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Glucose-stimulated lipolysis in rainbow trout, *Oncorhynchus mykiss*, liver[1]

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Abstract

Rainbow trout, *Oncorhynchus mykiss*, were used to characterize further the influence of glucose on hepatic lipolysis. Liver was removed from fed fish, cut into 1 mm^3 pieces and incubated for up to 5 h in Hanks medium containing either 2 mM, 5.5 mM, 10 mM, or 25 mM glucose. Glucose-stimulated lipolysis was indicated by tissue triacylglycerol (TG) lipase activity and by medium concentrations of glycerol and fatty acids (FA). Triacylglycerol lipase activity in liver pieces incubated in the presence of higher concentrations (25 mM) of glucose was significantly higher than that in liver pieces incubated in lower concentrations (2 mM) of glucose, rising from 0.075 ± 0.002 (mean ± SEM) nmol FA released/h/mg protein to 0.092 ± 0.004 units. Similarly, higher concentrations of glucose stimulated significantly more FA release and glycerol release from liver pieces than that stimulated by lower concentrations of glucose. Glycerol release from liver pieces incubated in the presence of 10 mM glucose and 25 mM glucose was ca. 2-fold to 2.8-fold, respectively, higher than that from liver pieces incubated in the presence of either 2 mM or 5.5 mM glucose. Fatty acid release from liver pieces incubated in the presence of 10 mM or 25 mM glucose was ca. 1.8-fold higher than that from liver pieces incubated in the presence of either 2 mM or 5.5 mM glucose. Notably, increased glycerol release was not accompanied by a parallel increase in FA. Fatty acid reesterification was more pronounced in liver pieces exposed to higher glucose (10 mM and 25 mM) than in liver pieces exposed to lower glucose (2 mM and 5.5 mM). ^{14}C-incorporation studies indicated that glucose serves as a carbon source for reesterified FA in trout liver. The route of reesterification appears to be from glucose to glycerophosphate to phosphatidic acid to diacylglycerol to TG. Increasing concentrations of glucose did not affect glycerol kinase activity, indicating that glucose-stimulated lipolysis was not accompanied by increased glycerol recycling within the liver. These results suggest that glucose stimulates fatty acid reesterification and directly enhances net lipolysis in trout liver incubated *in vitro*.

Introduction

Lipolysis proceeds from the hydrolysis of stored triacylglycerol (TG) and results in the release of glycerol and FA. The extent of FA release may be limited by plasma albumin and a considerable amount of FA is reesterified (Fain 1980). Glycerol, however, is completely lost since intracellular recycling appears to be prevented by the absence of local glycerol phosphorylation in adipose tissue (Wie-

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land and Suyter 1957). In mammals and birds, most lipid is stored in adipose tissue. Details of adipose tissue lipolysis, mediated by TG or hormone-sensitive lipase, are generally well characterized (Fain 1980). Fish tend to store lipids among several depot organs, including mesenteric fat, liver, and dark muscle (Sheridan 1988). In salmonid fish, such as salmon and trout, lipid may comprise up to 6% (fresh weight basis) of the liver (Henderson and Tocher 1987). Hepatic lipolysis in trout proceeds in a manner similar to that in the adipose tissue of mammals and birds. Hydrolysis of TG is mediated by a neutral lipase which is catalytically activated upon phosphorylation (Harmon *et al.* 1991a). A number of hormones, including glucagon, appear to enhance lipolysis in trout liver (Sheridan 1988; Plisetskaya *et al.* 1989).

Recent studies in our laboratory have shown that metabolites such as glucose may play a role in modulating trout hepatic lipolysis. Lipolytic rates from liver incubated in the presence of glucose were higher than those observed in liver incubated in the absence of glucose (Harmon and Sheridan 1992). In addition, injection of glucose into glucose intolerant trout resulted in elevated plasma FA accompanied by increased hepatic lipolytic activity (Harmon *et al.* 1991b). Hyperlipidemia has also been reported in diabetic and obese (Garland and Randle 1962; Jansson *et al.* 1990) subjects.

In the present study, we used rainbow trout to characterize further the influence of glucose on hepatic lipolysis. Our hypothesis was that glucose directly enhances net lipolysis in the liver.

Materials and methods

Experimental animals

Yearling rainbow trout (mean weight ± SEM, 100 ± 10 g) of both sexes were obtained from Garrison National Fish Hatchery near Riverdale, ND and maintained at North Dakota State University in dechlorinated municipal water (11°C) under 12L: 12D photoperiod. Fish were fed *ad libitum* twice daily with Glencoe Mills Trout Grower (Glencoe, MN) except 24–40 h prior to experimentation. Animals were anesthetized in buffered 0.01% (w/v) tricaine methanesulfonate (MS 222) and the liver removed, perfused with 0.75% NaCl, and prepared for *in vitro* culture.

