COMPARATIVE STUDIES OF GLUCOSE METABOLISM AND GLYCOGENESIS IN VITRO BETWEEN C3H/He AND C58 MICE

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SUMMARY: The amount of glucose phosphorylated and recycled by hepatocytes prepared form C3H/He and C58 mice was measured as an indication of the difference between the hepatic glucokinase activity in one strain compared to the other. The glucokinase activity in one strain compared to the other. The glucokinase activity was determined spectrophotometrically and by an assessment of the in vitro consumption of (2-^{3}H) glucose by hepatocytes. The variance in glucokinase activity between the two strains does not apparently affect the minimal rate of glucose recycling as measured by the relative loss of ^{3}H and ^{14}C from (2-^{3}H, U-^{14}C) -glucose. The correlation between glycogen synthesis and glucokinase activity in isolated hepatocyte cells is being considered.

Key Words: Glucose metabolism, glycogenesis.

INTRODUCTION

Glucokinase (E.C.2.7.1.2), an enzyme localized in liver parenchymal cells, plays a significant role in the metabolism of glucose by the liver. The enzyme, which is often referred to as type IV isoenzyme, has a characteristic high Km for glucose. Although most studies on the physiological regulation and nature of this enzyme have been performed in rats, some studies were also made in mice. It was observed that different glucokinase activities were found in different strains of mice; a two-fold increase in activity was found in a "high-activity" strain when compared to a "low-activity" strain (1). Breeding experiments demonstrated that glucokinase activity is regulated by a single co-dominant gene. Both dietary glucose and insulin appear to be necessary for the synthesis of glucokinase *in vivo*.

The uptake of glucose by hepatocyte cells reflects a difference between the rate of phosphorylation of glucose by glucokinase and the rate of hydrolysis of glucose-6-phosphate by glucose-6-phosphatase. This uptake is expected to be zero at the normal level of glycemia and becomes positive when the activity of glucokinase increases more than that of the glucose-6-phosphatase or when the latter activity is decreased. When an increased synthesis of glycogen is the consequence of a rise in the level of glycemia, the increase in glucose uptake occurs by a push pull mechanism. Indeed, an increased concentration of glucose will obviously increase the glucokinase activity. It will also stimulate, to a variable degree, glycogen. Synthesis and therefore decrease the glucose-6-phosphate concentration (2).

In this paper, studies of the regulation of the glucose/glucose-6-phosphate cycling in hepatocytes isolated from C3H/He and C58 mice are described and the physiological significance of the results is discussed.

MATERIALS AND METHODS

Materials

The sources of most chemicals were as described before (3).

Radioactive glucose was purchased from Amersham International PLC (U.K). (2-^{3}H) glucose was purified by paper chromatography according to the method described in reference (4). Agarose and Dowex resins were purchased from Pharmacia (Sweden). Heparin was obtained from weddle Pharmaceuticals (U.K.).

Other chemicals were from B.D.H. Chemicals Ltd. (poole, Dorset, U.K) or from Fisons Ltd. (Lough Borough, Leics, U.K.) All chemicals were of the best available grade and, unless otherwise indicated, were used without further purification.

Animals

The mice used in these experiments were all males of tenth to fourteenth subsequent generations bred as described by lavender (5) from pedigree mating pairs of C3H/He and C58 inbred strains purchased from Bantin and Kingman (Hull, U.K) in 1978.

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Methods

Hepatocyte cells were isolated from C3H/He and C58 mice as described in references (6-8). Cell viability of preparations ranged from 80 to 90%. Such hepatocyte preparations were used for glucose phosphorylation and gly studies. Glucokinase activity was determined on the supernatant of hepatocyte homogenates (6) by an established spectrophotometric method (5) using a UV/visible Pye Unicam SP 8-100.

To determine glucose phosphorylation and glucose cycling hepatocytes, approximately 0.1 ml packed cells suspended in 2 ml incubation krebs-Henseleint bicarbonate buffer supplemented with 0.5/μci (U-^{14}C glucose and 0.25 μci (2-^{3}H) glucose per ml and with a physiological glucose concentration of containing O_2/CO_2 (19/1). At the end of the incubation period the reaction was stopped by the addition of per chloric acid to bring a final concentration of 6% (W/V). After centrifugation, the supernatant was desalated by passage through Dowex 50 (H^+form) and Dowex 1 (acetate form). The ^{3}H>O was then separated from the radioactive glucose by retention of the later on a column of Dowex 1 in the borate form. The $3H_2O$ was counted. The retained glucose was then eluted with in acetic acid and counted for H and C in a LKB 1217 Bounter.

