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Characterization of glycerol uptake and glycerol kinase activity in rat hepatocytes cultured under different hormonal conditions

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Abstract

Glycerol uptake and glycerol kinase activity were studied in primary cultures of rat hepatocytes in the presence of either 1 nM insulin, 1 nM glucagon, or 100 nM dexamethasone, alone or in combination in the culture medium. Glycerol uptake exhibited saturation kinetic with K_m values (μM) and V_{max} (nmol/min × mg protein) ranging from 250–402, and 7.9–10.1, respectively. The corresponding K_m and V_{max} values for glycerol kinase activity were 36–46 and 8.7–12,7. Using the metabolic uncoupler 2,4-dinitrophenol, glycerol uptake and the cellular content of glycerol phosphorylated metabolites were reduced 33% and 43%, respectively, whereas no decrease in the cellular content of glycerol was seen. The glycerol analogues monoacetin, monobutyrin and dihydroxypropyl dichloroacetate were able in a concentration-dependent manner to inhibit glycerol uptake into hepatocytes with the two latter having IC_{50} values of approximately 1 mM. Moreover, it was demonstrated that the three glycerol analogues were substrates for glycerol kinase, which indicates a competitive mode of inhibition. The kinetic parameters for these substrates were calculated by using glycerol kinase from Candida Mycoderma. Monobutyrin was found to be 4 times lees efficient as substrate compared to the other substrates. Overall, these results indicate that independently of the culture conditions, glycerol uptake is the rate-limiting step in glycerol metabolism, and that the investigated glycerol analogues are metabolized via the same route as glycerol. © 1998 Elsevier Science B.V.

Keywords: Glycerol uptake; Cultured hepatocyte; Glycerol kinase; Inhibitor; Hormone

1. Introduction

Plasma glycerol is made available from lipolysis of triglycerides in adipose tissues and from hydrolysis of dietary fat. In glycerol metabolism the liver plays a pivotal role, since it is responsible for 70–90% of the total body capacity for glycerol utilization [1,2]. After uptake, glycerol is irreversibly converted by glycerol kinase (GK) to glycerol-3-phosphate (G-3-P). This compound acts as a carrier for reducing equivalents from the cytosol to the mitochondria as part of the glycerol phosphate shuttle [1,3], constitute the backbone in triglycerides [2], and provides carbon skeleton for gluconeogenesis [4–6]. Glycerol is a unique gluconeogenic substrate as it provides new carbons for gluconeogenesis [6,7], in contrast to lactate and alanine whose carbons originate directly from plasma

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glucose [8,9]. In non-insulin-dependent diabetes mellitus (NIDDM), it has been shown that the appearance of glycerol in plasma is elevated due to increased lipolysis. Moreover, the conversion of glycerol to glucose accounts for approximately 80% of the total glycerol turnover in diabetes as opposed to only 40–60% under normal conditions [4,5]. However, the increase in glycerol-derived gluconeogenesis appears not to be exclusively a result of accelerated lipolysis, but also of altered intrahepatic handling of glycerol [4,5].

Glycerol uptake has previously been studied in tumor cells [10], freshly isolated rat hepatocytes [11], and in perfused liver [12]. However, remarkably little is known about glycerol uptake into liver cells, how uptake is regulated by hormones, and how its availability as a precursor for the synthesis of glucose might be dependent upon uptake or the activity of GK in the liver cells. To gain knowledge about this, hepatocytes were cultured in the presence of different concentrations of insulin, glucagon and glucocorticoids (dexamethasone). Glycerol uptake in the presence or absence of the metabolic uncoupler 2,4-dinitrophenol (DNP) and GK activity were then measured to see whether glycerol uptake or GK activity is rate-limiting for glycerol metabolism. In addition, it was investigated whether the structural analogues of glycerol, monoacetin (MA), monobutyrin (MB) and 2,3-dihydroxypropyl-dichloroacetate (dichloro-MA), had any effects on glycerol uptake and GK activity. Dichloro-MA and MA have previously been shown to alter glycerol metabolism [11,13].

