Glycerol Kinase Deficiency Inhibits Glycerol Utilization in Phosphoglyceride and Triacylglycerol Biosynthesis

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ABSTRACT. Glycerol 3-phosphate is an initial metabolite in the biosynthesis of phosphoglycerides and triacylglycerols. Both glycerol and glucose are precursors of glycerol 3-phosphate. Cultured skin fibroblasts from patients with glycerol kinase deficiency utilized glucose, but not glycerol in the biosynthesis of phosphoglycerides and triacylglycerols. Phosphoglyceride and triacylglycerol biosynthesis in glycerol kinase deficiency fibroblasts is not diminished by the inability to use glycerol as a precursor of glycerol 3-phosphate. (*Pediatr Res* 19: 313–314, 1985)

Abbreviation

GKD, glycerol kinase deficiency

Glycerol 3-phosphate is an important intermediate in phosphoglyceride and triacylglycerol biosynthesis. It is derived from glycerol and ATP by the activity of glycerol kinase (adenosine triphosphate: glycerol 3-phosphotransferase, EC 2.7.1.30), or from dihydroxyacetone phosphate and NADH + H^+ by the activity of glycerol 3-phosphate dehydrogenase (glycerol 3-phosphate: NAD^+ 2-oxidoreductase, EC 1.1.1.94) (7).

We and others have described several patients who have GKD. GKD is an X-linked disorder that has been identified in adults, juveniles, and infants. Adults with GKD have pseudohypertriglyceridemia secondary to hyperglycerolemia (4, 8, 11). They have no apparent clinical problems. Two juveniles with GKD began to have symptoms at 4 yr of age of episodic vomiting, acidemia and stupor (2, 3). Infants with GKD have hyperglycerolemia, glyceroluria, adrenal hypoplasia, psychomotor retardation, nonspecific myopathy, and X-linked inheritance (1, 5, 9). Guggenheim *et al.* (5) suggested the clinical phenotype in infantile GKD patients could be due to glycerol excess or glycerol 3-phosphate deficiency, or both. However, no one has identified the cause of the diverse clinical problems associated with infantile GKD.

Since GKD blocks one of the pathways for generating glycerol 3-phosphate, we studied phosphoglyceride and triacylglycerol biosynthesis in cultured fibroblasts from five children with infantile GKD.

MATERIALS AND METHODS

Biosynthesis of phosphoglyceride and triacylglycerol. Cultured skin fibroblasts from five patients with GKD and from six controls were incubated with [2-³H]glycerol (1 mCi/ml, 1 Ci/

mmol, Amersham) in 199 Earle's medium containing 10% fetal calf serum. Each ml of medium contained 6 μCi of [2-³H]glycerol. The fetal calf serum contained 1 mg/dl glycerol. The specific activity of [2-³H]glycerol in the culture medium was 0.5 Ci/mmol. After 18 h of incubation the cells were trypsinized from the culture flasks, suspended in phosphate buffered saline, pH 7.3, and extracted twice with chloroform::methanol (2::1, v/v). After the solvent was evaporated with nitrogen, the solute was solubilized in 150 μl chloroform::methanol (2::1, v/v) and spotted onto Silica Gel LK50 chromatography plates. The plates were developed in chloroform::methanol::acetic acid::water (50::35::4::2, v/v) (10), dried, and exposed to iodine vapor. The regions of the silica gel corresponding to iodide stained controls of phosphatidylcholine, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylglycerol, and triacylglycerols were scraped from the glass plates and counted in scintillation fluid. [3-³H]glucose (1 mCi/ml, 11.5 Ci/mmol New England Nuclear) was also used to label phosphoglycerides and triacylglycerols in cultured GKD fibroblasts and control fibroblasts. Each ml of culture medium contained 1 μCi of [3-³H] glucose. The specific activity of the [3-³H]glucose in the culture medium was 0.177 mCi/mmol. The rate the cells incorporated [2-³H]glycerol or [3-³H]glucose is expressed as fmol of phosphoglyceride, or triacylglycerols synthesized per mg of cellular protein per min of incubation. The means and SEM were compared by the two-sample unequal variance *t* test.

Efflux of glycerol from cultured fibroblasts. GKD fibroblasts and control fibroblasts were incubated in 199 Earle's medium with 10% fetal calf serum containing [2-³H]glycerol (0.3 μCi/ml of medium). After 6 h of incubation the cells were sequentially washed with fresh medium. Aliquots of each of the washes were counted in scintillation fluid.

RESULTS

GKD fibroblasts incorporated little glycerol into phosphoglycerides and triacylglycerols (Fig. 1). Most of the glycerol used by GKD fibroblasts was for triacylglycerol biosynthesis, 1.6 (SE 0.3) fmol/mg·min. This limited incorporation is not because GKD fibroblasts failed to transport glycerol across their cell membrane. GKD fibroblasts and control fibroblasts each had the same rate of efflux of glycerol. During the 1st min GKD fibroblasts effluxed 17,099 cpm/mg cellular protein (SD 2,490) and control fibroblasts effluxed 15,748 cpm/mg cellular protein (SD 3,058).

Nor was the limited incorporation due to a large intracellular glycerol concentration that decreased the specific acticity of [2-³H]glycerol after it entered the GKD fibroblasts. Intracellular glycerol concentration was the same in both GKD fibroblasts and control fibroblasts.

