# Steroids for free? No metabolic costs of elevated maternal androgen levels in the black-headed gull

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## Summary

Within- and between-clutch variation in yolk titres of hormones of maternal origin has been found in many avian species. So far, experiments have revealed mainly beneficial effects of maternal androgens. This would also apply to black-headed gulls (*Larus ridibundus*). Previous experiments have shown that chicks benefit from these higher levels since their competitive abilities are improved and growth and survival probabilities thus enhanced. However, not all females show the same increase in yolk hormones from first to last egg or invest equally high amounts of androgens in their clutches. Possibly, there is a trade-off between the beneficial effects of high androgen levels and potential costs, such as increased metabolic rates. We studied possible metabolic costs of experimentally elevated yolk androgen levels for chicks of several age classes, starting three days prior to hatching until fledging at an age of approximately 30 days. Daily energy expenditure in the field, measured using the doubly labelled water technique, did not differ between treatments or between sexes. Oxygen consumption measured in birds at rest in the lab (RMR) did not vary between chicks hatched from androgen-injected (T) or oil-injected (Oil) control eggs at any age in thermo-neutral or below thermo-neutral conditions. Males showed a lower RMR than females towards the fledging age. We conclude that it is unlikely that the costs of high maternal androgen levels can be found in higher energy expenditure in the chick.

Key words: yolk androgen, maternal hormone, metabolism, energy consumption, daily energy expenditure, sibling competition, parental investment, chick growth, black-headed gull, *Larus ridibundus*.

## Introduction

Variation in and functional consequences of avian maternal hormone deposition in the egg have recently been receiving much attention. Several studies have reported large variation in these steroids both within and among clutches (e.g. Schwabl, 1993; Gil, 1999; Whittingham and Schwabl, 2002). The majority of studies reported that enhanced yolk levels of maternal androgens appear to have beneficial effects on offspring growth and development (e.g. Schwabl, 1996; Lipar and Ketterson, 2000; Eising et al., 2001; Eising and Groothuis, in press).

These beneficial effects of yolk androgens have mainly been discussed in the context of hatching asynchrony. For instance, experimental elevation of yolk androgen levels decreased the time until hatching by half a day in black-headed gull chicks [*Larus ridibundus*; Eising et al., 2001; but see Sockman and Schwabl, 2000 for an opposite effect in American kestrels (*Falco sparverius*)]. This is in accordance with the data presented by Lipar and Ketterson (2000) and Lipar (2001), who showed that increasing levels of yolk testosterone according to laying order were associated with an enlarged size of the hatching muscle in European starling (*Sturnus vulgarus*) and red-winged blackbird (*Agelaius phoeniceus*) chicks.

Moreover, since body mass and tarsus length at hatching of gull chicks from androgen-treated eggs were equal to those of oil controls, maternal androgens appear to enhance overall embryonic growth. Growth after hatching can be significantly enhanced by yolk androgens (Schwabl, 1996; Eising et al., 2001). Also, long-term enhancing effects of maternal yolk androgens on competitive behaviour have been reported both in canaries (*Serinus canaria*; Schwabl, 1996) and in gulls (Eising and Groothuis, 2002).

However, the occurrence of large within- and between-clutch variation in the deposition of maternal androgens (e.g. Schwabl et al., 1997; Reed and Vleck, 2001; Groothuis and Schwabl, 2002) begs the question of why not all mothers invest high amounts of hormones into their eggs, unless there is a cost involved. This cost may either be incurred by the mother producing the androgens or by the chicks exposed to them.

Steroid hormones can entail immunosuppressive costs (e.g. Mooradian et al., 1987; Ketterson and Nolan, 1999; Peters, 2000; but see also Ros et al., 1997; Braude et al., 1999; Hasselquist et al., 1999), which is also suggested for maternal androgens (T. G. G. Groothuis, C. M. Eising, C. Dijkstra and W. Müller, manuscript submitted; Müller et al., in press). High

yolk androgens may also induce detrimental levels of sibling competition or metabolic costs. In the present study, we focus on the latter.