The rate of glucose dephosphorylation was calculated according to reference (9). The measurement of cell hepatocyte glycogen content was based on a rapid method in small quantities of isolated hepatocytes (IO). Aliquots of 200 μl of either glycogen in liver or hepatocyte homogenate were spread evenly on pieces of filter paper (what man 3M chromatography paper, 2.5x2.5 cm) in duplicate. Glucose content in the incubated samples was determined by a spectrophotometric method using hexokinase and glucose-6-phosphate dehydrogenase (11).

RESULTS

Table 1 shows the results obtained after cells from each of the two strains of mice, C3H/He and C58, had been incubated with physiological 7.5 mM glucose for 60 min. Those results indicate clear differences between the two strains. The difference found in glucokinase activity as measured spectrophotometrically parallels that found for the two strains, while glucose-6-phosphate activity depends on glucose-6-phosphate concentration (11).

The activity of glucose phosphorylated by the activity of glucokinase *in vivo* depends on the concentration of glucose which was the same in C3H/He and C58 mice (5). The increase of glucokinase activity in C3H/He will result in a high rate of glucose-6-phosphate formation. Since the concentration of glucose-6-phosphate, rates of recycling and glucose-6-phosphatase, rates of recycling and glucose in two strains were the same, it raises the question that the increase of glucokinase in C3H/He mice does not affect the above parameters. Since the decrease or inhibition of glucokinase activity *in vitro* in C3H/He mice did not appear *in vivo*, this showed that more product of glucose phosphorylation must be channelled to other pathways in the C3H/He mice.

Table 1: The hepatocytes were obtained from C3H/He and C58 mice. Hepatocytes were incubated in the presence of 7.5 mM glucose and [2-H, U-C] glucose for 1 hr. The results correspond to the means±SEM for five experiments.

	C3H/He mice	C58 mice
Glucose phosphorylation Determined spectrophotometrically (mol/min/gr of total protein)	6.05±0.23	2.87±0.6
Glucose-Phosphorylation obtained detritation (mol/min/gr of total protein)	3.15±0.21	1.34±0.04
Concentration of glucose-6-phosphate at 1 hr (nmol/gr of total protein)	545±13.25	563±9
Glucose dephosphorylation (mol/hr/gr of total protein)	42.7±14	35±25
Glucose-6-phosphatase activity (mmol/hr/gr of protein)	6.2±0.18	6.5±0.32

Compensatory regulatory mechanisms may influence processes such as glycogen and lipid synthesis in such a way that it could explain the differences observed between the two strains studied in this paper. If there were no strain differences in the amount of glycogen or lipid, then it could be that glucokinase is not the controlling factor in glycogen and lipid synthesis and the physiological role of glucokinase may not be significant.

Measured spectrophotometrically parallels that found for the rate of glucose phosphorylation as measured isotopically in the whole liver and in isolated hepatocytes. For each of these two parameters, the ratio of the values obtained in each strain is well over two. Thus glucokinase activity in C3H/He is twice as high as in C58 mice. However, the results also indicate that there is no significant difference in a number of other parameters measured. No significant difference was found in the glucose-6-phosphatase activity determined on cell homogenates from each strain, nor in the concentration of intracellular glucose-6-phosphatase at mid stage of the incubation period. Similarly, no significant difference was found in the minimal rate of recycling of glucose as determined by the decrease in the ^{3}H/HC ratio in the substrate (2-^{3}H, U^{14}-C) glucose during the 60 min incubation period. However, the increased rate of glucose phosphorlation apparently does not influence the other parameters assayed in this study.

As seen in Figure 1, in the isolated hepatocytes prepared from fed C3H/He and C58 mice, a major increase in glycogen content was observed. The rise in values was the same in the two strains. In both animals a small

