Figure 1. Body mass in relation to age in days from hatching to fledging in European starlings in 1999. Open circles represent body mass of

the measurements. Each chamber was sampled in a sequence for 1 h, and $\dot{V}o_2$ was calculated using the Datacan software package (Sable Systems). RMR was designated as the lowest $\dot{V}o_2$ for 10 consecutive minutes during the hour of measurements. Measurement sequence did not affect RMR ($F_{3,53} = 0.6$, $P = 0.6$). We do not have respiratory quotients, so we calculated a range of RMR values in kJ/d for each age group. To do so, we used the lowest and highest possible energy conversions: 18.4 kJ/L O_2 if the animal was consuming protein as fuel and 20.9 kJ/L O_2 if the animal consumed carbohydrates (Schmidt-Nielsen 1990). Body mass (0.00 g) was measured before and after metabolism measurements, and the average mass was used in the analysis.

Body-Composition Analysis

Following RMR measurements, each bird was killed by exsanguination under anesthesia (mixture of ketamine and xylasine 50 : 50) and dissected for body composition. The organs collected were liver, heart (only the ventricle, because earlier tests showed better constancy in the dissection technique), kidney, gizzard, pancreas, small intestine (hereafter called "intestine," connective tissues and fat manually removed and emptied of its contents by pressing along its length with a probe), pectoral muscles (left side only; data multiplied by 2), and the complete left leg (thigh and calf). Leg bones in 5-d-old nestlings were soft and proved very difficult to extract from muscle tissues in a consistent manner, so for comparability, we kept the bones in with the leg muscles at all age stages. In the early stages of growth, leg bones were growing together with total leg mass as indicated by positive relationships between tarsus length (an indicator of leg bone length) and leg mass (5 d old: $r^2 = 0.60$, $n = 17$, $P < 0.0005$; 11 d old: $r^2 = 0.57$, $n = 16$, $P < 0.001$). By 12 d of age, the structural growth of the leg was nearly completed (less than 2% increase in tarsus length per day; data not shown), and thus most of the mass gain between 11 and 20 d of age was due to muscle tissues (no significant relationship between tarsus length and leg mass at 20 d of age; $P = 0.4$). Therefore, given that most of the leg muscle growth appeared late in development (between 11 and 20 d of age; see "Results"), we consider leg mass as a good indicator of leg muscle growth. Feathers were plucked from 11- and 20-d-old nestlings. All organs, feathers, and the remaining carcass were then frozen (−20°C) for subsequent analysis. Organs were later freeze-dried (model 8ES, Virtis, New York, NY) to constant mass and fat extracted in petroleum ether using a Soxhlet apparatus. The carcasses were ground to form a homogenous powder before fat extraction. Dry body mass was calculated as dry carcass mass plus dry organ mass. Lean dry body mass was calculated as lean dry carcass mass plus the mass of the lean dry organs. Water mass was calculated as fresh body mass minus dry body mass for the 5-d-old group and fresh body mass minus dry body mass plus dry feather mass for the 11- and 20-d-old birds. Although water and fat (Scott and Evans 1992)—and, in the case of 5-d-old nestlings, yolk (Steen and Gabrielsen 1986)—have no or very low levels of energy consumption, they may nevertheless introduce a "dilution effect" in the analysis with regard to metabolic rate. We therefore analyzed lean dry body and lean dry organ mass data unless otherwise stated.

Energy Investment in Tissue Accretion

Body-composition data allowed us to calculate gross energy investment in tissue accretion during the periods of linear and plateau phases of growth. We used the measured changes in lean body mass and fat content between 5 and 11 d and between 11 and 20 d of age for these estimates. We calculated the approximate total amount of energy spent on protein and fat deposition using the costs measured by Pullar and Webster (1977). That is 1.36 kJ/kJ for fat and 2.25 kJ/kJ for proteins. These values include both the energy deposited in the tissue and the energy spent in the physiological processes of tissue accretion. One gram of fat and protein containing 39.36 and 17.79 kJ, respectively (Schmidt-Nielsen 1990)—the actual energy spent in tissue accretion independent of tissue energy content—is 14.17 kJ/g ($[39.36 \times 1.36] - 39.36$) for fat and 22.24 kJ/g ($[17.79 \times 2.25] - 17.79$) for proteins.

