Thyroid hormones correlate with resting metabolic rate, not daily energy expenditure, in two charadriiform seabirds

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Summary

Thyroid hormones affect *in vitro* metabolic intensity, increase basal metabolic rate (BMR) in the lab, and are sometimes correlated with basal and/or resting metabolic rate (RMR) in a field environment. Given the difficulty of measuring metabolic rate in the field—and the likelihood that capture and long-term restraint necessary to measure metabolic rate in the field jeopardizes other measurements—we examined the possibility that circulating thyroid hormone levels were correlated with RMR in two free-ranging bird species with high levels of energy expenditure (the black-legged kittiwake, *Rissa tridactyla*, and thick-billed murre, *Uria lomvia*). Because BMR and daily energy expenditure (DEE) are purported to be linked, we also tested for a correlation between thyroid hormones and DEE. We examined the relationships between free and bound levels of the thyroid hormones thyroxine (T4) and triiodothyronine (T3) with DEE and with 4-hour long measurements of post-absorptive and thermoneutral resting metabolism (resting metabolic rate; RMR). RMR but not DEE increased with T3 in both species; both metabolic rates were independent of T4. T3 and T4 were not correlated with one another. DEE correlated with body mass in kittiwakes but not in murres, presumably owing to the larger coefficient of variation in body mass during chick rearing for the more sexually dimorphic kittiwakes. We suggest T3 provides a good proxy for resting metabolism but not DEE in these seabird species.

Key words: Daily energy expenditure, Resting metabolic rate, Thyroxine, Triiodothyronine

Introduction

As pleiotropic hormones, thyroxine (T4) and triiodothyronine (T3) are involved in the regulation of a multitude of physiological traits, but they are best known for their role in the regulation of tissue oxygen consumption and thermogenesis *in vitro* (Bobek et al., 1977; McNabb, 2000; Hulbert, 2000). T4 and T3 are also well known for their *in vivo* association with basal metabolic rate (BMR) in homeotherms, at least in the laboratory (Nicol et al., 2000; Silvestri et al., 2005; Johnstone et al., 2005; Kim, 2008). By increasing cellular metabolism, T3 can increase BMR and promote thermoregulation. Nonetheless, regulatory heat, the extra heat produced in response to cool temperatures (thermoregulation), is primarily under nervous control via shivering (McNabb, 2007). It is obligatory heat, the additional heat increment above essential life processes necessary to maintain thermoneutrality and the primary constituent of BMR, which is controlled by T3 in homeotherms (McNabb, 2007).

Information on the role of thyroid hormones in the regulation of BMR in wild homeotherms, however, is limited and equivocal (Burger and Denver, 2002; Chastel et al., 2003; Vézina et al., 2009; Li et al., 2010; Welcker et al., 2013). For instance, the relative role of T4 (as opposed to the better established role of T3) in the regulation of metabolism in birds is debated. Administration of T4 can increase BMR and/or daily energy expenditure (DEE) (Al-Adsani et al., 1997; Banta and Holcombe, 2002; Johannsen et al., 2012), and it is often unclear whether there is a direct effect of T4 or whether T4 is activated via deiodination (McNabb, 2000; Johannsen et al., 2012). Furthermore, whereas T3 is the biologically active molecule in mammals, T3 and T4 have similar physiological potency in some birds, even though the affinity of the thyroid receptor for T3 is almost identical between birds and mammals (McNabb, 2007). The ratio of T3:T4 is sometimes used as a diagnostic for thyroid-related illnesses, as homeostasis in the presence of thyroid illness

can distort that ratio (Mortoglou and Candiloros, 2004). More recently, the T3:T4 ratio has been used as an indicator of toxic contamination in wild animals because some thyroid hormone mimics outcompete T3 at the binding site of the carrier molecules (Verreault et al., 2004; Brar et al., 2010).

BMR is the minimal energy cost for a post-absorptive, resting and thermoneutral animal. DEE is the average daily energy cost. Both BMR and DEE vary within species (Bech et al., 1999; Fyhn et al., 2001; Speakman et al., 2003). One potential cause of variation in BMR (or resting metabolic rate, RMR) is that some individuals obtain a large amount of energy regularly and therefore have large metabolically-active organs, such as a large gastro-intestinal tract, heart or skeletal muscles (Hammond and Diamond, 1997; Speakman et al., 2003). Those costly organs are required to sustain the high DEE required to obtain and assimilate that energy. Other individuals obtain less energy and therefore have smaller and less active organs. Variation in BMR can thus reflect the variation in energy expenditure required for maintaining internal organs at a resting state, and as a consequence, one might anticipate a correlation between BMR and DEE. Such correlations have been reported by some authors (Nilsson, 2002; Tieleman et al., 2008; Careau et al., 2013), but not others (Meerlo et al., 1997; Fyhn et al., 2001; Speakman et al., 2003). If high metabolic intensity is associated with high DEE, high BMR and high thyroid hormone levels, we might anticipate a relationship between those variables. Given the role of T3 and T4 or of the T3/T4 ratio in regulating resting metabolism, and the putative energetic link between maintenance metabolism and DEE, we predicted that these thyroid hormones may also be involved in regulating DEE. If that were the case, then circulating levels of T3 and T4 should correlate with both maintenance metabolism and DEE. In support, a previous study used thyroid hormones as indicators of DEE in a charadriiform bird (Duriez et al., 2004).

