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online data

SUPPLEMENTARY ONLINE DATA

Effects of a glucokinase activator on hepatic intermediary metabolism: study with ^{13}C-isotopomer-based metabolomics

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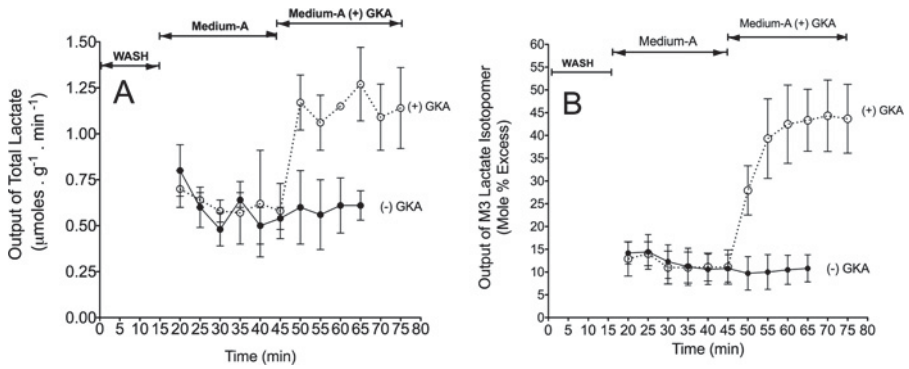

Figure S1 Output of total and M3 isotopomer of $^{13}C_3$ lactate during liver perfusion with [U-^{13}C]glucose

(**A**) Output of total lactate (μmol·g^{-1}·min^{-1}). (**B**) Output of M3 lactate (lactate labelled with ^{13}C at three carbons). Liver perfusions were carried out as detailed in the Experimental section of the main text. Briefly, after a 15 min conditioning of the liver with medium containing 0.1 % DMSO and 5 mM unlabelled glucose in Krebs buffer, pH 7.4 (WASH), the perfusate was replaced with medium containing 0.1 % DMSO, 5 mM [U-^{13}C]glucose, 0.3 mM $^{15}NH_4Cl$ and 1 mM glutamine (Medium-A). At 45 min, perfusion was continued with Medium-A plus 3 μmol/l Piragliatin, [(+) GKA]. Independent control perfusions without GKA [(-) GKA] were performed with perfusate containing Medium-A. Results are means ± S.D. of three independent liver perfusions per study group. M3 lactate isotopomer was chosen as a marker of direct metabolite of glycolysis from [U-^{13}C]glucose. M3 lactate must have been derived from [U-^{13}C]pyruvate, the product of [U-^{13}C]glucose glycolysis. These results demonstrate that: (i) GKA stimulated glycolysis of [U-^{13}C]glucose within 3–5 min after its addition to the perfusate; and (ii) the activation of glucokinase by 3 μmol/l Piragliatin is directly responsible for the remarkable elevation of total and ^{13}C-labelled lactate output from the perfusate [U-^{13}C]glucose.

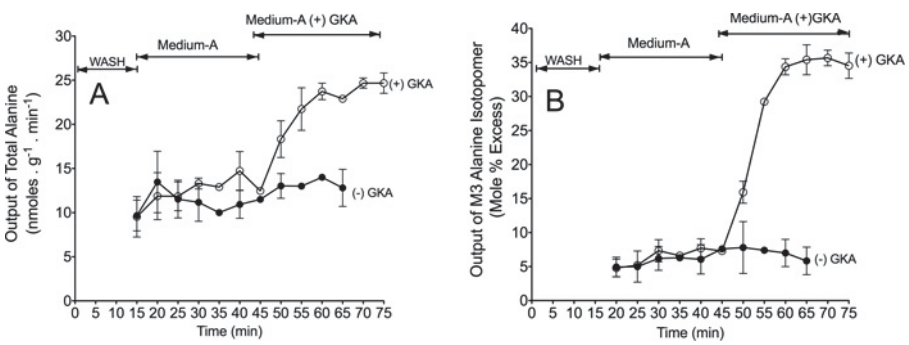

Figure S2 Output of total and M3 isotopomer of $^{13}C_3$ alanine during liver perfusion with [U-^{13}C]glucose

