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The effects of testosterone manipulation on the body condition of captive male yellow-legged gulls

Carlos Alonso-Alvarez[a,b,*], Miguel Ferrer[a], Jordi Figuerola[a], José A.R. Veira[b], Julio Estepa[c], Luis M. Torres[c]

[a] *Department of Applied Biology, Estación Biológica de Doñana, CSIC. Pabellón del Perú, Avda. Maria Luisa s / n 41013 Seville, Spain*

[b] *Departamento de Fisioloxía, Facultade de Veterinaria, Universidade de Santiago de Compostela, Campus de Lugo, 27002 Lugo, Spain*

[c] *Servicio de Análisis Clínico, Hospital Universitario de Valme, Ctra. de Cádiz s / n. 41014 Seville, Spain*

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Abstract

Persistently high testosterone levels are believed to be costly to males due to their negative effect on body condition. However, this assumption could not be validated when we analysed birds isolated from all social interactions. The hypothesis was tested on birds kept in isolation in order to analyse the effects of testosterone per se, and thereby exclude the influence of social interactions. Adult male yellow-legged gulls (*Larus cachinnans*) were captured, and after a period of adjustment, some individuals were subcutaneously implanted with testosterone, while the rest were used as controls. The gulls received ad libitum food for 10 days and were then fasted for 4 days. Thyroid hormones, body-mass change, daily food intake, hematocrit and several plasma biochemical parameters were analysed. Treated (T)-males maintained constant levels of plasma total protein throughout the experiment, whilst control (C)-males showed a decrease. We did not find any other differences between groups for the other variables analysed. Since the implanted birds sustained high testosterone levels for a number of days, any cost to body condition would have been revealed if these costs levels were actually important. Our results do not support the hypothesis that a reduction in body condition can be directly produced by plasma testosterone, although total protein changes do suggest different anabolic patterns in testosterone-treated gulls.

Keywords: Androgens; Birds; Daily intake; Hematocrit; Plasma chemistry; Thyroid hormones

1. Introduction

Testosterone influences many aspects of physiology and behaviour in male birds, and this androgen may affect physiological systems in a number of different ways. It may promote the performance of one system, whilst inhibiting the performance of others. This possibility has been proposed in terms of different trade-offs between physiological functions and/or between different modes of behaviour, all of which result in both benefits and costs to the bird (Wingfield et al., 1997; Ketterson and Nolan, 1999). Thus, as examples, we could cite the sustained aggressiveness during male–male conflicts (Wingfield, 1990), the

* Corresponding author. Tel.: +34-95-4232340; fax: +34-95-4621125.

E-mail address: alonso@ebd.csic.es (C. Alonso-Alvarez).

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increase in extra-pair copulation rates (Raouf et al., 1997), or the acquisition of large territories (Moss et al., 1994) as benefits of persistently high testosterone levels in plasma. On the other hand, testosterone costs may include the inhibition of incubation behaviour (Oring et al., 1989), a decrease in chick-feeding rates (Saino and Møller, 1995), decreased immunoresponse (Duffy et al., 2000) and low survival rates (Dufty, 1989; Nolan et al., 1992; Sockman and Schwabl, 2000). Lastly, a negative impact of testosterone on birds' body condition has been repeatedly suggested (Wingfield, 1990; Ketterson et al., 1991; Ros et al., 1997).

