

Regulation of Fetal Lung Phosphatidyl Choline Synthesis by Cortisol: Role of Glycogen and Glucose

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Summary

Twenty pregnant rabbits were studied in pairs. Half were given cortisol subcutaneously on days 24, 25, and 26 of gestation in dosage of 2 mg/kg/day. Half served as controls and received saline. The fetal lungs were studied on the 27th day of gestation by incubating lung slices in the presence of [6-¹⁴C]glucose. Glucose consumption significantly increased in the tissues from animals treated with cortisol, 17.61 ± 5.56 (SD) $\mu\text{mol/g}$ wet lung versus 14.28 ± 5.78 (SD) $\mu\text{mol/g}$ in the controls ($P < 0.05$). The glycogen content of tissues treated with cortisol was significantly reduced compared to the controls, 2.42 ± 0.97 (SD) mg/g wet lung versus 3.81 ± 1.05 (SD) mg/g ($P < 0.05$). Treatment with cortisol resulted in significantly enhanced incorporation of the ¹⁴C label into glycogen and phosphatidyl choline (Tables 3 and 4). These data suggest that glucocorticoids affect fetal lung phosphatidyl choline production by promoting glycogenolysis and increasing glucose incorporation into phosphatidyl choline.

Speculation

Diminished glycogen content of the fetal lung in the latter part of gestation may reflect increasing utilization for phosphatidyl choline synthesis. Adenosine 3',5'-monophosphate (cyclic AMP) increases which occur after glucocorticoid administration may be responsible for activation of glycogen phosphorylases in the lung as they are in the liver. If insulin inhibits glycogenolysis in the lung as it does in the liver, insulin may inhibit phosphatidyl choline synthesis in the lung by preventing glycogen and glucose from serving as precursors.

Glucocorticoid administration to pregnant sheep, rabbits, monkeys, and human beings can induce premature maturation of the pulmonary surfactant system in the fetus (6, 15, 17, 19, 20, 24, 35). The mechanism by which these agents induce premature maturation of fetal lungs is not clear. Farrell and Zachman (7, 8) have shown that antenatal administration of glucocorticoids to fetal rabbits results in increased activity of choline phosphotransferase, a rate-limiting enzyme for phosphatidyl choline synthesis through the choline incorporation pathway described by Kennedy (14). We have reported that in fetal rabbit lung, cortisol inhibits cyclic AMP phosphodiesterase, increases concentrations of cyclic AMP, and increases incorporation of choline and methionine into phosphatidyl choline (1). Glucocorticoids have multiple effects on carbohydrate metabolism through the *de novo* synthesis of a number of enzymes of the glycolytic pathway, including fructose-1,6-diphosphatase (23), phosphohexose isomerase (38), glucose-6-phosphatase (37), and phosphoenolpyruvate carboxykinase (29). In tissues such as liver and muscle, the glycolytic pathway is closely coupled to the

pathways involved in phospholipid synthesis (5). The widespread metabolic effects of glucocorticoids may also influence the availability and utilization of substrates for phosphatidyl choline synthesis.

Glucose has been shown to be an important precursor for phosphatidyl choline synthesis in adult rabbit lung (9), and there is indirect evidence that glycogen is a precursor for phosphatidyl choline in fetal rabbit lung (15, 24, 35). Between 25 and 28 days of gestation, the amount of glycogen, which had previously been high, decreases and disappears from the alveolar type II cells (15). Simultaneously, lamellar inclusion bodies, the storage sites for surfactant (16), appear in greater numbers and it has been suggested that this observation implies that glycogen may be a precursor for surfactant (15). Glucocorticoids also affect lipid metabolism, promoting lipolysis in adipose tissue with the release of glycerol and free fatty acids (25), both of which are precursors for phosphatidyl choline synthesis (22, 32, 33). It appears, therefore, that glucocorticoids have multiple effects upon pulmonary metabolism, which may result in increased phosphatidyl choline synthesis. The present study was undertaken to determine whether glucocorticoids affect fetal lung phosphatidyl choline production by modifying glucose utilization or conversion.

