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Original article

The effect of thyroid hormone status on plasma glucose-insulin interrelationship in broiler chickens

J Buyse [1], E Decuypere [1], J Simon [2]

[1] *Laboratory for Physiology of Domestic Animals, KU Leuven, Kardinaal Mercierlaan 92, 3030 Heverlee, Belgium;*
[2] *INRA, Station de Recherches Avicoles, Nouzilly, 37380 Monnaie, France*

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Summary — The aim of the present experiments was to study the plasma glucose–insulin relationship in eu-, hypo- or hyperthyroid broiler chickens. None of the thyroid states modified the fasting plasma glucose and insulin levels. Hypothyroid chickens exhibited a normal glucose tolerance and a normal glucose-induced insulin release after oral glucose (2 g/kg body weight) administration compared to euthyroid chickens. In contrast, hyperthyroid chickens exhibited an improved glucose tolerance accompanied by a lower insulin release. Insulin injections at a concentration of 0.1 U/kg body weight was only hypoglycemic in hyperthyroid chickens, which confirms the observation that these chickens are more sensitive to insulin. From this study it can be suggested that alterations in body composition according to thyroid status are at least partly mediated by changes in the control of carbohydrate metabolism by pancreatic hormones.

broiler / thyroid status / plasma glucose–insulin interrelationship

Résumé — Effets de l'état thryoïdien sur la relation glycémie–insulinémie chez les poulets de chair. *La relation glycémie-insulinémie a été étudiée chez des poulets de chair hyper-, hypo- et euthyroïdien. A jeun, l'état thyroïdien ne modifie pas la glycémie ou l'insulinémie. Après une surcharge orale de glucose (2 g/kg), les poulets hypothyroïdiens présentent une tolérance au glucose et une réponse insulinique normale. Par contre, les poulets hyperthyroïdiens ont une meilleure tolérance avec des insulinémies plus faibles. Une injection d'insuline (0,1 U/kg) n'est hypoglycémiante que chez les animaux hyperthyroïdiens, confirmant ainsi une sensibilité accrue à l'insuline en cas d'hyperthyroïdisme. Les changements de composition corporelle en fonction de l'état thyroïdien observés chez les poulets de chair sont donc partiellement liés à des modifications du métabolisme glucidique par les hormones pancréatiques.*

poulets de chair / état thyroïdien / relation plasmatique glucose-insuline

INTRODUCTION

Thyroid deficiency in the domestic fowl is associated with increased fatness, while hyperthyroidism induced by feeding protamone (1% T4 activity) or by long-term administration of thyroid hormones decreases fat deposition (Wilson *et al,* 1983; Decuypere *et al,* 1987). The decreased fat deposition associated with hyperthyroidism was not proportional in all body fat components, but the observed decreases were percentagewise greatest in abdominal fat, followed by total carcass, thighs and breast muscle fat content, respectively (Decuypere *et al,* 1989).

Besides changes induced in circulating levels of growth hormone and somatomedin C by thyroid status (Decuypere *et al*, 1987), it was reported that thyroid hormones also influence plasma pancreatic hormone concentrations (Cogburn *et al*, 1986) and *vice versa* (Mitchell and Raza, 1986).

Recently it was shown that in 14 week-old White Leghorn cockerels, T_4/T_3 injections reduced glucose-induced insulin concentrations while fasting plasma glucagon levels were elevated (Klandorf, 1988). Whether this is linked with a change in tissue sensitivity to insulin is not known.

In focussing research on thyroid–insulin interaction involvement in adiposity in meat-type chickens an investigation was made into the changes in insulin response upon glucose challenge in long term hypo- or hyperthyroid broilers and by the reciprocal test, the glucose response upon insulin challenge.

MATERIALS AND METHODS

Chickens, housing, management

Day-old broiler chicks (Hybro) were obtained from a local hatchery and equally divided among 4 floor pens (1.05 x 0.77 m). Temperature was set at 30°C during the first week and was gradually lowered by 2°C per week until a temperature of 20°C was reached. The lighting schedule provided 23 h of light each day, and wood shavings were used as litter.

Diets

A commercial broiler diet containing 3 150 kcal ME/kg and 22% crude protein was supplemented respectively with 0.1% MMI (2-mercapto-5-methylimidazole, methimazole; Janssens Pharmaceutica, Beerse, Belgium) or with T_3 (3, 3', 5 -triiodothyronine) at a concentration of 1 ppm (experiments 1 and 2) or 0.5 ppm (experiment 4). Diets were provided *ad libitum* from day 1 on. Control birds received the basal diet only.

