

Glycogen Concentration in Fetal Mouse Tissues

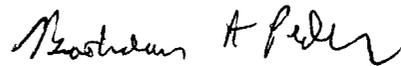
An Honors Thesis (Honors 399)

by

Michelle Huang

Thesis Advisor

Dr. Bartholomew Pederson

A handwritten signature in black ink, appearing to read "Bartholomew A. Pederson". The signature is written in a cursive style with a large initial 'B' and 'P'.

Ball State University
Muncie, IN

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Abstract

Glycogen is the main storage unit of glucose and is found in tissues throughout the body. Tissue development and the contribution from glycogen in the fetal mouse can be followed by measuring glycogen concentrations in fetal tissues. In the present work, glycogen concentrations in the brain, heart, lung, liver, and muscle of wild-type fetal mice are compared from 14.5 to 18.5 dpc. The results indicate that glycogen level reaches a maximum at 16.5 dpc in the brain and muscle, 15.5 dpc in the heart, 18.5 dpc in the liver, and 17.5 dpc in the lung.

Introduction

Glycogen, a branched polymer of glucose, is a storage form of carbohydrate and is present in almost all organisms (Pederson, 4, 6, 9, 12). Insulin induces the uptake of glucose into some tissues, and its subsequent conversion to glycogen (12); this conversion generally occurs during times of nutritional sufficiency (6). Muscle and liver are the main sites of glycogen storage (8, 12). In skeletal muscles, glycogen is broken down to produce ATP due to an energy demand; however, other tissues are supplied with glucose by the breakdown of liver glycogen (4). Liver glycogen is primarily used to maintain the glucose levels in the bloodstream (6, 7); it is also used to provide glucose to newborn mice until the liver is able to produce glucose by gluconeogenesis (7). Glycogen can also be made in other cells throughout the body (12).

Glycogen synthesis is initiated by the self-glycosylation of glycogenin (4). The first enzyme in glycogen synthesis is glycogenin, which is responsible for catalyzing the first part of the glycogen chain from UDP-glucose (12). After initiation, glycogen synthase and branching enzyme continue the catalysis of glycogen synthesis; glycogen synthase elongates the chain, and branching enzyme transfers a chain of about 7 glucose molecules to a chain of about 11 molecules (12, 4). The main linkage in glycogen is α -1,4-glycosidic link; however, the branch

points are introduced by α -1,6-glycosidic linkages (7, 9). There are two isoforms of glycogen synthase, GYS1 and GYS2; GYS1 is found in skeletal muscle, cardiac muscle, adipose tissue, kidney, lung, and brain cells, and GYS2 is only found in liver cells (12, 7). “Glycogen synthase activity is controlled by covalent modification, allosteric activation, and enzymatic translocation” (4).

In the fetal rat lung, glycogen reaches a peak concentration in the last third of gestation; this is just before changes in glycogen synthase activity levels (5). There is a subsequent drop in glycogen concentration (3). This drop, at about 18 days postcoitum (dpc), is related to the appearance of lamellar bodies and an increase in phospholipid synthesis and concentration (2, 3, 5). This had led to the belief that glycogen plays a role in the maturation of the developing lung (5). There are three roles that glycogen may play in the development of the lung: a source of precursors molecules in phospholipid synthesis; “as a source of reducing equivalents necessary for de novo fatty acid synthesis;” or an energy source (1).

Lung surfactant is used to “reduce surface tension at the air liquid interface in the alveolus of the mature lung;” it is a lipoprotein complex with phosphatidylcholine being the primary lipid (1, 11). Data from Risdale and Post suggests that glycogen stores from type II lung cells in fetal mice are the site of surfactant phosphatidylcholine synthesis (11). The glycogen stores in lung cells are depleted as surfactant is synthesized; this may be because glycogen acts as a carbon source for making the glycerol backbone and acyl chains of the lipids (2, 11). Glycogen may also act as an energy source of phosphatidylcholine synthesis (11).

In muscle, contractions are driven by glycogenolysis, the breakdown of glycogen—a phosphorolysis reaction that yields shortened glycogen and glucose-1-phosphate (4, 8, 12). Muscle glycogen can be manipulated by exercise and diet (8). While muscle glycogen may be

important for human exercise, this may not be the case in rodents (8). In mice and rats, liver glycogen may be used for exercise; therefore, muscle glycogen is only limiting when liver glycogen is low (8).

In the present work, glycogen concentrations in the brain, heart, lung, liver, and muscle of wild-type fetal mice are compared from 14.5 to 18.5 dpc. This is the third trimester of gestation; typical mouse gestation lasts 21 days. The pattern of glycogen production and use can be related to the period of time when organ tissues are developing in mice.