MATERIALS AND METHODS

Twenty pregnant New Zealand albino rabbits, conception timed to within 3 hr, were studied in pairs. Half were given cortisol (hydrocortisone phosphate) subcutaneously, 2 mg/kg/day in three equal doses on days 24, 25, and 26 of gestation. Half served as controls, received saline subcutaneously, but were otherwise treated identically.

On the 27th day of gestation the fetuses were delivered by cesarean section immediately after killing the doe with intravenous pentobarbital followed by KCl. The tracheas of the fetuses were clamped immediately after delivery and the spinal cord was severed. This prevented air breathing and loss of tracheal fluid. The lungs were excised and placed in cold Krebs-Ringer bicarbonate buffer, as modified by O'Neil and Tierney (26). The lungs were rinsed in the same buffer and blotted with a dry gauze sponge. Tissue slices of 1000 mm were then prepared using a Mellwain tissue slicer as described by O'Neil and Tierney (26). Approximately 300 mg tissue were added to 4.5 ml Krebs-Ringer buffer, pH 7.4, containing 0.563 mCi [6-¹⁴C]glucose (4.87 mCi/mmol, New England Nuclear Corp.) in incubation flasks with center wells. The flasks were weighed before and after the addition of tissue and the amount of tissue added to each flask was thus determined. After equilibration for 45 sec with 95% O₂ and 5% CO₂, the flasks were capped and incubated for 90 min in a Dubnoff metabolic shaker set at 38° and 120 oscillations/min. After the incubation, the flasks were placed on

ice. To some flasks in each group 0.5 ml quarternary amine (NCS, New England Nuclear Corp.) was placed into the center well and 2 ml 6% perchloric acid were placed in the outer compartment by means of a needle while the flasks remained sealed. These flasks were incubated again for 60 min to collect CO_2 in the quarternary amine. The quarternary amine containing $^{14}\text{CO}_2$ was transferred to vials to which an Aquasol-ethanol (New England Nuclear Corp.) based solution was added, and counted in a liquid scintillation counter (Nuclear Chicago Isocap 300). Glucose oxidation was calculated by multiplying the ratio of disintegrations per min of $^{14}\text{CO}_2$ to disintegrations per min of $[6\text{-}^{14}\text{C}]\text{glucose}$ originally added to the flask, by the amount of glucose initially present in the medium.

Tissue glycogen content was determined before and after incubation by the method of Van Handel (34). Glucose content in the incubation medium was measured before and after incubation by the method of Washko and Rice (36). Lactate content in the medium was determined by the method of Hohorst (13). Tissue DNA was determined by the method of Schneider (18).

Lipid analysis was performed after the slices were removed from the medium, washed in buffer solution, and lyophilized at -30° . Total lipids were extracted according to the method of Folch *et al.* (10) under nitrogen, using a Polytron homogenizer (Brinkman Instruments). Phospholipids were separated from neutral lipids by silicic acid column chromatography. The phospholipid fraction was then fractionated into various components by thin layer chromatography on Silica Gel H-coated plates. The plates were developed in chloroform-methanol-water (65:25:4). The phosphatidyl choline and phosphatidyl ethanolamine fractions were recovered and quantitated on the basis of phosphorus content determined by the method of Bartlett (2). Radioactivity was measured in a liquid scintillation counter. Molar incorporation of labeled glucose into the various products was determined on the basis of substrate specific activities. Statistical analysis was performed using a paired *t*-test.

RESULTS

Lung DNA concentrations before incubation in the control and cortisol groups were almost identical, 5.32 ± 0.94 (SD) mg/g lung in the controls and 5.35 ± 0.86 (SD) mg/g in the treated group. The wet weight to dry weight ratio was not significantly different between the groups, 9.17 ± 0.57 (SD) for the controls and 8.49 ± 0.69 (SD) for the cortisol group.

Table 1 demonstrates that glucose consumption calculated from disappearance of glucose from the medium during the 90-min incubation significantly increases in the tissues treated with cortisol *in vivo*, 17.61 ± 5.56 (SD) $\mu\text{mol/g}$ lung as compared to 14.28 ± 5.78 (SD) $\mu\text{mol/g}$ in the controls ($P < 0.05$). There are no significant differences in glucose oxidation or lactate production between control tissue and tissue treated with cortisol. Before incubation, glycogen content of tissues treated with cortisol is significantly reduced compared with the controls, 2.42 ± 0.97 (SD) mg/g lung versus 3.81 ± 1.05 (SD) mg/g ($P < 0.05$). This reduction persists after incubation.