Statistical Analysis

We constructed the body-mass growth curve presented in Figure 1 from 1,187 daily body-mass measurements collected on all nestlings from 29 broods. To illustrate the individual and brood-independent pattern of growth for this locality, the growth curve was generated through a repeated measure anal-

Table 1: Body composition, metabolic rate, and water index in nestlings collected at 5, 11, and 20 d

	Age (d)				
Variable	5	11	20	*F* (df)	*P*
Fresh body (g)	17.81 ± 3.72^{A}	59.13 ± 4.52^{B}	69.25 ± 4.88^{C}	677.9 (2, 53)	! .0001
Fat (g)	$.20 \pm .08^{A}$	$.99 \pm .34^{B}$	$1.38 \pm .54^{C}$		

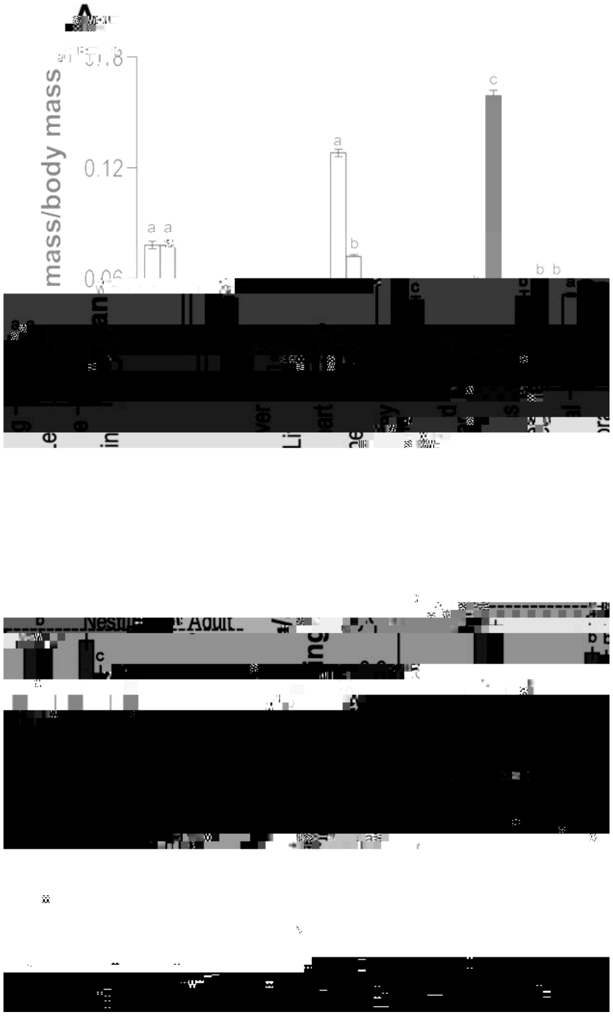

Figure 2. *A*, Proportion of nestling lean dry body mass occupied by the lean dry mass of specific organs within each age class. *B*, Nestling's lean dry organ mass relative to approximated adult target lean dry organ mass within age class. The dotted line represents equality between nestling and adult organ lean dry mass. Significant differences between ages within organs are represented by different letters. Statistical analyses were preformed on arcsine–square root transformed data, but the figures show untransformed values (see text for details). LD p lean dry. *White bars* p 5 d old, *gray bars* p 11 d old, *black bars* p 20 d old.

the largest organs in the body, but the relative contribution of the gizzard to total body mass had decreased such that both organs occupied the same proportion of total mass (gizzard 7.2% and liver 7.8%, of lean dry body mass; Fig. 2*A*). Most of the organ growth appeared during the linear phase of growth. Indeed, at 11 d of age, even though lean dry body mass was only 48.9% of adult target mass, the livers, kidneys, gizzards, pancreas, and intestine of nestlings had grown to 97.7%, 85.9%, 102.4%, 96.1%, and 68.4% of their final adult mass, respectively (Fig. 2*B*).

