Carcass glycogen repletion on carbohydrate re-feeding after starvation

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In mice, the response of carcass glycogen to glucose re-feeding after starvation is biphasic. The initial repletive phase is followed by partial (> 50%) glycogen mobilization. This turnover of carcass glycogen in response to carbohydrate re-feeding may play an important role in the provision of C₃ precursors for hepatic glycogen synthesis.

INTRODUCTION

It has been commonly stated that, in its role as the body's glucostat, the liver is the major site of glucose deposition on carbohydrate feeding and that glucose is a major direct precursor in hepatic glycogen synthesis. It is becoming increasingly apparent that this view is incorrect. The bulk (> 7%) of ingested glucose on carbohydrate re-feeding after starvation is metabolized initially at extrahepatic sites [1,2]. There is an intrinsic metabolic paradox (designated the 'glucose paradox' [see 3,4]), namely that, although the liver seemingly has a restricted capacity to metabolize glucose, glucose re-feeding nonetheless promotes rapid hepatic glycogen repletion and stimulates hepatic lipogenesis. Recent work [5–11] indicates that the metabolic basis for this paradox is that hepatic glycogen synthesis on carbohydrate re-feeding is predominantly from C₃ precursors (presumably mainly lactate) via sustained gluconeogenic flux. Conversion of dietary glucose into hepatic glycogen therefore proceeds indirectly via preliminary cleavage of glucose to C₃ intermediates. The site of conversion of glucose into lactate (and other C₃ compounds) is not known, but may involve skeletal muscle, erythrocytes and the gastrointestinal tract. There is also evidence that metabolic zonation of the liver allows glycolysis and gluconeogenesis (and associated glycogen synthesis from C₃ compounds) to proceed simultaneously in different hepatocyte populations [12,13]. The possibility therefore exists [13] that glucose is taken up and metabolized to lactate in the perivenous zone of the liver. This lactate released into the hepatic vein may act as a precursor in hepatic glycogen synthesis in periportal hepatocytes.

The present paper seeks to ascertain the contribution of carcass glycogen to glucose disposal on carbohydrate re-feeding after starvation in the mouse. It is shown that carcass glycogen may be an important source of lactate for hepatic glycogen synthesis.

EXPERIMENTAL

Male BALB/c mice (25 ± 0.2 g) were maintained at approx. 17 °C on a 12 h-dark/12 h-light cycle (day started at 08:00 h). Animals, starved for 24 h before the experiments, were administered glucose (2 mmol/100 g body wt.; 2 M solution) intragastrically under light anaesthesia [6]. After recovery from anaesthesia, which was rapid, mice congregated in groups and displayed

little movement. At intervals thereafter, each animal (six at each time point after glucose feeding) was anaesthetized with diethyl ether, and an arterial blood sample was withdrawn from the determination of blood metabolites (see [6]). The stomach and small intestine were removed intact for the determination of unabsorbed glucose [1], and the total liver mass was freeze-clamped in tongs cooled with liquid N₂ and weighed. The remaining carcass was weighed and also frozen in liquid N₂.

Frozen liver powder and fragmented carcass were digested in 30% (w/v) KOH, and glycogen content (measured as glucose equivalents and corrected to a body weight of 25 g) was determined enzymically [6] after precipitation in 66% (v/v) ethanol. Triacylglycerol was determined in a portion of the liver powder [14].

Results are expressed as means ± S.E.M. for the numbers of observations in parentheses. Statistical analysis was by the Student's *t* test.

RESULTS AND DISCUSSION

Glucose was rapidly absorbed from the gastrointestinal tract after oral glucose administration to 24 h-starved mice (Fig. 1). Blood concentrations of glucose and lactate rose in response to glucose feeding, but, whereas the rise in blood glucose concentration was transient, blood lactate concentrations remained elevated (> 3 mM) over 3 h after glucose administration. To quantify the contribution of liver and carcass glycogen deposition to glucose disposal *in vivo*, it was imperative that total glycogen content in liver and carcass be determined. These values and their response to glucose re-feeding are shown in Fig. 2. An important finding is that the initial response (at up to 30 min) to oral glucose administration was repletion approximately in parallel of both liver and carcass glycogen. Moreover, the amounts of glycogen stored in carcass and liver were approximately equivalent. At 30 min after glucose re-feeding, total carcass and total liver glycogen were 115 ± 10 μmol and 118 ± 16 μmol of glucose equivalents respectively. Total glycogen deposited (81 μmol into carcass + 103 μmol into liver) at 30 min after glucose administration accounts for 48% of the glucose absorbed.

