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Other Supplementary Files

Navarrete_SupplData.xls

Note for all tables: λ : ML-estimate of lambda, β : PGLS estimate of the effect size. If the lambda value is not significantly different from both 0 and 1 (*italics*), both classic independent contrasts and raw species regressions are shown additionally. P values <0.05 are shown in bold face.

Supplementary Information 1: Evaluation of the original anthropoid dataset to establish the Expensive Tissue Hypothesis

Introduction

In addition to compiling and analyzing our own dataset, we also conducted a detailed evaluation of the original study that supported the Expensive Tissue Hypothesis in anthropoid primates¹. Their original analysis was based on combining the digestive tract and body mass data from Chivers and Hladik³⁶ with brain size and body mass data from Harvey and Clutton-Brock³⁷ for anthropoid primates. The Chivers dataset included individual mass and surface area measurements from several components of the digestive tract (stomach, ileum, caecum and colon) as well as body mass in a sample of 157 mammalian specimens, including 37 primate species (the published data were corrected for a layout error, and complemented by the full list of measurements made available by D. Chivers to R.D. Martin and L. Aiello). Aiello and Wheeler analyzed the anthropoid species from this dataset and published their species averages several years later⁵ (Suppl. Table 2). The original analysis of Aiello and Wheeler yielded a negative correlation between brain size and digestive tract mass (the sum of stomach, ileum, caecum and colon mass), controlling for the effect of body mass ($N = 18$, $r = -0.69$, $P < 0.001$). This negative correlation persisted when *Homo sapiens* was excluded from the sample⁵ ($n = 17$, $r = -0.62$, $P = 0.007$).

In this evaluation, we use the Chivers and Hladik dataset to test the Expensive Tissue Hypothesis, making full use of current phylogenetic methodology³⁴ and more accurate sex-specific brain volume and body mass data that have become available in recent years^{15,32}.

Methods

Individual measurements of digestive tract mass were taken from the full Chivers dataset, after the exclusion of individuals suspected to be emaciated or immature. Brain volume and body mass from wild-caught adult primates were obtained from published sources^{15,32} and additional measurements by J. van Woerden (pers. comm.).

The original study correlated brain mass with gut mass, i.e. digestive tract mass, described as the sum of the masses of stomach, ileum, caecum and colon. In this evaluation, we correlated brain mass with stomach mass, intestine mass (described as the sum of ileum, caecum and colon) and digestive tract mass. Because brains and digestive tract measures were obtained from

different specimens, a major challenge is to deal with the variability in body mass within species and the resulting error of estimating the relative size of organs. In order to obtain an optimum correspondence between digestive tract and brain specimens, we included only adults that were not emaciated as compared to average body mass values for the respective sex (body mass more than 75% of the average mass). To preclude confounding effects of body mass dimorphism between the sexes, we adhered to the following rationale: first, we calculated the averages for the digestive tract variables for each species, while recording whether the average included only females, only males or both sexes. Second, brain mass was paired to the digestive tract values as follows: for species with no sexual dimorphism in body mass (less than 10% difference between males and females), we took the average brain mass for both sexes; otherwise, we took the average brain mass for the same sex or the whole species depending on the sex of the individuals included in the digestive tract averages. Our final sample included N=23 anthropoid species and N=2 strepsirrhines (Suppl. Table 2).

All variables were log-transformed and phylogenetic regressions (PGLS) were run using `pglmEstLambda` in the `CAIC` package³⁴ in R³⁵ with the consensus tree from the 10kTrees project³⁸. As the small sample size yielded unstable estimates of lambda in some cases (lambda not significantly different from either 0 or 1), we also ran all analyses with lambda set to 0 (raw data) or 1 (classic independent contrasts). The phylogenetic least-squares regression model included both body masses and the digestive tract mass as independent variables and brain mass as the dependent variable.

Results and discussion

In contrast to the original result of Aiello and Wheeler¹, our revised sample did not yield any significantly negative correlations between brain and one of the digestive tract variables or the combined digestive tract mass (Suppl. Table 1). Results did not differ according to whether phylogenetic information was taken into account or not, and whether the two strepsirrhine species were included or not.