Control fibroblasts incorporated glycerol into phosphoglyceride and triacylglyerol at rates that ranged from 42.7 (SE 8.1)
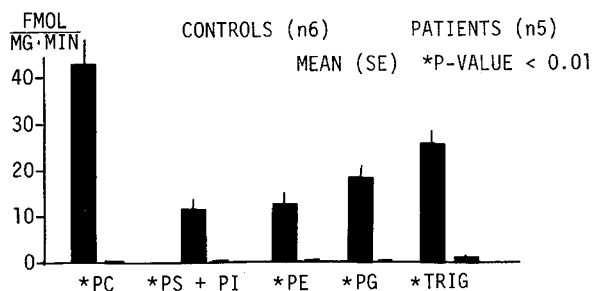
Fig. 1. [2-³H]glycerol incorporation into phosphoglycerides (PPG) and triacylglycerols (TRIG) of cultured fibroblasts from six controls (on the left of each pair) and from five patients with glycerol kinase deficiency (on the right of each pair). Phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and triacylglycerols.
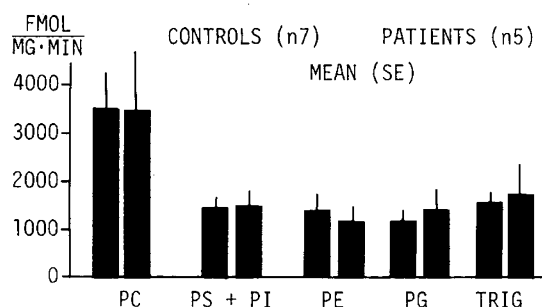

Fig. 2. [3-³H]glucose incorporation into phosphoglycerides (PPG) and triacylglycerol (TRIG) of cultured fibroblasts from seven controls (on the left of each pair) and from five patients with glycerol kinase deficiency (on the right of each pair). Phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and triacylglycerols.

fmol/mg·min for phosphatidylcholine to 11.3 (SE 2.2) fmol/mg·min for phosphatidylserine and phosphatidylinositol.

Overall, GKD fibroblasts utilized glycerol for phosphoglyceride and triacylglycerol biosynthesis at the rate which was 2% of the rate in controls. The p value was <0.01 for each comparison between GKD fibroblasts and control fibroblasts of the rate of glycerol incorporation into phosphatidylcholine, phosphatidylserine plus phosphatidylinositol, phosphatidylethanolamine, phosphatidylglycerol, or triacylglycerols.

Using [3-³H]glucose, we found GKD fibroblasts and control fibroblasts incorporated glucose equally well into phosphoglycerides and triacylglycerols (Fig. 2).

Control fibroblasts readily utilized glycerol for a precursor of glycerol 3-phosphate. The molar ratio of glucose to glycerol in the culture medium was 275::1 (550 × 10⁻⁵ mmol/ml glucose::2.0 × 10⁻⁵ mmol/ml glycerol) and the rate of glucose incorporation (1000 to 3400 fmol/mg·min) into phosphoglyceride or triacylglycerol was approximately 100 times that of glycerol (10 to 40 fmol/mg·min).

DISCUSSION

Cultured GKD fibroblasts incorporated little glycerol into phosphoglycerides and triacylglycerols. Glycerol kinase activity was not necessary for these fibroblasts to synthesize phosphoglycerides and triacylglycerols from glucose and to grow as well as control fibroblasts.

The GKD fibroblasts do not utilize glycerol in phosphoglyceride and triacylglycerol biosynthesis because the enzyme, glycerol kinase, that converts glycerol to glycerol 3-phosphate does not function. It is not because glycerol fails to enter the cell. GKD fibroblasts transport glycerol across the cellular membrane as well as control fibroblasts did.

Control fibroblasts incorporated glycerol at 1/100 the rate of glucose under conditions where the molar ratio of glycerol to glucose in the culture medium was 1::275. This apparent 2.75-fold increase in the incorporation of glycerol relative to glucose does not consider the intracellular metabolism of glycerol or glucose. The intracellular concentration of glycerol 3-phosphate or dihydroxyacetone phosphate under these culture conditions may have influenced the rate of which glycerol or glucose was used as a precursor for phosphoglyceride or triacylglycerol biosynthesis.

However Haessler and Isselbacher (6) also noted glycerol was readily incorporated into tissue lipids by hamster intestinal slices. They found 3-fold more glycerol than glucose was incorporated into lipids even though four times more glucose than glycerol was metabolized to carbon dioxide.

GKD fibroblasts are not dependent on glycerol for glycerol 3-phosphate biosynthesis. They use glucose for glycerol 3-phosphate biosynthesis and subsequent phosphoglyceride and triacylglycerol biosynthesis. Other tissues of GKD patients may be more dependent than fibroblasts on glycerol as a precursor for glycerol 3-phosphate. This could cause selective organ deficiencies of essential phosphoglycerides and triacylglycerols and contribute to the diverse clinical symptoms in patients with infantile GKD.

CONCLUSION

GKD fibroblasts utilize glucose but not glycerol for phosphoglyceride and triacylglycerol biosynthesis. GKD blocks one of two pathways for the biosynthesis of an initial metabolite, glycerol 3-phosphate, in phosphoglyceride and triacylglycerol biosynthesis.

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