We examined the strength of correlations between post-absorptive, thermoneutral and unstressed RMR, DEE and thyroid hormone levels in free-living, breeding thick-billed murres (*Uria lomvia*) and black-legged kittiwakes (*Rissa tridactyla*). We treat unstressed RMR as a surrogate for BMR. Both kittiwakes and murres are piscivorous seabirds of the order Charadriiformes that are characterized by high levels of energy expenditure relative to other seabirds (Shaffer, 2011). Kittiwakes are sexually dimorphic (males are ~10% larger than females), whereas murres are largely monomorphic (males are <5% larger than females with seasonal variation in size obscuring sex-specific trends). Kittiwakes are small (~400 g) gulls that spend most of their time at sea flying and foraging at the surface while murres are large (~1000 g) auks that forage by diving to depths of up to 150 metres. We examined RMR during incubation because RMR likely peaks at that time, at least for kittiwakes (Bech et al., 2002). We examined DEE during chick rearing because time spent flying, and thus DEE, likely peaks during chick rearing, at least for murres (D.A. Croll, Diving and energetics of the thick-billed murre, *Uria lomvia*, University of California, 1990; Elliott and Gaston, 2005; Elliott et al., 2008). We considered both bound and unbound hormone levels of T4 and T3 to examine three predictions: (1) RMR and DEE would both correlate with thyroid hormone levels, (2) that relationships would be stronger with free than with total thyroid hormone levels, and (3) RMR and DEE would be correlated across individuals within each species.

Materials and Methods

Field methods

We studied RMR in adult kittiwakes from 15 to 30 June 2010 (N=32) at Middleton Island, Alaska, and murres from 13 to 24 July 2011 (N=53) at Coats Island, Nunavut. All birds were in the latter half of incubation (eggs 15–25 days old). We studied DEE of some of the same individual kittiwakes from 4 to 20 July 2010 (N=24) when kittiwakes had chicks between 10 and 20 days old. Likewise, we also studied the same individual murres from 25 July to 10 August 2009 when murres had chicks between 5 and 15 days old (N=22; same observations as Elliott et al. (Elliott et al., 2013)). At our Arctic study sites, air temperatures were usually moderate (10–20℃), the night was very brief and birds generally foraged actively throughout both day and night. Birds were captured at the nest with a noose pole (murres) or a hook (kittiwakes). Only one adult per nest was captured and we did not correct body mass for linear size as such corrections do not improve estimation of lean or lipid mass in either murres or kittiwakes (Jacobs et al., 2012). Murres were sexed using sex-specific PCR primers applied to a drop of blood on filter paper (Elliott et al., 2010) whereas kittiwakes were sexed behaviorally by position during copulation. Both parents share incubation and chick-rearing duties, at least during the period of time covered by the current studies. Research was approved by the Protocol Management and Review Committee of the University of Manitoba under protocol F11-020.

Post-absorptive, thermoneutral and unstressed RMR

We used open-flow respirometry to measure metabolic rate of incubating birds in a respirometry chamber. Birds were captured between 0600 and 2200 hours local time (0430–2030 solar time) as they were switching off from their incubation shift (~10 hours for kittiwakes and ~12 hours for murres) (Gill et al., 2002; Elliott et al., 2010). The ambient temperature (T_a) during the measurements was within the birds' thermoneutral zone (range=15.1–18.2℃; higher temperatures appeared to result in discomfort and increased metabolic rate) (for thermoneutral zone, see Gabrielsen et al., 1988).

