Simultaneous Synthesis and Degradation of Rat Liver Glycogen

An In Vivo Nuclear Magnetic Resonance Spectroscopic Study

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Abstract

Using ^{13}C nuclear magnetic resonance spectroscopic methods we examined in vivo the synthesis of liver glycogen during the infusion of D-[1-^{13}C]glucose and the turnover of labeled glycogen during subsequent infusion of D-[1-^{13}C]glucose. In fasted rats the processes of glycogen synthesis and degradation were observed to occur simultaneously with the rate of synthesis ≫ degradation leading to net glycogen synthesis. In fed rats, incorporation of infused D-[1-^{13}C]glucose occurred briskly; however, over 2 h there was no net glycogen accumulated. Degradation of labeled glycogen was greater in the fed versus the fasted rats ($P < 0.001$), and the lack of net glycogen synthesis in fed rats was due to degradation and synthesis occurring at similar rates throughout the infusion period. There was no indication that suppression of phosphorylase *a* or subsequent activation of glycogen synthase was involved in modulation of the flux of tracer into liver glycogen.

We conclude that in both fed and fasted rats, glycogen synthase and phosphorylase are active simultaneously and the levels of liver glycogen reached during refeeding are determined by the balance between ongoing synthetic and degradative processes. (*J. Clin. Invest.* 1990. 86:612–617.) Key words: liver glycogen • in vivo NMR • phosphorylase • glycogen synthase

Introduction

After an overnight fast the rat liver is nearly depleted of glycogen stores. Repletion begins promptly upon feeding with both glucose and gluconeogenic substrates contributing to the formation of UDP-glucose and glycogen (1–6). The factors that control the transition of the liver from the glucose-producing to the glycogen-storing mode have been extensively studied, and include release of insulin into portal blood (7), suppression of glucagon secretion (8), and a rise in portal glucose concentration (9, 10). When isolated hepatocytes are incubated with high concentrations of glucose (11), and when mice are given large doses of glucose parenterally (12), near complete inactivation of phosphorylase *a* (GP*a*)[1] precedes the activation of glycogen synthase (GS*i*) and the onset of glycogen synthesis. This activation of the glycogen synthetic machinery occurs by dephosphorylation of both phosphorylase and synthase and is thought to underlie the transition of the liver from the fasted to the fed state. This reciprocal control of synthase and phosphorylase by enzyme dephosphorylation should prevent futile cycling of glucose through the glycogen pool during periods of net synthesis (13). GP*a* is thought to play a key role in this sequence as an intracellular glucose "receptor" (12–15). Binding of glucose alters the conformation of GP*a*, facilitating dephosphorylation by a protein phosphatase. This phosphatase is then subsequently available to dephosphorylate and activate the synthase.

Recent in vivo studies have indicated that when physiologic loads of glucose are given to rats, liver GP*a* may not be completely inactivated (16–20) and GS*i* not significantly activated (21, 22). If both enzymes were simultaneously active in vivo, futile cycling of glucose in and out of glycogen would be expected. Several authors have suggested that futile cycling between glucose-1-phosphate and glycogen may occur in both liver (23) and muscle (24), but experimental evidence for the operation of this cycle is lacking.

We have examined this question in the intact rat heart in vivo using ^{13}C nuclear magnetic resonance (NMR) spectroscopic methods and observed that during periods of net glycogen synthesis stimulated by insulin infusion, there was little or no futile cycling at the glucose-1-phosphate–glycogen step of glucose metabolism (25). In contrast, in the perfused liver extensive futile cycling of glucose through glycogen has been observed (26). In the current study we extend the use of ^{13}C-NMR spectroscopic methods to noninvasively examine the temporal pattern of in vivo ^{13}C-glucose incorporation into liver glycogen in the intact fed and fasted rat.

Methods

Animal preparation and infusion protocols

Male Sprague-Dawley rats (300–400 g) were either fasted overnight (16 h) or fed ad lib before being anesthetized with intraperitoneal barbiturate (Inactin). Polyethylene catheters were then inserted into both internal jugular veins and used for infusion of insulin and substrates and for sampling of plasma glucose. In each of seven study protocols the substrate mixtures were given intravenously rather than orally or via a gastric tube to minimize potentially confounding effects of anesthesia on gastric emptying and therefore substrate delivery. The anesthesia is required to prevent animal movement during the acquisition of NMR spectra. In the NMR studies, rats were placed on a lucite platform with the abdomen positioned over an NMR surface coil and the entire assembly placed in the bore of a 4.7-T magnet. The position of the liver relative to the surface coil was ascertained and adjusted based on a proton NMR image as previously described.[2] The rats studied spectroscopically were then given one of three infusion protocols.