It has often been suggested that testosterone (T) enhances metabolic rate. Such T-dependent metabolic costs have, for instance, been suggested in gulls soon after hatching (Ros, 1999). However, data available in the literature are rather controversial. Studies in the late 1920s showed a reduction of diurnal metabolic rate in castrated chickens (*Gallus gallus*) compared with intact controls (Mitchell et al., 1927). Similar results were presented for Japanese quail (*Coturnix coturnix japonica*; Feuerbacher and Prinzinger, 1981) and for spotted munia (*Lonchura punctulata*; Gupta and Thapliyal, 1984). Yet, an increase in body mass usually accompanies castration and may have accounted for the reported differences. Ketterson et al. (1991) experimentally showed in adult male dark-eyed juncos (*Junco hyemalis*) that, at least prior to and during the breeding season, high circulating T levels were accompanied by a reduction in body mass and fat storage, suggesting that high T levels may be energetically costly. More directly, Buchanan et al. (2001) experimentally showed in male house sparrows (*Passer domesticus*) that elevated testosterone levels increased metabolic rate and development of an androgen-dependent sexual ornament, the bib size, suggesting a metabolic cost of dominance signalling. By contrast, testosterone implantation in intact birds did not affect mass-specific basal metabolic rates in captive male white-plumed honeyeaters (*Lichenostomus penicillatus*; Buttemer and Astheimer, 2000) and in dark-eyed juncos (Deviche, 1992). Unfortunately, no data were provided about the activity levels of the birds in relation to treatment in either study. T-treated white-crowned sparrows (*Zonotrichia leucophrys gambelii*) showed increased activity levels and reduced resting metabolism (Wikelski et al., 1999). The authors suggested that increased activity was compensated by a reduction of resting metabolic rate (RMR; analogous to Deerenberg et al., 1998). The effect of androgens on metabolism is thus yet far from clear.

So far, no data have been reported on the relationship between maternally derived yolk androgens and metabolism. Are chicks facing a trade-off between beneficial effects of maternal androgens on the one hand and costs in terms of energy expenditure on the other? Therefore, we studied whether metabolic rates and daily energy expenditure of black-headed gull chicks hatching from eggs injected with androgens differ from those of chicks from oil-injected eggs during different stages of development. Since the black-headed gull is a sexually dimorphic species and significant sex differences in growth occur around day 15 after hatching (W. Müller, C. M. Eising, C. Dijkstra and T. G. G. Groothuis, unpublished data), we also included a comparison between the sexes.

## Materials and methods
### Design
Lab-based oxygen consumption measurements of animals at rest were conducted on black-headed gull (*Larus ridibundus* L.) chicks in three consecutive years (2000–2002), with a different developmental stage measured each year. In addition, measurements of daily energy expenditure (DEE) in free-living chicks were conducted in 2000. In all studies, we compared chicks hatched from androgen-injected eggs (T chicks) with chicks hatched from oil-injected control eggs (Oil chicks). A detailed description of the procedures follows below for each year separately. Sex was determined from a small blood sample collected shortly after hatching using molecular techniques (Griffiths et al., 1998). Since not all birds were blood sampled, sample sizes for sex were sometimes slightly smaller than for treatment. All measurements were conducted under license DEC 2160 from the Dutch animal experimentation committee.

### Egg injections
Black-headed gulls lay a modal clutch of three eggs, in which androgen levels increase significantly with laying order (Eising et al., 2001; Groothuis and Schwabl, 2002). In all experiments, we used only first-laid eggs, since the eggs of a clutch not only differ in androgens but also in mass, protein and water content and vitamins (Heaney et al., 1998). This enabled us to elevate the hormone level by injections to the same as that of normal last-laid eggs, hence within the physiological range of the species. We used the same procedures to select, calculate the dose to be injected and inject our eggs as in a previous study (Eising et al., 2001). Androgen treatment consisted of injection of a mixture of 0.12 μg testosterone and 10.0 μg androstenedione per 50 μl of oil. After hatching, all chicks were marked with a small, numbered, plastic band for individual identification.