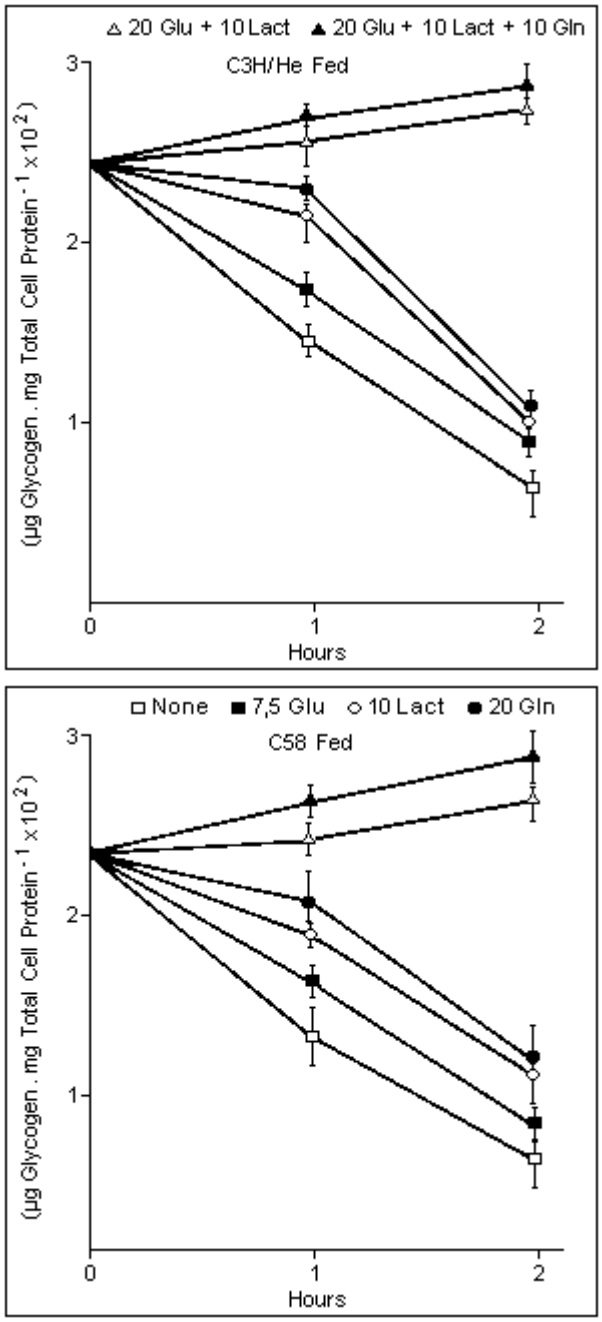


Figure 1: Effect of glucokinase on glycogen synthesis in hepatocytes. Hepatocytes were prepared from fed C3H/He and C58 mice. The cells 10 mg wet Wt) were incubated with the indicated sulistrates as described under methods and analyzed for glycogen content at the indicated times. The initial concentrations of glucose (glu) were 7.5, 20 mM, respectively. Further additions of lactate (10 mM) and of glutamine (10 mM) were new made at 1 hr and 2 hr values are means±SEM for there experiments.

amount of glycogen was formed without substrate or in the presence of 7.5 mM glucose and 10 mM lactate. In this study, glucose was a poor substrate for glycogen synthesis' glucose at a concentration of 20 M functioned poorly as a substrate for glycogen synthesis, and measurement of glucose in the hepatocyte at zero time and 120 min showed little, if any, uptake of glucose. Glutamine stimulated markedly glycogen synthesis from all substrates, but the most dramatic effect was observed with lactate plus glucose as substrate.

The results depicted in Figure 2 show that in fasting C3H/He and C58 mice, glucose at concentrations of 20 mM and 50 mM supplemented with 10 mM lactate and 10 mM glutamine appeared to support near maximal rates of glycogen synthesis.

On the other hand, after incubation for 2 hours deposition of glycogen was very sharp, but was not significantly different in the two strains, comparison with the experiments of glycogen synthesis in isolated cell hepatocyte in fed and starved C3H/He and C58 mice, showed no strain differences in the amount of glycogen concentration.

