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PLASMA B-ESTERASE ACTIVITIES IN EUROPEAN RAPTORS

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ABSTRACT: B-esterases are serine hydrolases composed of cholinesterases, including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), and carboxylesterase (CbE). These esterases, found in blood plasma, are inhibited by organophosphorus (OP) and carbamate (CB) insecticides and can be used as nondestructive biomarkers of exposure to anticholinesterase insecticides. Furthermore, B-esterases are involved in detoxification of these insecticides. In order to establish the level of these enzymes and to have reference values for their normal activities, total plasma cholinesterase (ChE), AChE and BChE activities, and plasma CbE activity were determined in 729 European raptors representing 20 species, four families, and two orders. The diurnal families of the Falconiforme order were represented by Accipitridae and Falconidae and the nocturnal families of the Strigiforme order by Tytonidae and Strigidae. Intraspecies differences in cholinesterase activities according to sex and/or age were investigated in buzzards (*Buteo buteo*), sparrowhawks (*Accipiter nisus*), kestrels (*Falco tinnunculus*), barn owls (*Tyto alba*), and tawny owls (*Strix aluco*). Sex-related differences affecting ChE and AChE activities were observed in young kestrels (2–3-mo-old) and age-related differences in kestrels (ChE and AChE), sparrowhawks (AChE), and tawny owls (ChE, AChE, and BChE). The interspecies analysis yielded a negative correlation between ChE activity and body mass taking into account the relative contribution of AChE and BChE to ChE activity, with the exception of the honey buzzard (*Pernis apivorus*). The lowest ChE activities were found in the two largest species, Bonelli's eagle (*Hieraaëtus fasciatus*) and Egyptian vulture (*Neophron percnopterus*) belonging to the Accipitridae family. The highest ChE activities were found in the relatively small species belonging to the Tytonidae and Strigidae families and in honey buzzard of the Accipitridae family. Species of the Accipitridae, Tytonidae, and Strigidae families were characterized by a BChE contribution that dominated the total ChE activity, while in the species of the Falconidae family, AChE activity dominated. With the exception of the barn owl, CbE activity (eserine-insensitive α-naphthyl acetate esterase [α-NAE] activity) in all species was almost absent or very low. The values obtained in this study for ChE, AChE, and BChE activities and the AChE:BChE ratios for buzzard, kestrel, barn owl, and tawny owl provide a good estimate of the normal values in free-living individuals of these European species. They can be used as a baseline to evaluate the effect of anticholinesterase insecticides in the field.

Key words: Acetylcholinesterase, biomarker, butyrylcholinesterase, carboxylesterase, cholinesterase, detoxification, European raptors, organophosphorus insecticide.

INTRODUCTION

In Europe and North America during the 1950s and 1960s, populations of many raptors, which are at the top of food chains, were affected by persistent and bioaccumulable organochlorine insecticides, especially 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE), a metabolite of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) (Ratcliffe, 1967, 1970; Cade et al., 1971; Newton and Bogan, 1978). After a ban on most organochlorine insecticides in the 1970s, organophosphorus (OP) and carbamate (CB) insecticides became the most widely used classes of insecticides. Thus, in 1999, OP and CB insecticides accounted for 21.0% and 5% by volume, respectively, of insecticides used in agriculture in the European Union (Eurostat, 2002). These insecticides only persist in the environment for a short time and they have a low potential for bioaccumulation and high acute toxicity. Many wildlife casualties have been associated with these compounds, primarily in raptor species (Goldstein et al., 1999a; Mineau et al., 1999; De Snoo et al., 1999). In many cases, wildlife is exposed to sub-

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lethal doses of insecticides. It is likely that the physiologic and behavioral effects of such doses have adverse consequences on population dynamics in free-living birds (Grue et al., 1991, 1997; Hill, 1995; Burkepile et al., 2002).

Organophosphorus and CB insecticides act by inhibition of acetylcholinesterase (AChE, International Enzyme Commission (EC) 3.1.1.7) in the central and peripheral nervous systems. This enzyme rapidly degrades the neurotransmitter acetylcholine in the synapse. The inhibition of AChE causes an accumulation of acetylcholine, which in turn alters cholinergic transmission, leading to subsequent physiologic disorders and ultimately causing respiratory failure and death (O'Brien, 1967; Matsumura, 1985; Mileson et al., 1998). According to Aldrige's classification (1953a), AChE is a serine hydrolase enzyme of the B-esterase group. This group is composed of the serine family of esterases inhibited by OP (and CB) compounds, that is, cholinesterases: AChE and butyrylcholinesterase (BChE, EC 3.1.1.8) and carboxylesterase (CbE, EC 3.1.1.1). Carboxylesterase represents a multigene family with broad substrate specificity (Satho and Hosokawa, 1998). In mammals, AChE is found in erythrocytes and BChE is mainly found in blood plasma. There is no AChE activity in avian erythrocytes. However, similar to mammals, AChE, BChE, and CbE are found in blood plasma with wide interspecies differences (Walker and Thompson, 1991). Because of their sensitivity to inhibition by OP and CB and their easy accessibility, blood B-esterases (primarily cholinesterases) can be used as nondestructive biomarkers. Plasma cholinesterase activities have been used to monitor bird exposure to anticholinesterase insecticides in the field (Hooper et al., 1989; Thompson, 1991; Wilson et al., 1991; Rainwater et al., 1995; Goldstein et al., 1999b; Parsons et al., 2000).

Furthermore, by binding OP and CB, plasma B-esterases are thought to play a role as scavengers to prevent these compounds from reaching cholinergic synapses. This role has been widely studied in mammals (Maxwell et al., 1991; Maxwell, 1992a; Chanda et al., 1997; Yang and Dettbarn, 1998) and more recently in birds (Parker and Goldstein, 2000). In mammals, this detoxification role is primarily attributed to CbE, which is found in large amounts compared with cholinesterases. Carboxylesterase is also involved in hydrolysis of OP and CB insecticides into nontoxic metabolites (see Jokanović [2001] and Sogorb and Vilanova [2002] for a review of the mechanisms involved in OP and CB detoxification).

Normal levels of plasma B-esterases were obtained from healthy birds by Westlake et al. (1983), who investigated total cholinesterase (ChE) activity and nitrophenyl acetate esterase activity. Hooper (1988) determined AChE and BChE activities in 11 North American raptor species, Wilson et al. (1991) and Goldstein et al. (1999b) determined normal values for ChE and AChE activities in raptors, and Bartkowiak and Wilson (1995) determined normal levels of CbE activity in several North American raptor species. Few data are available for European raptor species and information on AChE and BChE activities is not available.

Because plasma B-esterases are nondestructive biomarkers useful to assess sublethal exposure in species of high environmental value such as raptors, baseline values in healthy populations are necessary. Because these enzymes are involved in detoxification of anticholinesterase insecticides, they are likely to be determining factors in the sensitivity of species to these compounds and the knowledge of their normal levels in healthy bird populations is necessary. The aim of the present study was to determine the normal activities of CbE, ChE, AChE, and BChE and the relative contribution of AChE and BChE to ChE activity in a range of healthy European raptor species and to investigate, when possible, interspecies and intraspecies variations.

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MATERIALS AND METHODS

Animals

Most of the raptors were provided by the Wildlife Rehabilitation Center located at the Institut National de la Recherche Agronomique (INRA) in Versailles, France and by other wildlife rehabilitation centers of the nonprofit association Union française des centres de sauvegarde de la faune sauvage (UFCS), which form a network covering all of France. These centers receive injured birds for rehabilitation and release. They receive also young, healthy birds during the breeding season. All birds used in this study were kept in the rehabilitation center located at INRA-Versailles (2°05'E, 48°48'N). They were maintained in large, outdoor pens of a size (3×3×3 m, 6×3×3 m, 8×8×3 m, or 22×8×3 m) and density of birds adapted for the body size and biology of the various species. Raptors were sampled from March 1998 to February 2001. Blood was obtained from 729 birds representing two orders, four families, and 20 species. The largest sample sizes (Table 1) were for three diurnal species, buzzard (*Buteo buteo,* Accipitridae), sparrowhawk (*Accipiter nisus,* Accipitridae), and kestrel (*Falco tinnunculus,* Falconidae), and two nocturnal species, barn owl (*Tyto alba,* Tytonidae) and tawny owl (*Strix aluco,* Strigidae). Blood samples were collected as soon as the birds were rehabilitated and before their release. Physically disabled kestrels and buzzards, healthy but unsuitable for release (designated wild nonreleasable [wild NR]), were also sampled. Furthermore, during 1998–2000, fledged birds (2–3-mo-old) from captive pairs of kestrels and barn owls (designated captive bred) were also sampled after they had adjusted to separation from their parents. These young birds were raised by their parents in outdoor nest boxes and were fed a suitable diet (Table 1) with addition of vitamins and minerals. After separation from their parents, they were put in large pens and fed on live mice (10–15 days before sampling). All young raptors from the wild received in the rehabilitation center during the breeding season had a live-mouse diet before their release (also 10–15 days before sampling).

Among the 729 raptors in this study, 19% were captive bred and 46% came from the Ile de France area. The other raptors came from various areas of France and Egyptian vultures (*Neophron percnopterus*) and Bonelli's eagles (*Hieraaëtus fasciatus*) came from Mediterranean countries and were part of restoration programs. The kestrels, tawny owls, and barn owls sampled were mostly young birds (Table 1).

Developmental stage and, when possible, the age of the young birds were determined based on when they arrived at rehabilitation centers and morphologic characteristics or when they hatched. Determination of sex was based on morphologic and biometric characteristics (mass, width of tarsus) or by DNA test on feather samples (Vet-France, GENOPOLE, Evry, France) for Egyptian vultures (two females, one male), Bonelli's eagle (three females, five males) and 98 buzzards.