### DEE and resting metabolic rate (RMR) of older chicks
In 2000, we first measured DEE in the field and then RMR in the lab in 29 individuals between day 20 and day 32 after hatching. These 15 Oil and 14 T chicks were reared in the field by their foster parents and were part of an experiment focussing on the effect of maternal yolk hormone levels on begging behaviour (Eising and Groothuis, in press). For a description of the study site and the gull breeding system, see Eising et al. (2001). For this experiment, chicks from T- and oil-injected eggs (see below) were matched for age and body mass in two chick nests. Groups of these nests were located in enclosures (wire mesh covered with cloth; 60 m$^2$; 40–50 cm high) to facilitate re-capturing of chicks. To measure oxygen consumption, chicks were caught from their enclosures at around 19.00 h and were transported to the lab facilities, where the RMR protocol (see below) started at around 21.30 h. This was conducted on the day that the final blood sample for the DEE measurement was taken in the afternoon. Chicks were measured overnight and then returned to the field. Parents readily accepted the chicks and there was no effect on survival probabilities.

*Resting metabolism*

In 2001, we measured RMR of young chicks when they were presumably still consuming yolk and of older chicks under conditions at or below thermo-neutrality (15°C). Chicks from T- and oil-injected eggs were brought to the lab around hatching and were subsequently hand reared. During the first week after hatching, chicks of both treatments were matched for age and mass and, from then on, were housed in pairs in cages (0.9 m×0.75 m; 0.9 m high). Food and water were provided *ad libitum*. Between the age of 2 days and 10 days, oxygen consumption of 29 T and 24 Oil chicks was measured under thermo-neutral conditions. Another set of chicks was measured when they were between 20 days and 30 days old, either at thermo-neutral conditions (25°C; 14 T, 15 Oil) or below thermo-neutral (15°C; 10 T, 16 Oil).

In 2002, we measured metabolic rates just prior to and just after hatching, when chicks are still consuming yolk and are therefore exposed to the manipulated levels of androgens. Freshly laid eggs were brought to the lab, injected there (see below) and hatched in an incubator. Oxygen consumption of eggs was measured on the day aimed to be 2 days prior to hatching (32°C; 23 T, 23 Oil). Subsequently, the oxygen consumption of the chicks was measured on the day of hatching (32°C; 15 T, 19 Oil) or two days after hatching (30°C; 13 T, 11 Oil).

*Indirect calorimetry*

Oxygen consumption measurements were performed in darkness at temperatures ranging between 25°C and 32°C, which is thermo-neutral for gulls. In 2001, we also measured oxygen consumption of older chicks at 15°C, which is below thermo-neutral. In 2000 and 2001, oxygen consumption of chicks was measured overnight in individual airtight boxes (20 litre) equipped with a movement detector and a temperature sensor. In 2002, oxygen consumption of eggs and chicks was measured during the day for at least four hours in smaller respiration chambers (2 litre). Paper towels provided bedding. Food and water was not provided during the measurements but always directly thereafter. Just before and after the measurement, chicks were weighed to the nearest 0.1 g.

Measurements were conducted in an eight-channel open flow system. Dry outdoor air was passed through each of the eight chambers. Flow rates were measured and controlled with a mass flow control (Brooks, Model 5850S; Rosemount Inc., Veenendaal, The Netherlands) set to maintain the oxygen and carbon dioxide concentrations in the outlet air above 20% and below 1%, respectively. Oxygen and carbon dioxide concentrations of dried inlet and outlet air from each chamber were measured every 10 min with a paramagnetic oxide oxygen analyser (Xentra 4102; Servomex, Zoetermeer, The Netherlands) and an infrared carbon dioxide gas analyser (1440C1 STD; Servomex). Data points were collected from each metabolic chamber separately and stored by computer. Oxygen consumption was calculated according to Hill (1972) to correct for volume changes with respiratory quotient below

1 and expressed in standard temperature and pressure. The first hour and last 30 min of every measurement were excluded from the analyses.

Six measurements were taken from each chick per hour, and 30 min running means were calculated to determine the lowest average, which was taken as the measurement of RMR for each individual. To be able to compare DEE and RMR, oxygen consumption was expressed in kJ h$^{-1}$ using the gas-exchange conversion factors of Gessaman and Nagy (1988).