2. Materials and methods

2.1. Materials

Adult male Wistar rats (ca. 200 g) were obtained from Møllegård Breeding Center (Denmark). Plastic tissue culture dishes were purchased from Nunc (Denmark) and foetal calf serum, culture medium 199 (cat no. 3115-022), penicillin, and streptomycin from Life Technologies (Denmark). Collagen was obtained from Sigma Chemical Company, St. Louis, MO (USA), enzymes and co-enzymes from Boehringer, Mannheim (Germany) and Whatman filters DE-81 were obtained from Whatman, Maidstone (England). Insulin and glucagon were from Novo Nordisk, Bagsværd (Denmark), dexamethasone from Merck, Sharp and Dome, Haarlem (The Netherlands) and [^{3}H]glycerol (specific activity 72.5 Ci/mmol) was purchased from Dupont-New England Nuclear, Frankfurt (Germany). Monoacetin was purchased from Avocado, Lancashire (UK) and monobutyrin from Acros, Pittsburgh (USA). 2,3-Dihydroxypropyl dichloroacetate [13] was prepared via reaction of isopropylidene glycerol with dichloroacetyl chloride followed by hydrolysis (15% aq. formic acid/acetone, reflux 1 h).

2.2. Hepatocyte cultures

Hepatocytes were prepared essentially as described by Dich and Grunnet [14]. The isolated cells, of which more than 85% excluded trypan blue, were suspended in Medium 199 containing 5.5 mM glucose, 4% foetal calf serum and supplemented with 1 nM insulin plus 100 nM dexamethasone (condition A), 1 nM glucagon plus 100 nM dexamethasone (condition B), 1 nM insulin alone (condition C), 100 nM dexamethasone alone (condition D), 1 nM glucagon alone (condition E) or without addition of hormones (condition F). The cell suspension was plated onto collagen-coated 35 mm Petri dishes (2 ml/dish; 0.55 million cells/ml) or in 24 multiwell dishes (1.5 ml/dish; 0.15 million cells/ml). After 3 h, and subsequently every day, the medium was changed with the same medium except that the serum was omitted. Cells were cultured for 2 days.

2.3. Glycerol uptake

Hepatocytes for studies of glycerol uptake were cultured in 24 multiwell dishes. Transport studies were performed essentially as described for amino acids [15,16] by exchanging the culture medium with phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 7.3 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$ and 6 mM glucose, pH 7.35) containing different concentrations of radiolabelled glycerol (range 1–1000 μM). An incubation period of 1 min was used after demonstrating that uptake was linear for at least 2 min (results not shown). Uptake was terminated by rapid washes with 1 ml ice cold PBS twice. No radioactivity was lost

from the cells during washing. The effects of different glycerol analogues (range 100–2000 μM) on glycerol uptake were studied in the presence of 100 μM [^{3}H]glycerol. After termination of uptake, cellular levels of radioactivity and protein content were determined. In all experiments, radioactivity present in cultures briefly exposed (1–2 s) to [^{3}H]glycerol at 0°C was subtracted from the experimental values obtained at 37°C. In some experiments hepatocytes cultured in 35 mm Petri dishes were exposed to 100 μM DNP in PBS 5–10 min prior to uptake. The medium was then changed to the same medium added 0.5 mM [^{3}H]glycerol for 1 min. Uptake was terminated as described above by rapid washes with PBS followed by addition of 200 μl 70% ethanol. The culture dishes were placed at 37°C to evaporate the ethanol (10–15 min). Then 250 μl water was added to each dish and cells were scraped of and placed in test tubes and centrifuged at 10 000 rpm for 2 min. 50 μl of the supernatant was spotted onto a 2.5 mm diameter DE-81 Whatman filter. The filters were washed 4 times with 1 ml water. Radioactivity adhering to the filters was considered as glycerol phosphorylated metabolites (e.g. glycerol-3-phosphate, dihydroxyacetone-phosphate) and radioactivity passing through the filters was considered as glycerol. On the basis of the specific activity of glycerol in the incubation medium at the beginning of the experiment, the cellular content of glycerol and glycerol phosphorylated metabolites as well as the glycerol uptake were calculated.

The diffusion-related parts of the glycerol uptake were determined as previously described [17] using the equation: $v = (V_{max} \times [S])/(K_m + [S]) + k \times [S]$, where v indicates the velocity (nmol/min × mg protein), K_m the Michaelis–Menten constant (μM), k the rate constant of the non-saturable influx component (ml/min × mg protein), and [S] the substrate concentration (μM).