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1. *Abbreviations used in this paper:* GP*a*, phosphorylase *a*; GS*i*, glycogen synthase; NMR, nuclear magnetic resonance.

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Group 1. [1-^{13}C]Glucose (10 mg/min), fructose (5 mg/min), glutamine (2 mg/min), and regular insulin (20 mU/min) were given for 120 min. n = 3 for fasted rats; n = 4 for fed rats.

Group 2. Fructose, glutamine, and insulin were infused for 120 min as in group 1. [1-^{13}C]Glucose (10 mg/min) for 50 min was followed by [1-^{12}C]glucose (10 mg/min) for the final 70 min. n = 8 for fasted rats; n = 7 for fed rats.

Group 3. Fructose, glutamine, and insulin were given for 120 min as in group 1. [1-^{13}C]Glucose (10 mg/min) for 50 min was followed by [2-^{13}C]glucose (10 mg/min) for 70 min. n = 3 for fasted rats; n = 2 for fed rats.

Liver glycogen content was measured in the three groups described and in the four additional groups given below. The activities of GS and phosphorylase were measured in the latter four groups which were not studied spectroscopically.

Group 4. Fructose, glutamine, and insulin were given as in group 1 together with ^{12}C-glucose (10 mg/min) for 120 min, and small snip biopsies of the liver were obtained at the start and the completion of the infusion (n = 6 for fasted rats; n = 6 for fed rats.

Group 5. Rats either fasted overnight or fed ad lib were anesthetized and the livers excised 30 min later after receiving only a 0.9% NaCl infusion.

Group 6. After an overnight fast rats were provided free access to rat chow for 3 h and anesthetized, and their livers were excised.

Group 7. Fed rats were anesthetized and given an intravenous infusion of ^{12}C-glucose (20 mg/min) with fructose and glutamine and regular insulin (20 mU/min) and the livers were excised after 120 min. The higher dose of glucose was used in these rats to examine the effect of hyperglycemia on liver glycogen phosphorylase and synthase activity.

All rats were killed at the end of the infusions and their livers were immediately freeze-clamped in liquid N_2. Since fructose administration can affect liver ATP levels (27) in control experiments, we used in vivo ^{31}P-NMR methods to monitor liver ATP during substrate infusion and observed that the substrate mixture used in the current study had no effect on hepatocellular ATP levels. Increasing the fructose infusion rate by fourfold above that used in the current studies led to a reproducible fall of liver ATP of 50% or more.

In vivo NMR measurements

Details of the methods used to localize the liver and obtain high resolution NMR spectra will be described elsewhere.[2] In brief, the liver of the intact anesthetized rat was localized using the proton imaging capabilities of the 4.7-T Biospec spectrometer (Bruker Instruments, Inc., Billerica, MA) and a 3.8-cm surface coil tuned to the proton frequency (200.4 mHz). ^{13}C-NMR spectra were obtained at 50.4 mHz using a 2-cm receiver coil and a depth-pulse acquisition sequence (28) after shimming the field to a water line width of ~ 90 Hz. In the NMR spectroscopic studies the time course of glycogen synthesis in vivo was measured from the change of the peak height of the ^{13}C-glycogen signal over time observed in the proton-decoupled ^{13}C-NMR spectra. Spectra were obtained every 3.5 min during a 14-min basal period and throughout the 2-h infusion. [1-^{13}C]Glucose and [2-^{13}C]glucose were purchased from MSD Isotopes (Montreal, Quebec, Canada) and Omicron Biochemicals (Ithaca, NY), respectively.

Measurements of metabolites and enzyme activities

Glucose was measured using the glucose oxidase method in blood samples obtained at 15–30-min intervals during the infusion. Both the synthase and phosphorylase assays were run in the direction of glycogen synthesis as previously described (29–31). These assays involve the measurement of ^{14}C incorporation into newly formed glycogen from radiolabeled UDP-glucose (synthase) or glucose-1-phosphate (phosphorylase). Radiotracers were purchased from Amersham Corp. (Arlington Heights, IL), while all other reagents were obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification. The activity of GP*a* is defined as the activity seen in the absence of AMP and phosphorylase *b* that obtained with 5.0 mM AMP. GS*i* was defined as the activity observed when glucose-6-phosphate was present at a concentration of 500 μM and synthase *d* was the activity seen when the glucose-6-phosphate concentration was raised to 4.8 mM. The [UDP-glucose] in the final assay mixture (310 μM) was selected to approximate the concentration found in freeze-clamped tissue samples from rats given the combined glucose, fructose, and glutamine infusion.