*Daily energy expenditure*

Rates of DEE of free-living chicks were estimated with the doubly labelled water method (DLW; Lifson and McClintock, 1966; Speakman, 1997). During the field season of 2000, 28 chicks were injected intra-peritoneally with 0.8 ml of a mixture of H$_2$$^{18}$O and $^2$H$_2$O using calibrated 1-ml insulin syringes. Based on the principle of isotope dilution (Visser et al., 2000), the $^2$H and $^{18}$O concentrations in the DLW mixture were calculated to be 34.8% and 61.4%, respectively. After an equilibration period of 1 h (Speakman, 1997), the chick was weighed with an electronic balance to the nearest 0.1 g, and a blood sample ('initial') was taken by puncturing the brachial vein with a sterile needle and subsequently filling 4–6 glass capillaries each with 15 μl blood. Capillaries were flame-sealed immediately and stored at 4°C until further analysis. Thereafter, the chick was returned to its nest. After 48 h, the chick was recaptured, reweighed and another blood sample ('final') was taken following the same procedures as outlined above. Blood samples of six other chicks were taken for assessment of the natural abundances of $^2$H (152.3±0.98 atom percent) and $^{18}$O (2000.6±2.27 atom percent).

Blood samples were analysed in triplicate at the Centre for Isotope Research at the University of Groningen. For each individual, its amount of body water was determined following the principle of $^{18}$O dilution, using the quantity and $^{18}$O enrichment of the dose, the population-specific $^{18}$O background concentration and the individual-specific $^{18}$O concentration of the initial blood sample. The daily CO$_2$ production was calculated for each animal using Speakman's (1997) equation 7.17. DEE is expressed as kilojoules spent per day. For a detailed description of the analytical and calculation procedures, see Visser and Schekkerman (1999) and Visser et al. (2000).

*Statistical analyses*

All data on oxygen consumption and DEE were normally distributed for all age classes and could therefore be tested parametrically using General Linear Models (GLM) with or without repeated measures or by independent sample *t*-tests. The variables of interest – treatment, sex and age – and their possible interaction effects were only retained in the full models when they reached statistical significance ($P<0.05$). If no statistical significance was reached, single independent models are presented. Power analyses were performed using the program GPOWER (shareware; Erdfelder et al., 1996).

## Results

### Resting metabolic rate

RMR increased as chicks grew older and heavier (Fig. 1A; $r=0.945$, $F_{1,171}=1440$, $P<0.001$). Mass-specific RMR increased only for the first few days after hatching (Fig. 1B) and will be discussed below.

### Peri-natal RMR

#### Before hatching

Table 1A shows the mean egg mass-specific RMRs per treatment per age class. When tested in a GLM, including treatment and sex, mass-specific metabolic rates prior to hatching increased significantly with age ($F_{1,33}=37.87$, $P<0.001$). Since T treatment is known to decrease the time until hatching (this sample: T, $23.47\pm0.24$ days; Oil, $23.94\pm0.24$ days; $F_{1,33}=2.02$, $P=0.17$) and therefore age at hatching may not be similar, we also looked at the relationship between mass-specific RMR and the number of days after egg laying and found there was no such relationship ($F_{1,44}=0.02$, $P=0.89$).
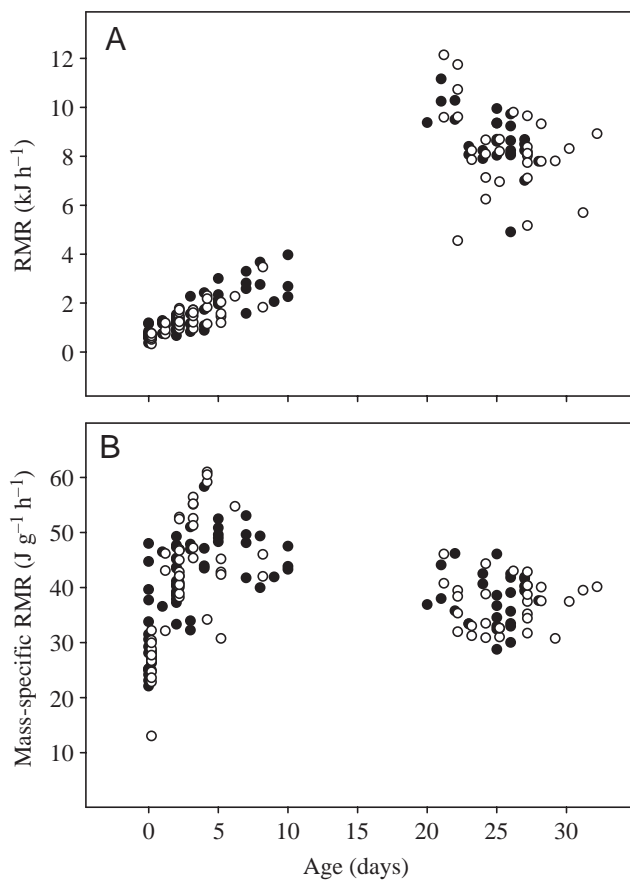


