- Tsou, A. Y., Ransom, S. C., Gerlt, J. A., Buechter, D. D., Babbitt, P. C. & Kenyon, G. L. (1990) *Biochemistry* **29**, 9856–9862
- Walker, J. E., Saraste, M., Runswick, M. J. & Gray, M. J. (1982) *EMBO J.* **1**, 945–951
- Ycas, M. (1976) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 2139–2140
- Yeh, W. K., Davis, G., Fletcher, P. & Ornston, L. N. (1978) *J. Biol. Chem.* **253**, 4920–4923

Received 23 January 1991

What determines the increase in liver cell volume in the fasted-to-fed transition: glycogen or insulin?

Liver size decreases during fasting and increases during refeeding in parallel with changes in glycogen content. It is traditionally assumed that an increase in cell volume occurs in association with, and as a consequence of, glycogen deposition. The recent evidence that changes in liver cell volume regulate glycogen metabolism [1–3] together with the well-established role of insulin in the regulation of potassium homeostasis [4–6] suggests an alternative hypothesis: that insulin regulates cell volume by stimulation of K⁺ uptake and that the increase in cell volume is the *cause* rather than the consequence of glycogen deposition.

Recent studies from four independent laboratories have established that changes in liver cell volume induced either by aniso-osmotic media or by Na⁺-driven substrate uptake cause co-ordinate changes in carbohydrate [1–3], amino acid [7–10] and protein [11] metabolism in the perfused liver and in isolated hepatocyte suspensions. This raises several questions on fundamental concepts of the control of metabolism. Häussinger, Lang and colleagues showed that glycogen degradation in the perfused rat liver is stimulated when liver cell volume is decreased

with hyper-osmotic media and inhibited when cell volume is increased [1,2]. Hue, Meijer and colleagues showed that glycogen deposition in hepatocyte suspensions correlates directly with cell volume irrespective of whether cell volume is increased by amino acids or by hypo-osmotic media [3]. Similar effects of changes in cell volume on the rates of glycogen synthesis are observed in hepatocyte cultures (M. Al-Habori, M. Peak & L. Agius, unpublished work).

The changes in glycogen metabolism induced by cell swelling cannot be explained by changes in cyclic AMP or cytosolic Ca²⁺ concentration [12] and it is currently not known what the mechanisms are that link changes in cell volume with glycogen deposition. Since glycogen deposition in the post-absorptive state involves an increase in the number of glycogen particles [13] it is possible that a cytoskeletal mechanism triggered by an increase in cell volume may be involved in the development of new glycogen particles. Regardless of the mechanism, the remarkable correlation *in vitro* between the rate of glycogen synthesis and cell volume, when the latter is altered experimentally [3], raises the issue whether the well-known increase in liver size in the fasted-to-fed transition is the cause rather than consequence of increased glycogen storage.

Liver size decreases during fasting and increases during refeeding (Table 1) [14]. In the rat the decrease in liver weight during a 48 h fast (44%) is associated with a proportional decrease in the total protein content ([14]; Table 1). However, during refeeding with carbohydrate, the initial increase in liver weight is proportionally greater than the small increase in protein content and consequently the protein concentration (per unit wet weight of liver) is diluted by about 36% relative to the fasted state (Table 1). During refeeding with carbohydrate and casein, the increase in liver weight (and in liver weight/body weight ratio) is greater than during refeeding with carbohydrate alone and consequently the protein concentration is diluted further (by about 44%; Table 1). The greater increase in liver weight during refeeding with carbohydrate and protein compared with carbohydrate alone can now be explained, at least in part, by amino-

Table 1. Changes in the total hepatic content and concentrations of glycogen, water and K⁺ during fasting and refeeding in the rat

Data are from [14]. Normal rats were fasted for 48 h or they were fasted for 48 h and then allowed to refeed *ad libitum* for 20 h either on carbohydrate (sucrose and starch) alone (Refed-1) or on carbohydrate and casein (Refed-2). All animals had access to water, but the refed rats also had access to a salts solution (10 × Ringer's salts solution). The water content represents intracellular and extracellular water. The values for total liver weight and content of water, protein, glycogen and K⁺ were determined from the concentrations (per unit wet wt.) and liver weight/body weight ratios in Table 1 in [14]. Values are means ± S.E.M. for the number of animals indicated, except for Refed-2 which are individual values of two rats. Statistics were by the unpaired *t*-test: **P* < 0.05, ***P* < 0.005 relative to values of fasted rats.