There are various reasons for the discrepancy between these results and the originally reported negative correlation. First, the Harvey dataset reported some brain size values that were not confirmed for some species in subsequent reports, and sometimes reported only male values without mentioning this fact. Second, sexual size dimorphism affects body mass more than brain mass³⁹, which may confound analyses where sex is not taken into account. Third, brain size data

have become available for more platyrrhine species in recent years, reducing the bias toward catarrhine species in the original analysis. Fourth, we excluded clearly emaciated individuals of the Chivers dataset in our analysis. In conclusion, matching the best available brain and body mass data with the Chivers dataset does not yield support for the Expensive Tissue Hypothesis in anthropoid primates.

Supplementary Table 1: Correlations between brain mass residuals and the residuals of mass of stomach, intestines and digestive tract (sum of stomach and intestines).

		A: dataset from Aiello and Wheeler, excluding <i>Homo sapiens</i>			B: revised dataset						
		Digestive tract mass			Stomach mass			Intestine mass		Digestive tract mass	
Sample		N	λ	p-value	N	λ	p-value	λ	p-value	λ	p-value
PGLS	Primates				25	0	0.24 (-)	0	0.17 (+)	0	0.86 (+)
	Anthropoidea	17	1	0.0001 (-)	23	0	0.23 (-)	0	0.16 (+)	0	0.94 (+)
IC	Primates				25	1	0.82 (-)	1	0.90 (+)	1	0.93 (-)
	Anthropoidea	17	1	0.0001 (-)	23	1	0.91 (-)	1	0.65 (+)	1	0.83 (+)
GLM	Primates				25	0	0.24 (-)	0	0.17 (+)	0	0.86 (+)
	Anthropoidea	17	0	0.004 (-)	23	0	0.23 (-)	0	0.16 (+)	0	0.94 (+)

PGLS: phylogenetic least-squares methods, IC: classic independent contrasts (lambda set to 1), GLM: linear model of logged species data (lambda set to 0). (+) indicates a positive correlation, (-) indicates a negative correlation. Significant p-values are shown in bold face.

Supplementary Table 2: The two datasets based on the digestive tract mass data from Chivers and Hladik³⁶.

Group	Species	A: Dataset used by Aiello and Wheeler		B: Revised dataset														
		Digestive tract data ³⁶		Brain data ³⁷		Digestive tract data ³⁶					Brain data ³²							
		BM	DT	BM	Brain	N _{ind}	BM	St	Int	DT	Female		Male		Dim	Sex	Adjusted sex	
											BM	Brain	BM	Brain			BM	Brain
Str	<i>Galago alleni</i>					1f	250	3	11	14	269	5	277	6	1.03	m/f	273	6
Str	<i>Galagoides demidoff</i>					1m	60	1	1	2	75	3	76	3	1.02	m/f	76	3
Pla	<i>Saguinus mystax</i>					1m	560	2	21	23	584	10	629	10	1.08	m/f	607	10
Pla	<i>Alouatta belzebul</i>					3f/1m	5050	94	274	368	5520	51	5525	55	1.00	m/f	5523	53
Pla	<i>A. seniculus</i>	4450	360	7250	58	1m	6150	116	325	441	5210	55	6668	55	1.28	m	6668	55
Pla	<i>Callicebus moloch</i>					2m	1165	7	32	39	887	17	935	18	1.05	m/f	911	17
Pla	<i>Cebus apella</i>	2890	110	2480	71	1f	2000	16	86	102	2489	64	3383	69	1.36	f	2489	64
Pla	<i>Lagothrix lagotricha</i>	8050	362	6300	96	1f/1m	8050	93	269	362	7020	89	7280	89	1.04	m/f	7150	89
Pla	<i>Saimiri sciureus</i>	740	39	665	24	1f/1m	990	6	39	45	743	24	860	24	1.16	m/f	802	24
Cat	<i>Cercopithecus cephus</i>	3338	120	3500	64	1f/2m	3567	26	102	127	2880	61	4290	66	1.49	m/f	3585	63
Cat	<i>C. neglectus</i>	4081	254	5480	71	1f/1m	7595	61	212	273	4130	61	8048	67	1.95	m/f	6089	64
Cat	<i>C. nictitans</i>					1m	6500	61	157	218	4260	67	6670	73	1.57	m	6670	73
Cat	<i>Erythrocebus patas</i>	11650	280	7800	107	1m	11650	54	226	280	6500	89	12400	97	1.91	m	12400	97
Cat	<i>Macaca fascicularis</i>	3175	149	5000	69	1f/2m	4400	38	167	205	3518	61	5360	65	1.52	m/f	4439	63
Cat	<i>Mandrillus sphinx</i>					1f	12300	123	641	764	12800	136	45000	159	3.52	f	12800	136
Cat	<i>Colobus polykomos</i>	7662	380	9400	77	2f	8465	190	173	363	6709	71	10600	78	1.58	f	6709	71
Cat	<i>Nasalis larvatus</i>	15880	598	15100	94	1m	15880	357	241	598	9730	85	19392	99	1.99	m	19392	99
Cat	<i>Presbytis melalophos</i>	6781	254	6650	80	3f/3m	6537	122	134	256	6567	61	6554	72	1.00	m/f	6561	66
Cat	<i>P. rubicunda</i>	6350	171	6300	93	1m	6350	105	66	171	6221	69	6310	75	1.01	m/f	6266	72
Cat	<i>Trachypithecus cristatus</i>	6850	433	8350	64	2f	6145	224	149	372	6060	58	6728	62	1.11	f	6060	58
Cat	<i>T. obscurus</i>	7580	315	7400	68	1f/2m	7170	182	133	314	6765	59	7347	64	1.09	m/f	7056	62
Cat	<i>Symphalangus syndactylus</i>	9300	490	10750	122	1f	11340	146	390	536	11295	124	11453	126	1.01	m/f	11374	125
Cat	<i>Hylobates pileatus</i>					1f	7260	56	238	294	5470	85	5500	95	1.01	m/f	5485	90
Cat	<i>Hylobates lar</i> *	5200	188	5500	108													
Cat	<i>Pongo pygmaeus</i>	64819	1591	53000	413	1f	56250	185	1095	1280	36948	338	80643	413	2.18	m/f	58796	375
Cat	<i>Gorilla gorilla</i>					1m	236000	595	4396	4991	71500	434	141500	528	1.98	m	141500	528
Cat	<i>Homo sapiens</i> **	60800	1107	65000	1300													