Margaret Brown (1996) reviewed definitions of the concept of body condition in birds and grouped them in two categories: conceptual definitions that "describe the degree to which an organism's physiological state influences its performance (i.e. production, activity or response to the environment)", and operational definitions "based on some aspects of body composition (i.e. levels of nutrient stores or indirect indicators of such levels)". In free-living birds, both a reduction (Wingfield, 1984; Ketterson et al., 1991) and an increase (Briganti et al., 1999; Hunt et al., 1999) in fat deposits or total body-mass have been described after testosterone implants. In order to explain the negative effects of testosterone on body condition, most studies (Ketterson et al., 1991; Chandler et al., 1994; Briganti et al., 1999; Ros, 1999) cite two old works on quail metabolism (Hänssler and Prinzinger, 1979; Feuerbacher and Prinzinger, 1981) involving castrated quails, which showed lower metabolic rates than intact males or castrated males with a testosterone supplement. Therefore, testosterone could impose a cost in terms of an increase in metabolism, and therefore in energetic demand. However, the effects of an increase in testosterone levels in intact quails were not analysed, and so the results were obscured by the increased thermal insulation caused by fat accumulation in castrated individuals. Moreover, the birds were not isolated to avoid behavioural interactions, and therefore the direct (metabolic) and indirect (behavioural) effects of testosterone were confused.

Testosterone increases aggressiveness (Wingfield et al., 1994) and song rate (e.g. Vallet et al., 1996; Hunt et al., 1997), both activities which suppose a strong energetic cost (Vehrencamp et al., 1989; Eberhardt, 1994). Therefore, the effects of testosterone on the organism could be influenced by the effects of testosterone on behaviour, and the intrinsic cost of this androgen on body condition (excluding behavioural interference) would remain unclear.

In the present study, we tested the hypothesis that testosterone produces a negative impact on body condition that is unrelated to increases in the frequency of social interactions. We experimentally increased the levels of plasma testosterone in a group of captive yellow-legged gulls *Larus cachinnans*, analysing their daily food intake and changes in body mass and hematocrit, as well as the levels of thyroid hormones, which reflect metabolic activity (e.g. McNabb, 2000). Moreover, we made an effort to increase our knowledge of the costs of testosterone, by analysing six biochemical parameters of plasma that are considered to be good body-condition indices for birds (glucose, urea, uric acid, total protein, triglycerides and cholesterol). Glucose is critical for homeotherms, because many tissues and cells depend upon its concentration in the blood (Castellini and Rea, 1992). Glucose levels in the blood are tightly regulated during fasting periods (Castellini and Rea, 1992), and thus a decrease in glucose plasma level will reflect a strong increase in a bird's energetic demand (Hazelwood, 1986). High values of uric acid and urea reflect the loss of reserves of muscular protein during starvation periods (Cherel and Le Maho, 1985). Their values increase with body-mass loss in yellow-legged gulls (Alonso-Alvarez and Ferrer, 2001; Alonso-Alvarez et al., in press) and are used as a body condition index in eagles (e.g. Ferrer, 1992). The plasma level of total protein has been proposed as a body condition index in other bird species (Dawson and Bortolotti, 1997; Ots et al., 1998), given the decrease it undergoes in situations of undernutrition (e.g. Totzke et al., 1999). Triglycerides are positively correlated with total body-fat content (Dabbert et al., 1997) and negatively with body-mass loss (Jenni-Eiermann and Jenni, 1994). Finally, cholesterol was the strongest correlated parameter with body-mass change during a food reduction experiment in yellow-legged gulls (Alonso-Alvarez et al., in press).