Methods and Materials

The mice used for this experiment were from the strain C57B1/6. All fetal glycogen concentrations were determined for wild-type mice.

For timed pregnancies, female mice were introduced into a cage with male mice late in the afternoon. The following morning the females were checked for vaginal plugs. The mice were sacrificed by cervical dislocation from 14.5 days postcoitum to 18.5 days postcoitum. Then, the embryos were harvested, and tissues were collected into liquid nitrogen. The tissues were stored at -80°C until their glycogen content was determined.

To determine glycogen content, powdered tissues samples from brain, muscle, liver, lung, or heart were boiled in 200 μl 30% (w/v) KOH. The tissues were then cooled on ice and 65 μl cold NaSO_4 was added followed by 540 μl cold 100% ethanol. The samples were subsequently heated at 100°C for 2 minutes and then centrifuged for 20 minutes at $17,500 \times g$ at 4°C . The supernatant was removed using a vacuum line and the pellet was then resuspended in 100 μl water. For all tissues with the exception of brain, 200 μl ethanol was added and then the

samples were centrifuged as above. This glycogen precipitation step was then repeated and the pellet was dried in a Speed-Vac.

For brain tissues, after resuspension, 1 ml 4:1 (v/v) methanol:chloroform was added and the samples were heated at 80°C for 5 minutes. The samples were centrifuged for 15 minutes at 17,500 x g at 4°C. The supernatant was discarded, and the pellet resuspended in 100 µl water. 1 ml 4:1 (v/v) methanol:chloroform was added, then the samples were centrifuged as before. After removing the supernatant and resuspending the pellet in 100 µl water, 200 µl 100% ethanol was added. The samples were heated at 100°C for 2 minutes, then centrifuged at 17,500 x g for 20 minutes at 4°C. After the supernatant was removed, the pellets were dried in a Speed-Vac.

All glycogen pellets were then suspended in amyloglucosidase (0.3 mg/ml in 0.2 M NaOAc pH 4.8); pellets from brain were suspended in 100 µl, all other tissues were suspended in 200 µl. The samples were then incubated at 40°C overnight to digest glycogen. To determine the concentration of glucose in the samples, 20 µl digested glycogen (diluted in 0.2 M NaOAc, pH 4.8, where appropriate) was added to 180 µl of a solution of 0.3 M triethanolamine, pH 7.5, 3 mM MgSO₄, 11.2 mM ATP, 0.9 mM NADP, and 1 µg/ml glucose-6-phosphate dehydrogenase. The absorbance was read at 340 nm before and after the addition of 5 µl diluted hexokinase [stock hexokinase (1500 u/ml) was diluted 1:25 with 0.3 M triethanolamine/3mM MgSO₄ pH 7.5].

Results

In the fetal brain, there is an increase in brain glycogen concentration at about 16.5 dpc. After this, glycogen concentration remained the same (Fig. 1); however, brain glycogen concentrations were consistently the lowest of any tissue. These fetal levels were higher than those we have measured in adult mouse brain (2-3 µmol/g).

The fetal heart glycogen concentrations rose to a peak at 15.5 days, and then trended towards slightly lower levels that were maintained throughout development. The level of glycogen in the developing heart is as much as 50 fold higher than we have measured in mouse adult heart (about 1 $\mu\text{mol/g}$).

Starting out at glycogen concentrations of zero, fetal liver glycogen increased significantly at 15.5 dpc. By 18.5 dpc, the glycogen concentration in the liver was much higher than the peak concentration seen in other fetal tissues (Fig. 3). The level at 18.5 dpc is similar to what we measure in fed adult mouse liver.

Muscle glycogen concentration has a large increase from 15.5 to 16.5 dpc, which remains steady through 18.5 dpc (Fig. 4). This glycogen level is 5-10 fold higher than what we measure in adult mouse muscle (5-10 $\mu\text{mol/g}$).

In the fetal lung, glycogen concentration rose to a peak at about 17.5 dpc before falling to about half the level at the peak (Fig. 5). Aside from the liver, lung glycogen concentrations were the highest of the tissues. At the peak of glycogen accumulation, the concentration of glycogen is as much as 60 fold higher than the level we measure in adult mouse lung (about 2 $\mu\text{mol/g}$).

