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Effect of Insulin *in vivo* on the Synthesis of Phospholipids in Different Chicken Tissues

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The incorporation of glucose U-^{14}C into phospholipids in one day old chicken has been studied. The incorporation was much greater in liver than in heart and skeletal muscle, while there was no such incorporation in adipose tissue.

There was a significant increase ($p < 0.01$) in labelled phospholipids in the liver, skeletal muscle and heart following insulin administration.

The role played by insulin in the regulation of glucose metabolism in chickens has not been sufficiently clarified.

It has been shown that insulin is ineficient in altering glucose metabolism in chicken tissues *in vitro* since it always requieres high concentrations to produce small effects, both in muscle and in adipose tissue (7, 9); neither does insulin have antilipolytic effects in pieces of adipose tissue or in adipocytes (8, 10). Up to now the antilipolytic effect in adipose tissue *in vitro* has only been produce by carbenoxolone (3).

On the other hand it has more recently been found that insulin at physiological doses stimulates the utilization of glucose in isolated fat cells (5) but not the utilization of acetate (11).

Following this it has further been shown that *in vivo* insulin also stimulates the synthesis from glucose of triglycerides in the liver, heart and skeletal muscle and adipose tissue (15), of fatty acids in heart and skeletal muscle (6) and of sterified cholesterol in the liver (12).

Therefore the possibility that insulin may also stimulate the synthesis of phospholipids from glucose has been further investigated. This paper describes the results.

Materials and Methods

One day old White Leghorn chicks were used. As a control group unanesthetized chicks were injected intracardially with 50 μl of a 0.9 % NaCl solution containing 10 μCi of glucose-U-^{14}C. The other group was injected with 50 μl of a 0.9 % NaCl solution containing 10 μCi of glucose-U-^{14}C plus insulin at a concentration of 0.75 I.U./kg body weight.

The animals were then decapitated at

10, 30, 60 and 120 min following injection and blood was collected from their necks in tubes containing fluroxalate. Subcutaneous abdominal adipose tissue, liver, heart and pectoral muscle pieces were quickly removed from the chickens and weighed immediately on a precision balance. The pieces were then digested in 5 ml of chloroform: methanol: 100 mM HCl (200:100:1) plus 5 ml of 100 mM HCl in order to extract the lipids (4).

Phospholipids were separated from the total lipid extract by thin layer chromatography on silica gel G. The plates were developed in chloroform: benzene (60:40). The purified and isolated phospholipids were then dissolved in 10 ml of toluene containing 0.5 % of 2.5-diphenyloxazole (PPO) and 0.01 % of p-bis-2-(5-phenyloxazol) (POPOP). The radioactive samples were counted in a LKB Wallac scintillation spectrometer.

Tissue samples for DNA analysis were defatted with 2.5 ml of chloroform:methanol (2:1) and assayed by BURTON's (1) method.

Chemicals. Glucose-U-^{14}C (328 mCi/mmol) was purchased from the Radio Chemical Centre, Amersham. Bovine insulin from Burroughs Wellcome Co., London. Highly polymerized calf thymus DNA was purchased from Sigma, and was used as a standard. Other chemicals were obtained from commercial sources.

Statistical analysis. Significance was determined using Student's t test to compare the means of unpaired samples.

Results

The accumulation of ^{14}C from glucose in the phospholipids of adipose tissue, liver, heart, skeletal muscle and plasma at 10, 30, 60 and 120 minutes after administration is shown in table 1.

The incorporation of radioactivity into phospholipids after the administration of labeled glucose was greater in the liver than in the other tissues after 10 min and also at each of the intervals studied.

Table I. Effect of insulin on the incorporation of glucose-U-^{14}C into phospholipids in different chicken tissues.

Data are presented as DPM/100 μg DNA for tissues and DPM/ml for plasma. Values are given as mean ± SEM of four chickens. Controls were injected with 50 μl of a 0.9 % NaCl solution containing 10 μCi of glucose U-^{14}C. Insulin was injected with 50 μl of saline solution containing 10 μCi of glucose U-^{14}C plus insulin (0.75 I.U/kg body wt). * $p < 0.05$; ** $p < 0.01$ vs. control.