Fig. 1. (A) Resting metabolic rates (RMR) as a function of chick age. Filled circles represent androgen-injected (T) chicks; open circles represent oil-injected (Oil) chicks. For clarification, the open circles have been plotted slightly to the right of the closed circles. (B) Mass-specific metabolic rates for T (filled circles) and Oil (open circles) chicks as a function of their age.

In the first model, there was no effect of treatment or sex on the eggs' RMR. Unfortunately, sex was not always known, which diminishes the sample size in the full model. Therefore, the effect of treatment was also determined over the total sample size ($N=46$) independently of sex. There was again no treatment effect on the mass-specific RMR of the eggs. When RMR was corrected for chick hatching mass instead of egg mass, there was still no significant effect of treatment ($F_{1,37}=2.47$, $P=0.12$) or sex ($F_{1,35}=0.02$, $P=0.90$). Thus, during embryonic development, androgen treatment did not affect metabolic rates.

#### After hatching

19 chicks (9 T, 10 Oil) were measured both at hatching and two days later, and a repeated-measures analysis of variance (ANOVA) shows that there was a significant positive effect of age (the repeat) on mass-specific RMR (Fig. 1B; Table 1B). Treatment and sex had no effect on RMR.

The data for age 0 days and age 2 days have also been analysed separately in order to have a larger sample size at both ages. At age 0 days, there was no significant difference in RMR between the T and the Oil group or between males and females. Mass-specific RMR was higher at age 2 days than at age 0 days. Again, there was no statistical difference between the RMRs of both treatment groups or an effect of sex. In conclusion, around hatching there were no indications of an effect of treatment or sex on chick metabolic rates.

### Early post-hatching metabolism

Early post-hatching RMR under thermo-neutral conditions is presented in Table 1C. None of the tested variables (age, treatment and sex) or their interactions contributed significantly to the explained variance in RMR.

#### RMR of older chicks at and below thermo-neutral conditions

Metabolic rates of chicks measured below thermo-neutral at 15°C were significantly higher ($F_{1,53}=8.75$, $P<0.005$) than those of chicks measured at thermo-neutral conditions (Table 1C). Overall, treatment had no effect on RMR ($F_{1,53}=0.64$, $P=0.43$).

Also, within each temperature range there was no effect of treatment. Overall, we found an almost significant effect of sex ($F_{1,51}=3.77$, $P=0.06$) on oxygen consumption. Males ($N=30$, mean age 23.7 days) consume on average $35.10\pm0.65$ J $h^{-1}$ $g^{-1}$, whereas females ($N=25$, mean age 23.8 days) consume $37.46\pm0.90$ J $h^{-1}$ $g^{-1}$. However, there was also a significant interaction effect of sex and temperature ($F_{1,51}=4.00$, $P=0.05$). In conditions below thermo-neutral, oxygen consumption of females was significantly higher than that of males, whereas in thermo-neutral conditions oxygen consumption of males and females was similar (Table 1C). At this age range, there was also a significant difference in body mass (males, $279.4\pm4.36$ g; females, $246.36\pm5.04$ g; $F_{1,53}=24.83$, $P<0.001$) between males and females, which may have caused differences in thermo-regulatory costs (see Discussion).