Experimental design

Experiment 1

At 5 wk of age, 7 birds in each treatment group were fasted overnight and administered a glucose load of 2 g per kg body weight (BW) delivered by intubation in the crop. Just before and 10, 30, 45, 60 and 90 min after the glucose load, a blood sample was taken from a wing vein using a syringe and was transferred to iced tubes. After centrifugation at 4°C, plasma was frozen until assayed.

Experiment 2

At 7 wk of age, 9 birds from each treatment group were fasted overnight, then refed for 2 h before the start of the experiment, in which birds were injected intravenously with 0.1 U insulin (porcine insulin, Actrapid MC, SA Novo Ind, Brussels, Belgium) per kg BW. Birds were bled immediately before and again at 10, 30 and 60 min after injection.

Experiment 3

At 9 wk of age, 10 control and 9 MMI-fed birds were fasted overnight and refed for 2 h before injection. Birds in each treatment group were injected intravenously with 0.5 U insulin per kg BW, and the other half with an equal volume of physiological solution (saline, 0.9% NaCl). Blood samples were taken prior to and at 10, 30 and 60 min after injection.

Experiment 4

In this experiment, the same experimental design as in experiment 3 was used but with 8-wk old control birds and birds fed the 0.5 ppm T_3 diet.

Assay procedures

Plasma glucose concentrations were measured according to the method described by Caroll *et al* (1956). Plasma insulin was measured by radioimmunoassay by using a guinea pig antiporcine insulin serum (Ab 27–6, a gift of G Rosse-

lin, Hôpital Saint-Antoine, Paris) with chicken insulin as standard, as previously described (Simon *et al*, 1974).

Statistics

Plasma glucose and insulin data were analysed by means of multivariate analysis of variance (Manova) with time series as repeated measures [GLM procedure, repeated measures analysis of variance (SAS, 1985)]. For each treatment group separately, plasma variable levels according to time of sampling were contrasted with pretreatment levels using a 1-way Manova with repeated measures (GLM procedure, repeated measures analysis of variance option Contrast transformation). In addition, differences in plasma glucose and insulin concentrations among the treatment groups within each time of blood sampling were analysed by 1-way analysis of variance. Means were contrasted with Duncan's multiple range test.

RESULTS

Experiment 1

The effects of an oral glucose (2 g/kg BW) load on plasma glucose and insulin levels in fasted broilers with different thyroid status are illustrated in figures 1 and 2, respectively.

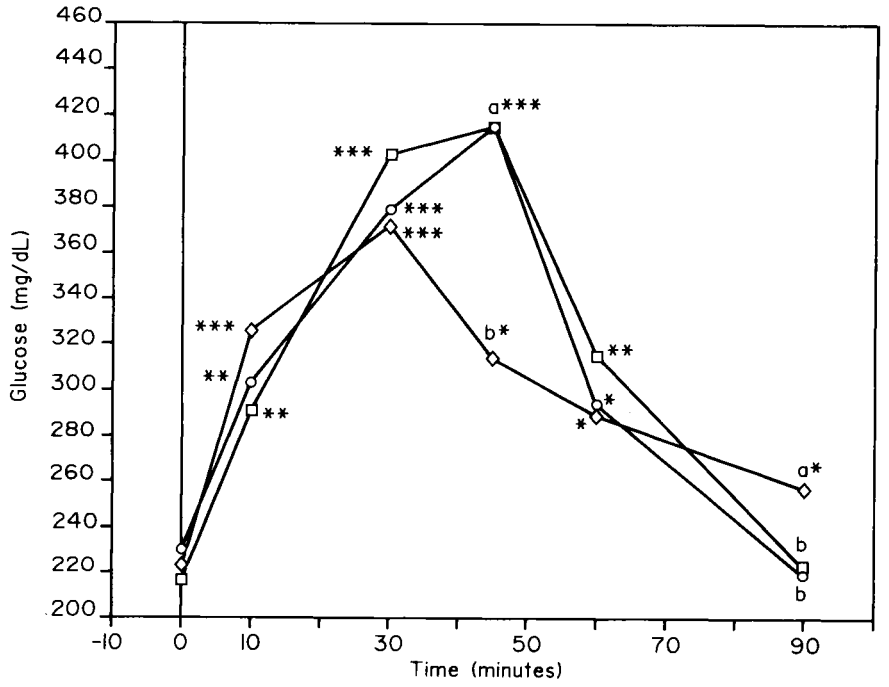

Fig 1. Effect of oral glucose load (2 g/kg BW) to fasted control (❑), T3-treated (◊) and MMI-treated (○) broilers on mean plasma glucose (n = 7). a, b : significant differences ($P < 0.05$ between treatment groups at the same sampling time (one-way Anova). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ significance level of difference with pretreatment level for each treatment group separately (one-way Manova for repeated measures).