State of rats ... n ...	Fed (5)	48 h-fasted (4)	Refed-1 (6)	Refed-2 (2)
Body wt. (g)	205 ± 11*	163 ± 10	181 ± 8	165, 165
Liver wt. (g)	7.5 ± 0.4**	4.2 ± 0.2	7.3 ± 0.4**	10.6, 9.7
Liver wt./body wt. (%)	3.7 ± 0.1**	2.6 ± 0.1	4.0 ± 0.2**	6.4, 5.9
Total liver content (per liver)				
Water content (g)	5.2 ± 0.3**	3.0 ± 0.1	5.0 ± 0.3**	7.4, 6.7
Dry weight (g)	2.3 ± 0.1**	1.2 ± 0.02	2.3 ± 0.3**	3.2, 3.0
Protein content (g)	1.65 ± 0.12*	0.96 ± 0.03	1.05 ± 0.08	1.3, 1.3
Glycogen content (g)	0.29 ± 0.03**	0.01 ± 0.01	0.83 ± 0.07**	1.49, 1.42
K ⁺ (mmol)	0.73 ± 0.04**	0.43 ± 0.02	0.70 ± 0.04**	1.0, 0.91
Concentrations (per 100 g wet wt. of liver)				
Water (% wet wt.)	69.1 ± 0.1	70.4 ± 0.7	69.0 ± 0.3	69.7, 68.8
Protein (% wet wt.)	21.8 ± 0.5	22.9 ± 0.4	14.6 ± 0.7**	12.1, 13.4
Glycogen (% wet wt.)	3.9 ± 0.4**	0.23 ± 0.10	11.3 ± 0.7**	14.1, 14.6
K ⁺ (mmol/100 g)	9.7 ± 0.1*	10.2 ± 0.2	9.6 ± 0.1*	9.4, 9.3
K ⁺ /protein	0.446 ± 0.013	0.444 ± 0.010	0.661 ± 0.033**	0.78, 0.70

acid-induced cell swelling [2,3,7]. A significant increase in weight during refeeding (16%) is observed within 90 min of access to food in rats previously fasted for 24 h (L. Agius, M. Peak & M. Al-Habori, unpublished work).

Early studies on the mechanism involved in the increase in liver size during refeeding were concerned with the amount of water accumulated in the liver per unit amount of glycogen deposited [14,15]. They established that the percentage water content (per g wet weight) of the liver remains relatively constant (69–70%; Table 1) irrespective of the increment in glycogen [14–16]. Consequently, it was estimated that the amount of water accumulated per g of glycogen deposited is about 2.3–2.4 ml (water content/dry weight [14,15]). Studies on the concentrations of phosphate and K^+ in liver during glycogen deposition (per g wet weight of tissue) established that the concentration of acid-soluble organic phosphate remains constant during glycogen deposition while the concentration of acid-insoluble phosphate decreases in parallel with the decline in protein concentration [14,16]. The concentration of K^+ (per unit wet weight of liver) remains relatively constant despite a large increase in liver weight (Table 1), indicating that uptake of K^+ parallels accumulation of water [14]. The K^+ /protein ratio increased by about 49% during refeeding with carbohydrate alone and by 67% during refeeding with carbohydrate and protein, relative to the fasted state, because the increase in total K^+ and water content are much greater than the increase in protein content [14,16].