To increase the power of the test regarding treatment and

Table 1. *Mass-specific metabolic rates in (A) androgen-treated (T) and oil-treated (Oil) eggs prior to hatching, (B) T and Oil chicks at the day of hatching and 2 days post-hatching, (C) chicks of several age classes in and below thermo-neutral conditions and (D) T and Oil chicks in the field*

| Experimental group (Age in days) | Temperature* (°C) | Metabolic rate (J h$^{-1}$ g$^{-1}$) | | | | Statistics |
| --- | --- | --- | --- | --- | --- | --- |
| | | T | Oil | Male | Female | |
| (A) 2002, Eggs | | | | | | |
| –3 | – | 8.62±0.72 (6) | 7.82±0.76 (6) | | | a |
| –2 | – | 9.98±0.55 (14) | 10.24±0.64 (10) | | | |
| –1 | – | 16.71±1.69 (3) | 15.91±1.48 (7) | | | |
| (B) 2002, Chicks | | | | | | |
| 0 | – | 29.01±1.28 (15) | 26.54±0.91 (19) | 26.81±1.12 (18) | 28.85±1.10 (15) | b |
| 2 | – | 41.86±1.21 (13) | 43.12±1.29 (11) | 42.58±1.22 (12) | 42.30±1.07 (12) | |
| (C) 2001, Early post-hatch | | | | | | |
| 2–10 | – | 47.51±1.72 (29), mean age 5.0 | 45.56±1.10 (24), mean age 3.7 | 45.79±1.31 (24), mean age 4.6 | 47.47±1.46 (24), mean age 4.6 | c |
| 20–30 | 25 | 34.70±1.00 (14), mean age 23.9 | 34.74±1.16 (15), mean age 23.6 | 34.73±0.95 (18), mean age 23.6 | 35.67±1.30 (11), mean age 23.9 | d |
| | 15 | 38.29±0.71 (10), mean age 23.1 | 37.04±1.50 (16), mean age 24.7 | 35.66±0.78, mean age 23.8 | 39.65±0.90, mean age 23.6 | e |
| (D) 2000, Field | | | | | | |
| 22–32 | – | 40.82±0.88 (14) | 39.99±0.78 (15) | 39.84±1.24 (9) | 40.11±0.78 (17) | f |

Values are means ± s.e.m.; *N* (sample size) is shown in parentheses.

The additional *p* value in the Statistics columns refers to the observed power of the test.

*–indicates that measurements were done in thermo-neutral conditions (see text).

[a]Treatment: $F_{1,33}=0.002$, $P=0.96$; sex: $F_{1,33}=0.003$, $P=0.96$; treatment (– sex): $F_{1,43}=0.04$, $P=0.84$, $p=0.06$.

[b]Whole group, Age (repeat): $F_{1,16}=81.24$, $P<0.001$; treatment (repeat): $F_{1,16}=0.03$, $P=0.86$; sex (repeat): $F_{1,16}=0.005$, $P=0.95$. Age 0, treatment: $F_{1,32}=2.59$, $P=0.12$, $p=0.06$; sex: $F_{1,31}=1.67$, $P=0.21$, $p=0.05$. Age 2, Treatment: $F_{1,22}=0.50$, $P=0.49$, $p=0.05$; sex: $F_{1,22}=0.03$, $P=0.87$, $p=0.08$.

[c]Age: $F_{1,52}=0.10$, $P=0.76$; treatment: $F_{1,51}=1.17$, $P=0.28$, $p=0.29$; sex: $F_{1,46}=0.73$, $P=0.40$, $p=0.13$.

[d]Treatment: $F_{1,27}=0.002$, $P=0.97$, $p=0.11$; sex: $F_{1,27}=0.001$, $P=0.97$, $p=0.71$.

[e]Treatment: $F_{1,24}=071$, $P=0.41$, $p=0.08$; sex: $F_{1,24}=10.28$, $P<0.005$, $p=0.07$.

[f]Age: $F_{1,27}=2.82$, $P=0.11$; treatment: $F_{1,27}=1.90$, $P=0.18$, $p=0.12$; sex: $F_{1,24}=0.04$, $P=0.85$, $p=0.22$.