Blood sampling

Blood samples were collected in the morning, between 5:30 and 9:30 AM to minimize possible diurnal variation in enzyme activities (Garcia-Rodriguez et al., 1987; Thompson et al., 1988). Blood samples (0.5–1 ml) were obtained from the jugular vein and occasionally from a brachial vein, using sterile 1-ml polypropylene syringes equipped with 26-gauge/12-mm or 27-gauge/20-mm needles. Blood samples were transferred to heparinized polypropylene microtubes and maintained cold (4–6 C). Plasma samples were obtained by centrifugation at 2,000 × G for 10 min at 4 C and stored in polypropylene microtubes at −32 C. They were assayed after 0.03–9 mo of storage with 90% of samples assayed before 5.6 mo. Time from blood collection to storage of plasma never exceeded 4 hr.

Esterase assays

Total plasma ChE activity was determined colorimetrically using the method of Ellman et al. (1961), as modified by Hill and Fleming (1982) for avian brain and plasma ChE. The assay was performed at 25 C in a single cuvette containing 20 μl of plasma, 5.0 mM acetylthiocholine iodide as substrate, and 0.24 mM 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) prepared in 0.05 M, pH 7.4, Tris buffer (Trizma® preset crystals, pH 7.4). The absorbances at 405 nm were read against air and the assay blank was subtracted. The activity was expressed as micromoles of substrate hydrolyzed per minute and per milliliter of plasma (the extinction coefficient for the colored product is 13.3×10^3 M^{-1} cm^{-1}). The measurements were performed on a programmable Shimadzu MPS-2000 spectrophotometer with a PR3 graphic printer and controlled temperature cell (Shimadzu Corporation, Kyoto, Japan).

Acetylcholinesterase activity was determined by preincubating plasma sample for 5 min at 25 C with the BChE selective inhibitor, iso-OMPA (tetraisopropylpyrophosphoramide), prepared in 0.05 M, pH 7.4, Tris buffer, at a reaction concentration of 10^{-5} M. Butyrylcholinesterase ac-

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TABLE 1. Raptor species included in this study, with the largest sample size, by age, origin,[a] sex,[b] and diet.[c]

Order Family	Species	Diet[c]	Age, origin, and sex: Young (<12-mo-old) ≤6 mo	Young (<12-mo-old) ≥6 mo	Young (<12-mo-old) Age not determined	Adults	Stage not determined	Total sample
Falconiforme								
Accipitridae	Buzzard (*Buteo buteo*)	Rat or chick[d]	9 (4 WNR, 5 W) (3 F, 2 M, 4?) 7%	22 (10 WNR, 12 W) (9 F, 10 M, 3?) 18%	—	72 (68 WNR, 4 W) (33 F, 38 M, 1?) 58%	21 (20 WNR, 1 W) (10 F, 10 M, 1?) 17%	124 (55 F, 60 M, 9?) 100%
	Sparrowhawk (*Accipiter nisus*)	Chick	9 (W) (8 F, 1 M) 33%	11 (W) (9 F, 2 M) 41%	—	7 (W) (5 F, 2 M) 26%	—	27 (22 F, 5 M) 100%
Falconidae	Kestrel (*Falco tinnonculus*)	Chick or live mouse[e]	126 (32 B, 91 W, 3WNR) (49 F, 48 M, 29?) 50%	58 (30 WNR, 28 W) (30 F, 21 M, 7?) 23%	1 (W) (M)	59 (20 WNR, 39 W) (19 F, 40 M) 24%	6 (4 WNR, 2 W) (6 F) 2%	250 (104 F, 110 M, 36?) 100%
Strigiforme								
Tytonidae	Barn owl (*Tyto alba*)	Mouse or live mouse	135 (105 B, 30 W) (?) 89%	1 (W) (?) 1%	4 (W) (?) 3%	11 (W) (?) 7%	1 (W) (?) 1%	152 (?) 100%
Strigidae	Tawny owl (*Strix aluco*)	Mouse or live mouse	88 (W) (?) 70%	2 (W) (?) 1%	11 (W) (1 F, 7 M, 3?) 9%	16 (W) (7 F, 4 M, 5?) 13%	9 (W) (1 M, 8?) 7%	126 (8 F, 12 M, 106?) 100%

[a] B = captive bred; W = wild; and WNR = wild nonreleasable.
[b] F = female; M = male; and ? = unknown.
[c] Diet in the rehabilitation center.
[d] Chicks for diet are 1 day old.
[e] Live mouse fed young after separation from parents.

tivity was calculated as the difference between ChE and AChE activities (Aldridge, 1953b; Fairbrother et al., 1991).

Carboxylesterase activity was determined according to the method of Gomori (1953) as adapted by Van Asperen (1962). The assay was conducted in a single cuvette, at 25 C, using 0.5 ml of 0.05 M phosphate buffer, pH 7.4, 10 μl of diluted plasma in buffer (or 10 μl buffer for the blank) and 5 μl of α-naphthyl acetate (NA) (in 95% ethanol) at a reaction concentration of 0.485 mM as substrate. The reaction was initiated by addition of the substrate and stopped after 10 min by addition of 2.5 ml of a solution of 0-dianiside, tetrazotized zinc chloride complex (fast blue salt BN) (1 mg/ml) freshly prepared in an aqueous solution of sodium dodecyl sulfate (1%). This reagent gives a blue-colored product in the presence of α-naphthol produced by the hydrolysis of α-NA. Its absorbance was measured at 600 nm after 15 min of storage in darkness. The measurements were performed on a programmable Shimadzu MPS-2000 spectrophotometer with a PR3 graphic printer. A calibration curve was obtained based on the absorbance of several dilutions of a solution of 0.1 mM α-naphthol (prepared from a 10 mM solution in 95% ethanol by dilution in 0.05 M phosphate buffer) in 0.05 M phosphate buffer containing 1% of 95% ethanol. The activity was expressed as micromoles of substrate hydrolyzed per minute and per milliliter of plasma. Because α-NA is also a substrate for ChE (Gomori, 1953; Van Asperen, 1962; Maxwell, 1992b), a second assay was performed with each plasma sample in the presence of eserine salicylate, a specific inhibitor of ChE, at a reaction concentration of 10^{-5} M (Gomori, 1953; Clement and Erhart, 1990; Fairbrother et al., 1991). The eserine salicylate solution was prepared by dilution with 0.05 M phosphate buffer of a solution of the substance in water.

Acetylthiocholine iodide, DTNB, Trizma® preset crystals, iso-OMPA, α-NA, Fast Blue salt BN, sodium dodecyl sulfate, α-naphthol, and eserine salicylate were obtained from Sigma Chemical Company (St. Louis, Missouri, USA) or Sigma-Aldrich Chemie Gmbh (Steinheim, Germany). Disodium hydrogen phosphate dodecahydrate and potassium dihydrogen phosphate used to make the phosphate buffer were R. P. Normapur™ analytical reagents Prolabo®, obtained from VWR International France, Fontenay-sous-Bois, France).

Quality control was done using a commercial universal control human serum, Precinorm® U (Roche Diagnostics GmbH, Mannheim, Germany) for ChE activity and a pool of buzzard plasmas prepared in our laboratory (several pools were prepared all throughout the duration of the study) for CbE, ChE, and AChE activities.

Statistical analysis

Statistical analysis was performed with SAS 8.1 for Windows software (SAS Institute Inc., Cary, North Carolina, USA) using descriptive statistic analysis; simple parametric analysis of variance (ANOVA), with Levene's test for homogeneity of variance testing, or nonparametric tests (Kruskal-Wallis test or Wilcoxon test for two classes) to a one-factorial design; the general linear model procedure for unbalanced ANOVA for a two- or three-factorial design with interactions; multiple comparisons by Scheffe's test; and correlation and regression analysis. A probability level of 0.05 was chosen as the level of significance. The normality of the distributions was assessed using the Kolmogorov-Smirnov statistic, with $P=0.15$ as critical value, and plotted data.

Plasma ChE, AChE, and BChE activities and the ratio between cholinesterase activities (AChE:BChE) were analyzed as a function of bird origin (captive bred, wild, or wild NR), sex, and developmental stage in the five species with the largest sample size. These analyses were performed depending on origins of the birds, data on sex and age, and number of animals (Table 1).

Interspecies differences were investigated for ChE activity and the AChE:BChE ratio. Analyses were made after logarithmic transformation to homogenize variances. Species with a sample size less than three individuals were not included in the ANOVA and the multiple comparison test. This analysis was performed on all the data collected 1) without any restrictions, 2) with respect to developmental stage (adults and young), and 3, 4) ignoring the data for wild NR kestrels and barn owls born in captivity (without and with respect to developmental stage).

Plasma α-naphthyl acetate esterase (α-NAE) assays were performed on fewer birds compared with the assays of cholinesterases. For each species, a descriptive statistical analysis of the two α-NAE activities was performed, with and without eserine. Analysis of the correlations between α-NAE activity, without eserine, and ChE activity was performed.

RESULTS

Cholinesterase activities

The origin (wild or wild NR), sex, and developmental stage of buzzards had no effect (Table 2) on cholinesterase activi-

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ties. The origin (wild or captive bred) of 2–3-mo-old kestrels had no significant effect on measured activities (Table 2). Except for BChE, these activities were significantly higher in females compared with males (Table 2, Table 3). In the 6–11-mo-old and adult kestrels, origin had a significant effect on ChE and BChE (Table 2). Activities were higher in wild NR birds than in wild birds (Table 3), but sex was not a significant factor. Cholinesterase and AChE activities and AChE:BChE ratios were significantly higher in adults compared with the 2–3-mo-old and the 6–11-mo-old young (Tables 2, 3). The developmental stage had no significant effect on BChE activity.