All organs were not growing at the same speed. Indeed, absolute growth (g lean dry mass/d) between 5 and 11 d of age was roughly three times faster in the liver, leg, and gizzard (0.07–0.09 g/d) and about two times faster in the intestine (0.04 g/d) compared with the kidney and pancreas (0.02–0.03 g/d). During the plateau phase of growth, most internal organs were already well developed, and much of the total lean dry mass gain was made up of growing muscles (Fig. 2*A*). Indeed, pectoral muscles showed the highest absolute growth rate during that period (0.10 g/d). Even though lean dry pectoral muscle mass was not yet comparable with adult pectoral muscle mass, at 20 d of age, they nevertheless comprised 15.9% of total lean dry body mass in nestlings (Fig. 2*A*). In the adult starlings, pectoral muscles represented 21% of lean dry body mass (data not sh[(not9.8(g/dt)-341(ta/dt)-341(tnep89.5(was)-26,5(was)-26,5Pts0 Tcl,r-21

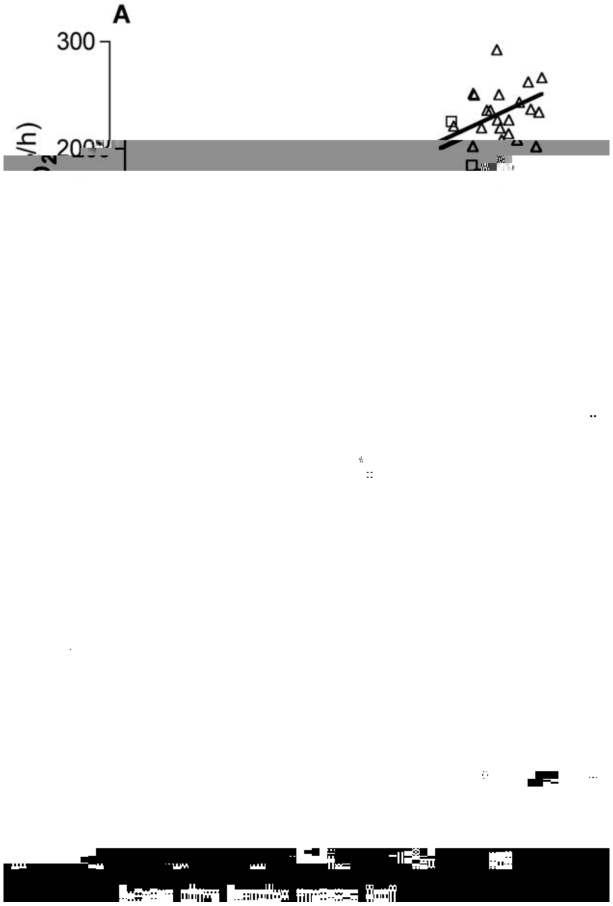

Figure 3. Relationship between resting metabolic rate (RMR) and (*A*) body mass and (*B*) lean dry body mass in nestling at 5 d (*circles*), 11 d (*squares*), and 20 d (*triangles*) of age. In *A*, the slopes do not differ significantly, but the intercepts are different. In *B*, both slopes and intercepts do not differ significantly. The dotted line represents the overall relationship across age classes. See text for details.

$P < 0.0001$, intercept $= -5.5$, slope $= 2.7$; 11 d: $r = 0.57$, $F_{1,14} = 6.2$, $P < 0.05$, intercept $= -77.2$, slope $= 3.9$; 20 d: $r = 0.48$, , , ,).