All values are in grams. Str: strepsirrhine, Pla: platyrrhines, Cat: catarrhines, BM: body mass, DT: digestive tract, St: stomach, Int: intestines, N_{ind}: number of individuals used in the average, Dim: index of sexual dimorphism in body mass (BM_{male}/BM_{female}). m/f: average of male and female values.

* *Hylobates lar*: excluded because the specimens were “fixed”, which may influence organ masses.

** Testing the Expensive Tissue Hypothesis as a general pattern to explain the *Pan-Homo* distinction requires that *Homo sapiens* is excluded from the comparison. The *Homo* values in the Aiello and Wheeler sample were taken from Ashoff et al.⁴⁰, presumably because the body mass value of the Chivers individuals is very low (45kg).

Supplementary Information 2: Supplementary Methods

This section contains a dissection protocol, the calculation of total adipose depots mass from abdominal adipose depots mass (Suppl. Figure 1), a rationale for how to control for the effect of body size (Suppl. Figure 2), and the phylogenetic tree (Suppl. Figure 3).

Specimens

Cadavers were collected from various museums, zoos and donors: Naturhistorisches Museum Basel, Naturhistorisches Museum Fribourg, Muséum d'Histoire Naturelle Genève, Muséum d'Histoire Naturelle Neuchâtel, Zoologisches Museum der Universität Zürich, Zoo Zürich, Knies Kinderzoo Rapperswil, Igelzentrum Zürich, Wildpark Langenberg, Dr. Marcus Clauss, Alexander Schweiger, Dr. Anna Lindholm, Dr. Carsten Schradin, Heinz Galli (all CH), Naturhistorisches Museum Mainz, Staatliches Museum für Naturkunde Stuttgart, Wilhelm and Helga Klemens, Elke Kissel (all DE), Zoo Antwerpen (NL), Royal Museum of African Fauna Tervuren (BE), National Museums of Scotland (UK), Hungarian Natural History Museum (HU), and the Field Museum of Natural History (USA).

Dissection protocol

Information about sex, age, origin, time and cause of death of the specimens was collected when available. Museum staff performed skinning and bone preparation if the specimens were to be prepared for their collections. Specimens from the National Museums of Scotland were prepared for dissection by G. Hantke by removing the bulk of all visceral organs, and keeping them frozen in a sealed plastic bag until A. Navarrete performed the dissections. All other cadavers were dissected by A. Navarrete.