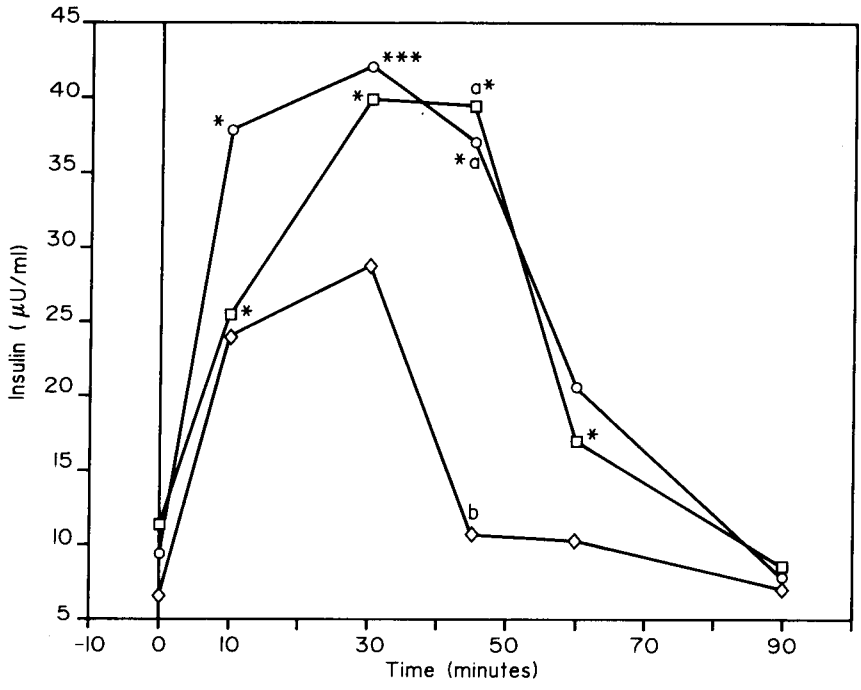

Fig 2. Effect of oral glucose load (2 g/kg BW) to fasted control (□), T3-treated (◊) and MMI-treated (○) broilers on mean plasma insulin levels ($n = 7$). a, b : See legend to fig 1; * : see legend to fig 1.

No difference in plasma baseline levels for both plasma variables between treatment groups were observed. Oral glucose administration induced a sharp increase in plasma glucose for all groups (fig 1). Multivariate analysis of variance for repeated measures revealed a significant effect of time ($P < 0.000\ 1$) and time x treatment group ($P < 0.05$) interaction. This interaction indicated a treatment group dependent plasma glucose disappearance pattern. At 45-min post-administration, plasma glucose levels in the T_3-fed birds had already declined and were significantly lower compared with both other treatment groups. At 90 min however, the mean plasma glucose level of hyperthyroid birds was significantly higher compared to both other treatment groups and remained higher than the fasting level.

For the plasma insulin data (fig 2), a significant time ($P < 0.000\ 1$) and treatment group effect ($P < 0.01$) was calculated. In MMI-fed and control birds, plasma insulin levels were significantly increased 10 min after the glucose load and remained high until 45 min post-treatment. Although the mean plasma insulin level of T_3-fed birds was also apparently increased at 10 and 30 min, differences were not statistically discernable at the 5% level from the pre-treatment level. At 45 min, the mean plasma insulin level was significantly lower in the T_3-fed group compared to both other groups.

Experiment 2

In this experiment, the effect of an intravenous injection of 0.1 U insulin per kg BW on plasma glucose levels was compared between the treatment groups (fig 3). Manova using data of all groups revealed a significant time ($P < 0.000\ 1$) and group ($P < 0.01$) effect. When performing a 1-way Manova to glucose data of the groups separately, a significant time effect ($P < 0.000\ 1$) was only present for the T_3-fed group. A significant decline in plasma glucose was already noticable at 10 min and persisted at 30 min post-injection. For the MMI-fed chickens, plasma glucose concentrations at 30 min post-injection were significantly lower than the corresponding level in control birds but not significantly different compared to pre-treatment values.