Table 2: Results of stepwise multiple regression on principal component (PC) variables explaining variability in resting metabolic rate within 5-d-olds

	PC1	PC8	PC9	PC6	PC4
Eigenvalue	7.805	.018	.007	.050	.140
Percent	86.7	.2	.1	.6	1.6
Slope	3.8	17.4	−22.6	−8.3	−4.9
Cumulative r^2	.77	.80	.83	.85	.88
Eigenvectors:					
LD carcass	.35	**−.82**	−.32	.03	.06
LD liver	.35	.23	**−.52**	.29	−.19
LD heart	.33	.14	−.02	**−.47**	**−.53**
LD kidney	.34	.16	−.01	.20	.19
LD gizzard	.34	.20	−.22	**−.62**	**.55**
LD pancreas	.30	−.08	.24	.37	.33
LD pectoral	.34	.39	−.02	.35	−.05
LD leg	.34	−.04	**.68**	−.09	.10
LD intestine	.32	−.16	.26	−.03	**−.46**

Note. Intercept = 42.7. LD = lean dry. Eigenvectors with values ≥0.4 are highlighted in bold.

Table 3: Results of stepwise multiple regression on principal component (PC) variables explaining variability in resting metabolic rate within 11-d-olds

	PC1	PC3	PC4	PC2
Eigenvalue	4.009	1.281	.954	1.649
Percent	44.5	14.2	10.6	18.3
Slope	7.2	8.9	12.0	−5.4
Cumulative r^2	.41	.52	.63	.70
Eigenvectors:				
LD carcass	.47	−.01	−.19	.00
LD liver	.30	**.41**	**.40**	.10
LD heart	.33	−.05	.05	−**.51**
LD kidney	−.003	**.81**	.13	−.14
LD gizzard	.20	.12	−**.51**	**.49**
LD pancreas	.29	−.39	**.58**	.15
LD pectoral	.44	−.03	−.03	−.29
LD leg	.46	−.02	−.33	.002
LD intestine	.21	.06	.28	**.60**

Note. Intercept p 156.5. LD p lean dry. Eigenvectors with values ≥0.4 are highlighted in bold.

intestines accounted for 22.2% of this value. Between 11 and 20 d of age, the energy spent on tissue growth was much lower, with an estimated value of 11.95 kJ/d. Pectoral and leg muscle growth accounted for 43.2% of this estimate. The approximated cost of tissue deposition during the period from 5 to 11 d of age therefore represented 140.0% and 38.4% of RMR in 5- and 11-d-old nestlings, respectively (RMR here is the average of calculated range in kJ/d; Table 1). During the plateau phase of growth, energy invested in tissue development represented only 16.3% and 10.9% of RMR at 11 and 20 d of age, respectively.

Discussion

Early postnatal body composition and development in altricial species is characterized by a disproportionate stomach size and rapid growth of the digestive organs such as the liver (O'Connor 1977; Ricklefs 1979; Lilja 1982, 1983; but see Ricklefs et al. 1998). Accordingly, in our study species, digestive organs (liver, pancreas, gizzard, and intestines) represented a substantial proportion (29%) of lean dry body mass in 5-d-old nestlings. At 11 d of age, although nestling body mass was roughly half of average adult mass, liver, kidney, gizzard, and pancreas were already at or very close (i.e., 86% for kidney) to the final adult target mass.

Relative water content decreased in growing nestlings, which indicates an increase in the proportion of ise multi936.8(muc)z2 1 Taccounted in(of)-2035(d,T*[(.6wt-295)-210(6.8(in)=)-200(thiura.5(opo128

Table 4: Results of stepwise multiple regression on principal component (PC) variables explaining variability in resting metabolic rate within 20-d-olds

