Studies on the substrate for hepatic lipogenesis in the rat

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The contribution of hepatic glycogen to lipogenesis was studied in isolated, intact rat hepatocytes. To establish its importance as a substrate for lipogenesis, the glycogen of isolated hepatocytes was prelabelled with ^{14}C from glucose. Evidence is presented that neither glucose nor glycogen constitute major sources of carbon for de novo synthesis of fatty acids and that less than 1% of glycogen is converted into fatty acids.

Isolated hepatocyte *Lipogenesis* *Fatty acid synthesis* *Lipogenic substrate* *Glycogenesis* *Glycogenolysis*

1. INTRODUCTION

It has been recognized for a long time that the capacity of the liver to store glycogen is rather limited. Glucose ingested in excess of immediate caloric needs and glycogen storage capacity is thought to be converted into fatty acids, which may be stored in large amounts as triacylglycerols in adipose tissue. However, several reports in the literature indicate that glucose makes only a minor contribution to the formation of glycogen [1–5] and fatty acids [6–9]. This has been documented both in vivo [1,5,6] and in vitro [2–4,7–9]. Unlike glucose, lactate can serve as a major substrate for fatty acid synthesis in isolated hepatocytes [7,8,10] as well as in perfused liver [8]. With the latter preparation it was shown that lactate could contribute up to 50% of the carbon for hepatic fatty acid synthesis [8]. The remainder of the carbon for lipogenesis is probably derived from an endogenous source, such as glycogen, protein or lipid. Of these carbon sources, glycogen seems the most likely. It has been postulated that glycogen probably contributes substantially to the carbon in fatty acids [7,8].

According to [8] the contribution of glycogen to the carbon requirement for lipid synthesis cannot be measured directly. However, in [11] the discovery was made that the liver synthesis and degradation of glycogen are not random processes but that formation and breakdown of glycogen molecules show a sequential order, viz., the last molecules synthesized will be degraded first and vice versa. With this information available, the possibility of directly measuring the contribution of glycogen to fatty acids is now open. Glycogen in hepatocytes can be prelabelled with ^{14}C from labelled glucose and the fate of the radioactive glucose derived from the prelabelled glycogen can be monitored in subsequent incubations, provided the extent of glycogenolysis is less than that of the preceding glycogenesis. In such experiments the specific radioactivity of the glucose formed from glycogen is equal to that of glucose in the newly synthesized glycogen molecules which in turn have the same specific radioactivity as the labelled glucose in the preincubation.

Here, we determine the contribution of glycogen to the carbon requirement of hepatic fatty acid synthesis. By prelabelling the glycogen of isolated hepatocytes with ^{14}C from glucose, it could be established that less than 20% of the carbon of newly synthesized fatty acids is derived from glycogen.

2. MATERIALS AND METHODS

2.1. Isolation and incubation of hepatocytes

Hepatocytes were obtained from male Wister rats (250–300 g), which had free access to water

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and were fed a stock, pelleted diet. Liver cells were prepared and purified essentially according to the procedure of [12] as described in [13].

Cells (6–8 mg protein/ml) were incubated for 1 h (unless indicated otherwise) in 25-ml Erlenmeyer flasks with Krebs-bicarbonate buffer (pH 7.4, final volume 3 ml) supplemented with 3.5% bovine serum albumin, which was charcoal-treated [14] and dialysed [15]. All incubations were carried out at 37° under an atmosphere of 95% oxygen, 5% carbon dioxide in a shaking water bath (90 strokes/min).

To measure the contribution of glucose to fatty acid synthesis, D-[U-^{14}C]glucose (5 μCi/ml) and $^{3}H_2O$ (1 mCi/ml) were added.

Glycogen in hepatocytes was labelled by incubation of the cells for 90 min with 50 mM D-[U-^{14}C]glucose (20 μCi/mmol). Afterwards the cells were washed twice by centrifugation at room temperature for 1 min at 100 × g and the pellet (intact hepatocytes) resuspended in bicarbonate buffer with 3.5% albumin.