Experiment 3

The comparison of the glucose response curves in a 2-factor Manova for repeated measures revealed a significant effect of time ($P < 0.001$) and a time x injection treatment (saline or insulin) effect, indicating that the plasma glucose levels were only affected when insulin was administered exogenously (fig 4). Iv injection of 0.5 U insulin per kg BW depressed plasma glucose values in control birds within 10 min. At this time, plasma glucose level for the MMI-fed birds were also apparently re-

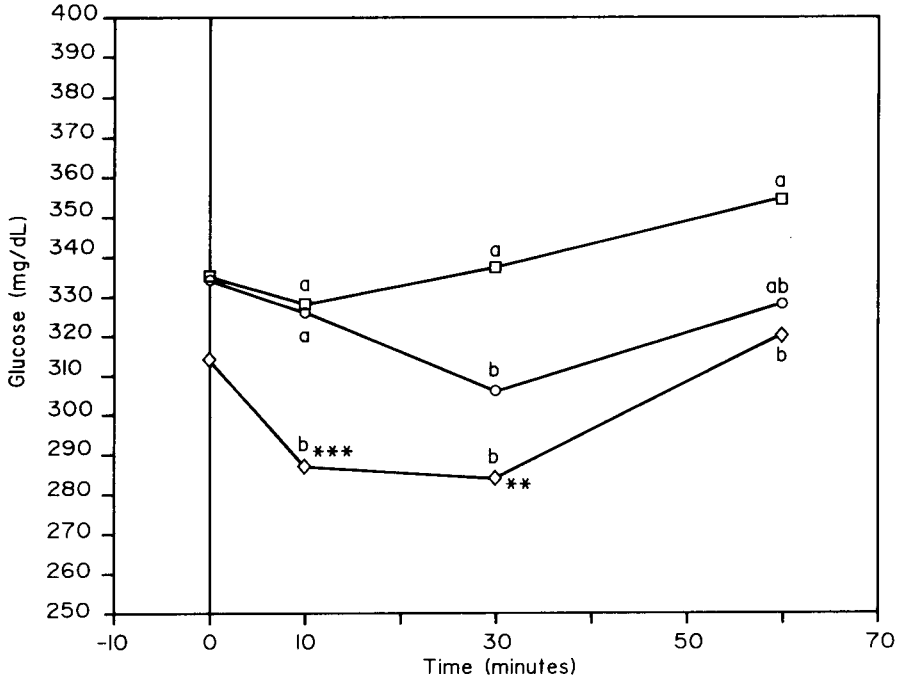

Fig 3. Effect of iv injection of 0.1 U insulin/kg RW to fed control (□), T3-treated (◊) and MMI-treated (○) broilers on plasma glucose levels ($n = 9$). a, b : See legend to fig 1; * : See legend to fig 1.

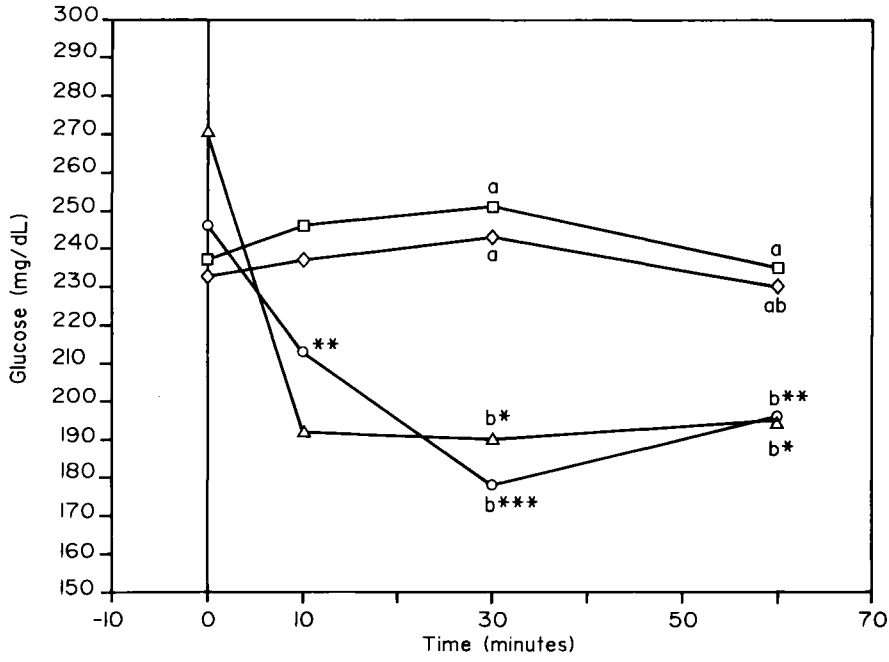

Fig 4. Effect of IV injection of 0.5 U insulin/kg BW to fed control (○) and MMI-treated (△) broilers or an equal volume of saline to control (□) and MMI-treated (◇) broilers, on plasma glucose levels (n = 4–5). a, b : See legend to fig 1; * : See legend to fig 1.

duced, but not statistically discernable (P = 0.054). For both groups, plasma glucose levels remained low for the duration of the sampling period.