	PC1	PC4	PC2	PC9
Eigenvalue	4.684	.649	1.706	.049
Percent	52.0	7.2	19.0	.5
Slope	4.9	9.2	5.5	−32.0
Cumulative r^2	.15	.22	.29	.35
Eigenvectors:				
LD carcass	.40	−.09	−.29	−**.76**
LD liver	.32	.06	.39	.04
LD heart	.38	.06	−.21	−.11
LD kidney	.30	**.79**	.15	.04
LD gizzard	.32	.15	.16	−.01
LD pancreas	.17	−.33	**.54**	.08
LD pectoral	.33	.12	−**.44**	**.53**
LD leg	.37	−**.40**	−.25	.34
LD intestine	.35	−.24	.34	.04

Note. Intercept p 231.5. LD p lean dry. Eigenvectors with values ≥0.4 are highlighted in bold.

the liver as the only organ having a significant effect on resting metabolism. Interestingly, a later study on the same species (Moe et al. 2004) revealed that when nestlings are food restricted during this period of rapid linear growth, there is a decline in the mass of several internal organs, including the liver, accompanied by a 37% decrease in RMR. Again, liver mass was found to be a significant contributor to the variation in RMR (Moe et al. 2004).

In 11-d-old starlings, when most internal organs have attained or are close to their final mass, and at 20 d, just before fledging, a point were body composition is very close to adult organ proportions (see Vézina and Williams 2003 for adult data), the pattern of organs related to RMR was different than at 5 d of age. In 11-d-old nestlings, we found a predominance of the kidney, pancreas, liver, and heart contributing to variation in RMR, while kidney, pancreas, and carcass were highlighted at 20 d. None of these internal organs were disproportionately large on a relative scale at this point, and by 11 d, except for the heart and carcass, growth was completed in all these organs. As with the heart, the kidneys have also been highlighted as a significant contributor to variation in BMR or RMR in adult animals, either separately or in association with other organs (Daan et al. 1990; Weber and Piersma 1996; Burness et al. 1998; Chappell et al. 1999). These organs, although typically representing only 1% of total body mass in adult starlings (based on Vézina and Williams 2003 data), show high metabolic intensity in vitro (Krebs 1950; Schmidt-Nielsen 1984).

Interestingly, no effect of pectoral muscles on variation in RMR was detected at 11 or 20 d of age, even though these organs were the fastest-growing body components between these time points. In European starlings, heat production by shivering attains adult levels by 12 d of age (Ricklefs 1979; Clark 1982), while flight capability develops rapidly between 15 and 20 d of age (Ricklefs 1979). These organs are therefore already functional before attaining adult size. It is possible that most of the pectoral muscle growth happened between our RMR measurements at 11 and 20 d of age, thus preventing us from detecting muscle growth effects on RMR. However, according to our estimates, tissue accretion during the plateau phase of growth might have only represented 10%–16% of RMR. This suggests that the energy investment in muscle development was not high enough to overshadow the functional costs of other organs. Accordingly, in 11- and 20-d-old nestlings, individual variation in RMR appeared to be affected by the size of high-metabolic-intensity organs, a pattern typical of adult animals (Piersma 2002), rather than by the energetic costs of fast-growing body components.

Based on these findings, we suggest that RMR variation of growing starling nestlings highlights a transition in metabolic costs over time. During the linear phase of growth, energy is consumed mostly by tissue-synthesis processes with fast-growing internal organs—such as liver, gizzard, and intestine—having a large influence on the total energy used. Later, when nestlings reach the plateau phase of growth, the energy used is transferred to functional costs more typical of adult condition, with variations in mass of high-metabolic-intensity organs likely to affect variations in RMR.

Context-Specific Nature of Relationships between Body Composition and Basal Metabolic Rate

Our data have important implications in the context of understanding the basis of variation in basal or resting metabolic rates in animals. They highlight the context-specific nature of relationships between organ mass and BMR or RMR. Here, our data suggest a transition in the underlying causes of RMR variation, from the metabolic costs of growing new tissues in developing nestlings to that of functional and maintenance costs of organs and physiological functions in fully grown birds. In adult animals, physiological systems are typically flexible and can change rapidly and reversibly in response to specific conditions (Piersma and Lindstro

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