## [COMMUNICATION]

# Flight Behaviour and Thyroid Hormone Regulation in Homing Pigeons

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ABSTRACT—Homing pigeons which were not given flight training for 3 months prior to the experiment, were flown the same distance of 48 km from the usual release site as reported in our earlier studies using pigeons which were in regular training. In these pigeons the flight lasted 90-160 min instead of the usual 60-80 min taken by pigeons which had regular training. This flight produced a more than two-fold increase in plasma levels of reverse triiodothyronine (rT<sub>3</sub>), concomitant with reductions in thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) levels and also in T<sub>3</sub>/T<sub>4</sub> ratio. The increase in plasma levels of rT<sub>3</sub> and the concomitant decrease in levels of T<sub>4</sub> and T<sub>3</sub> with no change in plasma osmolality, suggest inhibition of T<sub>4</sub> secretion and 5'-monodeiodination, and conversion of T<sub>4</sub> to rT<sub>3</sub>. The conversion of T<sub>4</sub> to rT<sub>3</sub>, the inactive form of T<sub>3</sub>, represents a mechanism of autoregulation of thyroid hormone function during strenuous and extended flight.

#### INTRODUCTION

Our recent studies with homing pigeons before and after natural homing flight, have shown significant post-flight (a distance of 48 km from the usual release site covered in 60–80 min) increases in plasma levels of glucose, free fatty acids (FFA), lactate, adrenaline, noradrenaline and growth hormone (GH), but not in the levels of corticosterone and the thyroid hormones, thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) [1, 2]. The increase in plasma adrenaline and noradrenaline was indicative of increased sympathetic activity and it was suggested that the flight-induced increase in plasma adrenaline could have stimulated the release of glucagon which in turn would have

brought about the increase in plasma glucose. The increase in plasma FFA was attributed to the increase in at least one adipokinetic hormone, GH, and the lack of any increase in plasma corticosterone to the stress-free nature of the flight.

In a subsequent study [3], the homing pigeons used, unlike those in the previous studies, did not receive the regular flight-training for a period of 3 months prior to the experiment, and so they took 90-160 min to fly the same distance. Significant increases in the levels of plasma glucagon (glucagonlike immunoreactivity) and presumably of glucagon-stimulated FFA, were observed. In marked contrast to the observations in the previous study [2], plasma levels of T<sub>4</sub>, T<sub>3</sub> and T<sub>3</sub>/T<sub>4</sub> ratio were significantly reduced. It occurred to us that this was due to the possible inhibition of T<sub>4</sub> secretion and 5'monodeiodination with the conversion of T<sub>4</sub> to reverse T<sub>3</sub> (3, 3', 5'-triiodothyronine or rT<sub>3</sub>), the inactive form of T<sub>3</sub>, as a mechanism for the regulation of thyroid hormone metabolism during a more strenuous and extended flight. In the present study, we have ventured to test this postulation by measuring levels of rT<sub>3</sub> in plasma samples obtained from the same birds used in the previous experiment [3].

### **MATERIALS AND METHODS**

Pigeons (*Columba livia*) used in our studies were from a colony of homing pigeons which were raised and maintained out-doors in lofts under natural photoperiod and temperature. They were

fed a daily ration of corn, wheat and barley. All studies [1–3] were conducted in the forenoon of a typical sunny mid-autumn day (6°C). The release site and distance flown (48 km) were the same in all studies. The control birds were given the usual 40 min car ride in order to simulate the 40 min car ride received by the experimental birds while being taken to the release site. Pigeons of both sexes weighing 350-400 g were used and blood samples (5 ml) were drawn from the brachial vein of each bird into heparizined syringes within 1-3 min of their arrival after the car ride (control birds) or flight (experimental birds). Blood samples were kept on ice and transported to the laboratory within 15 min and centrifuged (3000  $\times g$  for 10 min at 4°C). The separated plasma was stored at  $-20^{\circ}$ C in separate vials in duplicate. The present investigation is an extension of our previous study [3] and parts of plasma sampels obtained then are now used for the estimation of plasma rT3. In contrast to the previous studies [1, 2], these pigeons [3] had not received the regular flight training for a 3-month period between end of the racing season and time of the experiment.

The levels of rT<sub>3</sub> were measured in freshly thawed frozen plasma samples using a radioimmunoassay kit (code 10834) manufactured by BIODATA S.p.A., Italy. Osmolality was measured using a vapor pressure osmometer (Wescor, Utah).

Analysis of variance (ANOVA) was initially employed to test for sex differences and flight effect. Since sex did not prove to be a significant

variable from the ANOVA results obtained, values from both sexes were subsequently pooled and subjected to unpaired Student's *t*-test.