(**A**) Output of total alanine. (**B**) Output of M3 alanine (alanine labelled with ^{13}C at three carbons). Experimental details are as described in the legend to Figure S1. Results are means ± S.D. for three independent liver perfusions per study group. M3 alanine isotopomer must have been derived from [U-^{13}C]pyruvate, the product of [U-^{13}C]glucose glycolysis. As indicated in the legend to Figure S1, these results demonstrate that the activation of GK by 3 μmol/l Piragliatin was directly responsible for the remarkable elevation of total and ^{13}C-labelled alanine output from the perfusate [U-^{13}C]glucose.

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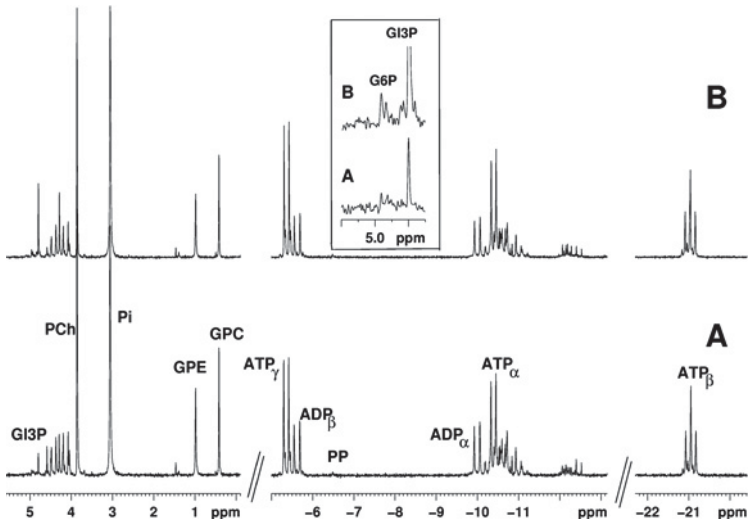

Figure S3 Representative ^{31}P-NMR spectra of freeze-clamped liver following perfusion without (A) or with (B) a GKA

Spectra demonstrate an approximately 2-fold higher peak of glycerol 3-phosphate (Gl3P) following perfusion with a GKA (**B**). In addition, the inset represents the expansion of the G6P peak at approximately 4.7 p.p.m., which was higher following perfusion with a GKA (**B**) compared with the control (**A**). PCh, phosphocholine; GPE, glycerophosphoryl ethanolamine; GPC, glycerophosphocholine.

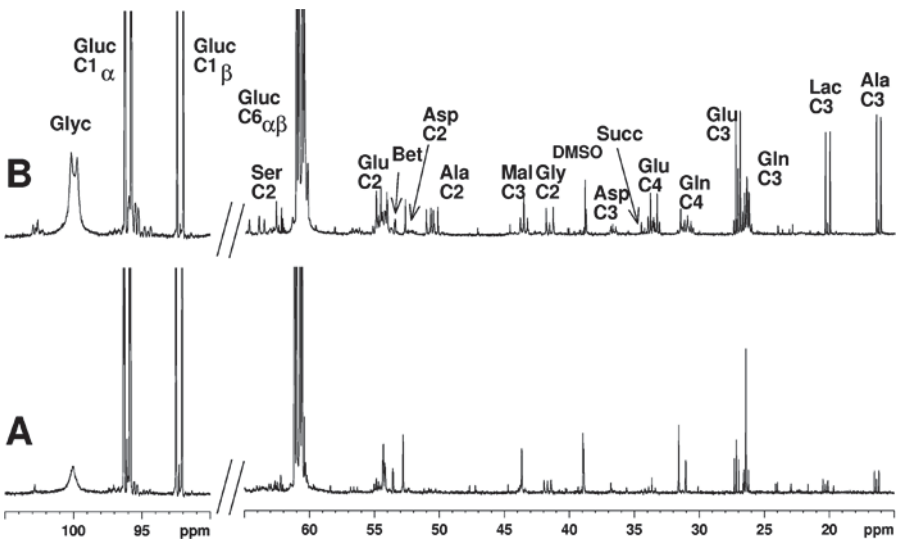

Figure S4 Representative ^{13}C-NMR spectrum of freeze-clamped liver following perfusion without (A) or with (B) GKA