The amount of K^+ accumulated in liver in the fasted-to-fed transition is much lower in molar terms than the amount of glucose polymerized to glycogen (0.27 mmol of K^+ and 5 mmol of glucosyl units; Table 1 and [14]). In contrast with these data in liver, in studies on diaphragm muscle incubated with insulin it was suggested that uptake of K^+ (determined using $^{86}Rb^+$) occurred in a molar ratio with the polymerization of glucose to glycogen [17,18]. It was inferred that K^+ is 'pumped up' as a consequence of glycogen synthesis. This has been described as the K^+ pump hypothesis [18]. The liver data [14] are not consistent with a hypothesis based on a 1:1 molar ratio of uptake of K^+ to glucose polymerized [18]. The difference in the K^+ /glucosyl-glycogen ratios between liver (Table 1) and muscle [17] may be due to tissue differences or to the use of $^{86}Rb^+$ in the muscle studies [17]. $^{86}Rb^+$ uptake provides an index of changes in K^+ flux but perhaps not a quantitative measure of absolute changes in K^+ content because of isotope discrimination in tissues with excitable membranes [19]. Direct measurement of uptake of K^+ and glucose by forearm muscle *in vivo* [6] showed that the ratio of glucose/ K^+ uptake is higher than would be consistent with the K^+ -pump hypothesis [17] but lower than the liver data [14]. If glycogen deposition is estimated from the glucose taken up, the lactate produced, the O_2 consumption and respiratory quotient data [6], the ratio of glucose conversion to glycogen/ K^+ uptake for the early stages of the insulin infusion is about 10:1. Regardless of the ratio, data showing parallel uptake of K^+ and glucose cannot distinguish between cause and effect. However, studies *in vitro* on muscle [20] or hepatocyte cultures (L. Agius & T. H. Thomas, unpublished work) have shown that stimulation of K^+ accumulation by insulin is independent of the availability of glucose, suggesting that it is not secondary to glycogen synthesis.

An alternative mechanism to account for the increase in the K^+ and water content of the liver during refeeding is 'direct' stimulation of cellular uptake of K^+ by insulin [4–6]. Insulin has a central role in the control of potassium homeostasis. Hypoinsulinaemia leads to a depletion of cellular K^+ and water and an increase in the plasma $[K^+]$ [21]. Hyperinsulinaemia causes uptake of K^+ by cells and a decline in plasma $[K^+]$ [5] as well as in plasma volume [22].

Insulin stimulates uptake of K^+ in hepatocyte suspensions by primary activation of Na^+_{out}/H^+_{in} exchange and secondary Na^+_{in}/K^+_{out} exchange [23]. The latter is driven by the ATP-dependent 'Na-pump' ($Na^+/K^+-ATPase$) and is electrogenic (3 Na^+ are exchanged for 2 K^+). The Na^+/H^+ antiporter exchanges extracellular Na^+ for intracellular protons and is important in the regulation of cell pH and cell volume [24]. The antiporter is quiescent above a certain cell pH and is activated by acidification of the cytosol, which is consistent with its postulated role in the control of pH homeostasis [24]. It is also activated by several growth factors as well as by insulin and by hyper-osmotic shrinking. Activation by these stimuli is not mediated by prior acidification of the cytosol but by an alkaline shift of the pH-dependence of the antiporter and leads to alkalization of the cytosol above the normal set point [24]. Insulin has been shown to increase cell pH in muscle [25] and to increase the total cation (K^+ plus Na^+) content in hepatocyte cultures (our unpublished work). Both these effects can be explained by the rapid activation of Na^+/H^+ exchange [23].

In man, splanchnic tissues and muscle are major sites of K^+ uptake during an insulin infusion [5,6]. Insulin-stimulated uptake of K^+ by splanchnic tissues is very rapid, reaching a peak within minutes, and does not correlate with glucose utilization [5]. This argues against uptake of K^+ being secondary to glycogen deposition. The stimulation of K^+ uptake during an insulin infusion is transient and involves a rapid increase followed by a decline in rate [5]. The latter is due, in part, to the fall in plasma $[K^+]$ and can be delayed by a simultaneous infusion of KCl, indicating that uptake of K^+ in response to insulin is a function of the extracellular $[K^+]$ [15]. Skeletal muscle loses potassium in the fasted state [20] but takes up K^+ during an insulin infusion [6]. Uptake of K^+ by muscle also does not correlate with utilization of glucose [6].

