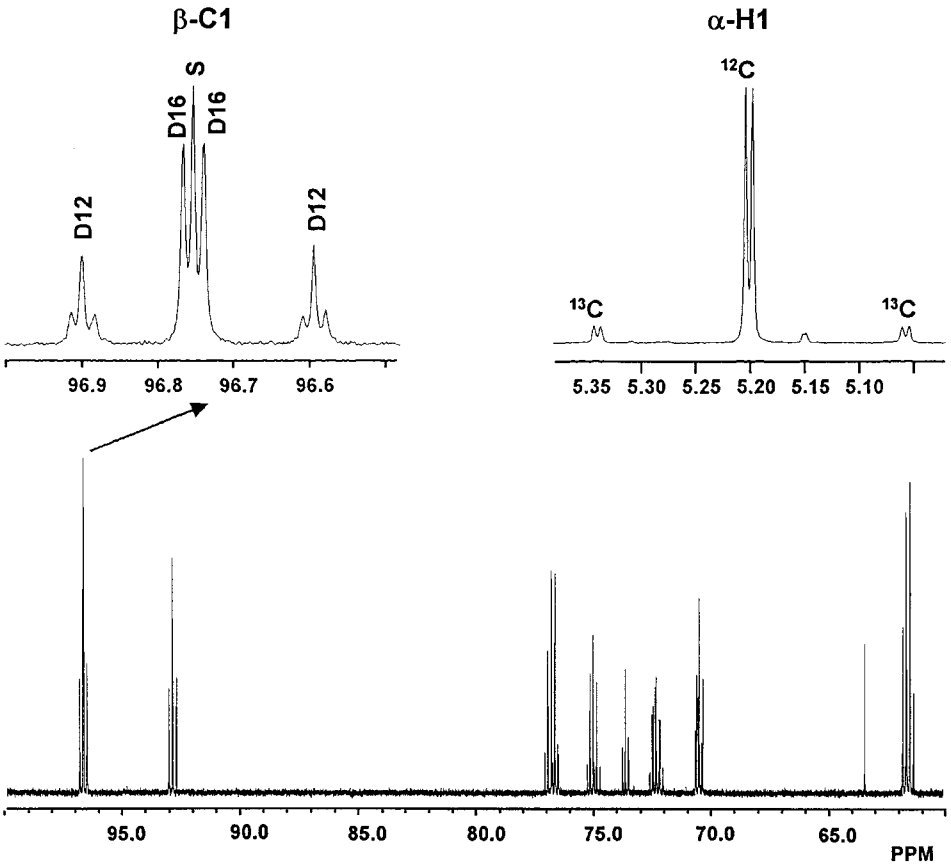

Figure 4. 14.1 T ^{13}C NMR spectra of blood glucose from a CCl_4 injured, fasted rat after continuous infusion of [1,6-$^{13}C_2$]glucose + [U-^{13}C]propionate. The β-C1 resonance of glucose is shown in the expansion. The α-H1 resonance from the ^1H NMR spectrum of the same sample is also shown

Table 2. Absolute hepatic fluxes (μmol/kg/min) for 24 h fasted control and CCl_4 injured rats

Group	PEP-ck flux (OAA → PEP)	PK flux (PEP → pyruvate)	Gluconeogenic flux (PEP → glucose)	CS flux (OAA → citrate)
Control ($n = 6$)	356.2 ± 33.6	231.5 ± 47.2	116.2 ± 4.8	90.6 ± 8.0
CCl_4 injured ($n = 8$)	239.2 ± 22.8	153.1 ± 26.0	85.6 ± 14.6*	47.6 ± 8.0**

* Significantly different from control ($p < 0.01$).
** Significantly different from control ($p < 0.005$).

integrated to provide absolute flux estimates for gluconeogenic and Krebs cycle fluxes in 24 h fasted rats.[8]