Immediately after capture, birds were weighed using a spring balance (Pesola, Switzerland) and transported to a small building and placed in a FoxBox II® (Sable Systems, Las Vegas, Nevada) respirometer for the measurement of RMR. We used magnesium perchlorate to dry outside air, which was pumped through a 15 L respiratory chamber with a flow rate of ~1.0 L/min (kittiwakes) or ~2.0 L/min (murres) using the pump and flow meter built into the FoxBox II respirometer. The effluent air was then passed through a FoxBox II® carbon dioxide analyzer. Carbon dioxide was then removed from the air using soda lime and the effluent was passed through a FoxBox II oxygen analyzer. We recorded effluent air concentrations every 15 seconds. To reduce drift, the oxygen analyzer was encased in Styrofoam. Both the oxygen and carbon dioxide analyzers were calibrated at the start and end of each field season using pure nitrogen and 30% oxygen-70% nitrogen stock gas. We measured baseline gas levels (scrubbed of water, and, for the oxygen analyzer, of carbon dioxide) for 1 hour before and after each measurement and calibrated the baselines to 20.6% oxygen and 0.04% carbon dioxide at the start of each measurement. Calibrating the baseline to 20.6%, instead of the traditional 20.95%, should have little effect on our calculations as those calculations use only the difference in percent oxygen between steady-state and ambient air, and both values would be similarly biased downward. We calculated RMR as the lowest 10-minute running average of instantaneous oxygen consumption using formula 3A in Withers (Withers, 2001), after accounting for washout delay, although as birds were in steady state, the effect of correcting for instantaneous oxygen consumption had less than 1% effect on oxygen and carbon dioxide measurements. RMR (W) was calculated from the value of oxygen consumption rate using a conversion factor of 19.9 J mL O_2^{-1}. Because carbon dioxide production rate can be less sensitive to drift than oxygen consumption rate, we repeated all analyses using carbon dioxide production rate.

DEE

We used the doubly-labeled water method to measure DEE during chick rearing. Upon capture, we weighed the birds and injected them intra-peritoneally with 0.5 mL doubly-labeled water (64.0 atom percent excess ^{18}O and 36.2 atom percent excess ^{2}H). Birds were then released and recaptured after 90 minutes (murres) or kept in a cloth bag for 60 minutes (kittiwakes), at which time we obtained an initial blood sample to allow measurement of equilibrium isotopic concentrations (Speakman, 1997). Murres that were released immediately returned to their breeding site and were inactive (brooding or incubating) until recaptured. Background blood samples were taken from 8 unlabeled adult kittiwakes and 9 unlabeled adult murres to determine the mean background level of isotopes. We recaptured, reweighed and resampled all injected individuals after an average of 48.0 hours (S.D.=2.4 hours, range=46–55 hours). All samples were flame-sealed into capillary tubes until analysis at the University of Aberdeen via isotope ratio mass spectrometry (Speakman and Król, 2005). Water for analysis of ^{2}H and ^{18}O was obtained by vacuum distilling blood samples into glass Pasteur pipettes. ^{2}H enrichment was determined from hydrogen gas derived from the distilled water by online chromium reduction. ^{18}O enrichment was analyzed by equilibration of

distilled water with CO_2 gas of known oxygen isotopic enrichment using the small-sample equilibration technique (Speakman and Król, 2005). Isotope ratios were then determined by gas source isotope mass spectrometry (IRMS) with isotopically characterized gases of H_2 and CO_2 in the reference channels. Enrichment of the injectate was estimated by a dilution series with tap water and mass spectrometric analysis of 5 subsamples of each solution (Speakman and Król, 2005).

Initial body water was determined using the plateau method from the ^{18}O dilution space, which agrees well with directly measured values for murres and other animals (Speakman, 1997; Jacobs et al., 2012). We assumed percent body water did not change over the course of our measurements. We calculated energy expenditure using a single pool model corrected for fractionation effects assuming a fixed evaporative water loss of 25% (equation 7.17) (Speakman, 1997). Based on direct examination of adult diet, the rate of CO_2 production was converted into estimates of DEE (W) using a caloric equivalent of 27.1 J mL CO_2^{-1} (murres: 81% protein, 14% fat, 5% carbohydrate) and 27.4 J mL CO_2^{-1} (kittiwakes: 80% protein, 15% fat, 5% carbohydrate). The doubly-labeled water method accurately estimates DEE at the individual level (uncertainty generally <10%) for charadriiform birds and other animals (Visser and Schekkerman, 1999; Van Trigt et al., 2002; Shirai et al., 2012). Furthermore, values derived from a number of alternative equations are all highly correlated ($R^2>0.95$), and as it is the relative (rather than absolute) value of DEE that is relevant to our results, the use of alternative equations would have little impact on our conclusions.