Preparations: Frozen specimens were allowed to thaw (2h for small specimens, overnight for large specimens) and external moisture on the skin was removed using a hair dryer. The total body mass was weighed when the body temperature equaled ambient temperature. The specimens were skinned and the skin was weighed. To avoid desiccation, the organs were put onto a wet paper towel immediately after removal. For weighing, individual organs were put onto small sheets of aluminum (small specimens) or into plastic containers (larger specimens), and net weights determined. All weights were recorded in grams.

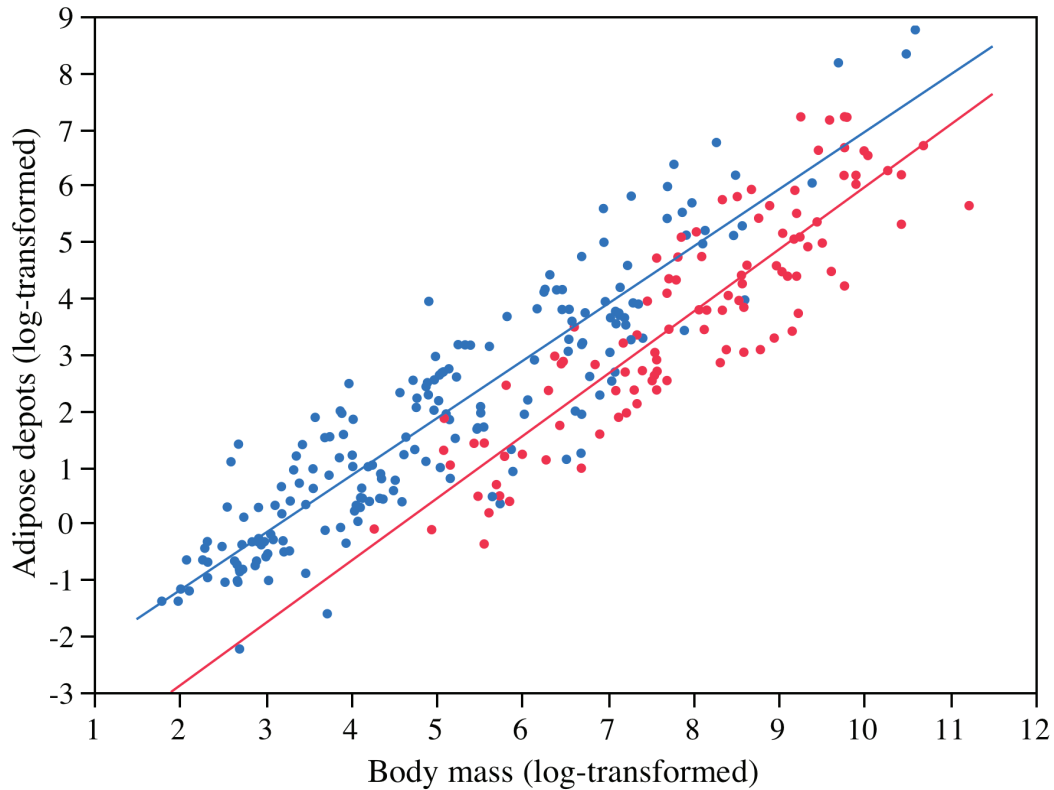
Abdominal cavity: The testes were removed in male specimens. The abdominal cavity was opened with a ventral cut from sternum to pubis and two transverse cuts along the distal end

of the rib cage. The spleen was removed. The small intestine was shifted aside, and the kidneys and additional male sexual structures (seminal vesicles) were cut off and removed. The adrenal glands were separated from the kidneys. The digestive tract was removed cutting the esophagus at the level of the diaphragm and cutting the anus and, in females, the vulva. The female sexual organs were removed. The liver and pancreas were removed. The digestive tract (stomach and intestines) was cleaned from connective and adipose tissue, laid on millimeter paper and photographed. Stomach, ileum, caecum and colon were separated, weighed a first time, cut open to remove contents, washed, dried with a paper towel after washing to remove excess water and weighed empty a second time. The other abdominal organs were weighed.