2. Materials and methods

2.1. Capture, housing conditions and experiment design

A total of 15 adult, male yellow-legged gulls were captured in the fish docks in Vigo (Galicia, Spain) in traps baited with fish. Birds were sexed by body size according to Bosch (1996). Individuals were trapped 1 month before egg-laying began in the gull colony (April 1998) to replicate gull body condition at its highest natural annual point of steroid activity throughout the year (Wingfield et al., 1982). The gulls were transported to 'La Cañada de los Pájaros' Wildlife Recovery Centre (Huelva, Spain) and housed in individual outdoor cages, which were visually isolated to avoid stress, but which received direct sunlight on their upper side ($4 \times 4 \times 4$ m^3; see also Alonso-Alvarez and Ferrer, 2001). The environmental temperature during the experiment oscillated between 18 and 35°C (min.–max.). We could assume that birds were not energetically stressed during the first 10 days of the experiment (see body-mass change in Table 1). Sex determination was verified by the PCR amplification of CHD gene fragment sequences following Griffiths et al. (1998). The gulls enjoyed unlimited access to water, and before the experiment began, were fed ad libitum for 2 weeks with sardines *Sardina pilchardus*. Fish is present in 32% of pellets in the original population of these gulls (Munilla, 1997), although the proportion by weight of fish in the gull's diet might be even higher. After this adaptation period, seven individuals were implanted subcutaneously with 38 mg of testosterone pellets (Organon Laboratories, Cambridge), while the remaining eight gulls received the same surgical treatment, but without an implant (T- and C-males, respectively). In order to avoid any random bias due to the small sample size, each gull was assigned to one treatment or another depending on its change in body mass (with respect to its weight at capture), thereby balancing out both groups. The birds were then fed ad libitum for 10 days (feeding period), after which their food was removed for 4 days (fasting period) to replicate the costs of testosterone under different situations of body mass change. Coulson et al. (1983) reported that the overall body-mass change throughout the year was 9% in the herring gull (*Larus argentatus*), a very closely related species, a value that was replicated in our experiment (Table 1). The length of the fasting period was based on a previous experiment (Alonso-Alvarez and Ferrer, 2001). An experiment using four groups to discriminate the effects of fasting was not possible due to the small sample size and space limitations in the facility. The individual daily food intake (g) was calculated by subtracting the weight of the non-consumed fish removed from the cage on the following day from the weight of fish provided. One control gull escaped from its cage on day 11 of the experiment and another bird lost its subcutaneous implant, probably due to problems with the skin-sealing method applied (a veterinary surgical glue; Vet-Seal, Braun, Melsungen, Germany). This fact was detected on the last day of the experiment, and thus the data for this gull from the fasting period were eliminated (after checking its testosterone plasma concentration). At the end of the experiment, the implants were removed and the gulls, once they had recovered their initial body weight, were set free in the original place of capture.

2.2. Blood sampling and body mass

Blood samples (always 2.5 ml, except on day 12; see below) were taken from the humeral vein on the implant day (day 0) and again on days 5, 10 (feeding period), 12 and 14 (fasting). In order to avoid any variation in blood biochemical parameters related to circadian rhythms (Ferrer, 1993), samples were taken in the middle of the day (between 11:00 and 15:00 h). Manipulation lasted for less than 2 min. Winged infusion sets (Valu-Set, Becton Dickinson, Utah, USA) were used to prevent damage to veins and were applied on alternative wings on each sampling day. Blood was stored in tubes with lithium–heparin as an anticoagulant. The plasma was separated by centrifugation ($550 \times g$ for 10 min) and was stored at -75°C until analysed. T_3 and T_4 concentrations were not determined for day 12 due to the small volume of plasma obtained.

The gulls and food were weighed with a dynamometer (Pesola, ± 5 g). The total body-mass change was defined as the proportion of mass lost with respect to the weight at the beginning of the experiment (day 0). The daily body-mass change was the proportion of mass lost with respect to the previous sampling day. These measurements were taken on days 5, 9, 10, 12 and 14.

Table 1
Mean daily intake and total body-mass change (mean ± S.E.) in control and testosterone-treated male gulls (C- and T-males, respectively) throughout a 14-day experiment

	C-males				T-males				*t*-test *P*
	Mean	S.E.	*n*	Range	Mean	S.E.	*n*	Range	
Mean daily intake at 9 days (g)	144.4	7.51	8	(113.3–188.9)	155.9	10.57	7	(121.1–192.8)	0.38
Total body-mass change (%)									
After feeding (0–10 days)	0.13	1.31	8	(−6.29–4.29)	1.73	1.24	7	(−2.34–6.86)	0.40
After fasting (10–14 days)	−9.73	1.55	7	(−15.06–4.29)	−8.40	1.09	6	(−12.36– −4.91)	0.51
After experiment (0–14 days)	−10.07	2.50	7	(−20.00– −1.89)	−6.66	1.63	6	(−11.56–1.27)	0.31