To estimate the contribution of glycogen to lipogenesis, two sets of incubations were run simultaneously. In the first set hepatocyte glycogen was prelabelled. In the second set hepatocytes were preincubated in the presence of 50 mM unlabelled glucose. The first set was then incubated in radioisotope-free buffer, whereas the incubation medium of the second set contained $^{3}H_2O$ (2 mCi/ml). After various time intervals aliquots were removed from the incubations for the measurement of the glycogen content and the incorporation of ^{3}H and ^{14}C into fatty acids.

2.2. *Analytical methods*

Glycogen was extracted from the cells according to [16]. Aliquots (1.5 ml) of the hepatocyte incubations were digested in hot KOH. Glycogen was precipitated with ethanol. The pellet was washed 3 times by dissolving it in distilled water followed by reprecipitation with ethanol. After glycogen was hydrolysed with acid, glucose and ^{14}C radioactivity were determined in the hydrolysate. Glucose was assayed by the GOD-Perid method (Boehringer, Mannheim). Lipids were extracted in aliquots (1.0 ml) of the incubations as described previously [17]. Lipogenesis in these cells was terminated by addition of 0.38 ml 2 N $HClO_4$. Total lipids were saponified at 75°C with 0.3 M NaOH in 90% (v/v) methanol. The non-saponifiable fraction was extracted and discarded. Following acidification, fatty acids were extracted and counted for radioactivity. Protein was determined as in [18].

In isolated hepatocytes absolute rates of fatty acid and glycogen synthesis vary from experiment to experiment. However, the calculated percentages of contribution were very reproducible between experiments. Therefore, in the figures and tables representative experiments are presented which have been reproduced with at least two other preparations of hepatocytes.

Table 1

Effect of glucose concentration on the rate of fatty acid synthesis by isolated rat hepatocytes

Glucose (mM)		Fatty acid synthesis (nmol acetyl units incorporated/h per mg protein)		Contribution of glucose to fatty acid synthesis (%)
Added	At t = 60 min	Derived from D-[U-^{14}C]glucose	Total, as measured by $^{3}H_2O$ incorporation	
0	10.4	0.72	37.9	1.9
2.6	11.8	1.22	42.5	2.9
5.2	13.0	2.09	44;4	4.7
7.9	15.3	3.69	56.5	6.5
10.5	17.8	5.30	61.0	8.7
26.2	31.4	17.0	101.3	16.7
52.4	55.7	28.1	114.1	24.6

Since glucose was transferred with the hepatocyte suspension, the initial glucose concentration was 6.4 mM above the added one

2.3. Sources of materials

D-[U-^{14}C]glucose and $^{3}H_2O$ were purchased from the Radiochemical Centre, Amersham; bovine serum albumin (fraction V) and collagenase were from Sigma; other chemicals were from Baker.

3. RESULTS

The dependence of hepatic fatty acid synthesis on the concentration of glucose was investigated in hepatocytes derived from fed donor rats. The total rate of fatty acid synthesis (measured by $^{3}H_2O$ incorporation) increased with increasing concentrations of glucose. At glucose levels above 30 mM the increase appeared to level off (table 1). In parallel incubations the contribution of glucose to de novo fatty acid synthesis was studied with D-[U-^{14}C]glucose. In the physiological range (up to 10 mM glucose) the contribution of glucose to the total rate of fatty acid synthesis was minor and amounted to only a few percent. At a very high and unphysiological glucose concentration of 50 mM the contribution of glucose to fatty acid synthesis was approximately 25% (table 1).

To establish optimal conditions for prelabelling cellular glycogen, the dependence of hepatic glycogen synthesis on the concentration of glucose was studied. With increasing concentrations of added glucose glycogenolysis gradually diminished and concentrations of added glucose above 25 mM rendered the hepatocyte into a glycogenic cell. In the presence of 50 mM added glucose a significant deposition of glycogen occurred (fig.1).