In the barn owl, ChE and AChE activities were significantly higher in captive-bred young compared with wild young of the same age. No significant difference was noted between wild adult and young birds (Table 4).

Cholinesterase, AChE, and BChE activities and AChE:BChE ratios in tawny owls and AChE activity and AChE:BChE ratios in sparrowhawks were significantly higher in adults than in the young (Table 4). Tables 3–6 summarize factors affecting plasma cholinesterase activities and AChE:BChE ratios and show the statistics for all species of this study.

Analysis of interspecies differences, ignoring variations within a species, gave the same results with or without values for wild NR kestrels and captive-bred barn owls. This is explained by the fact that interspecies variation was greater than the intraspecies variation linked to bird origin. Interspecies differences in ChE activity yielded the following order (Table 7): Bonelli's eagle <buzzard<sparrowhawk<tawny owl=barn owl=little owl (*Athene noctua*)=honey buzzard (*Pernis apivorus*). Cholinesterase activity in the Egyptian vulture and booted eagle (*Hieraaëtus pennatus*) was low but not significantly different from that in the buzzard. Plasma ChE activities in the buzzard and other species mentioned above, except Bonelli's eagle, were significantly higher than in the kestrel. For adult birds, plasma ChE activity was significantly higher in kestrel compared with Bonelli's eagle (this difference was not revealed for the total kestrel sample, which contained a majority of young and therefore had a smaller mean). In young birds, ChE activity was significantly higher in little owls compared with tawny owls. The data for the individual contribution of each cholinesterase showed that only kestrels and other Falconidae had a predominant AChE activity, with an AChE:BChE ratio>1 (2.77 –7.02) (Tables 5, 6). Thus, AChE in wild adult kestrels accounted for 76% (7%) (mean [SD]) of total ChE activity. The AChE:BChE ratio in other raptor families, Accipitridae, Tytonidae, and Strigidae, was close to one for the booted eagle and <1 (0.07–0.62) in other species, BChE being the main ChE activity. Acetylcholinesterase accounted for 24% (7%) of total ChE activity in buzzard, 23% (5.)%) in young sparrowhawk, 23% (8%) in wild barn owl, and 7% (3%) in adult tawny owl. The classification of species according to the AChE:BChE ratio for all birds showed the following order (Table 7): little owl=tawny owl<long-eared owl=honey buzzard=barn owl=buzzard=sparrowhawk=Bonelli's eagle<hobby (*Falco subbuteo*)=kestrel and Egyptian vulture<kestrel. When only adult bird data were considered, the following classification was obtained: Bonelli's eagle and other equal (AChE:BChE ratio) above-mentioned species<booted eagle<kestrel. Because the majority of kestrels were juveniles, the mean was lower and no difference was found compared with booted eagle.

Carboxylesterase (α-naphthyl acetate esterase activity)

Analysis of the correlation between ChE activity and plasma α-NAE activity in the absence of eserine along with the data on eserine-dependent α-NAE activity revealed the importance of ChE activity to-

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TABLE 2. Results of unbalanced analysis of variance for the effects of different factors on cholinesterase activities and AChE:BChE ratios in buzzards and kestrels.

	ChE[a]	AChE[a]	BChE[a]	AChE:BChE
Buzzards				
Origin and sex factor effects with interaction in young buzzards				
Origin[b]	1; 1.52; 0.23[c]	1; 0.11; 0.74	1; 1.53; 0.23	1; 0.87; 0.36
Sex	1; 0.10; 0.76	1; 0.19; 0.67	1; 0.24; 0.63	1; 0.32; 0.58
Interaction origin × sex	1; 0.30; 0.59	1; 1.04; 0.32	1; 0.06; 0.81	1; 0.25; 0.62
Residual	DF=20	DF=20	DF=20	DF=20
Developmental stage and sex factor effects with interaction in total sample				
Developmental stage	1; 0.37; 0.54	1; 0.41; 0.52	1; 1.08; 0.30	1; 3.05; 0.08
Sex	1; 0.04; 0.84	1; 0.00; 0.99	1; 0.06; 0.80	1; 0.06; 0.80
Interaction stage × sex	1; 0.28; 0.60	1; 0.14; 0.71	1; 0.65; 0.42	1; 0.44; 0.51
Residual	DF=91	DF=91	DF=91	DF=91
Kestrels				
Origin and sex factor effects with interaction in 2–3-mo-old kestrels				
Origin[d]	1; 0.12; 0.72	1; 0.16; 0.69	1; 0.01; 0.93	1; 0.45; 0.51
Sex	1; 6.07; 0.02*[e]	1; 7.58; 0.01*	1; 0.07; 0.79	1; 6.94; 0.01*
Interaction origin × sex	1; 0.03; 0.86	1; 0.00; 0.98	1; 0.30; 0.58	1; 0.07; 0.79
Residual	DF=88	DF=88	DF=88	DF=88
Developmental stage and sex factor effects with interaction in 2–3-mo-old and wild adult kestrels				
Developmental stage	1; 16.23; <0.0001*	1; 21.94; <0.0001*	1; 0.97; 0.33	1; 28.35; <0.0001*
Sex	1; 1.58; 0.21	1; 3.05; 0.08	1; 1.56; 0.21	1; 4.41; 0.04*
Interaction stage × sex	1; 2.16; 0.14	1; 1.93; 0.17	1; 0.49; 0.48	1; 0.32; 058
Residual	DF=127	DF=127	DF=127	DF=127
Origin, sex, and developmental stage factor effects with interactions in 6–11-mo-old and adult kestrels				
Origin[b]	1; 5.41; 0.02*	1; 3.50; 0.06	1; 4.41; 0.04*	1; 0.00; 0.95
Sex	1; 0.07; 0.79	1; 0.34; 0.56	1; 0.91; 0.34	1; 0.49; 0.48
Developmental stage	1; 4.73; 0.03*	1; 5.63; 0.02*	1; 0.01; 0.93	1; 4.61; 0.03*
Interaction origin × sex	1; 1.23; 0.27	1; 1.48; 0.23	1; 0.00; 0.95	1; 0.54; 0.46
Interaction stage × sex	1; 0.00; 0.95	1; 0.01; 0.92	1; 0.01; 0.91	1; 0.02; 0.89
Interaction stage × origin	1; 0.06; 0.80	1; 0.30; 0.58	1; 0.85; 0.36	1; 1.70; 0.20
Residual	DF=103	DF=103	DF=103	DF=103

[a] ChE = cholinesterase; AChE = acetylcholinesterase; BChE = butyrylcholinesterase.
[b] Wild and wild nonreleasable.
[c] DF = degree of freedom; value of *F* test; *P*.
[d] Captive bred and wild.
[e] * = factor effect is significant, $P<0.05$.

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TABLE 3. Plasma cholinesterase activities[a] and AChE:BChE ratios in kestrels.

		Age					
		2–3 mo old		6–11 mo old		Adults (≥12-mo-old)	
Enzyme[b]	Parameters[c]	Female $n=46$	Male $n=46$	Wild $n=22$	Wild nonreleasable $n=29$	Wild $n=39$	Wild nonreleasable $n=20$
ChE	Mean (SD)	1.014 (0.278)	0.862 (0.264)	1.004 (0.290)	1.174 (0.328)	1.165 (0.281)	1.268 (0.317)
	Med	0.960	0.784	0.983	1.150	1.107	1.226
	P10–P90	0.695–1.382	0.600–1.300	0.642–1.306	0.694–1.658	0.855–1.632	0.840–1.702
	Min–max	0.460–1.832	0.515–1.681	0.478–1.663	0.652–1.887	0.660–1.899	0.735–1.973
AChE	Mean (SD)	0.731 (0.250)	0.571 (0.248)	0.711 (0.262)	0.864 (0.315)	0.887 (0.257)	0.938 (0.295)
	Med	0.709	0.483	0.709	0.867	0.831	0.943
	P10–P90	0.417–1.081	0.304–0.845	0.363–0.927	0.454–1.385	0.602–1.288	0.612–1.357
	Min–max	0.284–1.540	0.210–1.332	0.226–1.448	0.396–1.671	0.456–1.536	0.410–1.599
BChE	Mean (SD)	0.283 (0.065)	0.292 (0.078)	0.293 (0.077)	0.310 (0.076)	0.278 (0.086)	0.330 (0.103)
	Med	0.292	0.275	0.262	0.293	0.260	0.302
	P10–P90	0.198–0.355	0.216–0.395	0.226–0.368	0.204–0.425	0.171–0.384	0.219–0.460
	Min–max	0.123–0.413	0.163–0.525	0.193–0.536	0.181–0.526	0.139–0.507	0.206–0.598
AChE:BChE	Mean (SD)	2.65 (0.88)	2.06 (0.94)	2.52 (1.14)	2.95 (1.41)	3.47 (1.44)	3.07 (1.25)
	Med	2.63	1.73	2.56	2.57	3.25	2.83
	P10–P90	1.52–3.72	1.06–3.68	1.44–3.11	1.40–5.07	1.74–5.81	1.43–4.54
	Min–max	0.84–5.27	0.58–4.15	0.90–6.73	1.34–7.74	1.47–7.41	1.26–6.21

[a] Activities are shown as μmoles substrate hydrolyzed/min/ml.
[b] ChE = cholinesterase; AChE = acetylcholinesterase; BChE = butyrylcholinesterase.
[c] SD = standard deviation; Med = median; P10–P90 = 10th–90th percentiles.