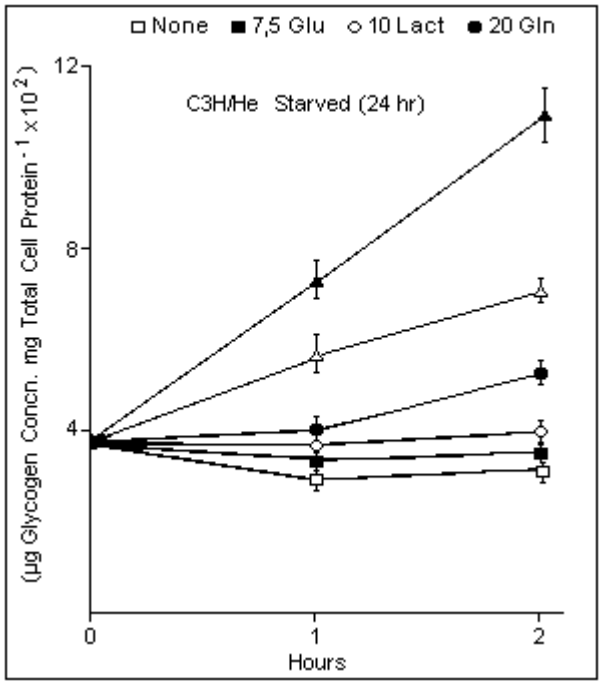
DISCUSSION

The present study demonstrates the difference. In glucokinase activities between the two mice strains under physiological conditions (Table 1). The activity of glucose phosphorylation in the two mice analyzed spectrophotometrically under physiological conditions is reflected by similar differences in the rates of glucokinase activity measured isotopically in isolated hepatocytes. At the same time, the concentration of glucose-6-phosphate and the rates of glucose recycling are not significant in glycogen synthesis in isolated cell hepatocyte in CH3/He and C58 mice have indicated that glucose was a poor substrate for the synthesis of glycogen. This so called "glucose paradox" is as yet unexplained, although several groups did observe glycogen synthesis from low concentrations of glucose (12).

It appears that glucose somehow signals the cell to direct glucose 6-phosphate formed from gluconeogenic intermediates away from the glucose-6-phosphatase reaction and into the pathway of glycogen synthesis while not being used directly itself (13).

The fact that, although glucose was of poor use for glycogen synthesis, gluconeogenesis could be stimulated to near normal rates in hepatocytes of mice C3H/He and C58 by lactate or fructose. This is in agreement with the results of Hemes *et al.* (14) for isolated perfused rat liver.

It was reported (13) that in rat hepatocyte, glutamine increased glycogen formation approximately 3-fold from glucose. At high concentration (50 mM), glucose became more effective as a substrate for glycogen synthesis. It is not clear why exogenous glucose at physiological



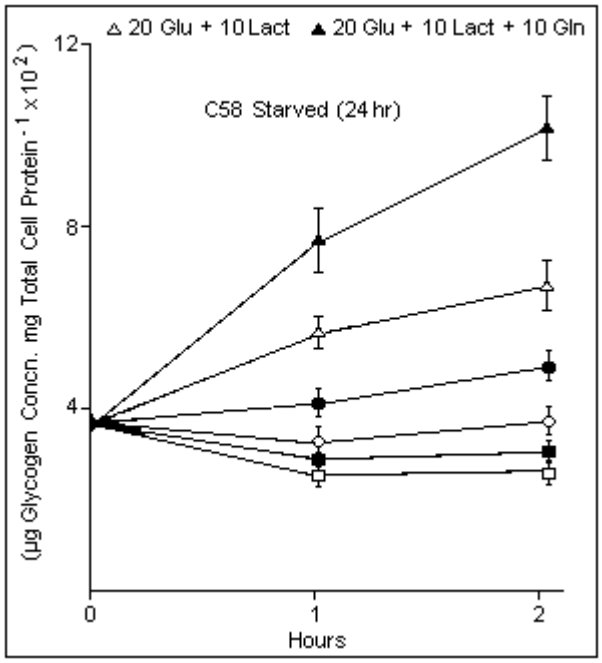


Figure 2: Effect of glucokinase in glycogen deposition in isolated cell hepatocyte, 10 mg of hepatoytes from 24 hr fasted C3H/He (Panel A) and C58 (Panel B) were incubated with the indicated sulistrates as described under method. Maximum glycogen synthesis for 50 mM Glutamine as compared to Figure 1 values are means±SEM for three experiments.

concentration does not function well as a substrate for glycogen synthesis, unlike other precursors of glucose-6-phosphate in the presence of glucose. It is known that when mouse hepatocytes are incubated with glucose alone in concentration up to 20 mM, a significant fraction of the glucose is converted to glucose-6-phosphate, but that the latter is immediately hydrolyzed via the glucose-6-phosphatase reaction which results in that little glycogen is formed.

Finally, the question arises as to how glucose initiates for glycogen synthesis in mice C3H/He or what is the rate of glucokinase in this condition. Although our results indicated there were no strain differences in glycogen synthesis, hence glucokinase is not the key enzyme in glycogen deposition. Hepatic glucokinase activity is not always sufficient to account for the uptake of glucose to form glycogen after post starvation ingestion of glucose in the rate (14). The present results suggest that gluconeogenesis could make a major contribution to hepatic glycogen synthesis, immediately after the ingestion of glucose after starvation. Thus, there is no requirement for glucokinase activity to be as rapid as the rate of glycogen deposition.

ACKNOWLEDGEMENT

The authors thank Prof. E Keyhani and Prof. J Keyhani for scientific and very fruitful advices, and Mrs. J Noor Mohammad for editing this manuscript.

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