A representative ^{13}C spectrum of plasma glucose from a rat infused with [1,6-$^{13}C_2$]glucose and [U-^{13}C]propionate is shown in Fig. 4. The spectrum has well-resolved contributions from [1,6-$^{13}C_2$]glucose as seen by the doublet signal in the C1β resonance (D16). Also, the glucose C1 fractional enrichment was well characterized by ^1H NMR. This allowed accurate quantitation of plasma [1,6-$^{13}C_2$]glucose enrichment and dilution from endogenous glucose production.[8] The remaining multiplet signals are derived from the incorporation of [U-^{13}C]propionate into glucose via the Krebs cycle and gluconeogenesis. Hepatic glucose output rates were calculated to be 58.1 ± 2.4 μmol/kg/min for the control group, corresponding to a PEP → glucose flux of 116 ± 4.8 μmol/kg/min.[8] These rates are consistent with our previous NMR measurements and the GC/MS measurements of Katz *et al.* with 24 h-fasted rats.[8,20] At the Krebs cycle level, absolute fluxes through citrate synthase, PEP-ck and pyruvate kinase were consistent with those measured in our earlier study.[8] As previously observed, there was extensive recycling of PEP carbons via pyruvate and oxaloacetate.[8,16,20]

Compared to controls, hepatic glucose output for the CCl_4-injured group was significantly lower (42.8 ± 7.3 μmol/kg/min, $p < 0.01$) reflecting a 26% reduction in PEP outflow to glucose (Table 2). The reduction

occurred despite the increase in the ratio of gluconeogenic to oxidative flux, suggesting a loss of hepatic metabolic capacity. Oxidative Krebs cycle activity suffered the greatest impact from CCl_4 injury with a 47% reduction in citrate synthase flux. Despite this significant loss of ATP synthesis capacity, the extent of PEP recycling through the so-called 'futile cycle' remained high.

DISCUSSION

Our measurements indicate that gluconeogenesis and Krebs cycle fluxes are altered in rat livers following CCl_4 intoxication. These alterations correlated with significant elevations in serum transaminase levels, standard clinical markers of liver failure. Both relative and absolute flux measurements were applied to determine their efficacy and sensitivity as metabolic indices of liver failure. Relative flux measurements have the advantage of being the simplest to implement in the clinical setting since they require a single tracer, [U-^{13}C]propionate. In humans this can be given orally, and when combined with phenylacetate and acetaminophen ingestion, the NMR analysis can be non-invasively performed on urinary glucuronide and phenylacetylglutamine.[9] However, while relative flux measurements provide a detailed picture of carbon distribution between oxidative and gluconeogenic pathways, they provide no information on the metabolic capacity of the system. These limitations are significant given that cirrhosis is characterized by a loss of functional hepatic mass in addition to possible alterations in metabolic fluxes. Absolute metabolic flux measurement reflects both carbon flux distribution and the metabolic capacity of the Krebs cycle and gluconeogenic network. This measurement requires an additional primed infusion of [1,6-$^{13}C_2$]glucose plus sampling of plasma glucose. Since the measurement does not account for possible contributions of glycogenolytic flux to hepatic glucose output, it can only be applied under conditions where hepatic glycogenolysis is known to be negligible. To this end, we are developing strategies that combine current ^{13}C NMR measurements with independent ^{2}H NMR tracer assays of hepatic glycogenolysis.[21,22]

Absolute metabolic flux analysis of 24 h-fasted rats revealed that hepatic citrate synthase flux was impaired to a much greater extent than gluconeogenic and PEP recycling fluxes following CCl_4 injury. CCl_4 is specifically activated in mitochondria[23] and is known to inhibit components of mitochondrial electron transport.[24] Studies on isolated mitochondria from livers exposed to acute CCl_4 intoxication show reduced oxygen consumption, ATP synthesis and reduction in respiratory control ratio.[25–27] These are all consistent with our observation of reduced acetyl-CoA oxidation *in situ*. There is scant information on the effects of CCl_4 injury on gluconeogenic outflow from the Krebs cycle. While one study reported an increase in pyruvate carboxylase activity following CCl_4 administration,[28] little is known about changes in PEP-ck and pyruvate kinase activities. Although the cytosolic location of PEP-ck and pyruvate kinase might afford some protection from the direct effects of CCl_4 toxicity, gluconeogenesis is ultimately dependent on a functional Krebs cycle and malate–aspartate shuttle to supply the bulk of substrate and reducing equivalents. To the extent that these processes are damaged by mitochondrial CCl_4 injury, gluconeogenesis will also inhibited. Finally, CCl_4 injury causes significant reductions in hepatic blood flow and perfusion,[29,30] resulting in reduced substrate and oxygen delivery. This is also likely to have a significant role in constraining absolute Krebs cycle and gluconeogenic fluxes.