In vitro *experiments*

In vitro experiments were carried out on rainbow trout liver pieces prepared as described previously (Harmon and Sheridan 1992a). Briefly, liver was delicately sliced into 1-mm^3 pieces, placed into Hanks medium, and preincubated in darkness with a gyratory shaker (150 rpm) under 100% O_2 at 14°C. After 1 h, the pieces were centrifuged (270 × g for 5 min at 14°C) and washed three times by resuspension-centrifugation. Finally, liver pieces were transferred to plastic multiwell (6-well, Falcon 3846 or 24-well, Falcon 3047) culture plates for experimentation and incubated at 14°C under 100% O_2. Liver pieces remain viable, based on O_2 consumption studies, for well over 5 h and have proved to be suitable for short-term culture based upon hormone responsiveness (Harmon and Sheridan 1992a).

One set of experiments involved the incubation of liver pieces (ca. 45 mg) in 1 ml (per well; 24 well plates) of Hanks solution [5.4 mM KCl, 4.0 mM $NaHCO_3$, 1.7 mM $CaCl_2$, 0.8 mM $MgSO_4$, 0.5 mM KH_2PO_4, 0.3 mM Na_2HPO_4, 10 mM HEPES, pH 7.6, 0.24% (w/v) bovine serum albumin] containing one of the following: 2 mM glucose and 138.25 mM NaCl, 5.5 mM glucose and 137 mM NaCl, 10 mM glucose and 134.75 mM NaCl, or 25 mM glucose and 127.25 mM NaCl. The incubation occurred over a period of 5 h in which the medium was removed and replaced with fresh at 0, 1, 2, 3, and 5 h. The medium and liver pieces were stored at −90°C for later analyses, usually within one week.

Another set of experiments involved the incubation of liver pieces (ca. 262 mg) in 2 ml (per well; 6-well plates) of Hanks solution containing one of the varying glucose-NaCl combinations plus the addition of 0.5 μCi/ml U-^{14}C-glucose (specific activity 340 mCi/mmol; New England Nuclear, Boston, MA) (cumulative incorporation studies) or 1.0

μCi/ml U-^{14}C-glucose (pulse-chase studies). In cumulative incorporation studies, liver pieces were incubated in the presence of label for a period of up to 5 h. In pulse-chase studies, liver pieces were incubated in the presence of label for a period of 45 min (pulse); after which, the medium from each well was removed, and the pieces were thoroughly rinsed and the wells refilled with Hanks medium without label (t_0). Incubation in the presence of cold medium proceeded for a period of up to 5 h (chase). In all studies, incubation was terminated by rinsing liver pieces thoroughly with the medium in which they were incubated (without label) followed by lipid extraction.

Biochemical analyses

Prior to analyses of medium metabolites, medium samples were deproteinated by heat treatment (65°C for 10 min) followed by centrifugation (16,000 × g for 10 min). Medium FA levels were measured by the micromethod of Noma *et al.* (1973). Glycerol release was measured by a modification of the glycerol dehydrogenase method (Worthington 1988). Thio-NAD reduction (A_{405}) was determined with a Bio-Rad (Richmond, CA) microplate reader. Assay conditions were as follows: sample or standard (50 μl), 100 μl assay buffer [potassium-carbonate-bicarbonate buffer (0.205 M, pH 10.0): ammonium sulfate (0.65 M); freshly made in the ratio of 98.4:1.6], 25 μl of enzyme (glycerol dehydrogenase, 7 units activity/ml in 4.76 mM potassium phosphate buffer containing 9.52 mM ammonium sulfate and 9.52 mM manganese chloride, pH 7.6) and 25 μl of thio-NAD (10 mM in assay buffer).

Lipid extraction was performed essentially as described by Bligh and Dyer (1959). Analyses of ^{14}C-incorporation products into the aqueous and organic phases were performed by thin-layer chromatography (TLC; silica gel 60, Merck). Components of the organic phase were separated using a 2 solvent system [solvent 1: diethyl ether, benzene, ethanol, acetic acid (40:50:2:0.2), and solvent 2: diethyl ether, hexane (5:95)] (modified from Freeman and West, 1966). Polar lipids (PL) were separated by TLC using chloroform: methanol: H_2O (65:25:4) (Allen, 1974). Components of the aqueous phase were separated using a solvent system containing n-propanol, acetic acid, water (8:1:1) (Block *et al.* 1955). Components containing ^{14}C were identified by autoradiography. Kodak X-OMAT RP (XRP-5) film was placed over TLC plates and the film was exposed in darkness for 4 weeks. Polar lipid autoradiograms were quantified using one-dimensional laser densitometry (Biomed Instruments, Fullerton, CA). Following autoradiography, all components were visualized by charring the TLC plates with sulfuric acid:H_2O (50:50, v/v). In some experiments, organic components were scraped individually (after identification under UV; sprayed lightly with 0.06% [w/v, in methanol] 2,7-dichlorofluoroscein) from TLC plates into glass vials, redissolved, dried, and quantified by liquid scintillation counting (LSC; after dissolution in 11.9 ml Scintisol, Isolab Inc., Akron, OH) or saponified and quantified by LSC according to Sheridan *et al.* (1985).