Results

Fig. 1 displays the natural abundance ^{13}C-NMR spectrum of the liver and the changes that occurred during a 2-h infusion of [1-^{13}C]glucose to a fasted rat (group 1). In these spectra only peaks attributable to lipids were seen before the ^{13}C infusion. Spectra with excellent signal-to-noise were obtained at 3.5-min intervals and the time course of the change of height of the [1-^{13}C]glycogen and [1-^{13}C]glucose peaks in the fasted group 1 rats are shown in Fig. 2. The signals attributable to the α and β peaks of [1-^{13}C]glucose rose promptly, reaching a maximum by 30 min, and then remained relatively constant until the end

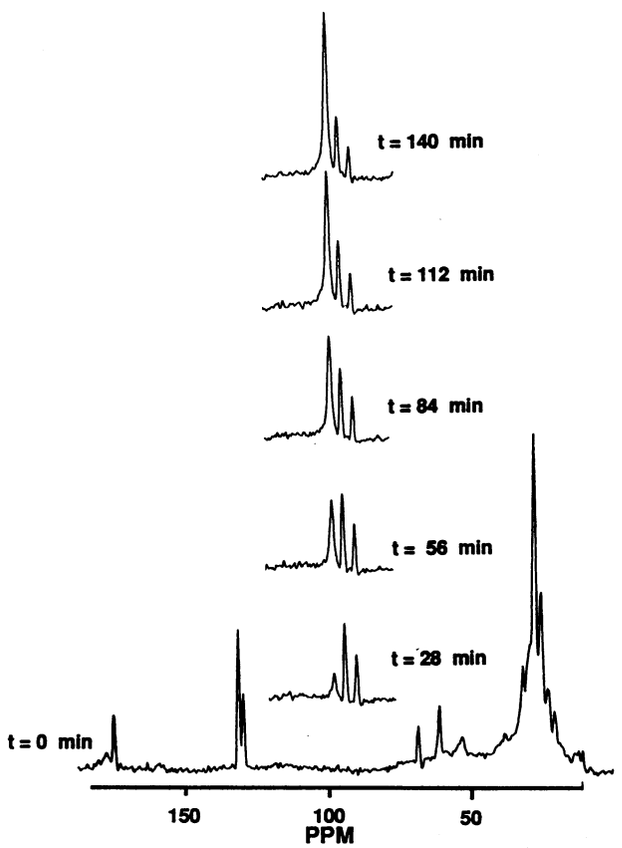


Figure 1. The in vivo natural abundance ^{13}C-NMR spectrum of the liver of a fasted rat (group 1) is shown in the lower panel. During a 160-min infusion of D-[1-^{13}C]glucose, sequential 14-min spectra were obtained. The region of the spectra containing the α and β peaks of [1-^{13}C]glucose and the peak from [1-^{13}C]glycogen (100.4 ppm) are shown in the insets. The remaining peaks are attributable to the carbons of fat and their choline and ethanolamine esters. The spectra consisted of 4,000 data points zero-filled to 8,000. Spectra were processed by convolution difference to remove broad components, followed by 10 Hz of Gaussian line broadening before Fourier transformation.

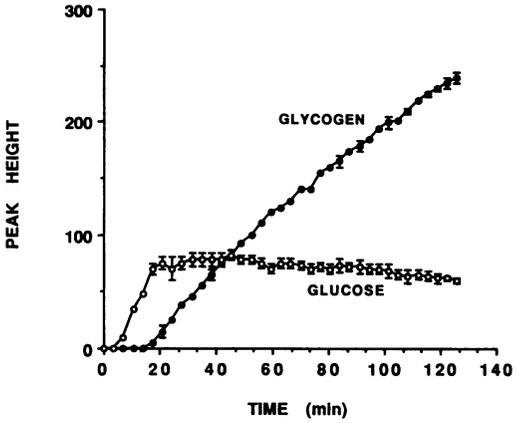


Figure 2. The time course for the changes in the height of the β-glucose peak and the [1-^{13}C]glycogen peak during a continuous infusion of labeled glucose, fructose, and glutamine (group 1, fasted). Spectra were obtained every 3.5 min and the heights of the glucose and glycogen peaks normalized to the height of the glycogen peak at 52.5 min. The error bars indicate ±1 SEM, n = 3.