To determine the contribution of glycogen to fatty acid synthesis hepatocyte glycogen was prelabelled with ^{14}C from glucose (see section 2). The prelabelled cells were further incubated in the absence or presence of low concentrations of unlabelled glucose. Fig.2 confirms the results presented in fig.1, that in the presence of up to 10 mM unlabelled glucose glycogen was broken down. Fig.2 also shows that degradation of glycogen and disappearance of radioactivity from glycogen occurred concurrently. This result confirms the observation in [11] that glycogen molecules which are synthesized last are hydrolysed first.

Fatty acid synthesis from prelabelled glycogen was determined and compared with the total rate of fatty acid synthesis as measured by the incorporation of tritium from tritiated water into fatty acids (table 2). The data indicate (i) that glycogen does not make a major contribution to de novo fatty acid synthesis (less than 20%) and (ii) that not more than 1% of glucose produced from glycogen is converted into fatty acids (table 2).

4. DISCUSSION

It has long been held that following a carbohydrate-rich meal glucose absorbed from the intestine is readily taken up by the liver and converted into glycogen. Glucose taken up in excess of hepatic storage capacity is believed to be transformed into

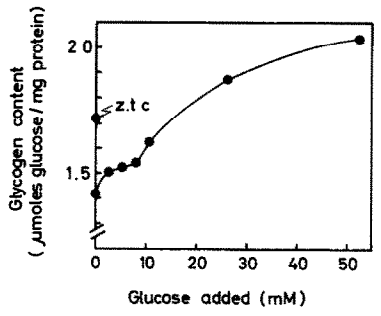

Fig.1. Effect of glucose concentration on the glycogen of hepatocytes. Hepatocytes were incubated for 60 min in the presence of various concentrations of glucose. z.t.c. (zero-time control) indicates the glycogen content at the start of the incubations. Since glucose was transferred with the hepatocyte suspension, the initial glucose concentration was 6.4 mM above the added one.

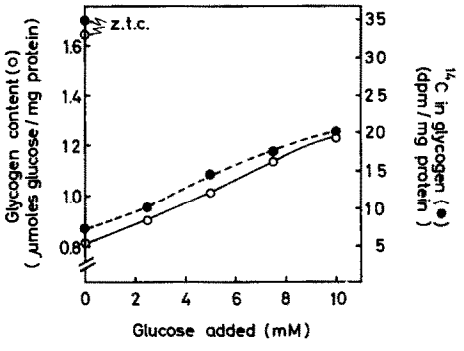

Fig.2. Glycogen breakdown and disappearance of radioactivity from prelabelled glycogen as a function of the concentration of glucose. Hepatocytes, containing prelabelled glycogen, were incubated for 60 min in radioisotope-free buffer in the absence or presence of glucose. z.t.c., zero-time control.

fatty acids which are secreted by the liver in lipoproteins. However, authors in [5] recently demonstrated that the bulk of glycogen, deposited in the liver after the ingestion of a carbohydrate load, is not synthesized from glucose but from C_3 units. The lack of glucose utilization for glycogen synthesis was explained by their observation that the liver has only a limited capacity to phosphorylate glucose at physiological concentrations [5]. This limited glucose phosphorylation prevents the massive conversion of glucose into liver glycogen.

The observed defect in glucose-phosphorylating potential in liver may also resolve the question why glucose is such a poor substrate for lipogenesis [6–9]. It leaves us with the intriguing dilemma as to what substrate is actually used for hepatic fatty acid synthesis. Authors in [7,8] have postulated that glycogen probably provides a significant amount of carbon for hepatic fatty acid synthesis. In an attempt to quantify the extent to which liver glycogen contributes to hepatic fatty acid synthesis we have prelabelled hepatic glycogen with ^{14}C from glucose and monitored the subsequent appearance of ^{14}C label in fatty acids. The findings of the present investigation indicate that, like glucose, glycogen is not a major substrate for fatty acid synthesis. Less than 20% of the total carbon used in fatty acid synthesis is derived from glycogen.