2.3. Hormone assays

Testosterone plus small amounts of dihydrotestosterone and androstenodione (total plasma androgens) were measured by enzyme immunoassay with commercial kits (Biosource, Nivelles, Belgium). The cross-reactivity with dihydrotestosterone and androstenodione was 0.61 and 0.76%, respectively. The minimum detectable concentration of plasma androgen was 0.05 ± 0.02 ng ml^{-1} (mean ± S.D.). For each plasma sample, 50 μl was assayed in duplicate. All samples were assayed in the same series. The intra-assay coefficient of variation (intra-assay CV) was 7.17%.

Triiodothyronine (T_3) and thyroxine (T_4) were analysed by means of chemoluminiscence immunoassay with commercial kits (Chiron Diagnostics, Essex, UK). Only their metabolically active unbounded fractions were measured with these techniques (FrT_3 and FrT_4). However, for simplicity, we have named these parameters T_3 and T_4. The cross-reactivity of our T_3 analysis with L-thyroxine (T_4) was 0.23%. The cross-reactivity of T_4 with L-triiodothyronine (T_3) was $< 1\%$. Aliquots of 50 and 25 μl of plasma samples in duplicate were needed for the T_3 and T_4 analyses, respectively. All samples were assayed in the same series (intra-assay CV, 2.6 and 3.91% for T_3 and T_4, respectively).

2.4. Plasma biochemistry

The biochemical parameters of plasma were measured in duplicate using a spectrophotometer (Hitachi 747, Tokyo, Japan) and commercial kits (Boehringer-Mannheim Biochemica, Mannheim, Germany). All samples were assayed in the same series (intra-assay CV always below 2%). The biochemical parameters analysed were (abbreviations and methods indicated in parentheses): glucose (GLUC; hexokinase method); uric acid (URIC; uricase method); urea (urease method); total protein (TP; biuret reaction); triglycerides (TRIG; enzymatic method); and cholesterol (CHOL; cholesterol esterase). The validity of these methods in yellow-legged gulls has already been demonstrated (Alonso-Alvarez and Ferrer, 2001).

2.5. Data analysis

The experimental effects were examined with repeated-measures ANOVA (ANOVAR), where the group (T- or C-males) was used as a factor (between-subject effect) and the effect of time (within-subject effect) was controlled (Zar, 1996). Student *t*-tests were used to compare values between the groups from the same day. Normality was tested with the Shapiro–Wilk test and homoscedasticity with the Levene *F*-test. Tests were performed using the SPSS statistical package (Norusis, 1993).

3. Results

3.1. The effects of implants on androgen levels in plasma

The initial plasma androgen concentrations (1 h after capture and on day 0) were not detectable

in all birds (under 0.05 ng ml^{-1}). After 5 days, androgen levels had increased in T-males (means $\pm$ S.E.: 7.70 ± 0.54, range 5.82–10.64 ng ml^{-1}; $n = 8$), but remained undetectable in C-males. On day 10 of the experiment, only one control bird showed a detectable concentration of the hormone (0.68 ng ml^{-1}), whereas implanted birds still had high levels (8.58 ± 0.82, range 5.72–12.05 ng ml^{-1}; $n = 7$). After fasting (day 14), the plasma androgen concentration of C-males had increased (1.06 ± 0.53, range 0.05–3.61 ng ml^{-1}; $n = 7$), although this increase was not enough to exceed levels in T-males (5.56 ± 1.38, range 2.93–12.02 ng ml^{-1}; $n = 6$; t-test: $t = 3.37$; d.f. = 11; $P = 0.006$).