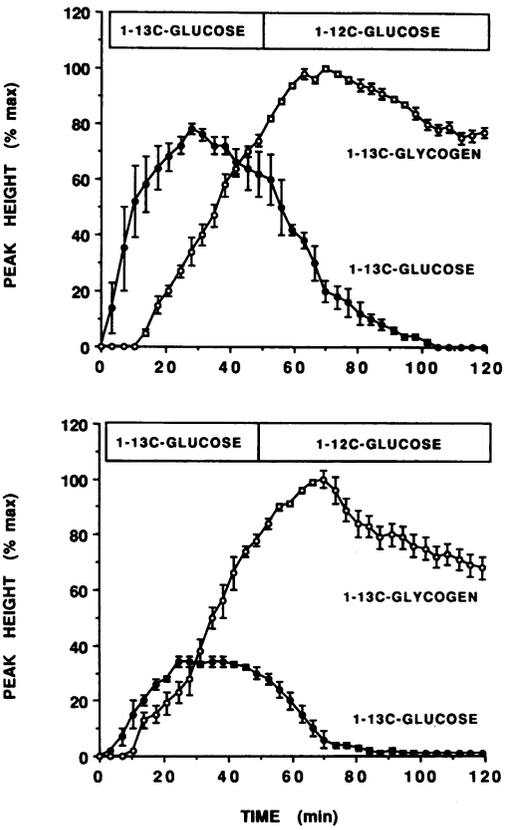


Figure 3. Top, Time course of the change of the height of the [1-^{13}C]-glycogen and [1-^{13}C]glucose peaks observed in the fasted group 2 rats studied using the pulse-chase protocol. Peak heights were normalized to the maximal observed height of the glycogen peak in each rat. Error bars indicate ±1 SEM. *Bottom,* Results obtained for the fed group 2 rats studied using the same infusion protocol.

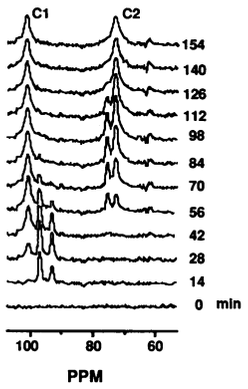
of the study. To facilitate comparison of the glycogen signals obtained in the different animals, values for the peak height of both the glucose and glycogen signals were normalized to the size of the glycogen peak present in each rat at 52.5 min after starting the infusion. After an initial delay the glycogen signal increased in an approximately linear fashion over the next 140 min in the fasted rats. The initial delay may reflect the time required to fully label the liver glucose pool, the time required for insulin to act, or both. In fed rats from group 1 (data not shown) the glycogen peak height rose in a similar manner for the first 80–100 min of the labeled glucose infusion, then either plateaued (n = 2) or even declined (n = 2), despite continued infusion of [1-^{13}C]glucose.

The mean blood glucose concentrations (±SEM) in the fasted rats studied in groups 1–3 averaged 54±3, 114±13, 82±7, and 120±17 mg/dl at 0, 30, 60, and 120 min, respectively. Compared with the fasted rats, the mean blood glucose concentrations in the fed rats were higher basally (79±9 mg/dl), but comparable during the infusion period (90±8, 90±23, and 194±49 mg/dl, at 30, 60, and 120 min).

The time course of the change in the ^{13}C-glycogen and ^{13}C-glucose peak heights seen in the ^{13}C-NMR spectra during a 50-min infusion of [1-^{13}C]glucose followed by a 70-min infusion of [1-^{12}C]glucose (group 2) is shown in Fig. 3. The ^{13}C-1-glycogen peak declined over the last 70 min of the study by an average of 17±3%/h in the fasted (*top*) and 33±9%/h in the fed rats (*bottom*), respectively. Despite identical [1-^{13}C]glucose infusions, the intensity of the signal attributable to circulating glucose was only approximately half as great in the fed compared with the fasted rats throughout the infusion period. Presumably this reflected more rapid clearance of glucose by the fed rats. The heights of the [1-^{13}C]glycogen peaks were comparable in the two groups. In both groups of animals the glucose signal declined abruptly after discontinuing the label infusion and disappeared by 85 min.