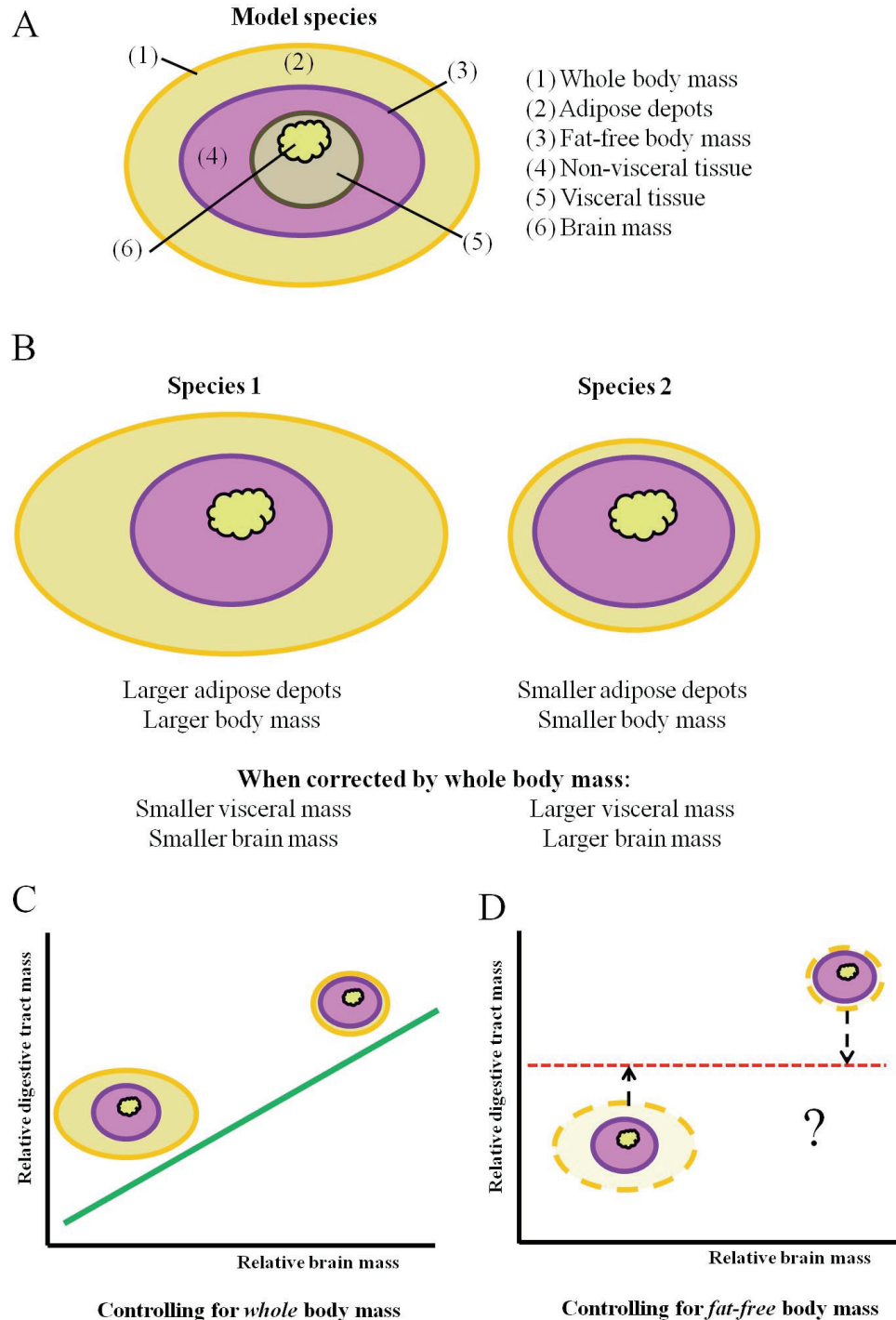
Thoracic cavity: The diaphragm was cut open following the inner rib cage line. Trachea and esophagus were cut above the sternum, and connective tissue was loosened to allow the thoracic organs to be pulled out distally. The heart was separated, opened and the chambers were cleaned of blood coagulations by rinsing. The lungs were separated by cutting the bronchi. The thoracic organs were weighed.