TABLE 4. Plasma cholinesterase activities[a] and AChE:BChE ratios in barn owls, tawny owls, and sparrowhawks.

		Barn owl			Tawny owl		Sparrowhawk	
Enzyme[b]	Parameters[c]	Captive bred 2 to 3-mo-old n=104	Wild 2 to 3-mo-old n=25	Wild adults[d] n=11	2 to 3-mo-old n=81	Adults n=16	Young[e] n=20	Adults n=7
ChE	Mean (SD)	3.106[f] (0.409)	2.869[f] (0.385)	3.142 (0.720)	2.654[g] (0.715)	3.361[g] (0.844)	1.952 (0.382)	2.071 (0.433)
	Med	3.061	3.007	2.977	2.615	3.432	1.988	2.167
	P10–P90	2.599–3.693	2.328–3.331	2.495–4.164	1.826–3.437	2.266–4.287	1.421–2.395	—
	Min–max	2.349–4.162	2.208–3.651	2.320–4.628	0.578–4.985	1.294–4.539	1.337–2.766	1.299–2.506
AChE	Mean (SD)	0.797[f] (0.208)	0.667[f] (0.258)	0.730 (0.322)	0.147[g] (0.042)	0.230[g] (0.078)	0.444[g] (0.114)	0.586[g] (0.159)
	Med	0.794	0.623	0.702	0.140	0.205	0.441	0.555
	P10–P90	0.540–1.075	0.372–1.033	0.454–0.874	0.100–0.206	0.148–0.321	0.297–0.608	—
	Min–max	0.237–1.344	0.215–1.182	0.407–1.585	0.070–0.286	0.123–0.395	0.244–0.662	0.382–0.815
BChE	Mean (SD)	2.309 (0.357)	2.202 (0.361)	2.412 (0.560)	2.507[g] (0.705)	3.131[g] (0.819)	1.509 (0.338)	1.485 (0.359)
	Med	2.298	2.096	2.178	2.484	3.113	1.535	1.599
	P10–P90	1.890–2.777	1.846–2.744	1.774–3.043	1.667–3.326	2.112–4.120	1.048–1.944	—
	Min–max	1.516–3.380	1.547–2.925	1.684–3.365	0.449–4.821	1.146–4.324	0.999–2.229	0.834–1.951
AChE:BChE	Mean (SD)	0.35 (0.11)	0.32 (0.15)	0.31 (0.12)	0.06[g] (0.03)	0.08[g] (0.03)	0.31[g] (0.10)	0.41[g] (0.13)
	Med	0.34	0.31	0.33	0.06	0.07	0.30	0.36
	P10–P90	0.22–0.47	0.15–0.50	0.20–0.41	0.04–0.10	0.04–0.13	0.21–0.46	—
	Min–max	0.09–0.69	0.10–0.64	0.14–0.52	0.03–0.29	0.04–0.13	0.16–0.53	0.28–0.59

[a] Activities are shown as μmoles substrate hydrolyzed/min/ml.
[b] ChE = cholinesterase; AChE = acetylcholinesterase; BChE = butyrylcholinesterase.
[c] SD=standard deviation; Med = median; P10–P90 = 10th–90th percentiles.
[d] Adult = 12-mo-old.
[e] Young = <12-mo-old.
[f] Significant difference between captive bred and wild young birds, $P<0.05$, nonparametric tests.
[g] Significant difference between young and adult birds, $P<0.05$, nonparametric tests.

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TABLE 5. Plasma cholinesterase activities[a] and AChE:BChE ratios in European raptor species of Accipitridae.

Family Species	*n*	Parameter[b]	ChE[c]	AChE[c]	BChE[c]	AChE:BChE
Accipitridae						
Egyptian vulture	3	Mean (SD)	0.709 (0.192)	0.269 (0.081)	0.441 (0.132)	0.62 (0.15)
(*Neophron percnopterus*)		CV	27.0	30.2	30.0	23.6
		Min–max	0.490–0.846	0.184–0.346	0.306–0.570	0.48–0.78
Bonelli's eagle	8	Mean (SD)	0.727 (0.054)	0.213 (0.023)	0.514 (0.058)	0.42 (0.07)
(*Hieraaëtus fasciatus*)		CV	7.4	10.6	11.2	17.1
		Min–max	0.626–0.785	0.180–0.247	0.436–0.602	0.30–0.52
Booted eagle	4	Mean (SD)	0.839 (0.175)	0.458 (0.112)	0.381 (0.065)	1.19 (0.12)
(*Hieraaëtus pennatus*)		CV	20.9	24.4	17.0	10.1
		Min–max	0.610–1.016	0.313–0.562	0.297–0.454	1.05–1.33
Marsh harrier	1		1.076	0.288	0.788	0.37
(*Circus aeruginosus*)						
Hen harrier	1		1.152	0.222	0.930	0.24
(*Circus cyaneus*)						
Long-legged buzzard	4	Mean (SD)	1.190 (0.132)	0.285 (0.086)	0.905 (0.080)	0.32 (0.09)
(*Buteo rufinus*)		CV	11.1	30.2	8.9	28.0
		Min–max	1.043–1.362	0.220–0.412	0.785–0.950	0.23–0.43
Buzzard	124	Mean (SD)	1.412 (0.321)	0.337 (0.130)	1.075 (0.264)	0.32 (0.13)
(*Buteo buteo*)		CV	22.7	38.6	24.5	40.1
		Med	1.367	0.324	1.032	0.29
		P10–P90	1.059–1.841	0.176–0.492	0.781–1.387	0.18–0.50
		Min–max	0.651–2.446	0.121–0.825	0.505–2.105	0.11–0.74
Black kite	2	Mean (SD)	1.263 (0.297)	0.263 (0.056)	1.001 (0.241)	0.26 (0.01)
(*Milvus migrans*)		CV	23.5	21.3	24.1	2.9
		Min–max	1.053–1.473	0.223–0.302	0.830–1.171	0.26–0.27
Goshawk	2	Mean (SD)	1.302 (0.303)	0.231 (0.038)	1.071 (0.265)	0.22 (0.02)
(*Accipiter gentilis*)		CV	23.3	16.5	24.8	8.4
		Min–max	1.087–1.516	0.204–0.258	0.883–1.258	0.21–0.23
Sparrowhawk	27	Mean (SD)	1.983 (0.391)	0.481 (01.39)	1.502 (0.337)	0.33 (0.11)
(*Accipiter nisus*)		CV	19.7	29.0	22.4	34.1
(total[d])		Med	2.080	0.479	1.559	0.31
		P10–P90	1.414–2.506	0.332–0.662	1.024–1.951	0.22–0.53
		Min–max	1.299–2.766	0.244–0.815	0.834–2.229	0.16–0.59
Honey buzzard	4	Mean (SD)	4.593 (0.740)	0.974 (0.431)	3.620 (0.583)	0.27 (0.11)
(*Pernis apivorus*)		CV	16.1	44.3	16.1	40.9
		Min–max	3.661–5.471	0.471–1.488	2.835–4.132	0.11–0.37

[a] Activities are shown as μmoles substrate hydrolyzed/min/ml.
[b] SD = standard deviation; CV = coefficient of variation (%); Med = median; P10–P90 = 10th–90th percentiles.
[c] ChE = cholinesterase; AChE = acetylcholinesterase; BChE = butyrylcholinesterase.
[d] Total sample without taking any factor into account.

TABLE 6. Plasma cholinesterase activities[a] and AChE:BChE ratios in European raptor species of Falconidae, Tytonidae, and Strigidae.

Family Species	*n*	Parameters[b]	ChE[c]	AChE[c]	BChE[c]	AChE:BChE
Falconidae						
Lanner falcon (*Falco biarmicus*)	1		0.658	0.576	0.082	7.02
Kestrel (*Falco tinnunculus*)	250	Mean (SD)	1.035 (0.313)	0.750 (0.288)	0.286 (0.079)	2.77 (1.20)
		CV	30.3	38.4	27.8	43.3
(total[d])		Med	0.994	0.732	0.278	2.66
		P10–P90	0.652–1.463	0.397–1.123	0.194–0.379	1.41–4.16
		Min–max	0.354–1.973	0.147–1.671	0.123–0.598	0.38–7.74
Hobby (*Falco subbuteo*)	3	Mean (SD)	1.251 (0.113)	0.931 (0.180)	0.320 (0.068)	3.08 (1.24)
		CV	9.0	19.3	21.1	40.3
		Min–max	1.153–1.375	0.764–1.121	0.254–0.389	1.96–4.41
Merlin (*Falco columbarius*)	1		1.308	1.007	0.300	3.35
Tytonidae						
Barn owl (*Tyto alba*)	152	Mean (SD)	3.069 (0.454)	0.761 (0.239)	2.308 (0.388)	0.34 (0.12)
		CV	14.8	31.4	16.8	35.3
(total[d])		Med	3.026	0.747	2.264	0.34
		P10–P90	2.520–3.651	0.472–1.069	1.865–2.879	0.20–0.47
		Min–max	2.208–4.628	0.177–1.585	1.516–3.762	0.08–0.69
Barn owl (total wild)	47	Mean (SD)	2.995 (0.540)	0.689 (0.285)	2.306 (0.456)	0.31 (0.14)
		CV	18.0	41.4	19.8	45.2
		Med	3.007	0.636	2.217	0.31
		P10–P90	2.343–3.651	0.381–1.033	1.846–2.925	0.14–0.50
		Min–max	2.208–4.628	0.177–1.585	1.547–3.762	0.08–0.64
Strigidae						
Short-eared owl (*Asio flammeus*)	1		1.775	0.437	1.338	0.33
Long-eared owl (*Asio otus*)	8	Mean (SD)	2.028 (0.230)	0.368 (0.106)	1.660 (0.207)	0.23 (0.08)
		CV	11.4	28.7	12.4	33.7
		Min–max	1.765–2.446	0.205–0.485	1.363–1.968	0.13–0.35
Tawny owl (*Strix aluco*)	126	Mean (SD)	2.897 (0.869)	0.167 (0.069)	2.730 (0.843)	0.07 (0.04)
		CV	30.0	41.0	30.9	58.3
(total[d])		Med	2.838	0.148	2.692	0.06
		P10–P90	1.827–4.018	0.102–0.276	1.684–3.787	0.04–0.11
		Min–max	0.521–5.513	0.070–0.435	0.399–5.391	0.02–0.31
Little owl (*Athene noctua*)	7	Mean (SD)	4.238 (0.962)	0.250 (0.091)	3.989 (0.993)	0.07 (0.03)
		CV	22.7	36.5	24.9	38.8
		Min–max	3.282–6.079	0.104–0.329	3.139–5.975	0.02–0.09