2.4. Glycerol kinase activity

After removal of the culture medium and rinsing with ice-cold PBS individual cultures were scraped of the 35 mm Petri dishes in a solution containing 1% KCl and 1 mM EDTA. Subsequently, the cells were homogenized and centrifuged for 2 min at 10,000 rpm and 50–100 μl aliquots the of supernatant were assayed for glycerol kinase activity at 37°C in a total assay volume of 350 μl using two different assay methods. Method I was a spectrophotometric assay running at pH 9.1 [18] employing glycerol-3-phosphate dehydrogenase and NAD^+ to convert glycerol 3-phosphate to dihydroxyacetone-phosphate and NADH after glycerol had been converted to glycerol-

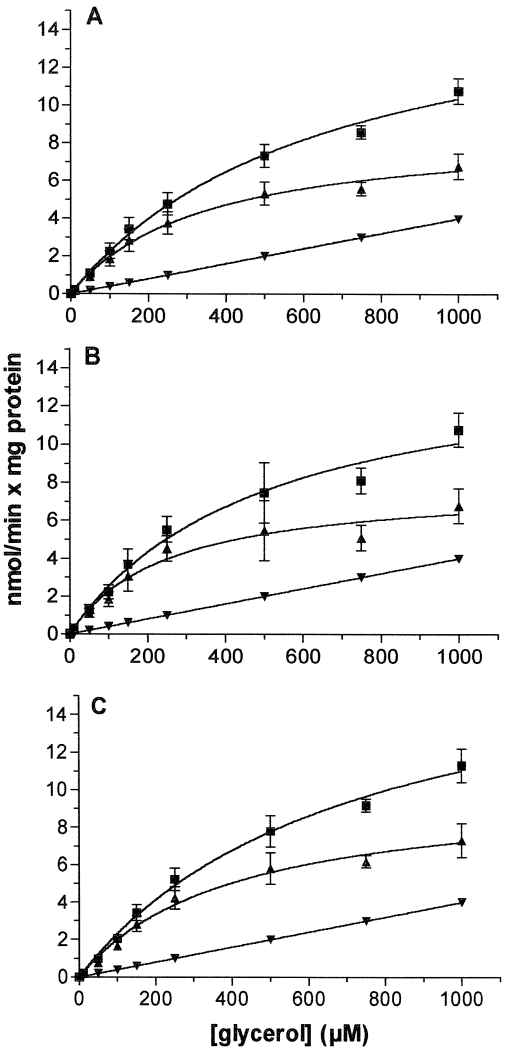

Fig. 1. Velocity of glycerol uptake (nmol/min × mg protein) in hepatocytes cultured in the presence of (A) 1 nM insulin (Ins) plus 100 nM dexamethasone (Dex), (B) 1 nM glucagon plus 100 nM Dex, or (C) 1 nM Ins alone. The experimentally obtained values for glycerol uptake (■) were resolved into saturable (▲) and non-saturable components of glycerol uptake (▼). The diffusion constant did not exceeded 0.004 ml/min × mg protein. Results are averages of four individual cell preparations with SEM indicated by vertical bars.

3-phosphate by GK. The structural analogues of glycerol, MA, MB and dichloro-MA (range 100–600 μM), were studied in the presence of 100 μM glycerol. Blanks were run in the absence of ATP. In some cases a 2% solution of the commercially available glycerol kinase from Candida Mycoderma was used at 30°C. Method II was a radioisotopic assay [11,19]. The assay mixture contained 25 mM HEPES-buffer (pH 7.4), 3 mM ATP, 2.5 mM $MgCl_2$ and varying concentrations of [^{3}H]glycerol (range 25–150 μM). The reaction was initiated at 37°C by addition of aliquots of the supernatants. After 5 min (blanks 1–5 s) the reaction was terminated by adding 150 μl of a 2 M glycerol solution (90°C) immediately followed by placing the reaction vials into a 90°C water bath for 5 min. The vials were then centrifuged at 10,000 rpm for 2 min and 20 μl of the supernatant spotted onto a 2.5 mm diameter DE-81 Whatman filter. The filters were washed 4 times with 1 ml water. Radioactivity adhering to the filters (G-3-P) was determined by liquid scintillation. Method II was used to study glycerol kinase activity vs. glycerol uptake, whereas Method I was used to characterize the glycerol analogues, since these could not be obtained in radio labelled form.