In summary, NMR analysis of glucose ^{13}C-enrichment following the administration of ^{13}C-tracers was shown to be an effective tool for documenting alterations in Krebs cycle and gluconeogenic fluxes following hepatic injury by CCl_4. These measurements provide a direct evaluation of the total hepatic capacity for glucose synthesis and acetyl-CoA oxidation.

Acknowledgements

This work was supported by grants NSF/AR1 DB1-9612144 from the National Science Foundation and NIH/SIG 1-S10-RR11488 from the National Institute of Health. R.A.C. was a recipient of a PhD Grant from JNICT-Portugal (PRAXIS XXI; BD-3604/94).

REFERENCES

1. Krahenbuhl S, Reichen J. Decreased hepatic glucose production in rats with carbon tetrachloride-induced cirrhosis. *J. Hepatol.* 1993; **19**: 64–70.
2. Petersen KF, Jacob R, West AB, Sherwin RS, Shulman GI. Effects of insulin-like growth factor I on glucose metabolism in rats with liver cirrhosis. *Am. J. Physiol.* 1997; **273**: E1189–E1193.
3. Petrides AS, Vogt C, Schulze-Berge D, Matthews D, Strohmeyer G. Pathogenesis of glucose intolerance and diabetes mellitus in cirrhosis. *Hepatology* 1994; **19**: 616–627.
4. Haagsma EB, Smit GP, Niezen-Koning KE, Gouw AS, Meerman L, Slooff MJ. Type IIIb glycogen storage disease associated with end-stage cirrhosis and hepatocellular carcinoma. The Liver Transplant Group. *Hepatology* 1997; **25**: 537–540.
5. Petersen KF, Krssak M, Navarro V, Chandramouli V, Hundal R, Schumann WC, Landau BR, Shulman GI. Contributions of net hepatic glycogenolysis and gluconeogenesis to glucose production in cirrhosis. *Am. J. Physiol.* 1999; **276**: E529–E535.
6. Bugianesi E, Kalhan S, Burkett E, Marchesini G, McCullough A. Quantification of gluconeogenesis in cirrhosis: Response to glucagon. *Gastroenterology* 1998; **115**: 1530–1540.
7. Jones JG, Naidoo R, Sherry AD, Jeffrey FM, Cottam GL, Malloy CR. Measurement of gluconeogenesis and pyruvate recycling in the rat liver: a simple analysis of glucose and glutamate isotopomers during metabolism of [1,2,3-$^{13}C_3$]propionate. *FEBS Lett.* 1997; **412**: 131–137.
8. Jones JG, Carvalho RA, Franco B, Sherry AD, Malloy CR.