The findings illustrated in Fig. 3 suggested that some degradation of labeled glycogen was occurring while the synthesis was continuing. However, since this evidence relies on a comparison between different groups of rats, we could not discount the possibility that animals studied in group 2 were in some manner more stressed than those in group 1 and were in fact no longer making significant amounts of glycogen after the first hour of [1-^{13}C]glucose infusion. To address this issue we used a double-label infusion protocol (group 3). Fig. 4 (*top*) shows a stack plot of the spectra obtained during the sequential infusion of [1-^{13}C]glucose followed by [2-^{13}C]glucose to a fasted rat, and the temporal pattern of change in the glycogen peak height is shown during the label infusion and the subsequent chase period during which ^{12}C-glucose was infused (*bottom*). The signals arising from glycogen labeled in the C_1 carbon were readily distinguished from C_2 glycogen and their intensities were quantitated separately. Note that the [1-^{13}C]-glycogen signal declined during the entire time the [2-^{13}C]-glycogen intensity was rising, indicating simultaneous synthesis and degradation of liver glycogen particles. In two fed rats we used the same infusion protocol and observed that the glycogen labeled in the initial 50-min glucose labeling period turned over at an average rate of 50%/h during the 70 min required for infusion of the second label. During this period of rapid glucose turnover incorporation of label from the second tracer occurred at a rate similar to that in the first labeling period.

These studies indicated that turnover of ^{13}C-glycogen occurred during periods when incorporation of ^{12}C-glucose into



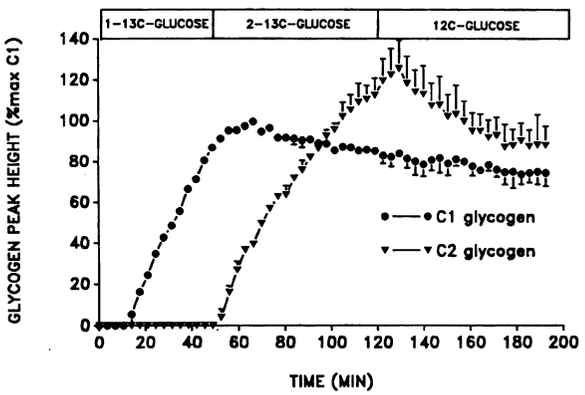


Figure 4. Top, A series of sequential spectra obtained in the fasted group 3 rats. Spectra were accumulated and processed as described in Fig. 1. Peaks assigned to the α and β peaks of the infused labeled glucose are best seen in the spectra labeled 14 min and 56 min. The C1 and C2 glycogen signals are clearly labeled in the 154-min spectrum. *Bottom,* The complete time course for the heights of the two species of labeled glycogen observed in the group 3 fasted rats. The glycogen peak heights were normalized to the maximal value obtained for the [1-^{13}C]glycogen. Error bars indicate ±1 SEM.

glycogen persisted. When the rate of turnover of the [1-^{13}C]-glycogen signal present at the end of the first hour was compared between the fed and the fasted rats the mean rate (percent per hour) was significantly greater ($P < 0.001$) in the fed (40±6%/h) compared with the fasted (18±3%/h) rats. However, the relationship between the incorporation (or release) of labeled glucose in glycogen and the net rate of glycogen synthesis could not be deduced from these tracer experiments alone. The glycogen contents of the liver at the end of the study in each of the seven groups of rats are given in Table I. We observed that fasted rats given only saline (group 5) have a very low glycogen content and infusion of the glucose-fructose-glutamine-insulin mixture (groups 1–3 and 4) and refeeding (group 6) led to significant increases in glycogen content. In contrast in the ad lib fed animals the liver had abundant glycogen at the outset of the study (group 4 basal and group 5) and this did not increase when either the glucose-fructose-glutamine-insulin mixture or high dose glucose was infused (groups 4 and 7).

The fed animals in the basal state had a lower GS*i* activity than the fasted rats (group 5), and there was no significant increase in the activity of GS*i* at the end of the 2-h infusion

Table I. Liver Glycogen Concentrations

	Fed	Fasted
	μmol/g wet weight	
Groups 1–3	211±25	46±9
Group 4		
Basal	276±44	13±4
+60 min	301±40	62±7
+120 min	268±37	102±11
Group 5	310±50	13±6
Group 6		170±6
Group 7	310±40	

The concentrations of glycogen were measured at the end of the infusion period. Values expressed are the mean±SEM.

period in either group. The highest GS*i* activities were observed in the fasted-refed rats and in the rats given a high dose of glucose and insulin (Table II).