2.5. Protein and statistics

Protein contents were measured using the Bio-Rad DC Protein Assay using human serum albumin as the standard.

V_{max}, K_m and statistics (*t*-test, unpaired) were calculated using GraphPad Prism (GraphPad).

3. Results

3.1. Glycerol uptake

The kinetic characteristics of the uptake of glycerol into rat hepatocytes cultured for two days in the presence of either 1 nM insulin plus 100 nM dexamethasone (Dex) (condition A), 1 nM glucagon plus 100 nM Dex (condition B) or 1 nM insulin alone (condition C) are shown in Fig. 1. Glycerol uptake could be resolved into a saturable process, from which V_{max} and K_m were calculated (Table 1), and a non-saturable diffusion process. The diffusion process constituted less than 25% of the saturable uptake at glycerol concentrations below 250 μM. No differences among K_m and V_{max} values were observed. The Hill coefficients for glycerol uptake were (A) 1.1 ± 0.1, (B) 1.1 ± 0.1 and (C) 1.0 ± 0.1 for the different culture conditions.

In order to also compare the effect of Dex alone (condition D), glucagon alone (condition E) and no addition of hormones (condition F), uptake were also measured for conditions A–F at a fixed glycerol concentration of 0.5 mM. From Table 1 it can be seen, that glucagon and Dex alone but not in combination caused a slight decrease in uptake.

Table 1
Kinetic parameters for glycerol uptake and glycerol kinase activity in cultured rat hepatocytes

Condition	Glycerol uptake			Glycerol kinase activity		
	K_m (μM)	Vmax (nmol/min × mg protein)	$V_{0.5\ mM\ glycerol}$	K_m (μM)	V_{max} (nmol/min × mg protein)	V_{max} *
A: Ins + Dex	346 ± 79	8.8 ± 0.8	5.3 ± 0.3	36 ± 16	8.7 ± 1.3	11.9 ± 1.1
B: Gluc + Dex	250 ± 91	7.9 ± 1.1	5.8 ± 0.7	46 ± 22	9.3 ± 1.7	9.5 ± 1.6
C: Ins	402 ± 96	10.1 ± 1.1	5.8 ± 1.3	36 ± 15	10.0 ± 1.4	10.9 ± 1.2
D: Dex	nd	nd	3.4 ± 0.2 **	nd	nd	9.8 ± 0.8
E: Gluc	nd	nd	3.8 ± 0.5 **	nd	nd	10.6 ± 1.4
F:	nd	nd	4.9 ± 0.3	nd	nd	12.7 ± 0.9

K_m and V_{max} for glycerol kinase activity were determined by Method II and V_{max} * by Method I (see Section 2) in cultured hepatocytes. Cells were cultured for two days as described in Section 2 in the presence of 1 nM insulin plus 100 nM dexamethasone (condition A), 1 nM glucagon plus 100 nM dexamethasone (condition B), 1 nM Insulin alone (condition C), 100 nM dexamethasone alone (condition D), 1 nM glucagon alone (E) or without addition of hormones (condition F).

$V_{0.5\ mM\ glycerol}$ indicates uptake using 0.5 mM glycerol. Results are averages of three to eight individual cell preparations ± SEM. Statistically significant differences from conditions A (** $P < 0.01$) are given.

nd = not determined.

To see whether a reduction in cellular ATP content and thereby glycerol kinase activity could affect the glycerol uptake and the cellular contents of glycerol and its phosphorylated metabolites, assuming that uptake does not demand energy, cells were incubated in the presence or absence of 100 μM DNP. From Fig. 2 it can be seen, that in the presence of DNP glycerol uptake and the cellular content of phosphorylated metabolites were reduced by 33% and 44%, respectively. No effect on the cellular content of glycerol was observed.

The effects of the glycerol analogues MA, MB and dichloro-MA on glycerol uptake in the presence of 100 μM glycerol were also investigated in order to obtain information about the specificity of the transport system. These uptake experiments were performed using hepatocytes cultured as described for condition A. All three analogues (Fig. 3) were able to inhibit glycerol uptake. MB and dichloro-MA were the most potent with IC_{50} values of approximately 1 mM.