Measurement of hepatic glucose output, krebs cycle, and gluconeogenic fluxes by NMR analysis of a single plasma glucose sample. *Anal. Biochem.* 1998; **263**: 39–45.
9. Jones JG, Solomon MA, Sherry AD, Jeffrey FM, Malloy CR. ^{13}C NMR measurements of human gluconeogenic fluxes after ingestion of [U-^{13}C]propionate, phenylacetate, and acetaminophen. *Am. J. Physiol.* 1998; **275**: E843–E852.
10. Jones JG, Cottam GL, Miller BC, Sherry AD, Malloy CR. A method for obtaining ^{13}C isotopomer populations in ^{13}C-enriched glucose. *Anal. Biochem.* 1994; **217**: 148–152.
11. Magnusson I, Schumann WC, Bartsch GE, Chandramouli V, Kumaran K, Wahren J, Landau BR. Noninvasive tracing of Krebs cycle metabolism in liver. *J. Biol. Chem.* 1991; **266**: 6975–6984.
12. Large V, Beylot M. Modifications of citric acid cycle activity and gluconeogenesis in streptozotocin-induced diabetes and effects of metformin. *Diabetes* 1999; **48**: 1251–1257.
13. Jones CG, Titheradge MA. Measurement of metabolic fluxes through pyruvate kinase, phosphoenolpyruvate carboxykinase, pyruvate dehydrogenase, and pyruvate carboxylate in hepatocytes of different acinar origin. *Arch. Biochem. Biophys.* 1996; **326**: 202–206.
14. Rognstad R, Clark DG, Katz J. Relationship between isotopic reversibility and futile cycles in isolated rat liver parenchymal cells. *Biochem. Biophys. Res. Commun.* 1973; **54**: 1149–1156.
15. Clark DG, Rognstad R, Katz J. Isotopic evidence for futile cycles in liver cells. *Biochem. Biophys. Res. Commun.* 1973; **54**: 1141–1148.
16. Beylot M, Soloviev MV, David F, Landau BR, Brunengraber H. Tracing hepatic gluconeogenesis relative to citric acid cycle activity *in vitro* and *in vivo*. Comparisons in the use of [3-^{13}C]lactate, [2-^{13}C]acetate, and alpha-keto[3-^{13}C]isocaproate. *J. Biol. Chem.* 1995; **270**: 1509–1514.
17. Fernandez CA, Des Rosiers C. Modeling of liver citric acid cycle and gluconeogenesis based on ^{13}C mass isotopomer distribution analysis of intermediates. *J. Biol. Chem.* 1995; **270**: 10037–10042.
18. Des Rosiers C, Di Donato L, Comte B, Laplante A, Marcoux C, David F, Fernandez CA, Brunengraber H. Isotopomer analysis of citric acid cycle and gluconeogenesis in rat liver. Reversibility of isocitrate dehydrogenase and involvement of ATP-citrate lyase in gluconeogenesis. *J. Biol. Chem.* 1995; **270**: 10027–10036.
19. Large V, Brunengraber H, Odeon M, Beylot M. Use of labeling pattern of liver glutamate to calculate rates of citric acid cycle and gluconeogenesis. *Am. J. Physiol.* 1997; **272**: E51–E58.
20. Katz J, Wals P, Lee WN. Isotopomer studies of gluconeogenesis and the Krebs cycle with ^{13}C-labeled lactate. *J. Biol. Chem.* 1993; **268**: 25509–25521.
21. Jones JG, Solomon MA, Carvalho RA, Sherry AD, Malloy CR. Quantitation of tracer levels of deuterium for human metabolic studies by ^{2}H NMR at 92.1 MHz (14.1 T). *ENC 40th Meeting*, 1999; Abstract no. 149.
22. Jones JG, Carvalho RA, Sherry AD, Malloy CR. Quantitation of gluconeogenesis by ^{2}H nuclear magnetic resonance analysis of plasma glucose following ingestion of $^{2}H_2O$. *Anal. Biochem.* 2000; **277**: 121–126.
23. Tomasi A, Albano E, Banni S, Botti B, Corongiu F, Dessi MA, Iannone A, Vannini V, Dianzani MU. Free-radical metabolism of carbon tetrachloride in rat liver mitochondria. A study of the mechanism of activation. *Biochem. J.* 1987; **246**: 313–317.
24. Ikeda K, Toda M, Tanaka K, Tokumaru S, Kojo S. Increase of lipid hydroperoxides in liver mitochondria and inhibition of cytochrome oxidase by carbon tetrachloride intoxication in rats. *Free Rad. Res.* 1998; **28**: 403–410.
25. Krahenbuhl S, Stucki J, Reichen J. Mitochondrial function in carbon tetrachloride-induced cirrhosis in the rat. Qualitative and quantitative defects. *Biochem. Pharmac.* 1989; **38**: 1583–1588.
26. Morimoto T, Tanaka A, Taki Y, Noguchi M, Yokoo N, Nishihira T, Nishikawa K, Yamamoto S, Nitta N, Jikkoh A *et al.* Changes in concentrations of respiratory components and cytochrome oxidase activity in mitochondria obtained from carbon tetrachloride-induced cirrhotic rat liver. *Clin. Sci.* 1988; **74**: 485–489.
27. Seya K, Ohkohchi N, Mori S. Changes in the ability of ATP synthesis in the mitochondrial membrane in the rat liver injured by carbon tetrachloride. *Tohoku J. Exp. Med.* 1996; **178**: 425–430.
28. Salto R, Sola M, Oliver FJ, Vargas AM. Effects of starvation, diabetes and carbon tetrachloride intoxication on rat kidney cortex and liver pyruvate carboxylase levels. *Arch. Physiol. Biochem.* 1996; **104**: 845–850.
29. Tanaka N, Tanaka K, Nagashima Y, Kondo M, Sekihara H. Nitric oxide increases hepatic arterial blood flow in rats with carbon tetrachloride-induced acute hepatic injury. *Gastroenterology* 1999; **117**: 173–180.
30. Kisauzi DN, Leek BF. Liver blood flow and volatile fatty acid utilization in sheep before and after carbon tetrachloride treatment. *J. Comp. Pathol.* 1988; **98**: 471–480.