Experiment 4

Two-factor Manova revealed a significant time effect ($P < 0.05$), but only when insulin was administered. No statistically significant differences between control and T_3-fed chickens were found either after saline injection or in pretreatment glucose level (fig 5). Iv injection of 0.5 U insulin per kg BW significantly depressed plasma glucose levels in T_3-fed birds within 10 min compared with the pretreatment level and with the saline-injected counterparts. For the control birds, the glucose level 10 min after insulin administration was not significantly different at the 5% level either from pretreatment level (P = 0.069) or from the saline-injected control group. At 30 min and at 60 min, plasma glucose levels were significantly depressed in both treatment groups.

DISCUSSION

The aim of the present experiments was to study the plasma glucose–insulin relationship in either hypo- or hyperthyroid broiler chickens.

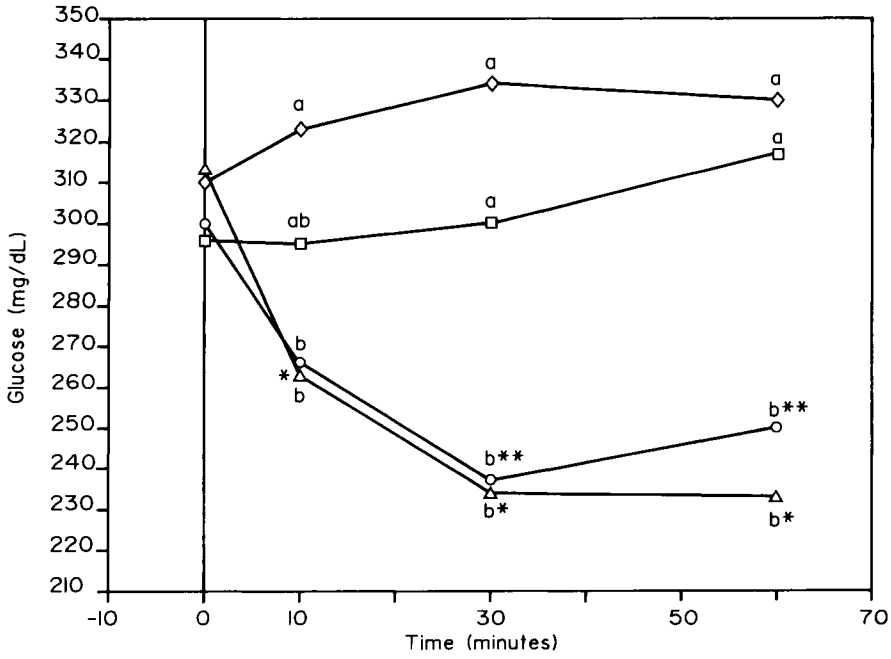

Fig 5. Effect of iv injection of 0.5 U insulin/kg BW to fed control (○) and T3-treated (△) broilers or an equal volume of saline to control (□) and T3-fed (◇) broilers, on plasma glucose levels (n = 5). a, b : see legend to fig 1. * : see legend to fig 1.

Although fasting resulted in decreased plasma glucose values as well as in decreased T_3 levels as a consequence of a decreased peripheral T_4 into T_3 conversion (Decuypere and Kühn, 1984), a long-term change in thyroid status did not modify the fasting plasma glucose and insulin concentrations. In contrast, Klandorf (1988) observed significant alterations in fasting plasma insulin levels in White Leghorn chickens according to their thyroid status.