For further explanation of statistics, see text.

sex, data measured in older chicks in thermo-neutral conditions were combined for both years (2000 and 2001) in a full factorial GLM. The result confirmed that none of the variables tested significantly affected RMR (treatment: $F_{1,50}=0.36$, $P=0.55$; sex: $F_{1,50}=1.85$, $P=0.18$; age: $F_{1,50}=0.25$, $P=0.62$).

The above results confirm the finding of the previous experiment that exposure to androgens during embryonic development does not affect oxygen consumption post-hatching in thermo-neutral conditions.

### *RMR of older chicks reared in the field*

The mean energy consumption in the T and Oil groups and for males and females is presented in Table 1D. None of the variables tested (age, treatment, sex or interactions) using backward GLM contributed significantly to the explained variance in mass-specific RMR.

### *Daily energy expenditure*

DEE averaged 341.2±14.8 kJ day$^{-1}$ (mean ± s.e.m., *N*=15) in the Oil group and 346.2± 4.9 kJ day$^{-1}$ (*N*=13) in the T group, which was not statistically different ($F_{1,23}=0.17$, $P=0.68$). Over the age range studied (22–30 days), age did not affect DEE ($F_{1,26}=0.74$, $P=0.40$). There was also no effect of sex (males, 349.0±7.0 kJ day$^{-1}$, *N*=8; females, 346.7±10.2 kJ day$^{-1}$, *N*=19; $F_{1,23}=0.06$, $P=0.81$) nor an interaction effect of treatment and sex ($F_{1,23}=0.34$, $P=0.56$). It is unlikely that body mass differences obscured an effect of treatment since there was no significant difference in body mass between treatment groups (T, 204.79±7.19 g; Oil, 207.67±8.74 g; $t_{27}=0.25$, $P=0.80$; males, 206.0±7.79 g; females, 208.9±7.14 g; $t_{26}=0.24$, $P=0.81$).

### *Activity-related metabolism*

Activity-related metabolism (which consists primarily of activity and thermo-regulatory costs) can be estimated roughly from the combination of DEE and RMR data. By subtracting the RMR in kJ day$^{-1}$ (Gessaman and Nagy 1988) from the DEE for each individual, we obtain an estimate for the activity metabolism of each chick. The effect of treatment on this estimate was far from significant (T, 184.37±11.88 kJ day$^{-1}$; Oil, 176.94±5.88 kJ day$^{-1}$; $F_{1,26}=0.29$, $P=0.60$) and so was the

effect of sex (males, 181.74±10.54 kJ day$^{-1}$; females, 182.99±8.88 kJ day$^{-1}$; $F_{1,25}$=0.01, $P$=0.94). Also, age did not affect activity metabolism ($F_{1,26}$=2.94, $P$=0.10).

The above results show that there was no effect of androgen treatment or sex on metabolic rates of black-headed gull chicks during the third and fourth week after hatching under natural conditions. Since the mean metabolic rates were very similar for both treatments and sexes throughout the age ranges over which we performed our measurements, we would have needed huge sample sizes (130–4350) to obtain any statistically significant differences, which are then likely to be biologically irrelevant. Thus, we have obtained no indications that energy expenditure differs between chicks from eggs with low or high androgen content.

## Discussion

We aimed to determine whether beneficial effects of maternal yolk androgens on chick development might be counterbalanced by increased energetic costs in black-headed gulls. We manipulated yolk hormone levels of first-laid eggs by means of androgen or oil injections, thereby mimicking the natural within-clutch variation between first- and last-laid eggs. Since maternal androgens stimulate pre- and post-natal growth as well as alertness and begging behaviour (Eising et al., 2001; Eising and Groothuis, in press), we expected the chicks to have a higher daily energy expenditure and RMR. In other species, a high growth rate is associated with high mass-specific RMR, pre-natally as well as post-natally (Vleck et al., 1980; Dietz and Drent, 1997). However, it is also possible that RMR is lowered by testosterone, as reduced RMR has been found in testosterone-treated male birds, possibly as a compensation for higher activity levels (Wikelski et al., 1999). We did not, however, find any increase or decrease pre- or post-hatching in RMR in response to our treatment.