[a] Activities are shown as μmoles substrate hydrolyzed/min/ml.
[b] SD = standard deviation; CV = coefficient of variation (%); Med = median; P10–P90 = 10th–90th percentiles.
[c] ChE = cholinesterase; AChE = acetylcholinesterase; BChE = butyrylcholinesterase.
[d] Total sample without taking any factor into account.

ward α-NA relative to other esterases that are responsible for α-NAE activity (Tables 8 and 9). Species of Accipitridae and Falconidae had very low or no α-NAE activity in the presence of eserine and a strong linear relationship (r^2=0.9583) between ChE and α-NAE activities (Fig. 1). Therefore, ChE accounted for almost total measured α-NAE activity in these species. Only the sparrowhawk had a very low residual eserine-insensitive α-NAE activity, which accounted for about 8% (median values) of plasma α-NAE activity with a high variability (range=2–27%; CV=69%). In the

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TABLE 7. Interspecies differences of total plasma cholinesterase (ChE) activity and AChE:BChE[a] ratio in a range of European raptor species.

	ChE						AChE:BChE					
	All birds[b]		Adults[c]		Young[d]		All birds		Adults		Young	
Family Species	*n*	Significance[e]	*n*	Significance[e]	*n*	Significance[e]	*n*	Significance[e]	*n*	Significance[e]	*n*	Significance[e]
Accipitridae												
Egyptian vulture	3	AB	3	AB	0		3	BCD	3	BC	0	
Bonelli's eagle	8	A	8	A	0		8	BC	8	B	0	
Booted eagle	4	AB	4	AB	0		4	CDE	4	C	0	
Long-legged buzzard	4	ABC	2		2		4	BC	2		2	
Buzzard	124	B	72	C	31	B	124	B	72	B	31	B
Sparrowhawk	27	C	7	CD	20	C	27	B	7	B	20	B
Honey buzzard	4	E	3	F	1		4	B	3	B	1	
Falconidae												
Kestrel	250	A	39	B	152	A	250	E	39	D	152	C
Hobby	3	ABC	2		1		3	DE	2		1	
Tytonidae												
Barn owl	152	DE	11	DEF	35	DE	152	B	11	B	35	B
Strigidae												
Long-eared owl	8	BD	5	CDE	2		8	B	5	B	2	
Tawny owl	126	DE	16	EF	101	D	126	A	16	A	101	A
Little owl	7	E	0		7	E	7	A	0		7	A

[a] AChE = acetylcholinesterase; BChE = butyrylcholinesterase.
[b] Total sample of each species; only species with $n>3$ are shown.
[c] Adult birds not including the wild nonreleasable birds for kestrels.
[d] Young birds (<12-mo-old) not including wild nonreleasable birds for kestrels and not including captive-bred birds for barn owls.
[e] Letters indicate the results of the multiple comparison test between means of log-transformed values; means having a common letter are not significantly different ($P>0.05$); letters are in ascending order, A corresponds with the smallest means.

TABLE 8. Plasma α-naphthyl acetate esterase (α-NAE) activity[a] and correlation with cholinesterase (ChE) activity in European raptor species of Accipitridae.

Family Species	Parameters[b]	Correlation		Eserine comparison	
		ChE	α-NAE	α-NAE	α-NAE with eserine
Accipitridae					
Egyptian vulture	Mean (SD)	0.709 (0.192)	0.343 (0.068)	0.343 (0.068)	0.023 (0.004)
(*Neophron percnopterus*)	Min–max	0.490–0.846	0.264–0.386	0.264–0.386	0.019–0.026
	N	3	3	3	3
	r (*P*)	0.996 (NS[c])			
Bonelli's eagle	Mean (SD)	0.727 (0.054)	0.408 (0.027)	0.374	0.021
(*Hieraaëtus fasciatus*)	Min–max	0.626–0.785	0.369–0.441		
	N	8	8	1	1
	r (*P*)	0.950 (0.0003)			
Booted eagle	Mean (SD)	0.811 (0.203)	0.428 (0.118)	0.428 (0.118)	0.032 (0.009)
(*Hieraaëtus pennatus*)	Min–max	0.610–1.016	0.319–0.554	0.319–0.554	0.026–0.042
	N	3	3	3	3
	r (*P*)	0.994 (NS)			
Long-legged buzzard	Mean (SD)	1.190 (0.132)	0.683 (0.036)	0.666 (0.017)	0.047 (0.036)
(*Buteo rufinus*)	Min–max	1.043–1.362	0.647–0.732	0.647–0.681	0.024–0.088
	N	4	4	3	3
	r (*P*)	0.968 (0.032)			
Buzzard	Mean (SD)	1.436 (0.329)	0.799 (0.183)	0.840 (0.203)	0.022 (0.013)
(*Buteo buteo*)	Med	1.357	0.766	0.799	0.021
	P10–P90	1.101–1.848	0.616–1.030	0.659–1.085	0.012–0.027
	Min–max	0.881–2.446	0.480–1.399	0.480–1.399	0.010–0.085
	N	54	54	32	32
	r (*P*)	0.973 (<0.0001)			
Black kite	Value	1.053		0.645	0.068
(*Milvus migrans*)	*N*	1		1	1
Goshawk	Value	1.087		0.633	0.049
(*Accipiter gentilis*)	*N*	1		1	1
Sparrowhawk	Mean (SD)	1.962 (0.384)	1.140 (0.268)	1.137 (0.249)	0.131 (0.117)
(*Accipiter nisus*)	Med	2.034	1.162	1.162	0.094
	P10–P90	1.414–2.506	0.865–1.462	0.867–1.396	0.040–0.321
	Min–max	1.299–2.766	0.642–1.850	0.772–1.850	0.019–0.463
	N	24	24	20	20
	r (*P*)	0.936 (<0.0001)			
Honey buzzard	Mean (SD)	4.578 (0.905)	2.683 (0.652)	2.683 (0.652)	0.030 (0.013)
(*Pernis apivorus*)	Min–max	3.661–5.471	1.932–3.099	1.932–3.099	0.022–0.045
	N	3	3	3	3
	r (*P*)	0.905 (NS)			

[a] Activities are shown as μmoles substrate hydrolyzed/min/ml.
[b] SD = standard deviation; Med = median; P10–P90 = 10th–90th percentiles; *r* = Pearson correlation coefficient; (*P*) = probability.
[c] NS = not significant, $P>0.05$.

Strigidae, all species had residual eserine-insensitive α-NAE activity, with the exception of the little owl, for which activity was almost absent. This level of activity represented about 41–47% (median values) of plasma α-NAE activity for the short-eared owl (*Asio flammeus*) and the long-eared owl (*Asio otus*) and about 17% (median values) for the tawny owl, with a high variability (range=5–55%; CV=58%). A special case was that of the barn owl, where α-NAE activity was high (22 times higher than in tawny owl) and remained high after addition of eserine. In this species, the contribution of other esterases to α-NAE activity was higher than that of ChE. By

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TABLE 9. Plasma α-naphthyl acetate esterase (α-NAE) activity[a] and correlation with cholinesterase (ChE) activity in European raptor species of Falconidae, Tytonidae, and Strigidae.