3.2. Glycerol kinase activity

Glycerol kinase is responsible for the conversion of glycerol to G-3-P. In order to study if GK activity rather than glycerol uptake is the rate limiting step in liver glycerol metabolism, the kinetic parameters K_m and V_{max} for GK were measured using Method II under the same culture conditions as described for glycerol uptake (conditions A, B, and C). As can be seen from Table 1, either the V_{max} or the K_m values were significantly affected by the different culture conditions. In addition, the specific activity ($V_{max}*$) was measured for culture conditions A–F using Method I (Table 1) and no differences in GK activities were observed.

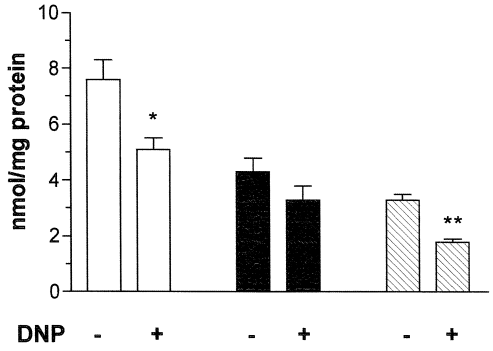

Fig. 2. Glycerol uptake (open bars), content of glycerol (black bars) and content of glycerol phosphorylated metabolites (hatched bars) in the absence or presence of 100 μM of the metabolic uncoupler 2,4 dinitrophenol (DNP). Glycerol uptake was determined in the presence of 500 μM [^{3}H]glycerol and the cellular contents of glycerol and glycerol phosphorylated metabolites were calculated from the specific activity of glycerol in the incubation medium at the start of the incubation. Results are averages of three individual experiments with SEM indicated by vertical bars. Statistical significant differences from control (−DNP) are given ($* P < 0.05$; $** P < 0.01$).

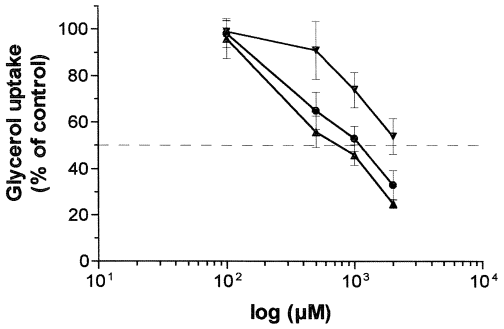

Fig. 3. Concentration–response curves for inhibition of glycerol uptake into cultured hepatocytes (condition A) by 2,3-dihydroxypropyl-dichloroacetate (●), monobutyrin (▲) and monoacetin (▼). Glycerol uptake was determined in the presence of 100 μM glycerol and increasing concentrations of inhibitors (range 0–2 mM). The control value (100%) corresponds to an uptake of 1.9 ± 0.4 nmol/min×mg protein. Results are averages of three to four individual cell preparations with SEM indicated by vertical bars. IC_{50} indicated by dashed line.

At 100 μM glycerol, the analogues MA and MB increased the velocity of GK concentration dependently (Table 2), suggesting that MA and MB either act as substrates for GK, or were able to activate the enzyme by increasing the affinity for glycerol. After excluding glycerol from the assay mixture, it was still possible to measure GK activity in the presence of MA or MB, in a concentration-dependent manner, although the activity was lower. Since the activity was measured as a coupled assay with glycerol-3-P dehydrogenase (GDH), control experiments revealed that MA and MB did not influence the activity of GDH (results not shown). On the basis of these observations, it was concluded that MA and MB act as substrates for GK, and that their phosphorylated products MA-P and MB-P act as substrates for GDH. These compounds were further characterized by us-

Table 2
Glycerol kinase activity in cultured rat hepatocytes in the presence of monoacetin and monobutyrin

	Glycerol kinase activity (nmol/min × mg protein)	
	100 μM glycerol	Minus glycerol
Control	6.0 ± 0.2	no activity
+ 100 μM MA	7.4 ± 0.3 *	5.7 ± 0.5
+ 200 μm MA	8.0 ± 0.3 * *	6.9 ± 0.5
+ 600 μm MA	9.2 ± 0.4 * * *	8.3 ± 0.6
+ 100 μM MB	7.1 ± 0.6	5.0 ± 0.2
+ 200 μM MB	8.0 ± 0.8	6.1 ± 0.7
+ 600 μM MB	8.9 ± 0.5 * *	8.2 ± 0.8