#### **RESULTS**

These pigeons, unlike the pigeons which received flight training prior to the experiment, took 90–160 min to fly "home" instead of the 60–80 min taken by the trained pigeons. Flight induced significant increases in plasma levels of rT<sub>3</sub> as opposed to decreases in T<sub>4</sub>, T<sub>3</sub> levels, and T<sub>3</sub>/T<sub>4</sub> ratio. The post-flight plasma rT<sub>3</sub> amounted to more than a two-fold increase over control values. Flight caused no significant change in plasma osmolality (Table 1).

#### **DISCUSSION**

The marked increase in plamsa levels of  $rT_3$  with no significant change in plasma osmolality, observed in the present study is indicative of increased conversion of  $T_4$  to  $rT_3$ . Similar increase in plasma  $rT_3$  as a possible regulatory mechanism to limit  $T_3$  activity by inhibiting  $T_4$  conversion to  $T_3$  has been observed in humans under increased physical exercise [4]. In flown homing pigeons the increase in  $rT_3$  and the concomitant reduction of plasma levels of  $T_4$ ,  $T_3$  and  $T_3/T_4$  ratio (Table 1) indicate inhibition of peripheral deiodination of  $T_4$  in order to limit the continued production of  $T_3$ . The peripheral conversion of  $T_4$  to  $T_3$  has been shown to be stimulated by GH in chickens [5]. In

Table 1. Plasma osmolality and levels of thyroid hormones in resting and flown homing pigeons

PA ANGELIANA COMO ANO MANTE PROPERTY	Control pigeons	Flown pigeons
Osmolality (mmol/kg)	303.5 ± 1.1 (10)	306.1 ± 3.8 (7)
Reverse triiodothyronine (rT <sub>3</sub> ) (pg/ml)	$211.9 \pm 16.9 \ (10)$	493.0±90.8 (7)**
Triiodothyronine (T <sub>3</sub> ) <sup>1</sup> (ng/ml)	$2.29 \pm 0.14$ (10)	$0.89 \pm 0.14 \ (8)^*$
Thyroxine $(T_4)^1$ $(ng/ml)$	$19.07 \pm 1.54 \ (10)$	12.54±2.36 (8)*
$T_3/T_4$ ratio <sup>1</sup>	$0.12 \pm 0.10 \ (10)$	0.08 ± 0.01 (8)**

Values are mean ± SEM. Figures in parentheses denote number of birds.

<sup>\*</sup> P<0.05; \*\* P<0.01

<sup>&</sup>lt;sup>1</sup> Data from previous study (George et al., 1989)

an earlier study using trained homing pigeons, post-flight circulating levels of GH were found to be significantly increased [2]. In more strenuous and extended flight such as was involved in the present investigation, an initial stimulation of T<sub>4</sub> release followed by an increase in rT3 levels should be expected. If so, the production of rT<sub>3</sub> could not be concomitant but should follow the release of T<sub>4</sub> so that excess T<sub>4</sub> could be eliminated by convertion to rT<sub>3</sub>. That this is so, has been indicated in experiments with tilapia [6] in which it was observed that rT<sub>3</sub> levels were the same as the low levels present in the control one hour after injection of T<sub>4</sub> despite the high concentrations of T<sub>4</sub> in the plasma. Since plasma T<sub>4</sub> and rT<sub>3</sub> increased following injection of T4, it was suggested that conversion of T4 into rT3 is independent of pituitary control. In light of these observations, it may be stated that the post-flight increase in plasma levels of GH observed in homing pigeons [2] could stimulate T<sub>4</sub> release in addition to releasing FFA from the fat depots. It is also possible that the inhibition of peripheral deiodination of T<sub>4</sub> to T<sub>3</sub> could have been brought about by the increased plasma levels of glucagon since it has been shown in the domestic fowl that glucagon inhibits 5'monodeiodination and may also cause initial reduction of T<sub>4</sub> secretion [7].

Rudas and Pethes [8] observed that rT<sub>3</sub> appears in the serum of chickens after warm exposure and suggested that cold exposure stimulates T<sub>3</sub> formation whereas heat exposure inactivates the T<sub>4</sub> secreted to produce rT<sub>3</sub>. During flight there is significant increase in body temperature of pigeons [9]. As flight becomes more strenuous and extended as observed in the present study, thermoregulation becomes crucial. Conversion of T<sub>4</sub> to rT<sub>3</sub> instead of T<sub>3</sub> would be a useful mechanism of thermoregulation and conservation of thyroid metabolism.

Since familiarity with the release site has been shown to reduce "release site bias", a behaviour characterized by deviation in the direction and better orientation of homeward flight [10–13], the longer time (90–160 min) taken by these pigeons

could be attributed to the lack of the flight training prior to the experiment.

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