GP*a* activity was similar in the basal state in fed and fasted rats (group 5). The GP*a* activity did not change after 2 h of glucose-fructose-glutamine and insulin infusion in the fasted rat, but rose in the fed animal (group 4). The lowest GP*a* activities were seen in the fed rats given the high dose glucose and insulin infusion (group 7, Table II). The blood glucose concentrations were very high in these rats, averaging 79 mg/100 ml basally and increasing to 134 at 15 min, and 302 at 45 min into the infusion. Interestingly, although groups 6 and 7 had comparable activities of GS*i* and GP*a*, there was no apparent synthesis of glycogen in the fed rats, but rapid (~ 0.9 μmol/min per g wet weight) net glycogen synthesis in the fasted-refed animals.

Discussion

The findings in the current study indicated that in the intact, overnight-fasted rat there was significant turnover of liver glycogen during periods of net glycogen synthesis. This process was, if anything, more prominent in the animals fed ad lib until the time of study. Thus, in considering the liver as an entire organ, glycogenolysis and glycogenesis clearly occurred simultaneously. The methods used do not allow us to exclude the possibility that synthesis and degradation were occurring in

Table II. Glycogen Synthase and Phosphorylase Activities

	Synthase			Phosphorylase		
	i	*d*	*i/d*	*a*	*b*	*a/b*
	μmol/min per g wet weight		%	*μmol/min per g wet weight*		%
a/b						
Group 4						
Fed	0.07 + 0.02	0.10 + 0.03	68±5	8.3±1	26±1	32±3
Fasted	0.22±0.05	0.25±0.05	82±6	3.2±0.5	18±1	18±1
Group 5						
Fed	0.06±0.01	0.14±0.01	43±1	3.8±0.8	17±1	23±2
Fasted	0.25±0.05	0.35±0.05	71±5	4.2±0.8	16±2	29±6
Group 6	0.36±0.05	0.38±0.05	94±3	1.9±0.4	17±1	12±2
Group 7	0.28±0.02	0.30±0.02	110±8	1.3±0.3	15±1	9±2

different cells. However, since in the pulse-chase experiments label loss during the chase must have involved cells that were actively making glycogen a brief time earlier, such an explanation necessitates that one postulate a time-dependent inactivation of glycogen synthesis and activation of glycogenolysis. Furthermore, at least in the fasted rats this process would have to have occurred when the liver glycogen stores are still quite low (< 70–80 μmol/g wet weight), a seemingly unlikely event.

Some quantitative feeling for the magnitude of the turnover process observed can be obtained from the pulse-chase data in the fasted rats. The rate of net glycogen synthesis in these rats averaged 0.3 μmol/min per g (see Table I) and the slope of the curve during the chase period was ~ 18% of the upsloping curve seen during the labeling interval. Since in these fasted rats there was almost no glycogen present at the beginning of the infusion period, the loss of labeled glycogen should correspond to the total rate of net glycogen loss and this would amount to ~ 0.045 μmol/min per g. It must be stressed that this would be a minimal estimate of the rate of glycogenolysis that occurred, since any of the labeled glucose-1-phosphate released from glycogen that was very rapidly converted to UDP-glucose could be reincorporated into glycogen and would not be detected as turnover. In addition, any turnover of the ^{12}C-glucose infused during the chase period would pass unobserved. In the fed animals the turnover of labeled glycogen was more rapid than in the fasted rats, as evidenced by the steeper slope of the declining glycogen signal during the pulse-chase and double-label experiments. Keeping in mind that the labeling technique used in the pulse-chase experiment only reports on the turnover of the glycogen labeled during the D-[1-^{13}C]glucose infusion period, it seems reasonable to assume that at least some of the unlabeled glycogen present in the fed rat liver before beginning the tracer infusion would be subject to the same process and hence the actual rate of glucose mobilization (labeled and unlabeled) from glycogen is underestimated to a greater extent in the fed compared with the fasted rats. Such a process would be consistent with our observed lack of significant net glycogen synthesis by the livers of fed rats despite infusion of a substrate mixture that supports brisk rates of glycogen synthesis in fasted rats.