Family Species	Parameters[b]	Correlation		Eserine comparison	
		ChE	α-NAE	α-NAE	α-NAE with eserine
Falconidae					
Lanner falcon	Value	0.658	0.533	0.290	0.014
(*Falco biarmicus*)	*N*	1		1	1
Kestrel	Mean (SD)	1.093 (0.287)	0.53 (0.127)	0.522 (0.119)	0.025 (0.008)
(*Falco tinnunculus*)	Med	1.085	0.523	0.521	0.024
	P10–P90	0.752–1.483	0.370–0.721	0.370–0.670	0.018–0.034
	Min–max	0.478–1.899	0.287–0.899	0.287–0.748	0.007–0.051
	N	93	93	42	42
	r (*P*)	0.964 (<0.001)			
	Mean (SD)	1.264 (0.157)		0.606 (0.053)	0.021 (0.002)
Hobby	Min–max	1.153–1.375		0.568–0.643	0.020–0.023
(*Falco subbuteo*)	*N*	2		2	2
Tytonidae					
Barn owl	Mean (SD)	3.093 (0.478)	53.5 (19.3)	44.5 (12.6)	42.1 (13.1)
(*Tyto alba*)	Med	3.063	49.3	42.8	39.8
	P10–P90	2.505–3.784	32.7–81.8	31.5–61.4	28.1–60.2
	Min–max	2.208–4.628	25.3–132.0	25.3–81.8	22.9–76.3
	N	126	126	70	70
	r (*P*)	0.426 (<0.0001)			
Strigidae					
Short-eared owl	Value	1.775		1.391	0.571
(*Asio flammeus*)	*n*	1		1	1
Long-eared owl	Mean (SD)	2.028 (0.230)	2.554 (0.634)	2.524 (0.679)	1.398 (0.841)
(*Asio otus*)	Med	1.976	2.517	2.268	1.073
	Min–max	1.765–2.446	1.731–3.495	1.731–3.495	0.423–2.675
	n	8	8	7	7
	r (*P*)	0.021 (NS[c])			
Tawny owl	Mean (SD)	2.894 (0.866)	1.936 (0.618)	1.985 (0.686)	0.410 (0.398)
(*Strix aluco*)	Med	2.831	1.883	1.884	0.263
	P10–P90	1.827–4.016	1.253–2.711	1.182–2.747	0.122–0.818
	Min–max	0.521–5.513	0.655–4.569	0.930–4.569	0.098–2.464
	n	123	123	70	70
	r (*P*)	0.752 (<0.0001)			
Little owl	Mean (SD)	4.238 (0.962)	2.103 (0.548)	2.103 (0.548)	0.043 (0.046)
(*Athene noctua*)	Min–max	3.282–6.079	1.530–3.225	1.530–3.225	0.000–0.138
	n	7	7	7	7
	r (*P*)	0.984 (<0.001)			

[a] Activities are shown as μmoles substrate hydrolyzed/min/ml.
[b] SD = standard deviation; Med = median; P10–P90 = 10th–90th percentiles; *r* = Pearson correlation coefficient; (*P*) = probability.
[c] NS = not significant, $P>0.05$.

comparison, α-NAE activities with or without eserine in other species were low.

Table 10 summarizes ChE plasma levels and eserine-insensitive α-NAE activities, mean body mass, and primary natural diet. Species were classified by family, order, and life style. The lowest ChE activities were found in two diurnal Accipitridae, Egyptian vulture and Bonelli's eagle, which were also the largest species. The highest ChE activities were mainly found in species of the nocturnal Strigiforme, which were relatively small species. There was a negative trend between ChE activity

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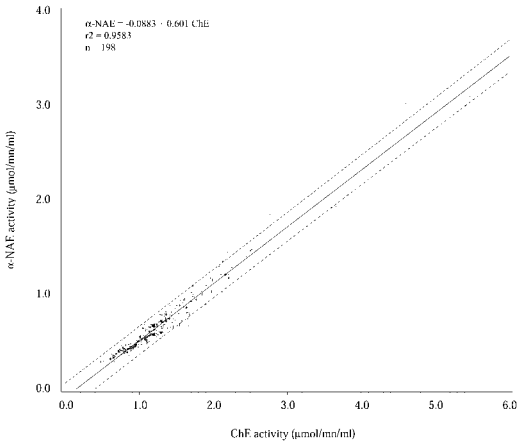

FIGURE 1. Regression of total plasma α-naphthyl acetate esterase (α-NAE) activity as a function of total plasma cholinesterase (ChE) activity in a range of European raptor species of Accipitridae and Falconidae. Lines represent predicted values for α-NAE activity and the 95% confidence interval.

and body mass in the Accipitridae, with the exception of the honey buzzard, as shown by the significant negative correlation ($r=-0.618$, $P<0.0001$, n=157). This negative correlation was also significant in the Accipitridae and the Strigiforme ($r=-0.614$, $P<0.0001$, $n=449$ without honey buzzard). In these species, AChE and BChE had a significant ($P<0.0001$) negative correlation with body mass, $r=-0.329$ and $r=-0.547$, respectively. This relationship between ChE activity and mass was very weak over the total range of species, including the Falconidae ($r=-0.078$, $P=0.038$, $n=701$). The small kestrel (200 g), which had a ChE activity at a level close to that of the larger buzzard (831 g), did not fit this relationship. However, for all species, the partial correlation, with the AChE:BChE ratio as a constant, was stronger ($r=-0.350$, $P<0.0001$, $n=701$). This relationship with body mass was significant for all species with respect to AChE ($r=-0.4053$, $P<0.0001$) but not BChE ($r=0.043$, $P>0.05$). Species of the Accipitridae and Strigiforme (the most numerous) mostly were characterized by a large BChE contribution to ChE activity, while the Falconidae were characterized by a dominant AChE contribution. The regression curves of ChE, AChE, and BChE activities relative to body mass, in the species of Accipitridae and Strigiforme, were linear after logarithmic transformation (primarily ChE and BChE; $r=-0.705$ and $r=-0.654$, respectively; $n=449$) and can be compared with the regression curve obtained on the whole range of species (Fig. 2, ChE). All species had no or low eserine-insensitive α-NAE activity except for the barn owl.

DISCUSSION

This study of factors that affect intraspecies variation in plasma cholinesterase activities revealed a sex-related difference in ChE activity in 2–3-mo-old kestrels, which was due to a sex-related difference in the main cholinesterase, AChE. There was no significant difference in BChE activity between the sexes. Rattner and Franson (1984) reported similar sex-dependent plasma ChE activity in American kestrels (*Falco sparverius*) in a small sample of birds at least 8 mo old. Total plasma ChE activity was higher in females than in males. Lanzarot et al. (2001) did not find significant sex-related difference in plasma ChE activity for 32 nestling (15–27-day-old) free-living peregrine falcons (*Falco peregrinus*).

Age-related differences in cholinesterase activities were observed in kestrels, sparrowhawks, and tawny owls. Activities were higher in adults than in young birds (2–3-mo-old and 6–11-mo-old for kestrels; 2–3-mo-old for tawny owls; <12 mo old for sparrowhawks). For these three species, age had an effect on plasma AChE activity and the AChE:BChE ratio. It affected plasma BChE activity in the tawny owl. In this species, BChE is the major contributor to total ChE activity. The overall mean AChE activity in tawny owls accounted only for 6% (3%) (mean [SD]) of ChE activity while it accounted for 25% (6)% and 71% (9%) of ChE activity in the sparrowhawks and kestrels, respectively. Higher AChE activity in adult kestrels and

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TABLE 10. Summary of esterase levels, main cholinesterase, body mass, and primary natural diet according to species, family, order, and life style in a range of European raptor species.

Life style Order Family	Species[a]	*n*	Body mass[b] (g)	Primary natural diet	Level[c] of ChE activity	Level[c] of AChE:BChE	Main cholinesterase	Level of α-NAE with eserine
Diurnal								
Falconiforme								
Accipitridae	Egyptian vulture (*Neophron percnopterus*)	3	1979 (170)	Carrion			BChE	Almost absent
	Bonelli's eagle (*Hieraaëtus fasciatus*)	8	2169 (316)	Birds, mammals, reptiles	1	2	BChE	Almost absent
	Booted eagle (*Hieraaëtus pennatus*)	4	792 (120)	Birds, reptiles, small mammals		3	Similar level	Almost absent
	Long-legged buzzard (*Buteo rufinus*)	4	873 (118)	Small mammals, lizards, snakes		2	BChE	Almost absent
	Buzzard (*Buteo buteo*)	124	831 (109)	Small mammals	3	2	BChE	Almost absent
	Sparrowhawk (*Accipiter nisus*)	27	238 (47)	Birds	4	2	BChE	Very low
	Honey buzzard (*Pernis apivorus*)	4	813 (241)	Wasp and bumblebee larvae	5	2	BChE	Almost absent
Falconidae	Kestrel (*Falco tinnunculus*)	250	200 (23)	Small mammals	2	4	AChE	Almost absent
	Hobby (*Falco subbuteo*)	3	220 (19)	Birds, insects		4	AChE	Almost absent
Nocturnal								
Strigiforme								
Tytonidae	Barn owl (*Tyto alba*)	152	299 (29)	Small mammals	5	2	BChE	High
Strigidae	Long-eared owl (*Asio otus*)	8	264 (26)	Small mammals		2	BChE	Low
	Tawny owl (*Strix aluco*)	126	426 (46)	Small mammals	5	1	BChE	Low
	Little owl (*Athene noctua*)	7	160 (8)	Small mammals	5	1	BChE	Almost absent

[a] The order of species by family is according to ascending order of cholinesterase (ChE) activity.
[b] Mean of total sample and (SD); for buzzard $n=105$, for kestrel $n=243$, and for barn owl $n=151$.
[c] Levels are shown by number in ascending order, only significantly distinct levels are shown. AChE = acetylcholinesterase; BChE = butyrylcholinesterase.