3.2. The effects of testosterone implants on body mass and intake

No differences in the averages of daily food intake or total body-mass change were found between T- and C-males during the experiment (Table 1). Moreover, no differences were detected when we analysed data using ANOVAR. Thus, no differences in food intake were found on analysing the nine daily measurements ($F_{1,13} = 0.97$; $P = 0.38$), nor when we analysed daily body-mass change on the three sampling days during the feeding period ($F_{1,13} = 0.72$; $P = 0.41$). The same occurred when we analysed the daily body-mass change on the five sampling days which included the fasting period ($F_{1,11} = 1.22$; $P = 0.29$). Likewise, no effects on daily body-mass change caused by the treatment during feeding period were found after controlling the total food intake (repeated-measures ANCOVA, factor treatment: $F_{1,11} = 0.71$; $P = 0.42$; covariate intake: $F_{1,13} = 1.59$; $P = 0.23$). Finally, no differences were detected in daily body-mass change when we only analysed the fasting period (two sampling days: $F_{1,11} = 0.74$; $P = 0.41$).

3.3. The effects of testosterone on blood parameters

No differences were found between the groups on the first day of the subcutaneous implant treatment (t-test: $P > 0.1$, in all the parameters analysed; Table 2). Moreover, in order to avoid any effects caused by differences not detected on day 0, data obtained from blood variables throughout the experiment were individually subtracted from the initial levels (see Tables 3 and 4). Hematocrit did not differ between the groups during the study period.

3.3.1. Thyroid hormones

The experiment did not show statistically significant differences between the two groups for T_3 and T_4 values during the feeding period, nor during the experiment or after the fasting period (see Table 3). Again, no tendencies were found in the two hormones after control of the initial values or of the value at day 10 during the fasting period (Table 4).

3.3.2. Plasma biochemistry

One statistically significant difference between the groups was found in the biochemical parameters (Table 3). This difference was present in the triglycerides value when we analysed the four measures during the experiment (including the fasting period; see Fig. 1). Levels were higher in

Table 2
Plasma concentrations of thyroid hormones and biochemical parameters in testosterone-treated (T-males) and control (C-males) male yellow-legged gulls on the start of the experiment (day 0)

	C-males			T-males			t	P
	Mean	S.E.	Range	Mean	S.E.	Range		
Hematocrit (%)	57.68	1.01	(53.6–60.0)	55.07	1.46	(47.2–60.5)	1.38	0.19
Glucose (mg dl^{-1})	349.88	10.39	(310–395)	347.4	10.68	(324–407)	0.16	0.87
Uric acid (mg dl^{-1})	15.01	3.47	(4.76–35.54)	10.76	2.77	(4.72–26.37)	0.94	0.37
Urea (mg dl^{-1})	7.75	0.86	(4.00–11.00)	6.00	0.85	(3.00–8.00)	1.79	0.10
Total protein (g dl^{-1})	3.73	0.19	(3.20–4.61)	3.59	0.31	(2.65–5.23)	0.28	0.78
Triglycerides (mg dl^{-1})	109.3	16.83	(51–210)	97.14	17.19	(50–194)	0.76	0.46
Cholesterol (mg dl^{-1})	393	19.25	(304–497)	332.0	25.11	(235–413)	1.75	0.11
T_3 (pg ml^{-1})	4.97	0.54	(3.57–7.39)	4.90	0.59	(3.06–6.66)	0.40	0.70
T_4 (ng dl^{-1})	0.78	0.13	(0.18–1.49)	0.54	0.11	(0.19–0.99)	1.14	0.27

C-males, $n = 8$; T-males, $n = 7$.