Thyroid assays

Upon capture and immediately after RMR measurements (murres) or only immediately after RMR measurements (kittiwakes), birds were blood sampled (1 mL from the alar vein into a heparinized syringe using a 25-gauge needle). Likewise, upon capture and immediately after DEE measurements (murres) or only immediately after DEE measurements (kittiwakes), birds were blood sampled in a similar manner. We obtained only one blood sample from kittiwakes because of their small size. Blood samples were stored on ice for <4 hours, centrifuged at 2000 *g* for 10 minutes, the plasma was removed and stored at −20℃ for the remainder of the field season (1–2 months) and then shipped to the University of Manitoba on dry ice and stored at −80℃ until analysis. We determined both total and free T3 and T4 concentrations in duplicate by radioimmunoassay using a commercially available kit on unextracted plasma with a slight modification (MP Biomedical kits 06B258710, 06B257214, 06B254216, 06B254029). The modification required longer incubation times for equilibration from the suggested 1–2.5 hours to 6 hours (J.W. and K.H.E., unpublished). Sample hormone concentrations in blood from 10 murres and 10 kittiwakes with a 6-hour incubation time were highly correlated with concentrations from the same samples with the recommended, shorter incubation period ($R^2>0.9$ for all hormones). Blood samples were also taken from 10 kittiwakes and 10 murres prior to placement in the FoxBox respirometer and following measurement of RMR. Strong positive correlations between hormone levels before and after measurement of RMR (murres: total T3: $R^2=0.89$, $t_9=3.69$, $P=0.005$; total T4: $R^2=0.84$, $t_9=3.25$, $P=0.01$; kittiwakes: total T3: $R^2=0.78$, $t_9=3.05$, $P=0.01$; total T4: $R^2=0.67$, $t_9=2.85$, $P=0.02$), suggest that hormone levels post-chamber were representative of circulating levels and not affected by stress associated with capture. Intra-assay variability was 4.5±1.1% (total T3), 4.9±0.9% (free T3), 3.9±0.6% (total T4) and 4.8±1.5% (free T4), inter-assay variability was 5.5% (total T3), 6.1% (free T3), 5.9% (total T4) and 7.1% (free T4) and all assays were parallel with the standard curve following serial dilution (ANCOVA: $P>0.2$).

Data analysis

We used simple, least-squares regression to examine the relationship between total and free T3 and T4 and between those hormones and RMR and DEE. We examined thyroid hormone levels and body mass simultaneously to eliminate the possibility that a correlation between energy expenditure and thyroid hormones was due to the correlation between thyroid hormones and body mass associated with thermoregulation. We also constructed a general linear model for both RMR and DEE with both free and total T3, free and total T4, sex, body mass, time of day (circularly transformed using the cosine of time since midnight; RMR only), ambient temperature and deviations from a 24-hour sampling interval (DEE only) as independent variables. To account for the effect of body mass, we also present data as residual of energy expenditure on body mass. We included ambient temperature as a covariate to test whether we were operating within the birds' thermoneutral zone. All statistical analyses were performed using R.2.4.1.

Results

Body mass and RMR were roughly double in murres compared to kittiwakes, whereas DEE was slightly less than three times as high (Table 1). Free hormone levels, but not total hormone levels were higher in murres implying lower levels of the binding proteins (Table 1). The respiratory quotient was slightly higher in kittiwakes than murres (Table 1). As is the case in most studies of any duration, RMR was generally recorded during the last half of measurements (average=3.1±0.4 hours after start of measurements).

Table 1. Average (±S.D.) values for metabolic rate and thyroid hormone levels for black-legged kittiwakes and thick-billed murres.

	Kittiwake	Murre
Incubation body mass (g)	432±38	980±78
Chick-rearing body mass (g)	420±41	998±51
RMR (W)	3.49±0.56	6.51±0.81
Respiratory quotient	0.74±0.03	0.71±0.04
DEE (kJ/d)	788±127	2036±552
Free T3 (pg/mL)	14.9±5.3	21.1±13.3
Total T3 (ng/dL)	396±173	360±314
Free T4 (ng/dL)	1.24±0.61	1.53±0.89
Total T4 (μg/dL)	2.79±0.85	2.41±0.91

RMR during incubation was not correlated with DEE during chick rearing in kittiwakes ($t_{23}=-1.09$, $P=0.30$, $R^2=0.10$) or murres ($t_{21}=0.51$, $P=0.67$, $R^2=0.03$). Total T3 and free T3 were correlated with each other in incubating kittiwakes ($t_{36}=6.06$, $P<0.0001$, $R^2=0.59$) and murres ($t_{52}=9.13$, $P<0.0001$, $R^2=0.62$). Total T4 and free T4 were correlated with each other in incubating kittiwakes ($t_{36}=3.79$, $P=0.0006$, $R^2=0.39$) and murres ($t_{51}=2.45$, $P=0.02$, $R^2=0.16$). Total T3 was not correlated with total T4 in murres ($t_{51}=0.11$, $P=0.91$, $R^2=0.00$) or in kittiwakes ($t_{36}=-0.65$, $P=0.52$, $R^2=0.02$). Free T3 was not correlated with free T4 in murres ($t_{51}=1.48$, $P=0.14$, $R^2=0.04$) or kittiwakes ($t_{36}=0.44$, $P=0.67$, $R^2=0.01$). The ratio of free T3 to total T3 was correlated with the ratio of free T4 to total T4 in incubating kittiwakes ($t_{36}=2.16$, $P=0.04$, $R^2=0.24$) and murres ($t_{41}=2.97$, $P=0.005$, $R^2=0.22$).

