CARBOHYDRATE METABOLISM

Measuring gluconeogenesis (GNG) with [2-^{13}C] glycerol and mass isotopomer distribution analysis (MIDA) in normal rats and in rats with streptozocin-induced diabetes. O Peroni, M Odeon, V Large, M Beylot *(Faculté de médecine, René-Laënnec, Inserm unité 197, rue Guillaume-Paradin, 69008 Lyon, France)*

MIDA is a new method for measuring, with stable isotope labelled precursors, the biosynthesis of compounds synthesized from the addition of several identical or comparable precursor molecules, such as glucose from two triosephosphates. It does this by allowing the calculation of the enrichment of the precursor pool.

In vitro, this method is valid. We have used it in vivo to quantify the total GNG in control rats ($n = 6$) and in rats with streptozocin-induced diabetes ($n = 6$). All rats were studied in the postabsorptive state. We used [3-^{3}H] glucose for measuring the hepatic glucose production (HGP) and [2-^{13}C] glycerol as a gluconeogenic tracer. Using GC-MS, the total ^{13}C glucose enrichment (IE) and glucose isotopomer distribution were measured to calculate the contribution of GNG to HGP.

In diabetic rats, glycaemia and HGP levels were higher than in control (26.4 ± 0.8 vs 8.6 ± 0.2 mM, $P < 0.05$ and 109 ± 6 vs 67 ± 3 µmol/kg/min, $P < 0.05$). The contribution of GNG to HGP was increased (67 ± 3 vs 53 ± 4%, $P < 0.05$) and the absolute GNG level was twice as high (73 ± 5 vs 37 ± 4 µmol/kg/min, $P < 0.05$) while nongluconeogenic glucose production remained unchanged (36 ± 3 vs 31 ± 2 µmol/kg/min, $P < 0.05$).

We conclude that i) the results obtained in control rats support, contrary to the recent report of Previs et al [*J Biol Chem* (1995) 270, 19806], the validity of this method for in vivo studies, and ii) in this experimental model of diabetes, the increase in HGP was due to an increase in total GNG.

Role of unsaturated fatty acids in the in vitro inhibition of glucose-6 phosphatase by glycogen. N Daniele [1], JC Bordet [2], G Mithieux [1] *([1] Inserm U 449; [2] 331, Faculté de médecine René-Laënnec, 69372 Lyon cedex 08, France)*

It has been suggested that glycogen, or an endogenous-associated compound, of molecular mass < 5 kDa and that can be dissociated by ion-exchange, could inhibit glucose-6 phosphatase (Glc6Pase) and could play a regulatory role in hepatic glucose production during nutritional transitions.

Glycogen has been purified from the liver of fed rats by KOH digestion and ethanol precipitation. It inhibited Glc6Pase activity in the microsomes isolated from 48 h-fasted rats (66 ± 14% of control activity in the presence of 30 mM glucosyl, mean ± SEM, $n = 4$). After filtration on DOWEX 1 (200–400 mesh), glycogen lost its inhibitory effect on Glc6Pase (101 ± 3% of control). The fractions bound to the column were eluted by 30 mM and 1 M HCl, successively. After evaporation to dryness and resuspension, these fractions inhibited Glc6Pase activity (80 ± 2 and 76 ± 3% of control, respectively).

Since glycogen is associated with the liver membranes, a hypothesis suggesting that the inhibitory compound could be of a lipidic nature was tested. Lipids were extracted from glycogen by Folch's method. Analysis by thin-layer chromatography revealed the presence of significant amounts of fatty acids (FA), but no complex lipids. Quantification by gas chromatography revealed that glycogen contained 4.9 ± 0.7 nmol FA/µmol glucosyl. After DOWEX filtration, 1.3 ± 0.7 nmol remained tightly bound to the glycogen and 1.2 ± 0.6 and