Table 3
Differences between testosterone-treated and control male gulls in hematocrit, several biochemical parameters and thyroid hormones during the experiment

	Feeding period		Whole experiment		Fasting period	
	$F_{1.13}$	P	$F_{1,11}$	P	$F_{1,11}$	P
Hematocrit	1.05	0.32	0.67	0.43	0.32	0.59
Glucose	2.07	0.17	0.69	0.42	0.11	0.75
Uric acid	0.32	0.58	0.01	0.98	0.31	0.59
Urea	0.41	0.54	0.68	0.63	0.06	0.80
Total protein	2.80	0.12	3.77	0.08	4.20	0.06
Triglycerides	3.14	0.10	11.04	0.01	3.59	0.09
Cholesterol	0.31	0.59	0.76	0.39	1.50	0.25
	$F_{1,13}$	P	$F_{1,11}$	P	t	P
T_3	0.23	0.64	0.53	0.48	0.97	0.35
T_4	1.83	0.20	1.27	0.28	1.52	0.16

ANOVAR and t-tests: feeding period, days 5 and 10; fasting period, days 12 and 14; whole experiment, days 5, 10, 12 and 14 (T_3 and T_4 were not analysed on day 12). T_3, free triiodothyronine; T_4, free thyroxine.

C-males (means $\pm$ S.E.: 79.43 ± 7.26 mg dl^{-1}) than in T-males (54.94 ± 6.38 mg dl^{-1}). A tendency towards statistical significance was detected for total protein (Table 3; Fig. 1). This variable showed lower mean values in C-males (3.09 ± 0.16 g dl^{-1}) than in T-males (3.68 ± 0.27 g dl^{-1}).

When data were subtracted from the initial values (day 0), and separately from the values present at the start of fasting (day 10; Table 4), there were no differences in triglyceride levels. However, total protein showed a stronger decrease in C-males (-0.71 ± 0.32 g dl^{-1}) than in T-males (-0.10 ± 0.20 g dl^{-1}; when we use the four sampling days; see Fig. 1).

4. Discussion

The aim of this study was to test whether high levels of plasma testosterone can produce any impact on body condition, as has been suggested by other studies on bird metabolism (Hänssler and Prinzinger, 1979; Feuerbacher and Prinzinger, 1981), but always excluding the influence of social interactions.

Body-mass change, daily food intake and glucose levels did not show any differences between the groups and did not suggest any different energetic demand. In mammals, androgens seem

Table 4
Differences between testosterone-treated and control male gulls in hematocrit, several biochemical parameters and thyroid hormones during the experiment after the control of the initial value (two first columns) or of the value at the day before fasting (last column)

	Feeding period		Whole experiment		After fasting	
	$F_{1,13}$	P	$F_{1,11}$	P	$F_{1,11}$	P
Hematocrit	0.03	0.86	0.02	0.90	0.12	0.74
Glucose	1.00	0.34	0.64	0.44	0.24	0.64
Uric acid	0.38	0.55	0.66	0.44	0.26	0.62
Urea	0.00	0.99	0.05	0.84	0.06	0.81
Total protein	3.20	0.10	4.50	0.05	1.41	0.26
Triglycerides	0.36	0.56	0.29	0.60	0.08	0.79
Cholesterol	1.81	0.20	1.74	0.21	0.08	0.79
	$F_{1,13}$	P	$F_{1,11}$	P	t	P
T_3	0.45	0.52	0.59	0.46	0.32	0.75
T_4	0.02	0.89	0.03	0.87	0.61	0.55

Feeding period, days 5 and 10; fasting period, days 12 and 14; whole experiment, days 5, 10, 12 and 14 (T_3 and T_4 were not analysed on day 12). Values on days 5, 10, 12 and 14 were subtracted from the day 0 value (ANOVAR). Values on days 12 and 14 were subtracted from the day 10 values (ANOVAR and t-test).