When considered simultaneously within a general linear model, RMR in kittiwakes increased with total T3 ($t_{36}=4.24$, $P<0.001$) and body mass ($t_{36}=3.32$, $P=0.002$), but was independent of free T4 ($t_{36}=-0.62$, $P=0.54$), total T4 ($t_{36}=0.27$, $P=0.79$), ambient temperature within the thermoneutral zone ($t_{36}=-0.07$, $P=0.95$), sex ($t_{36}=1.14$, $P=0.21$), time of day ($t_{36}=1.22$, $P=0.19$) and body mass ($t_{36}=1.88$, $P=0.07$). Using carbon dioxide production, rather than oxygen consumption, to calculate RMR did not alter the significance of any of those parameters. In contrast, DEE in kittiwakes increased with body mass ($t_{17}=4.20$, $P=0.002$), but was independent of free T3 ($t_{17}=-0.58$, $P=0.57$), free T4 ($t_{17}=-1.88$, $P=0.09$), total T3 ($t_{17}=-0.12$, $P=0.90$), total T4 ($t_{17}=-1.52$, $P=0.16$), sex ($t_{17}=0.84$, $P=0.42$) and ambient temperature ($t_{17}=0.11$, $P=0.93$). Similarly, univariate correlations showed that RMR in kittiwakes was most closely linked to T3, whereas DEE was most closely linked to body mass (Table 2; Figs 1, 2).

When considered simultaneously within a general linear model, RMR in murres increased with total T3 ($t_{50}=4.39$, $P<0.0001$) and body mass ($t_{50}=16.29$, $P<0.0001$) but was independent of free T4 ($t_{50}=-0.13$, $P=0.90$), total T4 ($t_{50}=0.53$, $P=0.60$), time of day ($t_{50}=1.01$, $P=0.32$), sex ($t_{50}=0.69$, $P=0.52$) and ambient temperature within the thermoneutral zone ($t_{50}=-0.17$, $P=0.90$). Using carbon dioxide production, rather than oxygen consumption, to calculate RMR did not alter the significance of any of those parameters. In contrast, DEE in

Table 2. Correlation coefficients for relationships between thyroid hormones and either DEE or post-absorptive, unstressed RMR. Correlations that are statistically significant ($P<0.05$) are shown in bold.

	Free T3	Total T3	Free T4	Total T4	Body mass
Kittiwakes					
RMR	**0.591**	**0.729**	−0.270	0.054	**0.458**
DEE	0.007	−0.247	0.270	−0.001	**0.618**
Murres					
RMR	**0.453**	**0.491**	0.015	0.145	**0.733**
DEE	−0.157	−0.261	0.204	0.102	0.027

murres was independent of free T3 ($t_{15}=0.58$, $P=0.57$), free T4 ($t_{15}=-1.88$, $P=0.09$), total T3 ($t_{15}=-0.12$, $P=0.90$), total T4 ($t_{15}=-1.52$, $P=0.16$), ambient temperature ($t_{15}=0.11$, $P=0.93$), sex ($t_{15}=0.56$, $P=0.58$) and body mass ($t_{15}=0.71$, $P=0.52$). Similarly, univariate correlations showed that RMR in murres was most closely linked to T3, whereas DEE was independent of all seven variables (Table 2; Figs 1, 3).