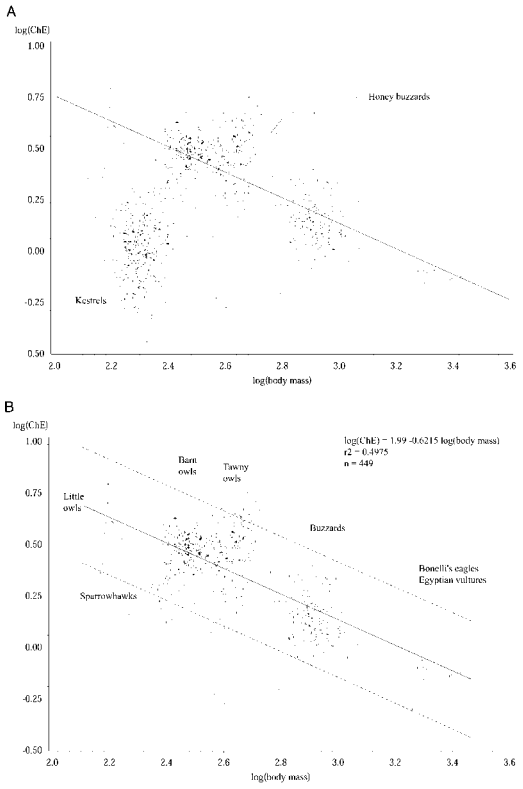

FIGURE 2. Log_{10}–log_{10} linear regression of cholinesterase (ChE) activity as a function of body mass. A. Data from the whole range of species (n=701) tested are shown. The line represents predicted values from Figure 2B. Kestrels (and also other Falconidae species, where the main cholinesterase is acetylcholinesterase) and honey buzzards mostly did not fit the linear model. B. Data from species of the Accipitridae family and the Strigiforme order (main cholinesterase is butyrylcholinesterase). Honey buzzard (n=4) was not included. Lines represent predicted values for log(ChE) and the 95% confidence interval.

higher AChE and BChE activities in adult tawny owls were reflected in the higher total ChE activity in adults of these two species. In sparrowhawks, the age-related difference for ChE activity was not significant, probably because of the small sample size. Gard and Hooper (1993) noted an increase in plasma ChE activity related to an increased BChE activity in two altricial passerine species, the eastern bluebird (*Sialia sialis*) and the European starling (*Sturnus vulgaris*), throughout the nestling period (>2–5 days posthatch) and also after the fledging period because BChE activity and BChE:AChE ratios were higher in adult birds than in fledglings. Butyrylcholinesterase was the main cholinesterase, and AChE activity represented 5% and 10% of ChE activity in the adult eastern bluebird and European starling, respectively. Wolfe and Kendall (1998) confirmed that, in the European starling, plasma ChE activity increases with age and that this increase is due to an increase in BChE activity. These authors found the same pattern in another passerine, the red-winged blackbird (*Agelaius phoeniceus*), in which AChE activity contributed only 6% to the ChE activity in adults. In contrast, a decrease in plasma ChE activity to adult values was observed in mallards (*Anas platyrhynchos*), a precocial species, between 5 and 40 days of age (Rattner and Fairbrother, 1991). In the present study, raptors, which are altricial species, had stable or increased ChE activity between juvenile and adult ages.

The slight differences in ChE and AChE activities found in this study for wild and captive-bred 2–3-mo-old barn owls could be explained by vitamin and mineral supplements in the diet of the captive-bred nestlings. However, this dietary difference did not affect BChE activity. In wild and captive-bred 2–3-mo-old kestrels, diet had no effect on ChE, AChE, and BChE activities. Plasma BChE is synthesized in the liver and is decreased in the blood of malnourished humans (Barclay, 1973). Therefore, a difference in the nutritional status of captive-bred and wild young could affect BChE activity, which is the predominant form in barn owls. Moreover, Van Lith et al. (1992) showed that plasma BChE activity in rats can be affected by the type of dietary fat. In accordance with these data, Goldstein et al. (1999b) evoked dietary change of migrant Swainson's hawks (*Buteo swainsoni*) between North America (where they eat vertebrates) and Argentina (where the diet is probably more insectivorous) to explain elevated plasma BChE activities in samples

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collected from birds in Argentina. In the present study, wild barn owls in the rehabilitation center were fed the same diet as the captive-bred birds, except for vitamin and mineral supplements. The absence of a difference in plasma BChE activity between wild and captive-bred barn owls suggests that the diet is not the cause of the observed differences in ChE and AChE activities. A plausible explanation could be a specific genetic factor in the pairs of barn owls kept for captive reproduction.

We have shown wide interspecies variation in the plasma ChE activity of European raptor species. Butrylcholinesterase was predominant in most species except in Falconidae, where AChE activity was about 3–7 times greater than BChE activity. In American Accipitridae, BChE contribution to plasma ChE activity was highest in red-tailed hawks (*Buteo jamaicensis*) (Hooper et al., 1989; Wilson et al., 1991) and Swainson's hawks (Goldstein et al., 1999b), while the highest AChE contribution to plasma ChE activity was reported in American species of Falconidae (Hooper, 1988 cited in Rattner and Fairbrother, 1991). For adult tawny owls, the values for ChE activity we found (mean=3.361, SD=0.844, n=16) were similar to those found by Westlake et al. (1983) using the method of Ellman et al. (1961) with the same substrate and temperature (mean=2.53, SD=1.00, n=6). For wild barn owls, the values obtained in this study (mean=2.995, SD=0.540, n=47) were similar to those found for captive American barn owls by Fleming and Grue (1981) using the same method and substrate (mean=2.707, SD=0.624, n=8).

The negative relationship observed between size and ChE activity is in agreement with results reported by Hill (1988) for bird-brain ChE activity. This author found lower levels in large Anseriforme and Galliforme species compared with smaller species such as passerines, but with some exceptions and differences observed for birds of the same genus and similar size. In our study, plasma from the Accipitridae had ChE activity three times higher in the honey buzzard than in the buzzard, although body mass and AChE: BChE ratios were similar. The *Pernis* genus is recognized as an ancestral group of the Accipitridae, which is phylogenetically distant from the genus *Buteo* (buzzard) (Seibold and Helbig, 1995; Wink et al., 1996; Mindell et al., 1997). Fossi et al. (1996) also found a negative relationship with body size for plasma CbE and brain ChE activities in seven different species of birds. It is well known that body size and many biologic variables, such as metabolic rate, are linked by an allometric scaling relationship with the metabolic demand per mass unit decreasing as body mass increases (West et al., 1997; Banavar et al., 2002). Esterases that are likely to contribute to metabolism are linked to the metabolic rate. If their total activity for the whole-blood volume follows a positive relationship with body mass (as does metabolic rate), their activity expressed by unit of blood will follow a negative relationship. The well-fitted linear regression curve of log-transformed ChE as a function of log-transformed body mass from the data of the present study is in agreement with the above law. In the raptor species studied here, there appears to be a trend toward a relationship between ChE activity and phylogenetic classification because the lowest activities were found in the largest diurnal Accipitridae species, vultures and eagles, and the highest in nocturnal Strigiforme species, which are rather small species. These species were characterized by a predominant BChE contribution to ChE activity, while for small Falconidae species, ChE activity was close to that of larger species, such as buzzard. In this case, AChE was the dominant contribution to ChE activity. Besides the level of ChE activity, the type of cholinesterase contributing to this activity should be considered in this relationship. Furthermore, these results suggest a higher efficiency of AChE compared with BChE in metabolic

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processes. Thus, body mass and phylogeny are factors that can explain in part the interspecies variability in ChE activity. Indeed, these observations need to be confirmed by other measurements from a wider range of raptor species.

Regarding CbE activity, there exist several assay methods and it is therefore difficult to compare data from various publications. As mentioned in the Introduction, CbE represents a multigene family with broad substrate specificity and several substrates have been used to measure its activity. Some substrates are hydrolyzed by other enzymes. Consequently, assay methods sometimes include selective inhibitors of other enzymes, such as ChE inhibitor (see above). Very few data are available on plasma CbE activity in raptors. Westlake et al. (1983) measured plasma esterase activity toward nitrophenyl acetate (NPA) in 27 avian species but only one raptor, the tawny owl. Like the activity toward the NPA substrate, the activity toward the α-α-NA substrate measures general plasma esterase activity. However, α-NA is a more specific substrate than NPA for rat plasma CbE, which has a greater affinity to OP compounds (Yang and Dettbarn, 1998). In the present study, eserine was used to inhibit ChE activity toward α-NA. The level of eserine-insensitive esterase activity was high only in barn owls. The eserine-insensitive esterase activity could be due to B-esterase CbE (EC 3.1.1.1) as discussed above. It could also arise from a Ca^{2+}-dependent A-esterase, which hydrolyzes the active oxon form of OP compounds to form nontoxic metabolites and is not inhibited by these compounds (Aldridge, 1953a) or to a Ca^{2+}-dependent arylesterase, which hydrolyzes phenyl acetate (see Reiner et al., 1993; Walker, 1993; Jokanović, 2001). A-esterases are abundant in the liver and blood of mammals, but birds lack these detoxifying enzymes (Brealey et al., 1980). Mackness et al. (1987) found that blood A-esterase (paraoxonase) activity was absent or at a low level in birds and that arylesterase (phenyl acetate as substrate) activity was similar in birds and mammals. These two studies did not include raptor species. Yawetz et al. (1979) compared the metabolism of the OP insecticide parathion in four species of birds and observed that hepatic A-esterase (paraoxonase) activity was higher in barn owl and blackbird (*Turdus merula*) than in African bulbul (*Pycnonotus capensis*) and house sparrow (*Passer domesticus*). This observation is consistent with the presence of A-esterase in the blood of barn owl. Because α-NA can be hydrolyzed by various esterases, when a Ca^{2+}-dependent A-esterase or a Ca^{2+}-dependent arylesterase activity is present in plasma, the use of a chelating agent in the assay is needed to inhibit them (Chanda et al., 1997). By comparison with other raptor species, the presence of A-esterase activity in the blood of barn owl could be an efficient mechanism to detoxify the active oxon form of OP insecticides. A high level of CbE could also give a more effective protection against anticholinesterase compounds (see Introduction). Nevertheless, Fleming and Grue (1981) have found that barn owls are more sensitive to the OP insecticide dicrotophos, with a higher mortality and a lower dose to produce 50% inhibition of brain ChE, than bobwhite quail (*Colinus virginianus*), starling, grackle (*Quiscalus quiscula*) and mallard.