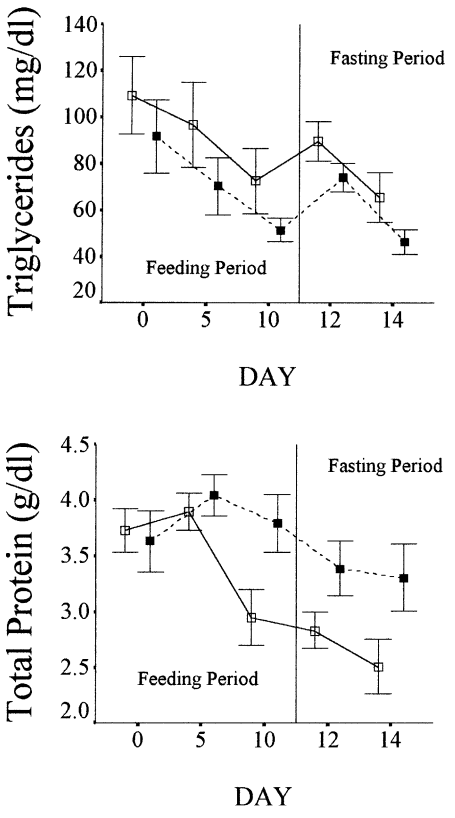

Fig. 1. Means (±S.E.) of triglycerides and total protein plasma concentrations in control (open squares, continuous line) and testosterone-treated (solid squares, dotted line) male yellow-legged gulls during the experiment.

to influence feeding behaviour and favour high intake rates (e.g. Gentry and Wade, 1976; Morley et al., 1985). In birds, Das (1991) did not find changes in total food consumption in the Indian weaver bird (*Ploceus philippinus*). Deviche (1992) found an increase in the feeding rate of isolated dark-eyed juncos (*Junco hyemalis*) implanted with testosterone. Nevertheless, he did not find changes in body-mass, body fat content or standard metabolic rates. Our results concerning thyroid hormones, which have an important role in the regulation of the metabolic rate, body temperature and oxygen consumption (reviewed in McNabb, 2000), do not support the hypothesis that androgens affect metabolic activity. Although metabolic rates were not measured, the correlation coefficient between oxygen consumption and T_3 or T_4 plasma concentrations in chickens (range 0.78–0.98; Bobek et al., 1977) suggests that any difference in metabolic rates will be reflected in thyroid hormone levels.

Two other studies (Thapliyal et al., 1983; Gupta and Thapliyal, 1984) failed to find any increase in metabolism after testosterone treatment on intact birds of two passerine species. Moreover, Gupta and Thapliyal (1984) found an increase in body mass in their treated birds. Despite their results, neither study kept birds in isolation, and so did not separate the direct effects of testosterone from effects derived from behavioural interactions. Recently, Wikelski et al. (1999) found in white crowned-sparrows (*Zonotrichia leucophrys gambelii*) that high testosterone levels induce an increase in food intake, body-mass loss and locomotor activity, but a reduction in the resting metabolic rate. Their birds were maintained in separate cages, but in the same indoor chamber, which probably affected behaviour and endocrinology due to visual or song communication (see e.g. Wingfield et al., 1994). In contrast, Buttemer and Astheimer (2000) did not find that testosterone affected the basal metabolic rate or body mass in captive and visually isolated white-plumed honeyeaters (*Lichenostomus penicillatus*). Therefore, none of these studies, nor the results of the present experiment, provide any evidence for a direct influence of testosterone plasma concentration on bird metabolism.

The majority of blood parameters analysed failed to show any between-group differences. Only triglyceride concentration was lower in T-males during the experiment, perhaps reflecting a reduction in the amount of body fat. Dabbert et al. (1997) found a strong positive correlation between total body fat and plasma triglycerides in male mallards (*Anas platyrhynchos*). Nevertheless, when we controlled triglycerides with respect to their initial concentrations (Table 4), this difference disappeared. In fact, changes throughout the experiment followed a similar pattern in both groups (see Fig. 1).

However, total protein did show a trend towards lower values in C-males in the first analysis (Table 3; Fig. 1), which was consistent when initial values were controlled (Table 4). There are four possible explanations for this:

1. A state of undernutrition in C-males. A decrease in the plasma levels of total protein (hypoproteinaemia) is observed in almost all diseases, but especially in the case of malnutrition (Augustine, 1982; Brugère-Picoux et al., 1987; Totzke et al., 1999). However, there

were no differences between the two groups in daily food intake or in body mass. Moreover, there were no differences in the values of uric acid or urea, which are both related to muscle protein catabolism during fasting periods (Cherel and Le Maho, 1985).