Hepatic lipolytic enzyme activity was determined as described by Harmon *et al.* (1991a). In this assay, partially purified (resuspended 20% ammonium sulfate fraction) lipase enzyme was incubated in the presence of substrate ([carboxyl-^{14}C]-triolein; New England Nuclear, Boston, MA) for 2 h at 14°C. Glycerol kinase was assayed by a coupled enzymatic method essentially as described by Bublitz and Wieland (1962) which results in NADPH production. To each cuvette was added 1 ml assay buffer (0.002 M $MgCl_2$ in 1 M hydrazine-0.2 M glycine buffer, pH 9.8), 50 μl 10 mM ATP, 50 μl 10 mM NAD, 20 μl glycerophosphate dehydrogenase (3.7 units/ml), 100 μl enzyme solution [liver homogenized (1:5) in 50 mM Tris-HCl, with 5 mM EDTA, pH 7.6; centrifuged at 16,000 × g for 20 min]. The reaction was started by addition of 50 μl 0.1 M glycerol and A_{340} monitored continuously for 10 min. Protein was determined by the protein-dye binding method (Bradford 1976) using a Bio-Rad microtiter plate reader (*c.f.*, Bio-Rad Technical Bulletin 1177).

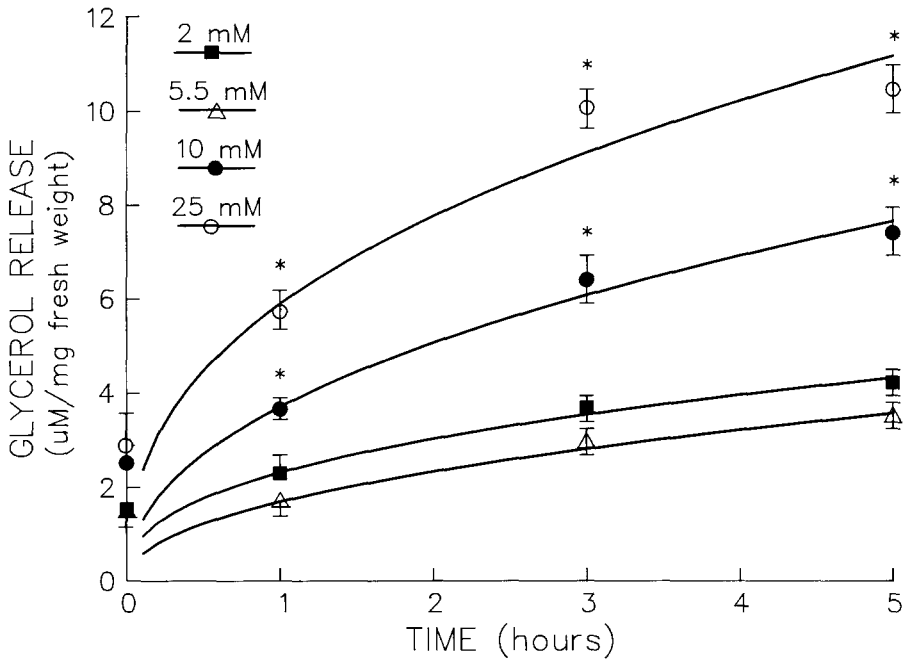

Fig. 1. Effects of various concentrations of glucose on glycerol release from liver removed from fed fish and incubated for 5 h. Data are presented as means ± SEM (n = 12); * $p < 0.05$ compared to 2 mM glucose group.

Table 1. Effects of various concentrations of glucose on the activities of triacylglycerol lipase and glycerol kinase[a]

Glucose concentration (mM)	Triacylglycerol lipase activity[b]	Glycerol kinase activity[c]
2	0.075 ± 0.002	0.23 ± 0.01
5.5	0.081 ± 0.003	0.26 ± 0.03
10	0.088 ± 0.003*	0.22 ± 0.02
25	0.092 ± 0.004*	0.18 ± 0.02

[a] Data presented as means ± SEM (n = 12); * $p < 0.05$ compared to 2 mM glucose group; [b] enzyme activity expressed as nmoles FA released/h/mg protein; [c] enzyme activity expressed as μmoles NADH formed/min/mg protein.

Statistics

Results are expressed as means ± SEM. Statistical differences were estimated by analysis of variance. Multiple comparisons among means were made by the Student-Newman-Keuls test; alpha was set at 0.05.