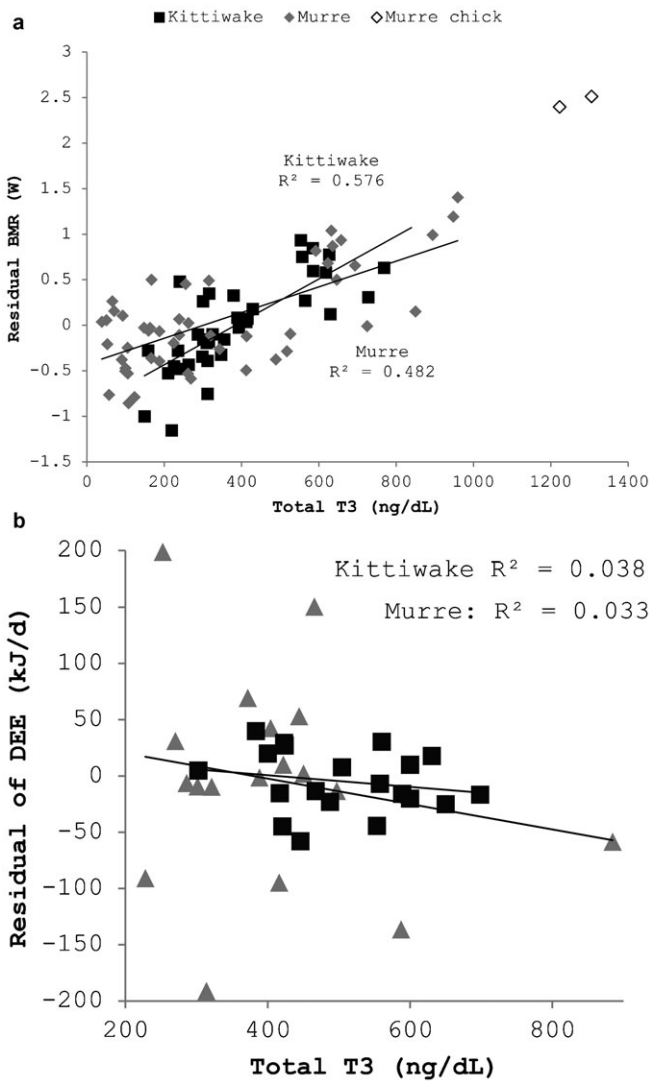

Fig. 1. (**a**) Residuals of post-absorptive, unstressed RMR on body mass during incubation increase with total T3 for both thick-billed murres and black-legged kittiwakes. (**b**) Residuals of DEE during chick rearing on body mass are not related to total T3 for thick-billed murres and black-legged kittiwakes. Values for two murre chicks are shown but not included in the regression.

Discussion

T3 strongly predicted RMR (Table 3). We suggest that T3 can be a useful indicator of RMR in some species of wild birds. Nonetheless, we caution that T3 probably proves useful only at a single location at a single time of year as both thyroid hormones and RMR likely vary non-linearly with environment (Burger and Denver, 2002). Our results challenge assertions, developed primarily from studies of non-charadriiform birds, that T4 is as biologically active as T3 in birds (McNabb, 2007). Comparisons of the likely thyroid receptor structure based on DNA sequences may help predict variation in receptor affinity to T3 and T4 among bird species, although thyroid receptors in birds are similar to mammals and do have higher affinity for T3 (Weirich and McNabb, 1984; Bellabarba et al., 1988; McNabb, 2007). In contrast, the absence of a relationship between T3 and DEE suggests that T3 is not an effective proxy for DEE as previously illustrated for a lizard (*Sceloporus undulatus*) (Joos and Johnalder, 1990) and humans (Starling et al., 1998). T3, however, may be a good indicator of DEE for inactive animals where BMR, or at least RMR, may constitute a substantial proportion of DEE (Toubro et al., 1996) or where thermoregulatory costs are high (Duriez et al., 2004). However, T3 is not a good indicator of DEE in humans (Starling et al., 1998), which would be a prime example of a species where RMR may constitute a substantial proportion of DEE. DEE did correlate with body mass (rather than T3), but only for kittiwakes, the species with greater sexual dimorphism, and consequently more extensive individual variation in body mass (Bech et al., 2002; Elliott et al., 2008; Jacobs et al., 2011).

The percent bound T3 was similar to the percent bound T4 suggesting that both hormones are similarly affected by carrying capacity, namely concentration of the binding proteins albumin and transthyretin in circulation (Hulbert, 2000). Birds lack specific T4-binding proteins and rely primarily on the less specific transthyretin (17–32%) and albumin (66–75%) carriers for transport (McNabb, 2007). Due to the absence of an effect of T4 on DEE or RMR, we suggest that T4 acts mainly as a reserve for rapid upregulation of T3 titers via cellular deiodinases when a rapid increase in BMR or other processes T3 regulates, is required. Possible exceptions are where T3:T4 ratios are influenced by exogenous thyroid mimics, such as polychlorinated biphenyls (McNabb, 2000). Interestingly, our correlations were higher between RMR and total T3 than free T3, further supporting the idea that metabolic rate is primarily regulated via increased deiodination (production of T3) than via changes in levels of binding proteins, such as transthyretin.