		Time after injection (min)			
		10	30	60	120
Adipose tissue	Control	—	—	—	—
	Insulin	—	—	—	—
Skeletal muscle	Control	32 ± 5.1	71 ± 8.8	68 ± 21.2	190 ± 28.8
	Insulin	38 ± 10.8	122 ± 12.9 **	104 ± 12.1 *	213 ± 5.9
Liver	Control	151 ± 8.2	521 ± 23.2	749 ± 63.8	503 ± 67.5
	Insulin	156 ± 43.6	710 ± 22.7 **	911 ± 59.9 *	833 ± 76.6 **
Heart	Control	95 ± 13.0	157 ± 8.3	202 ± 55.2	220 ± 33.1
	Insulin	91 ± 10.4	201 ± 8.0 **	231 ± 24.4	216 ± 8.9
Plasma	Control	606 ± 79.3	1,101 ± 88.5	816 ± 98.8	1,950 ± 546.3
	Insulin	602 ± 20.0	1,308 ± 132.0 *	1,030 ± 181.6	1,141 ± 275.4

The difference between the incorporation in the liver and the other tissues was at its maximum after 60 minutes, being 1,000 % greater in the liver than in the skeletal muscle and 270 % greater than in the heart.

At 120 min after glucose-U-^{14}C administration the radioactivity accumulated in the liver decreased significantly in comparison with that accumulated at 60 min. In the heart and skeletal muscle the opposite took place. Thus, at 120 min the radioactivity accumulated in the liver is only 165 % and 129 % more than that accumulated in the skeletal muscle and heart respectively.

At no interval studied was it possible to detect any incorporation of ^{14}C from glucose to phospholipids in adipose tissue.

There was a significant increase ($p < 0.01$) in labeled phospholipids in the liver, skeletal muscle and heart following insulin administration.

Discussion

These results indicate that the synthesis of phospholipids from glucose appears to take place in the liver of one day old chicks and that insulin can stimulate this process.

In chick embryo however it seems that intact phospholipids are transferred from the yolk to the embryo and its organs, and consequently substantial incorporation of glycerol-1-3-^{14}C or acetate-1-^{14}C into liver phospholipids has not been found (13).

Our *in vivo* experiments show that at least in the liver of one day old chicks, some capacity exists for the synthesis *de novo* of phospholipids from glucose-U-^{14}C. In adipose tissue there was no incorporation of ^{14}C from glucose at any interval studied while in skeletal muscle and heart the incorporation of ^{14}C was much less than that observed in liver.

Labeled phospholipids cease to accumulate in the liver when an increase of phospholipids occurs in heart and skeletal muscle. This may represent a balance between the rate of newly synthesized ^{14}C-phospholipids and their release from the liver to the blood and subsequent incorporation into heart and skeletal muscle. This suggestion is consistent with the increase of labeled phospholipids in plasma from 60 to 120 minutes.

These results confirm the hypothesis of DAVISON *et al.* (2) who suppose that phospholipids are synthetized in the liver and transported via the blood to other organs.

Injections of 0.75 I.U./kg body weight of insulin (which is the minimum dose that produced the maximum effect on the plasma glucose concentration [15]) produced a significant ($p < 0.01$) increase in the accumulation of ^{14}C phospholipids in liver, heart and skeletal muscle from 30 minutes after administration onwards.

From the discovery that physiological concentration of insulin can stimulate the transport and utilization of glucose in isolated chicken fat cells (5) evidence is now available which suggests the existence of other insulin sensitive tissues in chickens capable of removing glucose from the plasma (6, 12, 15).

Our results concerning the observed increase of ^{14}C-phospholipids produced by insulin may be discussed on the basis of a further contribution of the liver in the removal of glucose from the plasma which is consequent with the observed insulin-stimulated hypoglicemia (15).

These results are consistent with the discovery of specific binding of chicken insulin to isolated chicken liver plasma membranes (14).

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Resumen

Se estudia la incorporación de glucosa U-^{14}C a fosfolípidos en diversos tejidos del pollo de un día de edad, observándose mayor incorporación en el hígado que en otros tejidos estudiados (músculo esquelético, cardíaco y adiposo).

Igualmente, en pollitos tratados con insulina a dosis fisiológicas (0,75 I.U/kg de peso), se incrementa la incorporación de glucosa U-^{14}C a fosfolípidos en el hígado, músculo esquelético y cardíaco.

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