Results

Effects of glucose on hepatic lipid mobilization

The effects of glucose on hepatic lipid mobilization were determined on tissues removed from fed fish and incubated for 5 h. Lipolysis, determined by measuring hepatic TG lipase activity, was enhanced by the presence of high concentrations of glucose within the medium (Table 1).

Lipid mobilization was also indicated by the presence of medium metabolites. In general, the presence of high concentrations (10 mM and 25 mM) of glucose within the medium stimulated significantly more glycerol release and fatty acid release from the liver pieces than low concentrations (2 mM and 5.5 mM) of glucose. Glycerol release from liver pieces incubated in the presence of 10 mM glucose and 25 mM glucose was *ca.* 2-fold to 2.8-fold higher, respectively, than that of liver pieces incubated in the presence of either 2 mM or 5 mM glucose (Fig. 1). Fatty acid release from liver pieces incubated in the presence of 10 mM or 25 mM glucose was *ca.* 1.8-fold higher than that of liver pieces incubated in the presence of either 2 mM

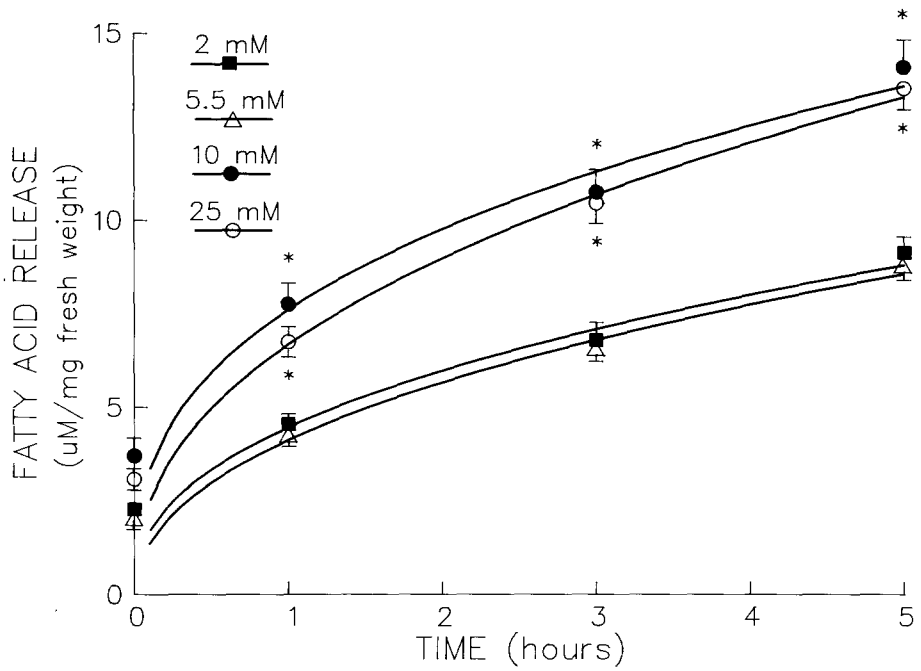

Fig. 2. Effects of various concentrations of glucose on fatty acid release from liver removed from fed fish and incubated for 5 h. Data are presented as means ± SEM (n = 12); * $p < 0.05$ compared to 2 mM glucose group.

Table 2. Fatty acid:glycerol ratios resulting from liver pieces incubated in various concentrations of glucose

Incubation period (h)	Medium glucose concentration (mM)			
	2	5	10	25
1	2.0:1	2.6:1	2:1	1.2:1
3	1.9:1	2.3:1	1.6:1	1.1:1
5	2.4:1	2.7:1	1.8:1	1.3:1

or 5.5 mM glucose (Fig. 2). Notably, glucose-stimulated FA release did not increase in parallel with glucose-stimulated glycerol release – indicative of increased FA reesterification, as estimated by FA:glycerol ratios, at higher glucose concentrations. Fatty acid:glycerol ratios resulting from the incubation of liver pieces in various concentrations of glucose are shown in Table 2.

Recycling of glycerol was evaluated by glycerol kinase activity. The presence of various concentrations of glucose within the medium appeared to have no effect on glycerol kinase activity – indicating that glucose was stimulating net lipolysis without appreciable glycerol recycling (Table 1)

Incorporation of ^{14}C-glucose into hepatic lipids

Glucose incorporation over time was determined in liver removed from fed fish and incubated in normal glucose-containing medium (5.5 mM). Fig. 3 indicates that ^{14}C-glucose incorporation into liver increases with incubation time for up to 3 h; after which, label incorporation into aqueous and organic components stabilizes. Within the total organic fraction, a majority of the ^{14}C-glucose was incorporated into nonsaponifiable elements (Fig. 3).