### Pre-hatching RMR

Mass-specific metabolic rates increased towards hatching. In birds, metabolism increases steeply during the last few days prior to hatching (Prinzinger et al., 1995), presumably associated with increased pre-hatching activity levels and accompanying maintenance cost or changes in the water balance (Vleck et al., 1980).

In the present study, oxygen consumption did not differ between the T and Oil eggs during the last days before hatching. Chicks from T-treated eggs hatched slightly sooner but were not smaller at hatching, indicating that testosterone stimulates pre-natal growth. This suggests we either measured metabolic rates during the plateau phase (Vleck et al., 1980; Prinzinger and Dietz, 1995; Prinzinger et al., 1995; Dietz et al., 1998), when growth had already levelled off, or the differences in growth are so small that they are not detectable in our RMR measurements.

### Post-hatching RMR

Post-hatching RMR increased for the first few days after hatching and then gradually decreased. This steep increase shortly after hatching is a common phenomenon in both precocial and altricial species and is associated with the development of mature function, including thermoregulation (Klaassen et al., 1994).

We found no indication that metabolic rates differed between T and Oil chicks after hatching. We expected to find such differences since T chicks are more active than Oil chicks (Eising et al., in press) even when maternal hormones are no longer present and such activity can be energetically costly (e.g. Ricklefs, 1974; Vehrencamp et al., 1989). Desai and Hales (1997) also suggested that maternal effects (nutritional status) play an important role in 'programming' the offspring's metabolism in later life. Moreover, testosterone stimulates muscle growth, and muscles are a relatively energetically demanding tissue (Piersma et al., 1996).

However, females had higher RMR than males below thermo-neutral conditions. Although RMR was expressed mass specifically, correcting for the significantly larger body mass in males relative to females (asymptotic body mass for males 261.4 g; females, 220.3 g; W. Müller, C. M. Eising, C. Dijkstra and T. G. G. Groothuis, unpublished data), this size difference may still account for the observed interaction effect of sex and temperature on RMR. Since heat conductance and insulating properties depend on surface-to-volume ratio, females might have to expend more energy to maintain body temperature.

### Post-hatching DEE

We did not find an effect of treatment on daily energy expenditure nor on activity metabolism. This suggests that the higher activity and begging frequencies in T chicks (Eising and Groothuis, in press) do not entail a detectable energetic cost or result in a compensation mechanism. McCarthy (1996) and Bachman and Chappell (1998) already suggested that the amount of energy allocated to begging behaviour in birds is slight compared with the amount of energy needed for growth. Similarly, Lynn et al. (2000) showed in adult dark-eyed juncos that testosterone increased activity but not daily energy expenditure. In that study, testosterone did increase locomotion and foraging behaviour whereas it decreased sleeping and preening. Lynn et al. proposed that this differential allocation suggests that there may be long-term costs associated with maintenance of elevated testosterone levels.

Overall, we have found no evidence that maternal androgens affect metabolism pre- or post-natally directly or indirectly *via* behaviour. There was neither an effect on RMR nor on DEE. This suggests that the behavioural changes evoked by maternal androgens are energetically cheap. There is no indication of an androgen-mediated trade-off between enhanced growth and begging behaviour on the one hand and metabolic costs in these chicks on the other.

The fact that we did not find differences in metabolic rates between T and Oil chicks is unlikely to be due to failure of the treatment. The technique of injecting androgens into eggs to mimic maternal variation has proven successful in earlier

studies, inducing faster growth and increased begging in gull chicks living in natural conditions (Eising et al., 2001; Eising and Groothuis, in press). Although most analyses show a very low power, it may be clear from the presented means that there is little scope for any statistical differences. The minimum required sample size to obtain a significant treatment effect with a power of 0.80 was 130, but most tests required close to 1000 individuals. Similarly, to obtain a significant sex difference we would need, at a minimum, sample sizes ranging between 580 and 4350, which suggests that there really are no biologically relevant differences in energy turnover.

Next, potential effects of yolk androgens on metabolism should be measured earlier in the ontogeny.

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