2. Differences in plasma volume due to dehydration. High total-protein values in birds have been related to dehydration (Arad et al., 1989; Brugère-Picoux et al., 1987). However, the hematocrit value, which is positively correlated with hydration levels in pigeons (Arad et al., 1989), did not differ between the groups.
3. Higher total protein concentrations in T-males could be related to anabolic activity. Puerta et al. (1995) found higher levels of plasma total protein in testosterone-implanted house sparrows (*Passer domesticus*). They argued that their results agreed with the known anabolic effect of testosterone. This androgen influences the development of the musculoskeletal system in mammals (Mooradian et al., 1987; Kawata, 1995; Sheffield-Moore, 2000). In birds, an increase in muscle weight (Fennel and Scanes, 1992a; Lipar and Ketterson, 2000) and a decrease in abdominal adipose tissue (Fennel and Scanes, 1992b) have been described in growing individuals during testosterone treatment. Thus, anabolic activity in muscles might demand higher concentrations of plasma proteins. This pattern might be reflected in our total protein values, although changes in body mass, daily intake or thyroid function were not detected.
4. Alternatively, the difference detected might be a statistical artefact. We have reported the results of 67 different tests (Tables 1–4). Using a statistical threshold of $P = 0.05$ to test for significance, we would expect three significant results to be produced by chance alone. Our study shows a similar result. In fact, a Bonferroni correction would reduce our threshold from 0.05 to 0.0007 for the *P*-value (Miller, 1981), a figure which was not reached in any test (see Tables 1–4). This reinforces the general first conclusion: high plasma concentrations of testosterone do not affect body condition in yellow-legged gulls. However, the adjustment for multiple tests is appropriate when significance in any individual test leads to the rejection of the broader null hypothesis (Chandler, 1995). Body condition may include different aspects of bird physiology. Therefore, the change in the plasma protein levels might be considered separately as a way of disentangling specific aspects of the effects of testosterone on physiology.

In summary, the present study does not support the hypothesis stating that a reduction in body condition is directly linked to plasma testosterone, although protein changes may suggest different anabolic patterns between groups. However, the small sample size might have prevented us from achieving a positive result; we were working with large birds, a fact which limited housing possibilities (see Section 2). In spite of this problem, the testosterone levels reached by T-males were higher than the levels reported for other bird species (reviewed in Beletsky et al., 1995) and for closely related species during the breeding season (*Larus occidentalis* approx. 3 ng ml^{-1} Wingfield et al., 1982; *Larus ridibundus* approx. 5 ng ml^{-1} Groothuis and Meeuwissen, 1992). Moreover, the dose was sustained for 14 days, sufficient time for effects to have manifested themselves if they were really important.

On the other hand, the objective of this experiment was only to test the effect of testosterone in the absence of behavioural interactions. In a lizard species (*Sceloporus jarrovi*), the more aggressive, T-implanted, free-living males experienced a lower ratio of energy intake to energy expenditure (Marler and Moore, 1989, 1991). Nevertheless, in a related study with captive, isolated animals, castrated, castrated but T-implanted and control lizards did not show any differences in body-mass change or oxygen consumption (Marler et al., 1995). In free-living, testosterone-implanted, yellow-legged gulls, the frequency of aggressive behaviour increased in comparison with control birds (Alonso-Alvarez and Velando, in press), but unfortunately these individuals were not recaptured to test the effects of the implants on body condition.

In the future, more studies are necessary in order to simultaneously differentiate the direct effects (those focussed on in the present study) from the indirect effects of testosterone on the physiological state of birds, and therefore to reappraise the role of this hormone on avian biology. Some findings, such as the low survival rate of free-living T-treated birds (Dufty, 1989; Nolan et

al., 1992), could then be considered from other perspectives when these issues are clarified.

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