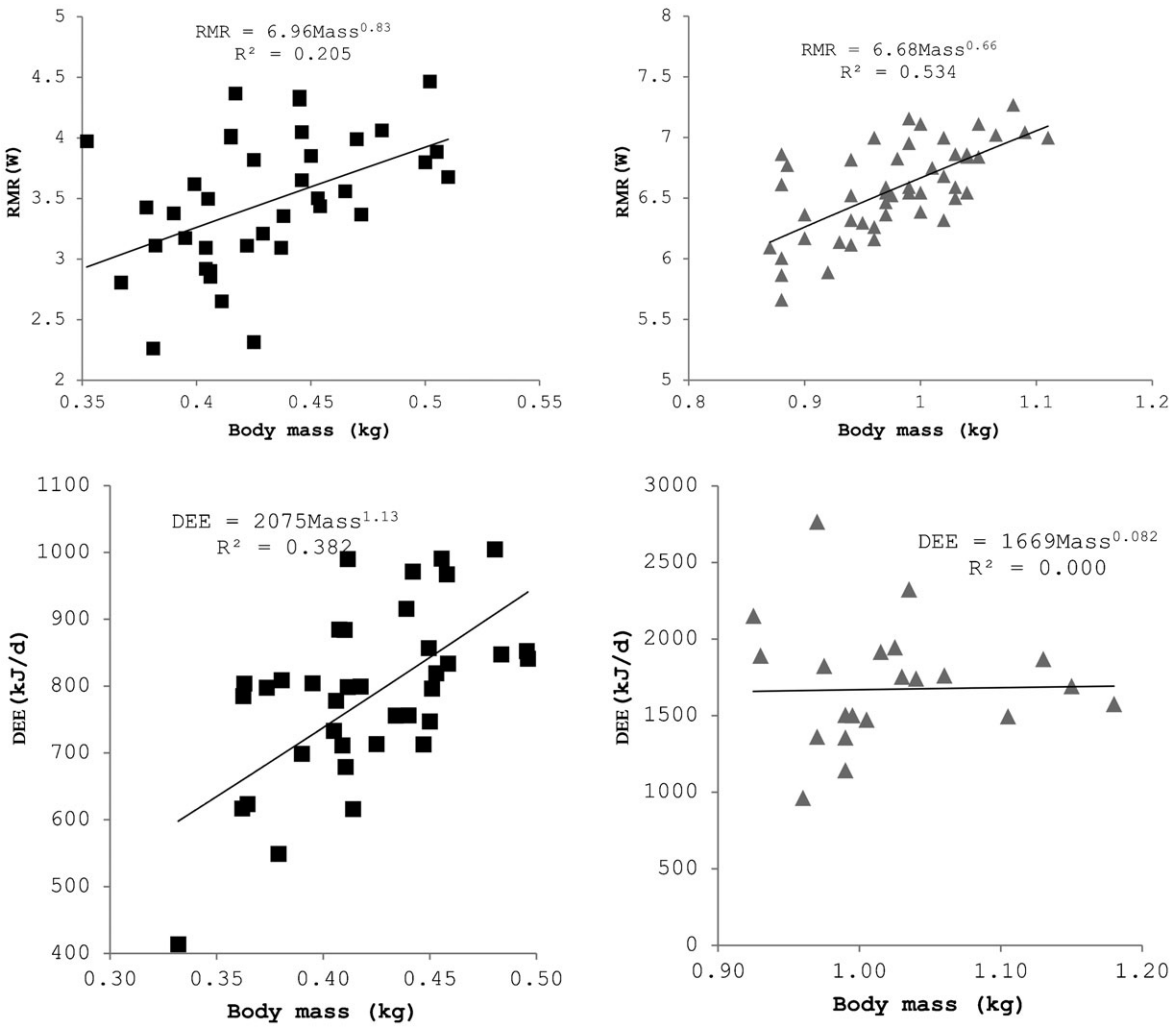

Fig. 2. RMR and DEE relative to body mass for kittiwakes (black symbols) and murres (gray symbols).

Our measurements of RMR were similar to those made by other authors, including those that attempted to measure BMR. Our value of 6.51 W for resting murres weighing 980 g during incubation compares favorably with the 6.9 W reported for post-absorptive, non-breeding murres weighing 803 g (Croll and McLaren, 1993), 7.0 W for post-absorptive murres weighing 1025 g (Hawkins et al., 1997) and 5.1 W for post-absorptive, breeding murres weighing 819 g (Gabrielsen et al., 1988). Our value of 3.49 W for resting kittiwakes weighing 432 g during incubation also compares favorably with the 3.34 W reported for kittiwakes weighing 365 g (Gabrielsen et al., 1988) and 3.31 W for "basal" metabolism in kittiwakes weighing 345 g during incubation (Bech et al., 1999).