The content of phosphatidyl choline, phosphatidyl ethanolamine, and neutral lipid in lung slices after 90 min of incubation is shown in Table 2. No significant changes are found after treatment with cortisol. Table 3 demonstrates that treatment

Table 1. Glucose consumption, glucose oxidation, and lactate production expressed as micromoles per g lung (mean \pm SD); glycogen content expressed as milligrams per g lung (mean \pm SD)

	<i>n</i>	Control	Cortisol	<i>P</i> value
Glucose consumption	8	14.28 ± 5.78	17.61 ± 5.56	<0.05
Glucose oxidation	9	0.272 ± 0.128	0.283 ± 0.144	NS
Lactate production	10	19.21 ± 6.85	18.43 ± 10.34	NS
Glycogen content				
Before incubation	9	3.81 ± 1.05	2.42 ± 0.97	<0.05
After incubation	10	3.07 ± 1.14	2.20 ± 0.84	<0.01

Table 2. Content of phosphatidyl choline and phosphatidyl ethanolamine expressed as micromoles per g lung (mean \pm SD); neutral lipid expressed as milligrams per g lung (mean \pm SD)

	<i>n</i>	Control	Cortisol	<i>P</i> value
Phosphatidyl choline	10	5.89 ± 1.24	6.22 ± 0.82	NS
Phosphatidyl ethanolamine	9	2.88 ± 0.43	2.76 ± 0.62	NS
Neutral lipid	9	2.06 ± 0.58	2.04 ± 0.68	NS

Table 3. Incorporation of $[6\text{-}^{14}\text{C}]\text{glucose}$ into various products, expressed as micromoles of glucose per g lung (mean \pm SD)

	<i>n</i>	Control	Cortisol	<i>P</i> value
Glycogen	10	0.116 ± 0.061	0.145 ± 0.099	<0.05
	9	0.054 ± 0.030^1	0.083 ± 0.028^1	<0.05
Phosphatidyl choline	7	0.039 ± 0.012	0.047 ± 0.013	<0.01
Phosphatidyl ethanolamine	7	0.023 ± 0.015	0.027 ± 0.014	NS
Neutral lipid	7	0.029 ± 0.018	0.032 ± 0.014	NS

¹ Expressed as micromoles of $[6\text{-}^{14}\text{C}]\text{glucose}$ per mg glycogen.

with cortisol results in significantly enhanced incorporation of the ^{14}C label into phosphatidyl choline ($P < 0.01$). No significant effect is seen in the incorporation of the ^{14}C label into phosphatidyl ethanolamine or neutral lipid. A significant increase in incorporation of the ^{14}C label into glycogen occurs after treatment with cortisol ($P < 0.05$). In Table 4 the amount of glucose converted into CO_2 , glycogen, phosphatidyl choline, phosphatidyl ethanolamine, and neutral lipid is expressed as a percentage of total glucose consumed. These products account for approximately 11% of glucose consumed. Treatment with cortisol does not modify the percentage of glucose utilized for the synthesis of CO_2 , glycogen, phosphatidyl ethanolamine, or neutral lipid. Incorporation of glucose into phosphatidyl choline is augmented after treatment with cortisol, resulting in a significant increase over the control, from $1.48 \pm 0.60\%$ (SD) to $1.72 \pm 0.63\%$ of total glucose consumption ($P < 0.01$).

Table 5 shows the distribution of ^{14}C in the fatty acid and glycerol moieties of phosphatidyl choline, phosphatidyl ethanolamine, and neutral lipid expressed as a ratio of fatty acids to glycerol. Cortisol treatment does not affect these ratios.