Bartkowiak and Wilson (1995) determined the level of plasma CbE in some species of American raptors of the Accipitridae and Falconidae families and in pigeon (*Columba livia*) with diethylsuccinate as substrate. The levels of diethylsuccinate esterase activity were 40 to 7 times lower in raptors than in pigeon. In our laboratory, total plasma α-NAE activity in two pigeons was 19.1 and 21.3 μmoles substrate hydrolyzed/min/ml and 18.2 for α-NAE activity in the presence of eserine in the first pigeon. This decrease in activity accounted for ChE activity, the level of which was 1.093. This result indicates a high eserine-insensitive α-NAE activity in pigeon, higher than ChE activity, and higher than the

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activities measured in any raptor species examined in this study except for the barn owl. The total plasma α-NAE activity of the pigeons was about 25-fold that of buzzards, 10-fold that of tawny owls, and less than 0.5-fold that of barn owls. Westlake et al. (1983) also found a two- to sevenfold higher NPA esterase activity in several species of pigeons (5.27–16.5) compared with tawny owl (2.53). Our results are consistent with those of Bartkowiak and Wilson (1995) for the same bird families.

Interspecies variability in esterase activities frequently is explained by diet, particularly for CbE activity. It seems reasonable to propose that mammalian and avian carnivores have lower activities than herbivorous and omnivorous species because their specialized diet leads them to encounter a smaller variety of compounds to detoxify (Bush et al., 1973; Westlake et al., 1983; Walker and Thompson, 1991). In raptors, Walker et al. (1987) found low levels of hepatic microsomal monooxygenase activities in kestrel and sparrowhawk compared with rat. Our results for plasma α-NAE activity in raptor species are in agreement with this trend. However, barn owls, which have the same diet (mainly small mammals) as buzzards, kestrels, and tawny owls, are obviously an exception. The particular diet of the honey buzzard is noteworthy. This species primarily eats larvae of wasps and bumblebees, the diets of which consist of insects or nectar and pollen, respectively. It is not clear if this dietary particularity can explain high ChE activity in honey buzzard, which lies off the regression line for ChE activity vs. body mass. To support the above hypothesis, we suggest that larvae of wasps and bumblebees have a weak capacity for detoxifying foreign compounds present in their food. Their predators would then be more exposed to nondetoxified compounds and, consequently, would need higher levels of plasma ChE than the buzzard, which has the same body mass but is less exposed, its prey having a more efficient detoxifying enzymatic system. Carboxylesterase can catalyze hydrolysis of a wide range of xenobiotic carboxylesters and aromatic amides (Satho and Hosokawa, 1998). Hence, they could be involved in detoxification of dietary lipophilic esters (Walker and Thompson, 1991). The role of ChE is unclear, but a similar role seems plausible. Alternatively, the dietary fat composition could explain elevated plasma BChE activities (Van Lith et al., 1992). As discussed by Goldstein et al. (1999b) for northern and southern Swainson's hawks (see above), the nutrient composition of the honey buzzard diet (insect larvae) is probably very different from that of the buzzard (small vertebrates). Thus, this dietary factor may have played an evolutionary role toward elevated BChE activity (and consequently, ChE activity) of this species compared with the buzzard.

In the absence of CbE and A-esterase, which are the main blood esterases contributing to anticholinesterase compound detoxification, the contribution of blood ChE to detoxification might become determinant, in addition to the activity of hepatic enzymes. This contribution varies with the level, the type, and the forms (isozymes) of the enzyme. In this context, the species displaying the highest ChE activity, such as those belonging to the Strigidae family, might be less sensitive to OP or CB insecticides. In a study of the acute toxicity of four anticholinesterase insecticides (ethyl 4-nitrophenyl phenylphosphonothioate [EPN], fenthion, carbofuran, and monocrotophos) on the American kestrel, eastern screech-owl (*Otus asio*), and northern bobwhite, Wiemeyer and Sparling (1991) found that the eastern screech-owl was highly tolerant to EPN but sensitive to other chemicals while the American kestrel was highly sensitive to all four chemicals. However, these authors did not determine the level of plasma ChE in these species. The level of ChE activity in American kestrel was measured and is similar to that of the European kestrel (Hunt et al., 1991) while, to our knowledge, ChE activ-

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ity in the eastern screech-owl has not been measured.

The nocturnal lifestyle does not seem to be a criterion for a high level of ChE activity because Buck et al. (1996) found 2.4–4.8 times lower activities in great horned owl (*Bubo virginianus*) than in nocturnal species in our study. However, this American species is larger and shows a greater AChE:BChE ratio (near one) than the Strigidae species of the present study. This is consistent with the contribution of body mass and the type of cholinesterase to the variability of ChE activity.

Indeed, it is well known that many factors play a role in the sensitivity of bird species to anticholinesterase insecticides. First, there are factors linked to the biotransformation process that depend, along with other enzyme activities, on the nature of blood and hepatic esterases (A- or B-esterase) of the species. The biotransformation activity of B-esterases depends on the level and the affinity of the enzyme for the toxic molecule. For many OP insecticides, the biotransformation process also depends on the rate of hepatic bioactivation into a toxic form, for example, the oxidative desulfuration of phosphorothionate insecticides by cytochrome P-450 monooxygenases to transform the parent compound to its active oxon form. Second, there are factors related to the affinity of the target cholinergic AChE for the toxic compound. Third, there are probably, for some anticholinesterase compounds, factors related to toxicity mechanisms other that just inhibition of cholinergic AChE (Pope, 1999). Finally, the sensitivity of a species can be modulated by factors such as sex, age, external temperature, in part by acting on the activities of several enzymes. Therefore, the level of B-esterase activities and the relative contribution of AChE and BChE to ChE activity are likely to be key factors in explaining the differential sensitivities of species to anticholinesterase insecticides, but they cannot account for all of the differences.

In this work, the levels of cholinesterase activities and eserine-insensitive α-NAE activity were determined in several European raptor species. Large samples of free-living raptor species are difficult to obtain and suitable statistical sampling methods are not easily realized. Although the raptors used in this study may not be fully representative of natural populations, the values for ChE, AChE, and BChE activities and AChE:BChE ratios obtained for the species with the largest sample sizes, that is buzzard, kestrel, barn owl, and tawny owl, should provide a suitable baseline to evaluate the exposure to anticholinesterase insecticides of birds of the same species caught in the field. This evaluation should be made using the same experimental conditions for the cholinesterase assays with respect to time of blood sample collection because of possible diurnal variation in enzyme activity. Sex and age should be taken into account for ChE, AChE activities, and the AChE:BChE ratio in kestrels. The groups of the present study that could be used as reference groups are the 2–3-mo-old male and female, wild 6–11-mo-old and wild adult kestrels. Age should be taken into account for all the tawny owl parameters. In wild barn owls, despite the absence of a significant difference between young and adult birds, there is a tendency toward higher values in adults and this difference might become significant with larger samples. The slight difference in ChE and AChE values between wild and captive-bred young barn owls and the possible bias in this sample, as discussed above, show that care is necessary before choosing captive-bred birds as reference populations. The sample of young sparrowhawks can be used as a control, keeping in mind that it contains a majority of female birds.

The use of these control values for monitoring sublethal anticholinesterase exposure in field studies would be more relevant for groups than for individuals. Mean values, obtained for birds of the concerned area, should be compared against the

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mean values obtained for the same species in the present study. This is possible whatever the statistical distribution of the biochemical parameter when the size of the sample is ≥30 individuals. When the statistical distribution of the parameter deviates from a Gaussian distribution, the comparison of the percent of individuals for which this parameter is under a predetermined threshold value, such as the 10th percentile, is an alternative method. A deviation from a Gaussian distribution was found for some of the parameters in buzzards (all parameters), barn owls (ChE and BChE), tawny owls (AChE and AChE:BChE ratio), adult kestrels (AChE: BChE ratio), wild 6–11-mo-old kestrels (BChE and AChE:BChE ratio), young male 2–3-mo-old kestrels (all parameters), and sparrowhawks (AChE:BChE ratio). The value of these parameters as references when monitoring exposure to anticholinesterase insecticides depends on the species. For example, AChE activity and the AChE:BChE ratio are not valuable for such a use in the tawny owl. Furthermore, the AChE:BChE ratio can be valuable only to account for a difference in the relative level of inhibition of the two cholinesterases.

Although a diagnosis of exposure to anticholinesterase insecticides in a group of birds is possible, the diagnosis for an individual is difficult to perform because of natural biological variability and overlapping of values between healthy and exposed populations. When a parameter has a Gaussian distribution, the normal bounds of this parameter for an individual can be determined by mean±(SD×t), where t is near two for P=0.05 and N≥30. In this case, 3% of the individuals have a value under the calculated lower bound and 3% above the calculated upper bound in the healthy population. When the distribution of the parameter deviates from a Gaussian distribution, observed percentiles can be used to determine the limits of the healthy population. A value such as the 10th percentile is a suitable threshold that can be used as a lower limit under which an inhibition of enzymatic activities can be suspected. For ChE, AChE activities, and the AChE:BChE ratio in buzzards, a logarithmic transformation of the data yielded a normal distribution. The limits of normal values (mean±2SD) calculated with the transformed data were (0.881–2.154) and (0.143–0.684) (μmoles/min/ml) for ChE and AChE activities, respectively, and (0.14–0.66) for the AChE:BChE ratio.

Buzzards and kestrels should be of particular interest as indicators of exposure in raptor wild populations because these two species are common and widespread in Europe. Furthermore, buzzards did not show any sex- or age-related difference, and adult kestrels did not show any sex-related differences. Also, in the latter species, sex dimorphism allows an easy distinction between male and female adult birds. Therefore, these diurnal species, which are primarily mammal eaters, could be used as relevant reference species to assess exposure of raptors to anticholinesterase insecticides.

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