Birds were captured as they were relieved by their partner at the nest site and kept for 4 hours. Assuming 10-hour (kittiwakes) and 12-hour (murres) incubation shifts, the birds were likely post-absorptive during measurements of RMR, as gut retention rates in charadriiform birds are <6 hours (Hilton et al., 2000) and murres, at least, seldom have anything in their guts when captured at the colony (A.J.G., unpublished). For birds that we

Table 3. Correlation coefficients for relationships between T3 and RMR or BMR across different species of adult birds.

Species	R	Reference
Black-legged kittiwake *Rissa tridactyla*	0.73	Our study
Black-legged kittiwake *Rissa tridactyla*	0.77	Welcker et al., 2013
Chicken *Gallus gallus* (8 weeks)	0.99	Bobek et al., 1977
House sparrow *Passer domesticus*	0.62	Chastel et al., 2003
Little bunting *Emberiza pusilla*	0.78	Liu et al., 2006; Zheng et al., 2013
Red knot *Calidris canutus*	0.28	Vezina et al., 2009
Thick-billed murre *Uria lomvia*	0.49	Our study

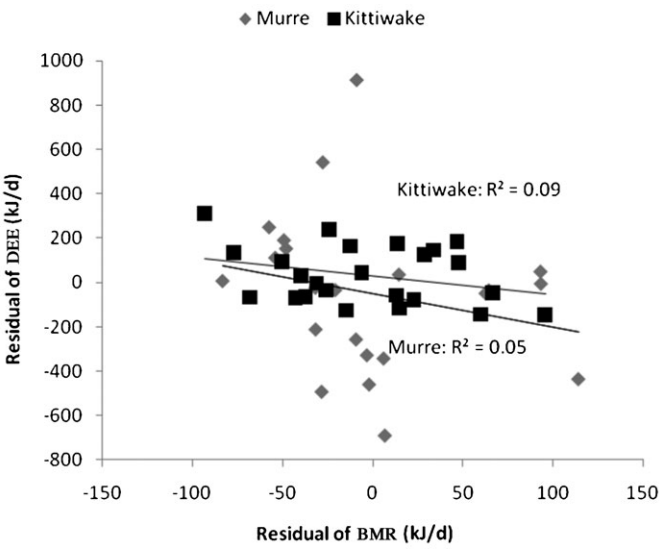

Fig. 3. Residuals of post-absorptive, unstressed RMR on body mass during incubation are not correlated with residual of DEE on mass during chick rearing.

measured pre- (<3 minutes from capture) and post-measurement hormone levels, there was no significant difference in corticosterone or thyroid hormone levels for either species (for kittiwakes, those birds were excluded from the current study, as the remaining kittiwakes were not sampled pre-measurement). As our birds were likely post-absorptive, we found no evidence for daily rhythms in RMR for our Arctic-nesting birds (see Results) and as our birds were unstressed, we believe that RMR closely approximates BMR.

DEE at the time of peak energy demands (chick rearing), and RMR at a time when birds spend a substantial proportion of their time resting (incubation) were not correlated within individual breeding kittiwakes and murres. RMR was correlated with T3, and T3 measured at the same time as the DEE measurements was, if anything, slightly inversely correlated with DEE. The absence of a relationship between DEE and T3 therefore provides additional support for the absence of a positive relationship between RMR and DEE – although DEE may be correlated with the component of RMR independent of T3. DEE increases between incubation and chick rearing for both kittiwakes and murres, as birds increase flying time twofold between those periods, and there is an increase in the size of some metabolically-active organs between these two breeding stages (Bech et al., 2002; Elliott et al., 2008; Jacobs et al., 2011). However, some metabolically-intensive organs decrease in size between incubation and chick rearing (Bech et al., 2002; Elliott et al., 2008) and BMR decreases between those two time periods, at least for kittiwakes (Bech et al., 2002). One potential mechanism underlying individual variation and seasonal trends in RMR could be that RMR is primarily determined by body temperature and metabolic intensity rather than by organ size, at least over the range of organ size variation apparent in nature outside of long-distance migration and similar extreme events (Rønning et al., 2008). Although BMR and DEE are sometimes correlated in interspecific comparisons (Daan et al., 1990), our results provide support for the conclusion that RMR/BMR and DEE are not directly linked at the level of the individual within a species (Meerlo et al., 1997; Speakman et al., 2003). Further study, directly examining the correlation between RMR and DEE within the same life-history stages, is required to conclusively demonstrate the absence of a correlation.

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Competing Interests

The authors have no competing interests to declare.

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