DISCUSSION

The results of this study demonstrate that antepartum cortisol administration is associated with a significant decrease in fetal lung glycogen content. Although lung glycogen depletion occurs normally during maturation, administration of cortisol significantly enhances the rate of depletion. In addition, the rate of incorporation of glucose into glycogen increases after cortisol administration implying increased glycogen turnover. Our finding of lung glycogen depletion with cortisol treatment is consistent with morphologic studies showing an accelerated decrease in glycogen content of alveolar type II cells in cortisol treated fetal rabbits (15, 24, 35).

Glucose, a product of glycogenolysis, undergoes a significant increase in consumption after cortisol administration. Cortisol significantly increases the amount of glucose utilized for glycogen and phosphatidyl choline. Cortisol also increases the amount of glucose utilized for CO_2 , phosphatidyl ethanolamine, and neutral lipid, but not significantly. The percentage of glucose consumed which is utilized for CO_2 , glycogen, phosphatidyl ethanolamine, and neutral lipid is not significantly changed with cortisol treatment. This percentage is significantly increased for phosphatidyl choline synthesis only. The increased amount as well as increased percentage of glucose consumed for phosphatidyl choline synthesis indicate that there is preferential incorpora-

tion of glucose into phosphatidyl choline as compared with other lipids after cortisol treatment. These products account for approximately 11% of glucose consumed. The remaining glucose is probably converted into other cellular components such as proteins, carbohydrates, nucleic acids, and other lipids. We have measured only a small fraction of the compounds involved in cellular metabolism.

Figure 1 demonstrates the metabolic pathways by which glucose may be incorporated into both the glycerol and fatty acid portions of the phosphatidyl choline molecule (3, 4, 9, 14, 30). Via the glycolytic pathway, the interconvertible trioses, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate are formed. Glyceraldehyde 3-phosphate continues in the glycolytic pathway to produce pyruvic acid which, when oxidized, results in the formation of acetyl-CoA. Acetyl-CoA is a precursor for fatty acids, and, bound to coenzyme A, fatty acids can enter the pathway for phosphatidyl choline synthesis. At least two pathways may be involved in the utilization of dihydroxyacetone phosphate in forming the glycerol moiety of phosphatidyl choline (21). In the major pathway, dihydroxyacetone phosphate is converted to glycerol 3-phosphate. This may be followed by the addition of two acyl groups, which results in the formation of phosphatidic acid, an important precursor of phosphatidyl choline. Dihydroxyacetone phosphate and glycerol 3-phosphate recently have been shown to be precursors for phosphatidyl choline in microsomal preparations of alveolar type II cells (31). These trioses provide acceptor molecules for fatty acids. If specific products generated during glycolysis are utilized for phospholipid synthesis in fetal lung, then the regulation of glycolysis by glucocorticoids may influence the rate of phosphatidyl choline production from glycolytic substrates. Glucose metabolism would also generate reduced pyridine nucleotides utilized for the reductive synthetic steps in phosphatidyl choline production.

The total content of phosphatidyl choline in lung tissue was found not to be appreciably affected by cortisol treatment. This confirms a previous report by Russell *et al.* (27), who suggested that a qualitative change with treatment, such as an increased proportion of saturated phosphatidyl choline, may occur in the phosphatidyl choline only at the alveolar surface. It is also possible that glucocorticoids increase secretion of surfactant onto the alveolar surface and also increase surfactant synthesis so that altered rates of lung phosphatidyl choline synthesis are not reflected by parenchymal phosphatidyl choline content. When lung phosphatidyl choline is measured after incubation, as was done in the study by Russell *et al.* and in the present study, it is probably mostly the parenchymal phosphatidyl choline that is measured if the medium is not analyzed. We have measured the content of phosphatidyl choline in lung slices from adult rats

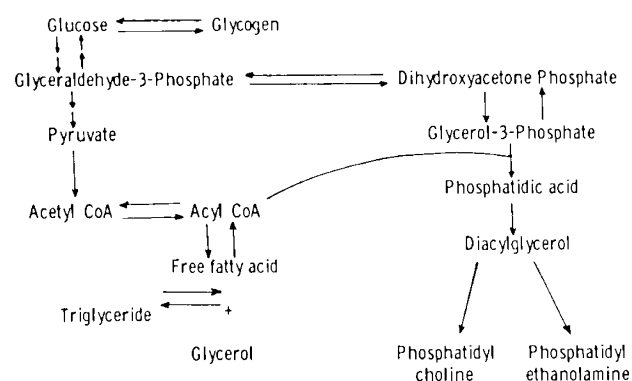


Fig. 1. Scheme by which glucose can be converted into phosphatidyl choline. Single arrowhead indicates one-step reactions and double arrow heads indicate multiple step reactions.