Analytical TLC followed by autoradiography of the total organic fraction revealed that label was incorporated into polar lipids (PL) and acylglycerols (Fig. 4); the only nonsaponifiable component detected was glycerol. ^{14}C-glucose was incorporated

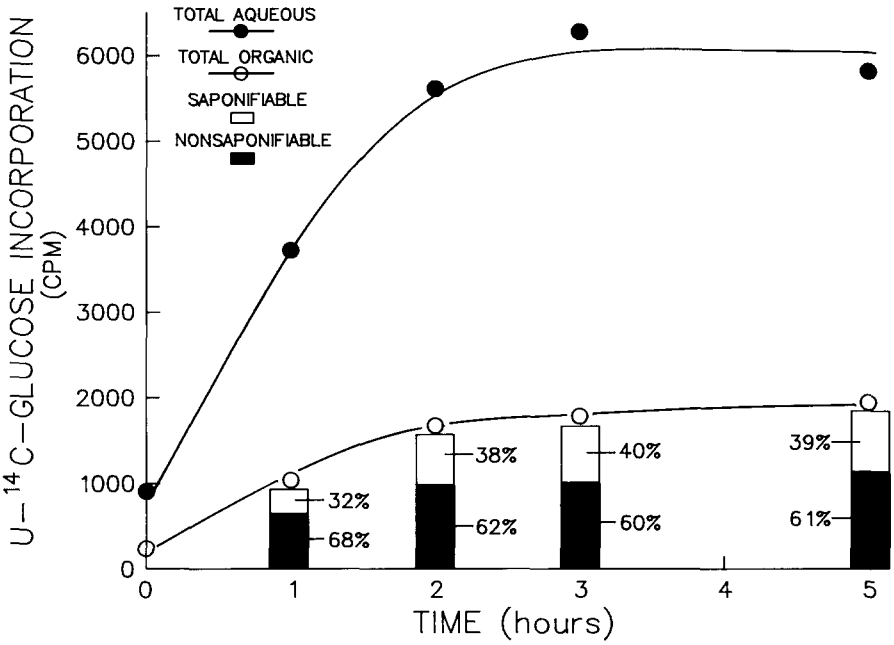

Fig. 3. Time-course of ^{14}C-glucose incorporation into total aqueous and total organic components of liver removed from fed fish and incubated *in vitro* (5.5 mM glucose). Liver pieces were incubated with 0.5 μCi U-^{14}C-glucose for the times indicated and extracted and analyzed as described in Materials and methods. Saponifiable and nonsaponifiable elements of the total organic fraction are expressed as percent of total organic incorporation. All data presented represent the means of duplicate determinations.

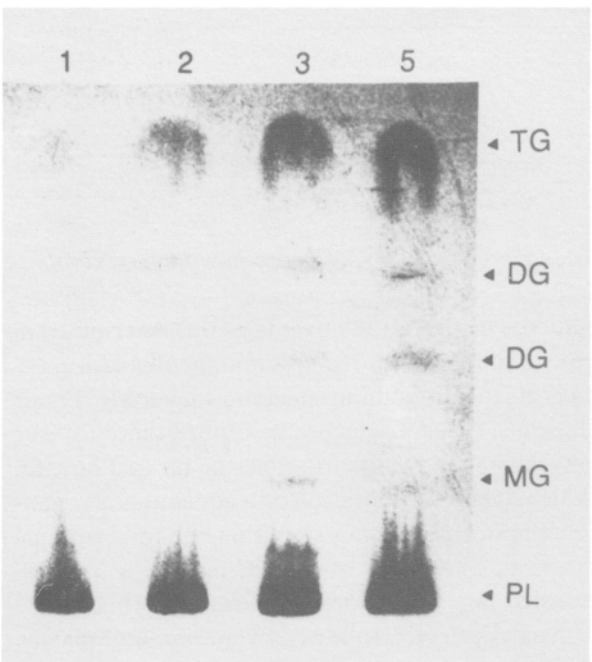

Fig. 4. Autoradiogram of organic phase components separated by thin-layer chromatography. Bands indicate the presence of ^{14}C-glucose incorporation into liver incubated *in vitro* (5.5 mM glucose with 0.5 μCi U-^{14}C-glucose) for the times indicated. TG, triacylglycerols; DG, diacylglycerols; MG, monoacylglycerols; PL, polar lipids.

into higher acylglycerols in a time-dependent manner (Fig. 4).

Analysis of the PL fraction indicated the presence of several polar components, including: phosphatidic acid, phosphatidyl serine, sphingomyelin, phosphatidyl choline, and lysophosphatidyl choline (Fig. 5). Label incorporation into phosphatidic acid represented 27.6% of the total ^{14}C-glucose incorporated into PL within 1 h of incubation.