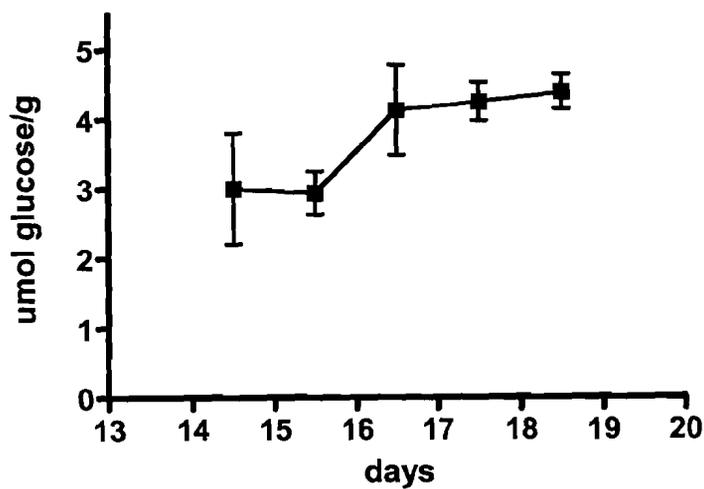


Figure 1: Fetal brain glycogen concentrations from 14.5 to 18.5 days postcoitum. Glycogen content was determined for brain from wild type embryos as described in methods and materials.

Values are reported as means and standard errors of the mean (n=6). $p=0.01$ when comparing values at 15.5 with values at 17.5 dpc. $p=0.005$ when comparing values at 15.5 with values at 18.5 dpc.

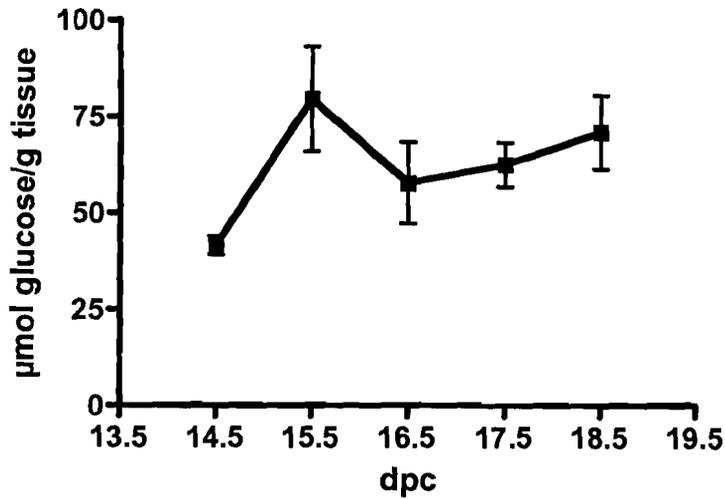


Figure 2: Fetal heart glycogen concentrations from 14.5 to 18.5 days postcoitum. Glycogen content was determined for heart from wild type embryos as described in methods and materials. Values are reported as means and standard errors of the mean (n=5-7). $p=0.03$ when comparing values at 14.5 with values at 15.5 dpc.

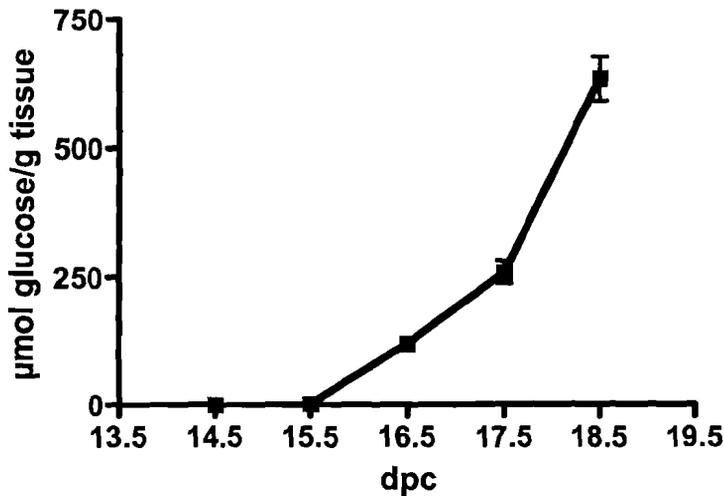


Figure 3: Fetal liver glycogen concentrations from 14.5 to 18.5 days postcoitum. Glycogen content was determined for liver from wild type embryos as described in methods and materials. Values are reported as means and standard errors of the mean (n=4). $p=0.0001$ when comparing values at 15.5 with values at 16.5 dpc. $p=0.002$ when comparing values at 16.5 with values at 17.5 dpc. $p=0.0002$ when comparing values at 17.5 with values at 18.5 dpc.