Very recently, Niewoehner and Nuttall (32) have reported that fed rats on either a chow diet or a high carbohydrate diet also fail to increase liver glycogen stores over the 2 h after a large (4 g/kg) oral glucose load, while fasted rats increase net hepatic glycogen content promptly. No tracer measurements were made in the latter study. Therefore, it could not be ascertained whether the lack of net glycogen accumulation in the fed rats resulted from failure of synthesis or, as suggested here, accelerated turnover.

One methodologic concern would be whether the decrement in the [1-^{13}C]glycogen signal seen during the chase period in both the pulse-chase and double-label experiments in fact represented a true loss of tracer from glycogen or simply a loss of NMR signal due to altered structural properties of the glycogen molecule as progressively more glucose residues are added to the glycogen chains. This appears unlikely since prior studies have demonstrated that despite the large size of the glycogen molecule (10^6–10^8 D), virtually all the glucose residues in the molecule contribute equally to the ^{13}C-glucose signal (33, 34).

The simultaneous synthesis and degradation of glycogen seen in the current study is qualitatively in accord with results observed in the isolated perfused liver (26). Net glycogen synthesis in the perfused liver occurred at a slower rate (~ 0.12 μmol/min per g) and during a pulse-chase experiment the rate of glycogen breakdown was nearly as rapid as the rate of synthesis, a finding similar to the current results in fed rats. Using hepatocytes isolated from fasted rats, Katz et al. (35) observed little or no breakdown of glycogen during periods of rapid net synthesis (> 1 μmol/min per g). Glycogen turnover was only studied in cells isolated from fasted rats since net glycogen synthesis did not occur in cells from fed rats. In addition, in those studies turnover of glycogen was observed for a briefer time (30–40 min) than in the current study. It should also be considered that the absence of glycogen turnover in the isolated cell preparations may relate to a variety of factors, including the high concentrations of substrates used, differences in the methods used to study turnover, or the absence of the neural and hormonal milieu present in the intact animal. In addition, it remains possible that under circumstances where net glycogen synthesis is very rapid, turnover may be less prominent.

It is of interest that in this study we observed no clear relationship between the activity of GP*a* assayed in tissue extracts and the turnover rate of glycogen in the intact rat. A similar lack of correspondence had been previously noted in both heart (25) and liver (35). In the current study we observed a rather wide range of GP*a* activities in the livers of rats treated under the different experimental protocols. In particular, those rats fed either a chow meal or the high dose glucose infusion (groups 6 and 7) appeared to have the lowest GP*a* activities. In group 7 this may be due to the high level of glycemia achieved (see above), with glucose acting as a signal for GP*a* inactivation (12, 14, 15). However, in the refed fasted rats blood glucose remained within the normal range and it is unlikely that glucose-induced inactivation of phosphorylase played a role in the lowered phosphorylase activity in this setting. Our data do not exclude the possibility that transient portal hyperglycemia, perhaps acting in concert with portal insulin, may have contributed to the phosphorylase inactivation. In all experimental groups the activity of GP*a* was substantially greater than synthase *a* activity in the same liver. It does not appear that the high GP*a* activity measured in these liver extracts is attributable to a nonspecific activation of phosphorylase by our assay conditions, since we observe differences in activity among the experimental groups and the expected inhibitory effect of glucose on GP*a* activity in group 7.

Since these rats received fructose together with insulin, glucose, and glutamine in their infusion mixtures, it is possible that the in vivo expression of GP*a* activity was suppressed either by the lowering of hepatocellular inorganic phosphate produced by fructose (27) or to an inhibitory effect of fructose-1-phosphate per se on GP*a* activity (36). To determine whether either of these mechanisms is playing a significant role will require more detailed study of the hepatic content of P_i and fructose-1-phosphate during periods of glycogen synthesis provoked by this or similar substrate infusions. Regardless of mechanism, these data suggest that the phosphorylation state of phosphorylase alone will not dictate the expression of phosphorylase activity in vivo.

In conclusion, we have presented evidence that in both the fed and fasted rat the processes of glycogen synthesis and degradation occur simultaneously in the liver during periods of net glycogen synthesis. The factors that control the extent of this substrate cycling, particularly in the fed rat, require further investigation. These findings suggest that the conventionally

held model for the regulation of hepatic glycogen synthesis by sequential inactivation of GP*a* and activation of glycogen synthase must be significantly revised.

Acknowledgments

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