prior to GnRH suggests that in diabetic rats there is some impairment in the ability to hypersecrete LH. However, the pituitary glands of these diabetic castrates were able to maintain serum levels of LH that were 10–20-fold higher than those found in intact animals (table 2). Similar data were reported in a previous study[5]. In this earlier study[5] pituitary LH concentration was measured in castrated diabetic rats and found to be not significantly different from that in castrated controls.

When treated with GnRH, the increment in serum LH level in diabetic rats was only about half that noted in controls. Although high serum LH levels were evident in both groups, the ability to secrete LH in response to GnRH stimulation was clearly impaired in the diabetic rats.

When intact rats were treated with GnRH (table 2) the diabetic animals had higher LH levels than controls ($p < 0.01$).

This was interpreted as indicating a greater release of stored hormone in response to GnRH in diabetic as compared to control rats. Pretreatment levels of LH in neither serum nor pituitary glands were obtained in this experiment. However, data from a previous study[5] indicate that diabetic rats have reduced serum levels of LH associated with elevated levels of LH in the pituitary glands, suggesting failure of release mechanisms. The LH levels after GnRH treatment were not as high as expected. The apparent loss of potency in the GnRH used was presumably due to prolonged (over 1 year) storage as a frozen solution.

The results of these experiments suggest that in the absence of gonadal steroids, the responsiveness of the pituitary gland of the diabetic rat to GnRH is impaired. Reduced serum levels of LH in intact diabetic rats[3,5], however, cannot be explained by reduced responsiveness to GnRH.

Table 2. Effect of diabetes on body weight and serum LH concentration following GnRH in intact male rats

Date	Treatment	Number of rats	Body weight (g)*	Serum LH (ng/ml)*
17 May	Control	6	579±13	48±5
	Diabetic	6	352±15	256±34
24 May	Control	6	580±12	47±6
	Diabetic	5	340±19	247±46
6 June	Control	6	600±12	29±6
	Diabetic	4	342±24	164±23

* Diabetic vs control, $p < 0.01$ for each date; values are mean ± SEM.

On the contrary, the responsiveness of the pituitary gland of the intact diabetic rat is many times greater than that of the intact control rat. A possible explanation for these findings is that the pituitary of the diabetic rat responds better because testosterone levels in diabetic rats are low[3,5]. It is known that pituitaries of castrated rats are more responsive to GnRH than those of intact rats[12] and that testosterone reduces the amount of LH released in response to GnRH[13]. If this explanation is correct then it must be assumed that the reduced inhibition by androgen is a more important factor in determining serum LH levels than the impairment of pituitary function related to insulin deficiency.

In addition these data suggest that GnRH secretion by the hypothalamus of the diabetic rat must be deficient. This conclusion is based on the assumption that normal secretion of GnRH coupled with reduced testosterone levels would lead to hypersecretion of LH. Obviously this is not the case as diabetic rats have normal or below normal serum levels of LH[3,5] but elevated levels of pituitary LH[5].

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Effect of insulin in vivo on the synthesis of free fatty acids (FFA) in chicken heart and skeletal muscle

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Summary. The effect of insulin on the synthesis of free fatty acids from glucose in the skeletal and heart muscles of chicken is examined. 10 min after glucose-(U-^{14}C) administration, labeled free fatty acids (FFA) appeared in both skeletal and heart muscles. 0.75 IU of insulin kg^{-1} b.wt significantly increased the labeled FFA at the 30, 60 and 120 min intervals, with a maximum at 60 min.

In chickens it has been difficult to demonstrate an insulin effect either in vitro or in vivo. A concentration far higher than that found in chicken plasma is always required[1–3]. Nevertheless Gomez-Capilla and Langslow[4] reported a clear effect of insulin (at physiological concentrations) on the glucose metabolism of isolated fat cells, althouht the extent of this stimulation was less pronounced than that produced in mammals.

This finding, together with references to the onset of hypoglycemia produced by insulin[5,6] suggests the existence of other insulin-sensitive tissues in the removal of glucose from the plasma. We have therefore examined the effects of insulin on the synthesis of free fatty acids (FFA) from glucose in the skeletal and heart muscles of chickens. This paper describes the results of this study.

Materials and methods. 1-day-old White Leghorn chicks were used. As a control group unanesthetized chicks were injected intracardiacally 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 IU/kg b.wt.