Activity of glycerol kinase in cultured hepatocytes determined by Method I (see Section 2) after addition of different concentrations of monoacetin (MA) and monobutyrin (MB) in the presence and absence of 100 μM glycerol. Results are average of three individual cell preparation ± SEM. Statistical significant differences from control are given for data where glycerol was present (mid column).
* $P < 0.05$; * * $P < 0.01$; * * * $P < 0.001$.

ing GK from Candida Mycoderma. From Fig. 4 it can be seen that using either dichloro-MA, MA or MB as substrates, the GK activity exhibited saturation kinetics with V_{max} values of 58–61 nmol/min × mg protein, which are higher than the V_{max} for glycerol (46 nmol/min mg protein). In contrast, the K_m value for glycerol (111 μM) was lower than the K_m values obtained with MA, MB and dichloro-MA as substrates (range 140–491 μM). All substrates resulted in Hill coefficients of 1.0 ± 0.1.

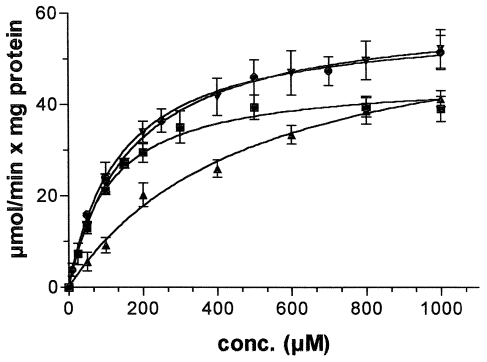

Fig. 4. Glycerol kinase (from Candida Mycoderma) activity using either glycerol (■), monoacetin (▼), dichloro-monoacetin (●) or monobutyrin (▲) as the substrate. The following kinetic parameters were calculated. V_{max} ± SEM (nmol/min × mg protein): 45.8 ± 1.3 (glycerol); 60.5 ± 0.8 * * * (monoacetin); 58.0 ± 0.7 * * * (dichloro-monoacetin); 61.5 ± 4.1 * * (monobutyrin). K_m ± SEM (μM): 111.4 ± 10.8 (glycerol);169.4 ± 8.0 * * (monoacetin); 140.5 ± 6.4 * (dichloro-monoacetin); 491.2 ± 72.7 * * * (monobutyrin). Results are averages of four to six individual experiments with SEM indicated by vertical bars. Statistical significant differences from glycerol are given (* $P < 0.05$; * * $P < 0.01$; * * * $P < 0.001$).

4. Discussion

The K_m values found for glycerol uptake are more than two fold higher than the range of normal plasma glycerol concentrations of about 60–100 μM [4,5]. This gives the liver a reserve capacity to take up transient increases of glycerol and support the notion that serum levels below 1 mM can be almost completely extracted by the liver [1,2]. The K_m values for uptake found in this study are two to four fold higher than the corresponding values found in Novikoff rat hepatoma cells [10], and freshly isolated hepatocytes [11]. The obtained K_m values for GK activity were approximately one order of magnitude lower than those for uptake, whereas V_{max} for GK and glycerol uptake were similar. If the efficiency of glycerol uptake under the different culture conditions is expressed as the ratio between V_{max} and K_m, a value of 0.03 is obtained. Comparing this value with similar ratios for glycerol kinase (0.20–0.28), it can be concluded that the efficiency of glycerol uptake only constitutes 10–15% of that of GK activity. This indicates that the rate limiting step for glycerol metabolism is glycerol uptake which, moreover, is independent of the culture conditions. However, to further support this notion in hepatocytes, one would assume that most of the glycerol taken up, if glycerol transport is rate limiting, should be metabolized leaving the intracellular glycerol content low. On the other hand, if glycerol kinase activity is rate limiting, one should expect a increase in the intracellular glycerol content. The finding, that glycerol uptake and the cellular content of phosphorylated metabolites in the presence of DNP were reduced without altering the cellular content of glycerol, does indeed substantiate, that glycerol uptake is rate-limiting. This is in agreement with that previously suggested by Li and Lin [11] using freshly isolated hepatocytes. However, based only on the obtained kinetic constants one