Whereas the peak of hyperglycemia was observed 10 min after intravenous glucose injection by Klandorf (1988), in our experiment peak levels were only observed after 45 min in control and hypothyroid birds. The way of glucose administration is responsible for the shift in occurrence of the glucose peak, as shown by Klandorf (1988). In our experiment, the disposal of an oral glucose load was clearly accelerated at 45 min in the hyperthyroid birds. This is in contrast with the impairment of glucose tolerance in hyperthyroid mammals (Lenzen and Bailey, 1988). Hyperthyroidism affected the course of glucose disappearance in our experiment, while this was not apparent in the data of Klandorf (1988). This is most likely accounted for by the fact that in the experiments of Klandorf (1988) glucose was administered intravenously, which caused a very rapid disappearance of glucose. However, in both studies after the initial plasma–glucose decline, glucose levels of hyperthyroid

birds remained high compared to pretreatment levels and to other treatment groups.

In T_3-treated chickens, the improvement of glucose tolerance is associated with slightly lower insulin levels at 30 min and a rapid normalization at 45 min. The fact that insulin increased only during a short time following glucose load in T_3-treated broiler chickens is in accordance with the results observed in hyperthyroid White Leghorn chickens following intravenous glucose load (Klandorf, 1988). The rapid disposal of glucose most likely accounts for the shorter duration of insulin release in hyperthyroid chickens. It would not result from the decreased feed transit time which was observed in T_4- or T_3-treated chickens (Decuypere and Siau, 1990) since glucose was taken up rapidly (10 and 30 min) and totally from the intestine and resulted in high plasma levels at an equally early time after ingestion in all groups.

Despite the fact that the chicken is relatively resistant to insulin compared to mammals, insulin sensitivity in chickens may also be modified by nutritional or genotypic factors (Simon, 1989). The rapid glucose disposal and low insulin levels in hyperthyroid broilers suggests a state of increased insulin sensitivity. This was effectively observed in experiment 2 where 0.1 U insulin/kg had a hypoglycemic effect only in hyperthyroid chickens. Furthermore, it is also possible that the high plasma levels of T_3 in T_3 supplemented broiler chickens (Decuypere *et al,* 1987) may increase glucose transport in insulin sensitive tissues. Such a positive synergy has been observed in isolated chicken embryo heart cells (Gordon *et al,* 1986).

As hyperthyroidism is associated with an increased metabolic rate, it is very likely that the available glucose is removed very rapidly from the glucose pool by means of enhanced transport in tissues and readily oxidized to maintain the high metabolic rate.

Hypothyroid chickens exhibited a normal glucose tolerance. The glucose-induced insulin release is rapid and slightly increased at 10 min compared to both other treatment groups. Therefore, hypothyroidism would increase insulin levels in chicken. In our MMI-treated chickens, the enhancement of plasma insulin levels is, however, less marked than in thyroidectomized (Klandorf, 1988) or in PTU-treated White Leghorn chickens (Raheja *et al,* 1980).

In the present study, insulin sensitivity was similar in hypothyroid and in control chickens. Therefore, it is possible that the enhanced insulin levels in hypothyroid accounts for their enhanced fatness (Decuypere *et al,* 1987). Insulin is effectively lipogenic in chicken hepatocytes (reviewed by Simon, 1989). In addition, the lipogenic effect of insulin may be further potentiated by a decrease in plasma glucagon levels as observed in PTU-treated White leghorn chickens (Raheja *et al,* 1980).

Whether glucagon sensitivity is dependent upon thyroid state remains unknown. This is currently under investigation *in vitro* using adipocytes isolated from eu-, hyper- and hypothyroid chickens.

When increased fat deposition induced by hypothyroidism or due to selection is compared with respect to the plasma glucose–insulin interrelationship, some aspects are changed in a similar manner such as the enhanced glucose-induced insulin levels in fat birds (reviewed by Simon, 1988). On the other hand, some discrepancies can be observed which do not allow equalizing of the selection effect with the effect of altered thyroid state to a parallel change in carbohydrate metabolism. Genetically fat chickens, compared to their lean counterparts, are slightly hypo-

glycemic, exhibit an improved glucose tolerance and are more sensitive to exogenous insulin at least in the fasted state (Simon, 1988, 1989). In contrast, our "fat" hypothyroid chickens were less sensitive to exogenous insulin and exhibited an impaired glucose tolerance compared to hyperthyroid chickens. Moreover, even though plasma T_3 levels are slightly lower in genetically fat chickens, dietary T_3 supplementation which permits near-normalization of plasma T_3 levels, has minimal effect on the difference between the 2 lines in fat content (Leclercq *et al,* 1988).

From the results reported herein and those obtained in genetically fat chickens, it is suggested that in chickens, alterations in body composition according to thyroid status or by means of selection, are at least partly mediated by changes in the control of carbohydrate metabolism by pancreatic hormones.

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