From 30 min after glucose administration onwards, total carcass glycogen decreased. Between 30 min and 3 h

Fig. 1. Response to oral glucose administration (2 mmol/100 g body wt.) of blood glucose (Δ) and lactate ($\blacksquare$)

Glucose remaining unabsorbed in the stomach and small intestine ($\circ$) is calculated as % of initial dose (0.5 mmol per 25 g mouse). Each point represents the mean of values from six mice, and S.E.M. is denoted as a vertical bar.

after glucose administration, this fall amounted to 61 μ mol (53%), equivalent to 75% of the newly synthesized glycogen deposited in response to glucose re-feeding. During this same period, hepatic glycogen continued to be deposited, albeit at a lower rate. This followed a brief lag in the rate of glycogen deposition between 30 min and 1 h after glucose administration, which may indicate biphasicity in the response of hepatic glycogen to glucose re-feeding. Between 30 min and 3 h after glucose administration, total hepatic glycogen increased by 45 μ mol (38%), equivalent to 44% of the newly synthesized glycogen deposited in response to glucose re-feeding. At 3 h after glucose re-feeding, total glycogen deposited (carcass + liver) accounts for 34% of the glucose absorbed. Liver triacylglycerol (mean 50 ± 3 mg/g wet wt.) did not increase in response to re-feeding.

The present findings support the view that the response of carcass glycogen to glucose re-feeding after starvation is biphasic. In the initial phase (up to approx. 30 min), there is a rapid carcass glycogen repletion. A major fate of absorbed glucose is metabolism at extrahepatic sites, and a significant proportion (up to 22% of absorbed glucose) appears to be converted into carcass glycogen. In this repletive phase, carcass is in quantitative terms approximately equal to liver as a site of glycogen deposition. Thereafter, in the secondary phase (approx. 30 min after glucose administration onwards), > 50% of carcass glycogen is mobilized, presumably mainly via glycolysis to generate pyruvate. The extent to which this pyruvate is oxidized to CO_2 *in*

Fig. 2. Response to oral glucose administration (2 mmol/100 g body wt.) of total liver glycogen ($\circ$) and total carcass glycogen (Δ)

Glycogen content is expressed as μ mol of glucose equivalents, corrected to a body weight of 25 g. Each point represents the mean of values from six mice, and S.E.M. is denoted as a vertical bar. Statistically significant changes in liver and carcass glycogen relative to the corresponding values at 30 min are denoted as * $P < 0.005$ and *** $P < 0.001$.

situ in carcass tissues is unknown, but delayed re-activation of the pyruvate dehydrogenase complex in extrahepatic tissues on carbohydrate re-feeding [11] may serve to limit pyruvate oxidation *in situ* and maintain the supply of C_3 metabolites (namely lactate, pyruvate and alanine) for hepatic glycogen synthesis. Thus extrahepatic production of C_3 metabolites on carbohydrate re-feeding may involve not only glycolytic conversion of glucose into pyruvate, but also glycogen turnover. Carcass glycogen has been known for some time [15] to play potentially an important role in starvation as a source of gluconeogenic precursors (see [16]). The present study indicates that the deposition of carcass glycogen and its subsequent mobilization in response to glucose re-feeding (see Fig. 3) may be an important source of C_3 precursors for hepatic glycogen synthesis. The extent to which carcass glycogen turnover is obligatorily linked to fuel utilization associated with muscle contraction is unknown, but it cannot be precluded that muscular activity

Fig. 3. Glucose disposal on carbohydrate re-feeding after starvation

Dietary glucose is presumed to be metabolized mainly at extrahepatic sites (1). Hepatic metabolism (2) is likely to be restricted. The fate of glucose in extrahepatic tissues involves conversion into glycogen (3), in addition to glycolytic conversion into pyruvate (4). Carcass glycogen is partially mobilized to generate pyruvate (5), and this pyruvate (and lactate and alanine: see [16]) may act as a precursor in hepatic glycogen synthesis (6).

(associated with the stress of the experiment?) may have contributed to the stimulation of glycogenolysis. It remains to be established whether turnover of carcass glycogen after glucose re-feeding after starvation occurs in species other than the mouse.

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