^{14}C-glucose flux through the various components of the organic fraction was analyzed in a pulse-chase fashion (Table 3). After an initial 45 min pulse, a majority of the label was incorporated into the phosphatidic acid-containing PL fraction. In the chase part of the experiment, label moved into the DG and TG fractions, and out of the PL fraction. After a 1 h chase, label incorporation in-

Table 3. ^{14}C-glucose flux through organic components of liver incubated *in vitro*[a]

Lipid class	Time after pulse (h)				
	0	1	2	3	5
TG[b]	14	32	39	39	42
DG	4	15	6	6	5
MG	2	2	2	2	2
FA	3	3	3	3	3
PL	77	48	49	49	47

[a] Liver pieces were incubated in U-^{14}C-glucose-containing Hanks medium (with 5.5 mM glucose) for 45 min (pulse); after which, the pieces were rinsed thoroughly and resuspended in Hanks medium without label, and incubated for the various times indicated (chase). Data presented as percent total incorporation into total organic components of duplicate determinations; [b] abbreviations: TG, triacylglycerols; DG, diacylglycerols, MG, monoacylglycerols; FA, fatty acids; PL, polar lipids.

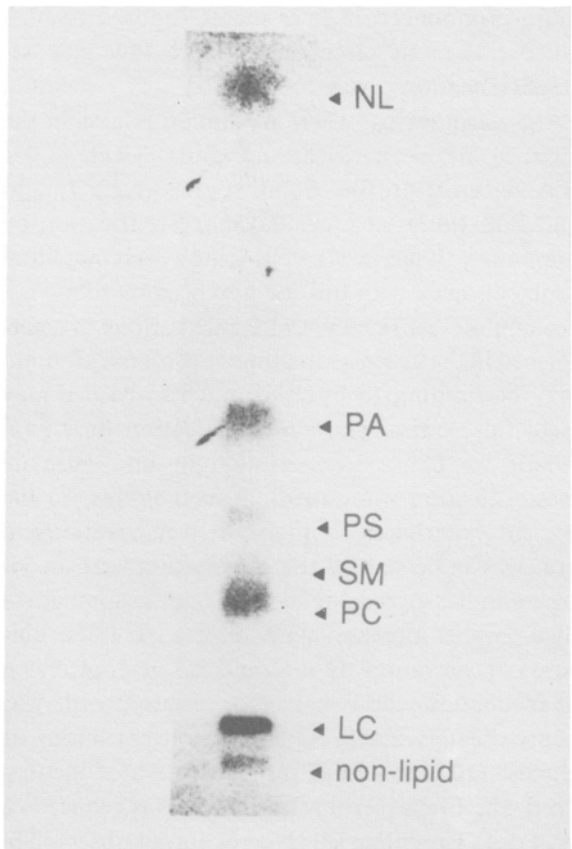

Fig. 5. Autoradiogram of polar lipid components separated by thin-layer chromatography. Bands indicate the presence of ^{14}C-glucose incorporation into liver incubated *in vitro* (5.5 mM glucose with 0.5 μCi U-^{14}C-glucose) for 1 h. NL, neutral lipid; PA, phosphatidic acid; PS,phosphatidyl serine; SM, sphingomyelin; PC, phosphatidyl choline; LC, lysophosphatidyl choline.

creased nearly 275% and 128%, respectively, into the DG and TG fraction. After a 2 h chase, label continued to move into the TG fractions, while label incorporation into the DG fraction decreased. Only slight label incorporation into the FA and monoacylglycerol (MG) fractions was observed; this pattern was unchanged over the course of the chase period.

The effects of the presence of various concentrations of glucose on the incorporation of ^{14}C-glucose into hepatic lipids were determined in liver removed from fed fish and incubated for 5 h. Components of the organic and aqueous phases of liver homogenates were separated by TLC and autoradiographed to detect the presence of ^{14}C-glucose incorporation (Figs. 6 and 7). In the presence of low concentrations of glucose (2 mM and 5.5 mM), the autoradiograms indicated that ^{14}C-glucose was still present in the tissue to a large extent, that considerable ^{14}C-incorporation into glycerophosphate was present, and that ^{14}C-incorporation into PL, including phosphatidic acid (PA), diacylglycerol (DG), and TG, was present in low amounts (Figs. 6 and 7). In the presence of high glucose concentrations (10 mM and 25 mM), the autoradiograms indicated that the ^{14}C-glucose had been cycled into the reesterification system and that increased reesterification products were observed upon increasing glucose concentrations. Notably, lower amounts of ^{14}C-incorporation into glycerophosphate was evident while substantial amounts of incorporation into PL was detected. Label incorpora-

