Authors' reply

To the Editor: When labelled glucose is used to determine endogenous glucose production (EGP) by infusing [6,6-$^2H_2$]glucose [1], the rate by which "new" glucose produced within organs (mainly the liver, but to some extent the kidney and perhaps the intestine) enters the systemic circulation can be assessed from dilution of the enrichment of glucose (or its specific activity if radioactive-labelled glucose is infused). Measuring that EGP provides answers, for example, on what contribution those glucose-producing organs make to the actual blood glucose concentration, and whether nutrients, e.g. amino acids, increase glucose production [1].

Of course, glucose-producing organs also use the glucose they produce, and to the extent that new glucose is used before entering the circulation, glucose production is greater than the amount measured by the dilution of labelled glucose in the systemic circulation. Moreover, when EGP is assessed from the arteriovenous balance technique, i.e. the difference between the quantity of glucose leaving and entering an organ, glucose produced and utilised within the organ is not measured. For clarity two terms could be used, one ("net glucose production") for the quantity of new glucose released into the circulation, the other ("total glucose production") for the quantity produced. The latter could also be referred to as EGP, and the former as endogenous glucose output, i.e. glucose produced and released into the circulation for use. At present these terms are generally used interchangeably and have not been employed previously in this manner to our knowledge.

If, as concluded by Burns and Cohen, glucose uptake by the liver were substantial, our estimation of gluconeogenesis (GNG) from the difference between (net) EGP and glycogenolysis measured by ^{13}C nuclear magnetic resonance (NMR) spectroscopy would have been an underestimate of (total) EGP. We appreciate the extensive literature on hepatic intralobular functional heterogeneity and the effect it could have on estimates of GNG [2]. Figure 1 summarises the concept that periportal hepatocytes primarily produce whereas perivenous hepatocytes remove glucose. Consequently, if all new unlabelled glucose produced in the periportal zone was taken up in the liver lobule, it would not appear in the hepatic vein and would not enter the peripheral blood which is sampled. As a result this unlabelled glucose would not dilute the labelled glucose. On the other hand, if the new glucose does enter the systemic circulation, returns to the liver and is taken up by hepatocytes, we will measure this glucose by the dilution of the labelled glucose. While the portal vein is usually not accessible for studies in humans, rates of glucose uptake in the splanchnic bed and in the kidney have been estimated by combining arteriovenous balance and labelled glucose techniques. Even if all glucose uptake by the splanchnic bed is attributed to the liver, whether from its perivenous zone or not, the amount taken up in normal subjects [3], as well as in Type 2 diabetic patients [4], is small compared to the overall amount of glucose produced. Moreover, there does not seem to be a major difference between the periportal and perivenous zones in the contribution of the gluconeogenic (indirect) and direct pathways to glycogen synthesis, at least in rats following a glucose load [5].

We also directly measured the contribution of GNG to glucose production from the ratio of ^2H enrichments from ^2H_2O in the hydrogens at carbons 5 and 2 of glucose [1]. That ratio provides the measure of the contribution of GNG to the production of the new glucose in the circulation. Rates of GNG were not different when calculated either by ^{13}C NMR spectroscopy or the ^2H_2O method (see Table 2 of [1]), providing further evidence that the estimate obtained by NMR spectroscopy was correct.

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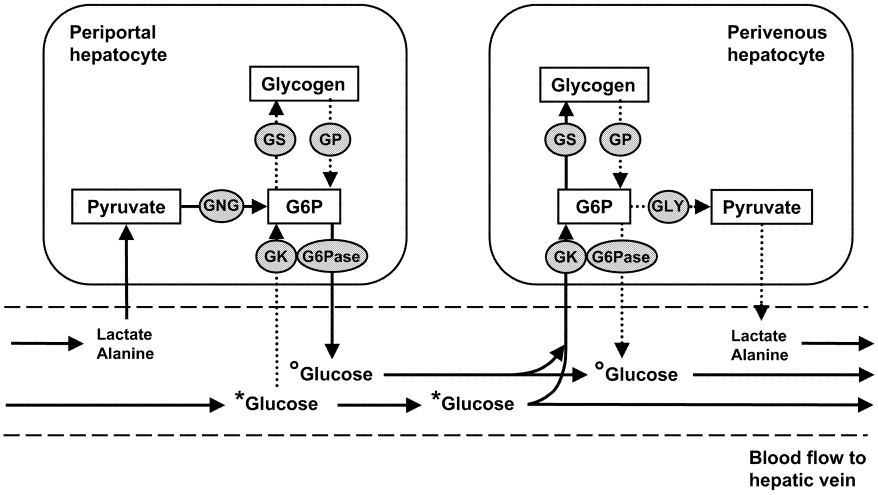

Fig. 1. The fate of labelled glucose (*Glucose) and new unlabelled glucose (°Glucose) within the liver lobule. Glucose production is ultimately regulated by the balance of fluxes through glucokinase (GK) and glucose-6-phosphatase (G6Pase). The intracellular fate of glucose metabolism depends on fluxes through the gluconeogenic pathway (GNG), glycogen synthase (GS) and glycogen phosphorylase (GP)

References

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Abbreviations: EGP, endogenous glucose production; GNG, gluconeogenesis; NMR, nuclear magnetic resonance.

Comment

—to: Walter U, Toepfer T, Dittmar KE et al. (2003) Pancreatic NOD beta cells express MHC class II protein and the frequency of I-A(g7) mRNA-expressing beta cells strongly increases during progression to autoimmune diabetes. Diabetologia 46:1106–1114

To the Editor: Walter et al. [1] are to be congratulated for having done what may eventually be recognized as the definitive study on the controversial topic of MHC Class II expression by beta cells during the autoimmune attack of Type 1 diabetes. Since I consider this to be a carefully performed and important piece of work, I feel almost churlish in having to point out deficiencies in their presentation and interpretation. Walter et al. have studied the time course of MHC Class II (specifically A^{g7}) expression in the beta cells of NOD mice, examining mRNA production, protein expression within the cell and surface expression compared with histological appearance at 3, 6, 9 and 11 weeks of age, before onset of overt diabetes and after diabetes has developed. I believe their findings are of considerable interest, but I wish to make the following points. Firstly, the title given to this paper is very misleading. Unless one reads the full manuscript carefully it sounds as though Walters et al. have found extensive and increasing expression of Class II MHC in the beta cells of the NOD mouse as it develops diabetes. However, from the data they present it can be seen that although MHC Class II mRNA becomes increasingly detectable from 6 weeks onwards, there is still no detectable MHC Class II protein at this time, despite the fact that the immune destructive process was well under way and local production of cytokines promoting MHC expression would be expected. Intracellular MHC Class protein was subsequently found only in samples taken at 11 weeks or more, shortly before the onset of actual diabetes. Most importantly, at no timepoint were they able to detect surface expression of IA^{g7}. I can understand the authors' excitement at finding the mRNA at the earlier timepoints, but considering MHC molecules are purposely designed for membrane insertion, it is still the lack of surface expression in the face of what must be a considerable cytokine storm that is surely most remarkable. Bearing in mind that the NOD mouse also has the gene for the other mouse MHC class II molecule (IE) inactive, the deficiency in surface MHC Class II is striking. Perhaps Walter et al. would be more excited by their negative data if they were aware that it is in precise agreement with a mechanism for the underlying aetiology of Type 1 diabetes suggested to be due to a deficient inhibitory signal given by MHC Class II [2].

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