would expect, that the cellular content of glycerol in hepatocytes should be much lower compared to the cellular content of phosphorylated metabolites, than that observed in this study. Possible explanations for this could be: (1) The cellular contents of glycerol and glycerol phosphorylated metabolites were calculated on the basis of the specific radioactivity of glycerol in the incubation media at the start of the incubation period. This may well differ from the specific radioactivity in the intracellular pool of glycerol from which its metabolites is produced. The content of phosphorylated metabolites (and glycerol) therefore represents nominal content which in all likelihood would be underestimated, if the glycerol taken up was diluted with the existing cellular pool of glycerol. (2) The K_i value for G-3-P on GK has been reported to be about 0.5 mM [20], which is in the same order as the concentration of G-3-P found in the liver (range 0.2–0.5 mM) [2]. Using these values together with the obtained K_m values for GK in Table 1 and assuming competitive inhibition [20,21] the apparent K_m for glycerol can be calculated to be 90–100 μM i.e. the glycerol turnover will decrease. (3) GK may be found either in the cytosol or bound to porin on the outer mitochondrial membrane depending on the metabolic state [3,22,23]. The apparent K_m values for ATP and glycerol are lower for the bound enzyme than for that in the supernatant [3]. Moreover, beside being a competitive inhibitor of GK glycerol 3-phosphate also promotes release of porin bound GK in the liver and brain [22,23]. Therefore, GK kinase activity will also be dependent on the metabolic status, which under these experimental conditions is not known.

The present study builds on the assumption, that glycerol uptake is a non-energy demanding facilitated process. However, it can not be excluded based on the present results, that uptake takes place against a glycerol concentration gradient. Altogether, further studies are needed to explore this uptake mechanism.

It has been shown that insulin is important for maintaining GK activity in cultured hepatocytes [24], while Ref. [25] failed to demonstrate such a dependency. This discrepancy may be explained by differences in concentrations and exposure times to insulin.

Gluconeogenesis from glycerol may be of major importance in both cancer and NIDDM due to elevated plasma glycerol levels and glycerol turnover [4,5,13,26,27]. Extrapolating the obtained kinetic values for glycerol uptake to the in vivo situation, and taking into account that uptake appears to be rate limiting for glycerol metabolism, an increase in plasma glycerol from 100 μM to 125 μM, which corresponds very well with the increased plasma glycerol seen in diabetes [4,5], will give rise to an increase in glycerol uptake of about 15–20%. This is compatible with the elevated glycerol clearance rates observed in diabetic subjects compared to non-diabetic subjects [4,5]. In NIDDM subjects there are an almost two fold increase in gluconeogenesis from glycerol. Therefore, in addition to increased glycerol uptake, altered intrahepatic handling of glycerol must take place as previously suggested [4,5].

Compounds that inhibit glycerol kinase or in particular glycerol uptake may be important tools in attempts to clarify the specific role of glycerol as a gluconeogenic substrate. All three glycerol analogues investigated in this study showed inhibition of glycerol uptake. Previously, it was shown that MA competitively inhibited glycerol uptake into freshly isolated hepatocytes [11] in the same order as found in this study. Both MA and dichloro-MA have been reported as inhibitors of GK with the latter one being non-competitive in nature [28]. Our data demonstrated that all three analogues were substrates for GK originating from hepatocytes and from Candida Mycoderma, which clearly indicates that the mode of inhibition was competitive. In this context it should be mentioned that none of the analogues are substrates for GDH i.e. phosphorylation of the analogues via GK is a requirement. Again, expressing the efficiency among the different substrates as the $V_{max}:K_m$ ratio, the following values were obtained: 0.4 (glycerol), 0.4 (dichloro-MA), 0.1 (MB) and 0.4 (MA), respectively. This indicates that MB was 4 times less efficient as substrate for GK compared to the other substrates.

Recently, MB, which is synthesized by adipocytes, has attracted considerably attention because of it's angiogenic activity [29,30]. It's biosynthesis appears to be tightly linked to lipolysis [31] and plasma concentrations in the lower μM range have been found [30]. However, nothing is known about the metabolic fate of this compound. On the basis of the present results, it is suggested that clearance of MB from plasma might occur via the glycerol transporter

and subsequently metabolism via GK and GDH. However, further experiments are needed.

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