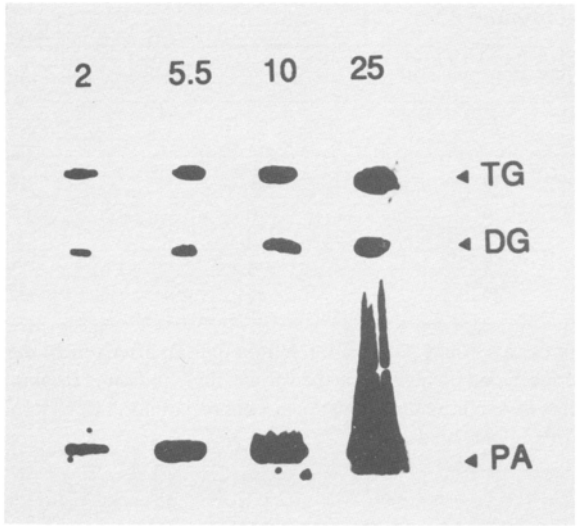

Fig. 6. Autoradiogram of organic phase components separated by thin-layer chromatography. Bands indicate the presence of ^{14}C-glucose incorporation into liver incubated (5 h) in various concentrations (2 mM, 5.5 mM, 10 mM and 25 mM) of glucose (with 0.5 μCi U-^{14}C-glucose). TG, triacylglycerols; DG, diacylglycerols; PL, polar lipids.

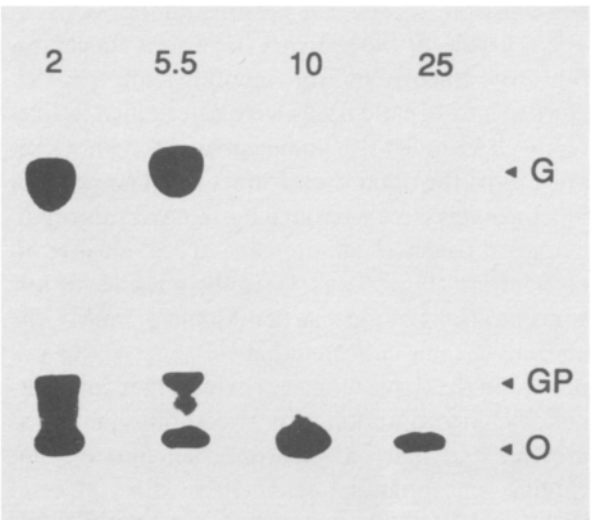

Fig. 7. Autoradiogram of aqueous phase components separated by thin-layer chromatography. Bands indicate the presence of ^{14}C-glucose incorporation into liver incubated (5 h) in various concentrations (2 mM, 5.5 mM, 10 mM and 25 mM) of glucose (with 0.5 μCi U-^{14}C-glucose). O, origin (unknown); GP, glycerophosphate; G, glucose.

tion into the DG and TG fractions also occurred in increased amounts (Fig. 7).

Discussion

The results of the present study support our starting hypothesis and indicate that glucose directly enhances lipolysis in the liver of rainbow trout. Glucose-stimulated lipolysis was manifested as increased glycerol and fatty acid release into culture medium as well as by increased TG lipase activity. These observations confirm our previous report that mobilization of lipid from trout liver involves two steps: hydrolysis of TG and release of lipolytic products (Harmon *et al.* 1991a).

Fatty acid reesterification in trout liver is promoted by glucose. This conclusion is based on the observation that fatty acid reesterification was more pronounced in liver pieces exposed to high glucose than in pieces exposed to low glucose. Reesterification was estimated by medium FA:glycerol ratios, where maximum release in the absence of reesterification would yield a 3:1 (FA:glycerol) profile. Some studies (*e.g.*, Jungas and Ball 1963) have evaluated reesterification by examining tissue levels of FA; however, potential problems arise with this method because tissue FA accumulate artificially, since incubations are performed in the absence of albumin. Because albumin may be limiting to lipolysis and its absence may yield false estimates of reesterification (*cf.*, Fain 1980), we chose to use albumin and estimate reesterification using medium metabolites. In the present experiments with trout liver, reesterification was indicated by the observation that an increase in net glycerol release was not accompanied by a parallel increase in FA release. At lower glucose concentrations (2 mM and 5.5 mM; after 5 h of incubation) the FA:glycerol release profile was approximately 2.5:1. At higher concentrations of glucose (10 mM and 25 mM; after 5 h of incubation), the FA:glycerol release ratio was reduced to *ca.* 1.5:1. The enhanced glycerol output observed in glucose-exposed cultures suggests that glucose is stimulating net lipolysis by favoring the hydrolysis of TG to glycerol. The importance of glycerol to net lipolysis has been addressed in a number of mammals, including humans (Shafrir and Gorin 1963; Jansson *et al.* 1990). Our current observations suggest that glycerol also is a good indicator of net

lipolysis in trout liver. The FA resulting from lipolysis appear retained to a greater extent in high glucose-exposed trout liver and are most likely reesterified. Several studies on mammalian adipose tissue also have suggested that glucose-stimulated lipolysis may be due to increase reesterification of FA with a subsequent decrease in the tissue FA pool (Jungas and Ball 1963; Bally *et al.* 1965; Carlson 1967), although other reports discount this possibility (Efendic and Ostman 1971; Ho *et al.* 1970; Schimmel and Goodman 1971).