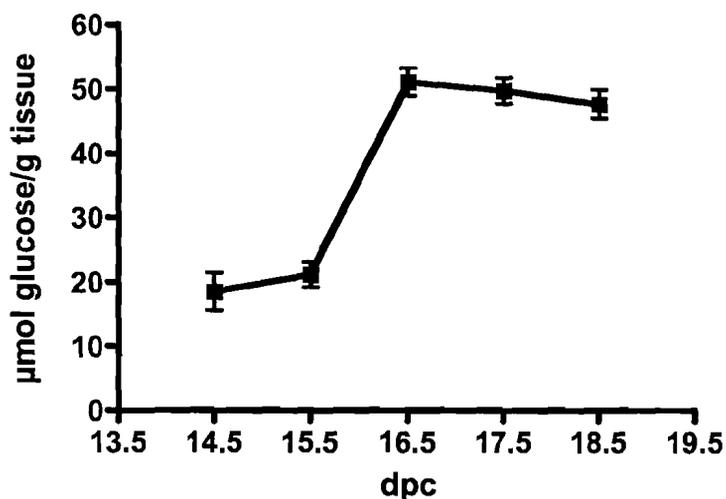


Figure 4: Fetal muscle glycogen concentrations from 14.5 to 18.5 days postcoitum. Glycogen content was determined for muscle from wild type embryos as described in methods and materials. Values are reported as means and standard errors of the mean (n=3-4). $p=0.002$ when comparing values at 15.5 with values at 16.5 dpc.

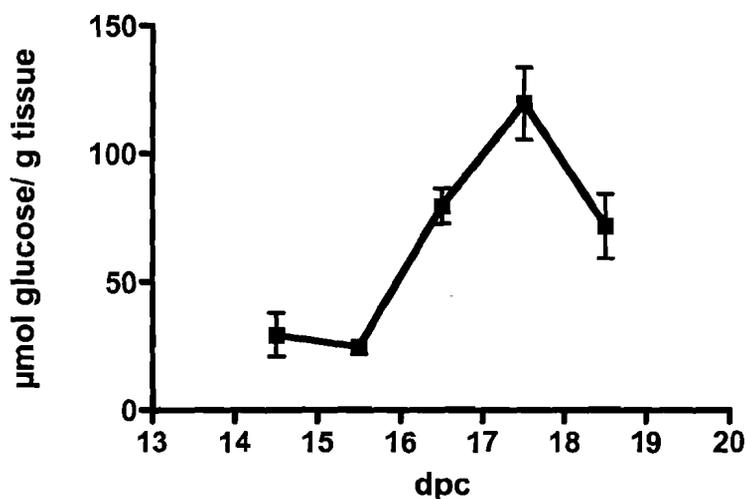


Figure 5: Fetal lung glycogen concentrations from 14.5 to 18.5 days postcoitum. Glycogen content was determined for lung from wild type embryos as described in methods and materials. Values are reported as means and standard errors of the mean (n=3-4). $p=0.001$ when comparing values at 15.5 with values at 16.5 dpc. $p=0.04$ when comparing values at 16.5 with values at 17.5 dpc. $p=0.07$ when comparing values at 17.5 with values at 18.5 dpc.

Discussion

The results from these studies in mouse embryos are largely consistent with findings in rabbit reported by other investigations (14).

The brain showed very low levels of glycogen throughout the experiment. The role of glycogen in the brain is not well understood. Suggestions of its functions include maintaining neuron viability during ischemia and hypoglycemia and participation in memory formation.

The liver is responsible for maintaining the glucose level of the bloodstream, which will provide glucose to the brain, as well as other tissues. After birth, before the liver begins gluconeogenesis, the liver is the main source of energy in the form of glucose (7); therefore, a large store must be built up while the fetus is still receiving nutrients from the mother.

Pederson et al. observed that fetal mice lacking glycogen synthase had abnormal heart morphology after about 14.5 dpc. This suggests that glycogen is needed for heart growth and development. This is also consistent with the peak in heart glycogen concentration at 15.5 dpc. The changes in mouse heart glycogen during development are not as dramatic as reported for rabbit.

Muscle glycogen has been considered a major energy source for muscle contractions. Once the glycogen stores are exhausted, fatigue and impaired muscle performance have been observed (8). Muscle contraction activates glycogen synthase (12); it has been observed that fetal mice begin to move at about day 14 after conception (13). The rise in muscle glycogen concentration seen from 14.5 to 16.5 dpc, may be due to the activation of glycogen synthase by motion.

The peak of glycogen concentration at about 17.5 dpc followed by a drop in concentration in the fetal lung confirms previous findings by Maniscalco et al. (5). This drop has also been linked to the appearance of lamellar bodies in the lung (2, 3, 5). Lamellar bodies are the storage sites of phospholipids produced in the lung; these phospholipids are used for the production of lung surfactant (5). Subsequent experiments could involve measuring the activity

of glycogen synthase and glycogen phosphorylase to aid in understanding the regulation of glycogen accumulation in this and other tissues. Most importantly we would like to measure lung surfactant levels in 17.5 dpc embryos and compare the values with those obtained from embryos that are unable to synthesize lung glycogen. This would allow us to determine if lung glycogen is important for surfactant synthesis.

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