Adipose depots: During dissection, visible subcutaneous and intermuscular accumulations of adipose depots were continuously removed and put in a separate container. Adipose depots were removed from the walls of the abdominal and thoracic cavities, and from between the visceral organs. The total adipose depots were weighed. For 45 species in our sample, adipose depots of the whole body were measured directly. For the other 55 species, only abdominal adipose depots were measured, from which we calculated a proxy of total adipose depots by scaling the abdominal depots mass with a factor 3.419. This scaling factor was derived from a comparison of the two measurements for all individuals for which body mass and one of the two adipose depots measurements was available (Suppl. Figure 1). Fat-free body mass was calculated as whole body mass minus total adipose depots mass. The rationale on the usage of fat-free body mass to control for differences in body size between species is illustrated in Suppl. Figure 2.

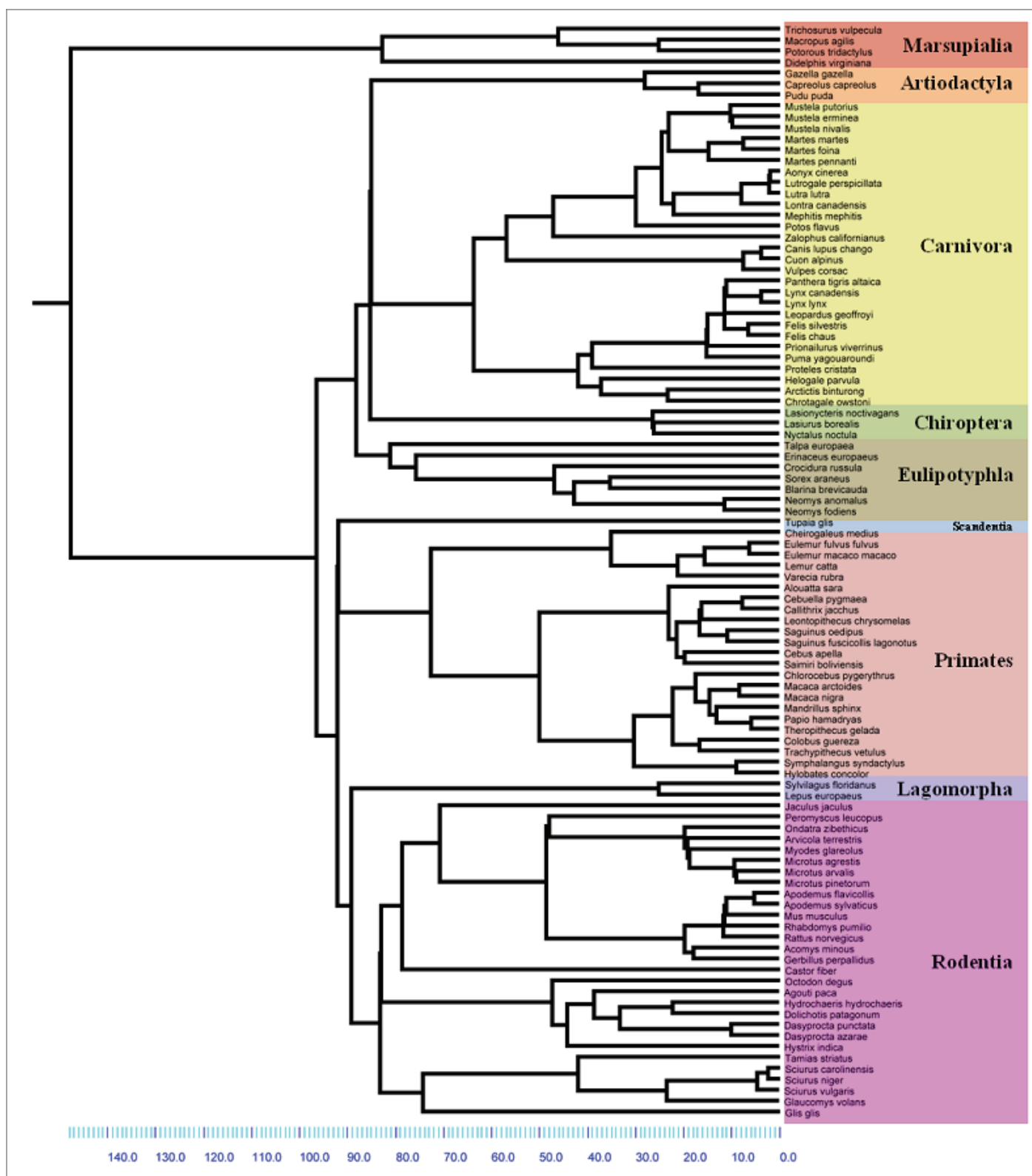
Skeletal measures: Some weeks to months later, the specimens were skeletonized (but not degreased), and the bones were dried and weighed. The cranial capacity was determined using the seed filling method³², and converted into an estimate of brain mass by multiplying the volume with 1.036³³.