These spectra demonstrate remarkably higher peaks of ^{13}C isotopomers of lactate, alanine, serine, glycine, malate, glutamate, aspartate and glycogen in liver perfused with GKA (**B**) compared with control (**A**). Bet, betaine; Gluc, glucose; Lac, lactate; Glyc, C1-glucosyl (glycogen); Mal, malate; Succ, succinate.

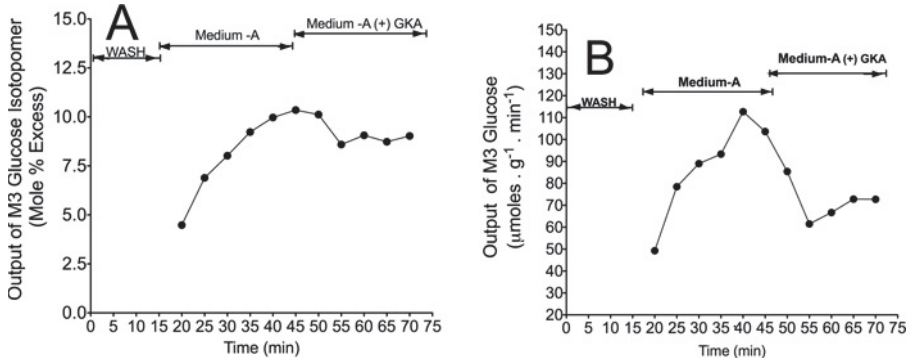

Figure S5 Output of total and ^{13}C-labelled glucose during liver perfusion with [U-^{13}C]-labelled pyruvate and lactate

(**A**) Output of M3 glucose (glucose labelled with ^{13}C at three carbons). (**B**) Rate of M3 glucose output calculated as the rate of total glucose output (μmol·g^{-1}·min^{-1}) multiplied by enrichment (MPE) of M3 isotopomer. Liver perfusion was carried out as detailed in the Experimental section in the main text. Briefly, after a 15 min conditioning of the liver (WASH), the perfusate was replaced with medium containing 0.1 % DMSO, 2.1 mM lactate and 0.3 mM pyruvate (both [U-^{13}C] labelled), 0.3 mM $^{15}NH_4Cl$ and 1mM glutamine (Medium-A). At 45 min, perfusion was continued with Medium-A plus 3 μmol/l Piragliatin [(+) GKA]. The production of M3 glucose isotopomer was chosen as representative of gluconeogenesis from [U-^{13}C]pyruvate plus lactate. M3 glucose must have been directly derived from [U-^{13}C]labelled pyruvate plus lactate. In addition, LC-MS analysis demonstrated the production of M2, M4, M5 and M6 glucose, but with remarkably lower ^{13}C enrichment than M3 glucose (results not shown). These data demonstrate that 3 μmol/l Piragliatin inhibited gluconeogenesis from pyruvate plus lactate within 3–5 min after its addition to the perfusate.

Table S1 ^{13}C enrichment in the glycolytic intermediate G6P in freeze-clamped liver following perfusion without (control) or with a GKA [(+) GKA]

Results are means ± S.D. of three livers per group.

^{13}C Isotopomer	Control (MPE)	(+) GKA (MPE)
M+1	0.4 ± 0.3	1.2 ± 1.0
M+2	2.9 ± 0.9	2.2 ± 0.4
M+3	5.1 ± 1.2	2.7 ± 1.6
M+4	2.4 ± 0.8	1.8 ± 0.6
M+5	4.4 ± 1.1	2.9 ± 1.4
M+6	40.8 ± 6.4	57.6 ± 15.1
Sum	55.9 ± 4.9	68.7 ± 14.7

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