If both glucose and glycogen do not contribute substantially to the carbon requirement for lipogenesis the question about the substrate for fatty acid synthesis is still open. In vitro observations [7,8,10] have suggested an important role for lactate in lipogenesis. The concentration of lactate in the blood following a glucose-containing meal [5,7] is sufficient to support the rapid rates of fatty acid synthesis observed in vivo. In analogy with the concept of a glucose → lactate → glycogen pathway for glycogen synthesis, put forward in [5], we propose a glucose → lactate → fatty acid pathway, which is consistent with observations in vivo [5,7] and in vitro [7,8,10] and also with the Cori cycle [19]. The site for the initial metabolism of ingested glucose still has to be identified. Intestine and/or muscle have been suggested as likely candidates [5]. Our studies with isolated hepatocytes rule out a third possibility vented by these workers, viz., that the liver itself might be the locus of glucose metabolism, if the concept of metabolic zonation of the tissue in glycolytic and gluconeogenic regions [20] can be firmly established. In preparations of isolated hepatocytes both 'glycolytic' and 'gluconeogenic' hepatocytes would be present. Contrary to what has been observed, such mixed preparations would not identify glucose as a poor lipogenic and glycogenic substrate.

According to [21] protein degradation in iso-

Table 2

Relationship between glycogen degradation and fatty acid synthesis in isolated hepatocytes

Incubation time (min)	Glucose added (mM)	Glycogen (nmol/mg protein)		Fatty acid synthesis (nmoles acetyl units/mg protein)		Fatty acids from glycogen (%)	Glycogen recovered in fatty acids (%)
		Level	Loss	Derived from ^{14}C glycogen	Total, as measured by $^{3}H_2O$ incorporation		
0	0	1097.5	–	–	–	–	–
10	0	971.0	126.5	0.9	9.3	9.6	0.23
15	0	950.8	146.7	1.6	13.5	11.8	0.36
20	0	859.8	237.7	2.9	16.2	17.9	0.40
10	10	1006.4	91.1	1.1	10.8	10.1	0.40
15	10	955.9	141.6	2.0	13.9	14.4	0.47
20	10	966.0	131.5	3.2	19.3	16.6	0.81

Hepatocytes were preincubated with 50 mM D-[U-^{14}C]glucose. After 90 min labelled glucose was removed and the cells were further incubated in the absence or presence of unlabelled glucose for the indicated periods of time. The values for fatty acid synthesis derived from labelled glycogen have been corrected for the 5.6 nmoles acetyl units/mg protein present in the hepatocytes at the start of the incubations

lated hepatocytes can amount to 4% of the total cellular protein per h, which means that amino acids, after conversion into acetyl-CoA, may provide substantial carbon for de novo synthesis of fatty acids.

The present studies also reveal that unlabelled glucose, added at a high but not unphysiological concentration of 10 mM, is unable to dilute the ^{14}C label derived from glycogen and incorporated in fatty acids. On the contrary, in the presence of unlabelled glucose there is a tendency of increased lipogenesis from labelled glycogen (table 2). Glucose probably impairs the glucose-6-phosphatase reaction [22] and as a result more glycogen carbon is channeled into glycolysis and subsequently into fatty acids. This indicates that glucose at that concentration is unable to reach the pool of labelled intermediates, which confirms the observation in [5] that liver is short of glucose-phosphorylating capacity and as a result does not metabolize glucose at physiological concentrations.

Studies are in progress to identify and quantify the products of glycogen metabolism. Preliminary results indicate that the bulk of glycogen is released by the liver as glucose, probably for the benefit of extra-hepatic tissues.

In conclusion, neither glucose nor hepatic glycogen constitute a major source of carbon for de novo synthesis of fatty acids by the liver. As is the case with glycogenesis, C_3 units produced by extrahepatic tissues probably furnish the carbon for hepatic lipogenesis.

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