The present ^{14}C incorporation studies indicate that glucose serves a carbon source for reesterified FA in trout liver. This contention is supported by several observations. First, labeled glucose is incorporated into the organic fraction in a time-dependent manner. Second, a majority of the labeled material is nonsaponifiable. The only nonsaponifiable organic material detected was glycerol, indicating that the glycerol-containing organic fraction was glucose-derived. Lastly, the abundance of ^{14}C-label in the TG fraction coupled with the observation that labeled TG was more abundant in liver cultured in high glucose, suggests that glucose-derived acylglycerol glycerol increases in the presence of high glucose concentrations. The route of reesterification in trout liver appears to be from glucose to glycerophosphate to PA to DG to TG. This contention is supported by labeled carbon flux from aqueous (glycerophosphate) to organic elements (PA-containing polar lipid fraction, DG, TG) as well as by the pulse-chase label flux studies (*cf.*, Table 3). This pathway has been shown to be a major pathway of TG synthesis in animals (Allen 1976). Fatty acids resulting from lipase-mediated hepatic hydrolysis in trout (Harmon *et al.* 1991a) at any of the steps from TG to DG, or DG to MG, or MG to glycerol and FA, could be reesterified to PA or DG. The presence of label observed in the PA-containing polar lipid and DG fractions further support such a reesterification route in trout liver. It should be noted that other carbon flux patterns could be operating in the liver of trout as well. Certainly, we did not evaluate all the fates of glucose (oxidation, etc.) in the present study and, of course, the liver is known to be quite lipogenic (*cf.*, Sheridan *et al.* 1985), utilizing glucose, acetate and lactate for fatty acid synthesis. This would account for the presence of label in the saponifiable organic elements observed in the present study.

Lipolysis is influenced by TG hydrolysis, FA reesterification and glycerol recycling within the cell. Glycerol recycling is regulated by glycerol kinase-mediated phosphorylation. In adipose tissue, glycerol recycling was initially thought to be negligible (Wieland and Suyter 1957) with most glycerol being released from the cell. As a result, glycerol is considered to be the best indicator of net lipolysis (*cf.*, Wolfe and Peters 1987). Some reports, however, have suggested that glycerol kinase activity is a significant factor in lipolysis (Robinson and Newsholme 1967). These researchers believe that the increased utilization of FA could be explained equally well by a decrease in glycerol kinase activity as by an increase in lipase activity (Robinson and Newsholme 1967). In trout liver we measured rates of glycerol kinase activity that were comparable to those obtained in rat adipose tissue (Robinson and Newsholme 1967). Notably, trout hepatic glycerol kinase activity was not affected by glucose concentration. This suggests that glucose was primarily affecting lipolysis and reesterification.

Glucose-stimulated lipolysis also has been reported in mammalian adipose tissue. The presence of glucose in culture medium was reported to promote glycerol release from human adipose tissue (Efendic and Ostman 1971) as well as from rat adipose tissue and adipocytes (Jungas and Ball 1963; Carlson 1967; Chlouverakis 1976; Ho *et al.* 1970; Naito and Okado 1975). Jungas and Ball (1963) observed that enhanced glycerol release was accompanied by diminished FA production and proposed that the rate of reesterification may result from the ability of glucose to supply glycerophosphate and PA. The present data confirm this hypothesis; this is particularly evident from the abundance of labeled PA-containing polar lipids in liver incubated in high concentrations of glucose. A major difference between the present results and those of Jungas and Ball (1963) is that in trout liver glucose-stimulated lipolysis results in both FA and glycerol release, whereas in rat epididymal adipose tissue, glucose-stimulated lipolysis resulted in enhanced

glycerol and in reduce FA production. The basis for the difference between these two systems is not known, but perhaps the presence of albumin in the trout system augmented FA release. The manner by which the lipolytic process is stimulated by glucose, however, remains an enigma. Jungas and Ball (1963) suggested "that by furnishing glycerophosphate for the reesterification process, glucose lowers the levels of free fatty acids within the tissue to such an extent that lipolysis and hence glycerol production are favored." The present data from trout liver support such a contention in view of the enhanced degree of FA reesterification accompanied by elevated net glycerol production.

Glucose-stimulated hepatic lipolysis in trout helps to explain the results of a number of previous experiments. For example, glucose was found to enhance basal lipolytic activity (Harmon and Sheridan 1992a) as well as enhance glucagon-stimulated lipolytic activity (Harmon and Sheridan 1992b). Glucose-derived glycerol enhancement of reesterification would contribute to the increased net glycerol output observed in these experiments. Similarly, glucose-stimulated lipolysis could, in part, underlie the hyperlipidemic pattern resulting from glucose injected into glucose-intolerant trout (Harmon *et al.* 1991b). Notably, hepatic TG lipase activity was elevated during the period of hyperglycemia (Harmon *et al.* 1991b).

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