About 98% of total body K^+ is intracellular. K^+ is the most abundant intracellular cation and is therefore a major determinant of intracellular osmolarity and thereby cell volume [26]. Since the extracellular K^+ pool (plasma and interstitial fluid) comprises only 2% of total body K^+ , a 50% decrease in plasma K^+ concentration during an insulin infusion could theoretically cause only a small increase (1%) in cellular K^+ content, assuming that insulin-responsive tissues (liver, skeletal muscle and adipose tissue) account for a high proportion (75%) of total cellular K^+ . However, the effects of an insulin infusion on K^+ balance between the intracellular (insulin-sensitive tissues) and the vascular compartment do not represent the physiological situation where insulin is secreted in response to a mixed meal. Potassium is a major dietary constituent. In man the dietary K^+ intake is about 50–150 mmol/day. K^+ is cleared rapidly from the plasma, largely by liver and muscle [5]. K^+ clearance is impaired in cirrhosis [27], indicating the importance of the liver in K^+ clearance, and also in insulin deficiency [28,29]. Dietary K^+ stimulates insulin secretion and only small rises in plasma $[K^+]$ are necessary to stimulate secretion by the pancreatic β -cells [30]. If a high proportion of dietary K^+ is initially cleared by extrarenal tissues (liver and muscle) [27], it can account for a substantial increase in the cellular K^+ content, particularly in liver which is the major site of K^+ clearance [5]. Assuming a liver weight of 1.7 kg in man and a total hepatocellular K^+ content of about 200 mmol, dietary K^+ (approx. 50 mmol) can account for a 15% increase in the hepatic K^+ content, if the liver clears 50% of the K^+ load [5]. Net uptake of K^+ by cells can occur by net substitution for Na^+ and/or by a net increase in the cellular K^+ and Na^+ content. Consequently, if the K^+ concentration of the liver cell remains relatively constant during clearance of dietary K^+ in man, as it does in the fasted-to-fed transition [14], uptake of K^+ would be associated with an increase in the total cationic

content, balanced with a corresponding increase in anions, and therefore with parallel uptake of water and an increase in cell volume.

Long-term insulin deficiency, as in untreated diabetes, results in ketoacidosis and severe cellular depletion of K^+ . The latter has frequently been attributed to the decline in cell pH which results in a fall in the cell concentration (and content) of dissociated forms of weak acids (carboxylates and phosphates) and thereby in the K^+ content. Studies involving acute acid-base perturbations by infusion of mineral or organic acids have established that cellular K^+ balance is not a simple function of pH and that insulin and glucagon have direct effects on hepatic uptake and release, respectively, of K^+ [31,32]. Cell pH reflects the balance between cellular acid loading (metabolic production or uptake of protons) and acid extrusion (Na^+/H^+ exchange or bicarbonate uptake). Diabetes is associated with an alkaline shift of the pH threshold of the Na^+/H^+ antiporter and with a slower rate of Na^+/H^+ exchange [33], suggesting that in insulin deficiency acid extrusion is impaired at a cell pH below the threshold of the Na^+/H^+ antiporter [33]. Administration of insulin during ketoacidosis results in rapid cellular uptake of K^+ . This is presumably in part due to the reversal of the metabolic changes that lead to excessive production of protons but also to direct activation of Na^+/H^+ exchange by insulin [23], an increase in cell pH [25] and net uptake of K^+ independent of the metabolic changes [5].

It is widely assumed that the correlation of liver cell volume and cellular glycogen content results from uptake of water and/or K^+ in association with glycogen deposition. All the evidence outlined above indicates that insulin can stimulate an increase in cell volume by rapid uptake of K^+ independently of glycogen deposition. The recent evidence that changes in cell volume regulate glycogen metabolism [1–3] suggests that an insulin-induced increase in cell volume may be a component of the post-receptor events in insulin action leading to stimulation of glycogen synthesis. The mechanisms that link changes in cell volume with changes in glycogen metabolism [1–3] and other metabolic functions [7–11] have not been identified [12]. After this letter was submitted, a paper by Häussinger & Lang [34] reported that glucagon causes cell shrinkage in the perfused liver and suggested that the decrease in cell volume could serve as a 'second messenger' in mediating the metabolic effects of glucagon [34]. Our own unpublished results show that the effects of insulin on glycogen synthesis and glycolysis are impaired when the activation of Na^+/H^+ exchange is prevented and suggest that an increase in cell volume is a component of the post-receptor events of insulin that is essential for the expression of the metabolic effects of insulin. The role of changes in cell volume in mediating the effects of hormones warrants detailed investigation.

We thank the Juvenile Diabetes Foundation and Nordisk UK for financial support.

Loranne AGIUS, Matthew PEAK and Molham Al-HABORI
Department of Medicine, The Medical School, The University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne NE2 4HH, U.K.