The animals were then decapitated at 10, 30, 60 and 120 min after the administration of the treatments and pieces of heart and pectoral muscle varying between 200 and 300 mg were quickly removed and placed in 5 ml of chloroform: methanol: HCl (200:100:1) plus 5 ml of a 100 mM HCl solution in order to extract the lipids[7].

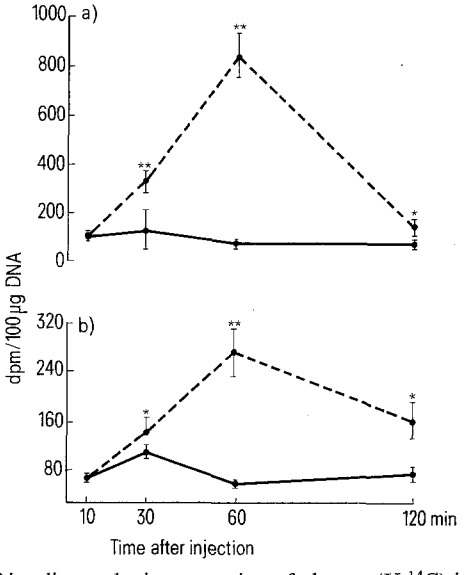
TLC was performed on the total lipid extract using silica gel G and chloroform: benzene (60:40) as the developer solvent. The purified and isolated fatty acids were then dissolved in 10 ml of toluene containing 0.5% of 2, 5-diphenyloxazole (PPO) and 0.01% of p-bis-2-(5-phenyloxazol) (POPOP). The radioactive samples were then counted in a LKB wallac Scintillation spectrometer. Tissue samples for DNA determination were treated and assesed as previously described by Burton[8].

Chemicals. Glucose-U-^{14}C (328 mCi/mmole) was purchased from the Radio Chemical Centre, Amersham. Bovine Insulin was purchased from Wellcome Research Laboratories, Beckenham, Kent. Highly polimerized calf thymus DNA, used as a standard, was obtained from the Sigma Chemical. Other chemicals were obtained from commercial sources.

Statistical analysis. All results were statistically evaluated by the Student t-test.

Results. The figure shows the accumulation of counts in the FFA of heart and skeletal muscle at 10, 30, 60 and 120 min after the glucose-U-^{14}C administration, and the effect of insulin. At the 10-min-period, labeled FFA appeared in the skeletal and heart muscle, the amount being some 54% less in the former than in the latter. In chicks receiving insulin, the labeled FFA increased significantly at the 30, 60 and 120-min intervals with the maximum increase occuring at 60 min (644% for skeletal muscle and 1128% for heart muscle).

Discussion. Although lipogenesis in birds has been extensively studied[9-11] the effects of insulin on this process still remain to be clarified. Our results show a clear insulin action on FFA synthesis in heart and skeletal muscle as well as a capacity of synthesis of FFA in these tissues. Previous studies have indicated that in birds, the liver is the predominant site of fatty acid synthesis[12,13]; the function of adipose tissue is primarily to store the fatty acids, in contrast to the situation in mammals[14,15]. More recently it has been suggested that in birds, other tissues such as the intestine, skin or muscle can contribute to the total synthesis of fatty acids[16,17]. Our results agree with this suggestion and point out the considerable significance of the synthetic activity of the muscle, taking into account the predominance of this tissue in the body in terms of mass. In chicken muscle, (obliquus abdominis externus and rectus abdominis) 870 nM of insulin was required in order to stimulate glucose uptake and glycogen synthesis[1], and the stimulation was minor. Our results, however, are in agreement with those of other authors, who were able to show that a 0.7 nM concentration of insulin was sufficient to stimulate amino acid and glucose transport into the heart cells of chickens embryos[18]. Our findings provide new evidence in support of the belief that there are other insulin-sensitive tissues besides the liver and adipose tissues[4,6]. Our suggestion is that all of these insulin-sensitive tissues together may contribute towards the removal of glucose from the plasma bringing about the consequent observed insulin-stimulated hypoglycemia.



Effect of insulin on the incorporation of glucose (U-^{14}C) into free fatty acids in chicken heart (a) and skeletal (b) muscle. Values are expressed as mean ± SE. Number of chicken 4. —— glucose-(U-^{14}C) in NaCl; - - - glucose-(U-^{14}C) plus insulin 0.75 IU/kg b.wt. * $p<0.05$, ** $p<0.01$.

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