after 90 min of incubation and in whole lung before incubation and found an average loss of 28% in saturated phosphatidyl choline content in 30 paired experiments. Thus, the slicing procedure or the incubation is accompanied by appreciable losses in phosphatidyl choline. During the incubation, phosphatidyl choline could be lost into the medium. Although the lung slice technique is valid in certain instances (26), it may not accurately reflect phospholipid secretion onto the alveolar surface. Gilder and McSherry (11) found that total phospholipid content was not well related to the level of phosphatidyl choline synthesis in lung slices from normal rabbits.

When lung slices from adult rats are incubated with labeled glucose, over 90% of the radioactivity recovered in the phospholipid fraction is found in the glycerol moiety of the molecule (9). We found that only about 60% of the radioactivity is in the glycerol portion. A similar finding was reported by Hamosh (12), who also used fetal rabbits. Possibly more glucose is utilized for fatty acid synthesis in the fetus than in the adult.

When glucose labeled in the 6th carbon atom is used as a tracer, one finds the minimum level of labeled CO_2 generated. Thus, the 6th carbon atom is more likely to be incorporated into phosphatidyl choline. In the pentose cycle, the first carbon atom of glucose is most readily oxidized, whereas in glycolysis carbon atom numbers 3 and 4 are most readily oxidized. Consequently, in both glycolysis or in the pentose cycle, the 6th carbon atom has the greatest tendency to become incorporated into dihydroxyacetone phosphate. Furthermore, the 6th carbon of glucose is most likely to become the methyl carbon of acetyl-CoA (18). Calculation of glucose oxidation using $[6\text{-}^{14}\text{C}]$ glucose has limited validity because of the necessary assumption of equal rates of oxidation of all glucose carbon atoms.

Because cortisol induces morphologic changes in the fetal lung, metabolic effects following cortisol treatment might not be direct effects of the glucocorticoids, but rather a consequence of the fact that numerous changes have occurred in the lung, such as maturation of the type II cells (15, 24, 35).

CONCLUSION

It can be concluded that cortisol administration to rabbits *in vivo* results in increased glucose consumption by fetal lung tissue slices. This is accompanied by depletion of glycogen and increased incorporation of glucose into phosphatidyl choline. Glucose serves as a substrate for phosphatidyl choline synthesis and, in the presence of glucocorticoids, the flux of glucose to phosphatidyl choline is specifically increased. Glycogen appears to be a source of glucose for phosphatidyl choline synthesis.

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Table 4. Incorporation of $[6\text{-}^{14}\text{C}]$ glucose into various products, expressed as percentage of total glucose consumption (mean \pm SD)

	n	Control	Cortisol	P value
Glycogen	6	4.24 \pm 2.01	4.23 \pm 1.87	NS
Phosphatidyl choline	4	1.48 \pm 0.60	1.72 \pm 0.63	<0.01
Phosphatidyl ethanolamine	4	0.92 \pm 0.53	1.08 \pm 0.75	NS
Neutral lipid	3	1.20 \pm 0.74	1.32 \pm 0.67	NS
Carbon dioxide	6	3.30 \pm 1.97	2.77 \pm 1.68	NS

Table 5. Incorporation of $[6\text{-}^{14}\text{C}]$ glucose into fatty acid and glycerol of various products, expressed as a ratio (mean \pm SD) of fatty acid to glycerol

	n	Control	Cortisol	P value
Phosphatidyl choline	10	0.62 \pm 0.22	0.64 \pm 0.19	NS
Phosphatidyl ethanolamine	9	0.73 \pm 0.24	0.79 \pm 0.22	NS
Neutral lipid	9	0.69 \pm 0.11	0.75 \pm 0.22	NS

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