- Graf, J., Haddad, P., Häussinger, D. & Lang, F. (1988) *Renal Physiol. Biochem.* **3–5**, 202–220
- Lang, F., Stehl, T. & Häussinger, D. (1989) *Pflügers Arch.* **413**, 209–216
- Baquet, A., Hue, L., Meijer, A. J., van Woerkom, G. M. & Plomp, P. J. A. M. (1990) *J. Biol. Chem.* **265**, 955–959
- Briggs, A. P., Koechig, I., Doisy, G. & Weber, C. J. (1923) *J. Biol. Chem.* **58**, 721–730
- DeFronzo, R. A., Felig, P., Ferrannini, E. & Wahren, J. (1980) *Am. J. Physiol.* **238**, E421–E427
- Andres, R., Baltzan, M. A., Cader, G. & Zierler, K. (1962) *J. Clin. Invest.* **41**, 108–115
- Wettstein, M., vom Dahl, S., Lang, F., Gerok, W. & Häussinger, D. (1990) *Biol. Chem. Hoppe-Seyler* **371**, 493–504
- Häussinger, D. & Lang, F. (1990) *J. Cell. Biochem.* **43**, 355–361
- Häussinger, D., Lang, F., Bauers, K. & Gerok, W. (1990) *Eur. J. Biochem.* **188**, 689–695
- Häussinger, D., Lang, F., Bauers, K. & Gerok, W. (1990) *Eur. J. Biochem.* **193**, 891–898
- Häussinger, D., Hallbrucker, C., vom Dahl, S., Lang, F. & Gerok, W. (1990) *Biochem. J.* **272**, 239–242
- Baquet, A., Meijer, A. J. & Hue, L. (1991) *FEBS Lett.* **278**, 103–106
- Devos, P., Baudhuin, P., Van Hoof, F. & Hers, H.-G. (1983) *Biochem. J.* **209**, 159–165
- Fenn, W. O. (1939) *J. Biol. Chem.* **128**, 297–307
- Puckett, H. L. & Wiley, F. H. (1932) *J. Biol. Chem.* **96**, 367–371
- Fenn, W. O. & Haege, L. F. (1940) *J. Biol. Chem.* **136**, 87–101
- Calkins, E., Taylor, I. M. & Hastings, A. B. (1954) *Am. J. Physiol.* **177**, 211–218
- Larner, J. (1990) *Adv. Enzymol.* **63**, 173–231
- Hille, B. (1973) *J. Gen. Physiol.* **61**, 669–686
- Zierler, K. L. (1960) *Am. J. Physiol.* **198**, 1066–1070
- Andres, R., Cader, G., Goldman, P. & Zierler, K. H. (1957) *J. Clin. Invest.* **36**, 723–729
- Hilsted, J., Bonde-Petersen, F., Madsbad, S., Parving, H. H., Christensen, N. J., Adelhoff, B., Bigler, D. & Sjontoft, E. (1985) *Clin. Sci.* **69**, 273–277
- Fehlmann, M. & Freychet, P. (1981) *J. Biol. Chem.* **256**, 7449–7453
- Grinstein, S. & Rothstein, A. (1986) *J. Membr. Biol.* **90**, 1–12
- Fidelman, M. L., Seehozler, S. H., Walsh, K. B. & Moore, R. D. (1982) *Am. J. Physiol.* **242**, C87–C93
- Jones, N. F. & Poston, L. (1990) in *The Metabolic and Molecular Basis of Acquired Disease* (Cohen, R. D., Lewis, B., Alberti, K. G. M. M. & Denman, A. M., eds.), vol. 1, pp. 1070–1095, Baillière Tindall, London
- Decaux, G., Soupart, A., Cauchie, P. & Delwiche, F. (1988) *Arch. Int. Med.* **148**, 547–548
- DeFronzo, R. A., Sherwin, R. S., Dillingham, M., Hendler, R., Tambourlane, W. V. & Felig, P. (1978) *J. Clin. Invest.* **61**, 472–479
- DeFronzo, R. A. (1988) *Metabolism* **37**, 105–108
- Hiatt, N., Davidson, M. B. & Bonorris, G. (1972) *Horm. Metab. Res.* **4**, 64–68
- Adroque, H. J., Chap, Z., Ishida, T. & Fleid, J. B. (1985) *J. Clin. Invest.* **75**, 798–808
- Adroque, H. J., Lederer, E., Suki, W. N. & Eknayan, G. (1986) *Medicine* **65**, 163–172
- Boyarisky, G., Rosenthal, Barrett, E. & Boron, W. F. (1991) *Am. J. Physiol.* **260**, C167–C175
- Häussinger, D. & Lang, F. (1991) *Biochem. Cell Biol.* **69**, 1–14

Received 11 February 1991