Supplementary Figure 1: Regression of adipose depots mass on body mass for 292 specimens. The slopes of the least-squares regression of total adipose depots (blue symbols) vs. body mass ($\ln(\text{total fat})$): $-3.233 + 1.017 \ln(\text{body mass})$, $N=188$) and the least-squares regression of abdominal adipose depots (red symbols) vs. body mass ($\ln(\text{abdominal fat})$): $-5.103 + 1.106 \ln(\text{body mass})$, $N=104$) did not differ significantly from each other. Thus, a common slope of 1.0401 was fitted to both subsamples ($\ln(\text{abdominal fat})$): $-4.578 + 1.0401 \ln(\text{body mass})$, $N=104$; $\ln(\text{total fat})$): $-3.348 + 1.0401 \ln(\text{body mass})$, $N=188$), and the scaling factor was derived from the intercept difference, $1/\exp(-4.5775 + 3.3482) = 3.419$. Using fat-free body mass would yield a very similar correction factor of 3.46. An alternative method is described in Section 3.3.



Supplementary Figure 2: The rationale for controlling for body size by using fat-free body mass. A species is depicted by its body composition (A). Two species may have the same fat-free body mass, but different amounts of adipose depots (B). Controlling for whole body mass yields positive correlations between organ masses (C). Only controlling for fat-free body mass allows us to detect a possible trade-off between the sizes of different organs (D).



Supplementary Figure 3: Phylogenetic tree of the 100 mammal species in our sample, mainly from Bininda-Emonds et al.⁴¹. Distances are given in million years before present. As the phylogenetic generalized least-squares method (PGLS) requires a completely resolved

phylogeny, polytomies were resolved using various sources. Within Chiroptera, *Lasiurus* and *Nyctalus* are more closely related to each other than to *Lasionycteris* according to Volleth and Heller⁴². Within Carnivora, polytomies in the Lutrinae group were resolved using Koepfli et al.⁴³. Within Rodentia, the phylogeny of Cricetidae follows Robovský et al.⁴⁴, the one of Muridae follows Lecompte et al.⁴⁵, and the distance between Cricetidae and Muridae was taken from Michaux et al.⁴⁶. Within Caviidae, distances were taken from Rowe and Honeycutt⁴⁷ and Veniaminova et al.⁴⁸. Distances within Primates were modified following Arnold et al.³⁸.

Supplementary Information 3: Supplementary results and discussion

3.1. Extended results, including the N=45 species subsample

This section contains the correlation matrix for all organs used for Figure 2 in the main text (Suppl. Table 3), full statistics for the results shown in Table 1 in the main text (Suppl. Table 4), and additional results for the 45-species sample with known total adipose depots mass (Suppl. Table 5).

Supplementary Table 3: Pair-wise correlation matrix including brain, visceral organs, and adipose depots mass. All correlations are controlled for fat-free body mass and calculated by PGLS.

	Heart	Lungs	Kidneys	Liver	Digestive tract	<i>Stomach</i>	<i>Intestines</i>	Spleen	Adipose depots
Brain	$\beta = 0.15$ $P = 0.13$	$\beta = -0.03$ $P = 0.73$	$\beta = 0.01$ $P = 0.92$	$\beta = -0.02$ $P = 0.85$	$\beta = 0.16$ $P = 0.06$	$\beta = 0.15$ $P = 0.042$	$\beta = 0.11$ $P = 0.15$	$\beta = -0.02$ $P = 0.60$	$\beta = -0.07$ $P = 0.017$
Heart		$\beta = 0.63$ $P < 0.0001$	$\beta = 0.15$ $P = 0.13$	$\beta = 0.18$ $P = 0.035$	$\beta = 0.16$ $P = 0.09$	$\beta = 0.08$ $P = 0.27$	$\beta = 0.12$ $P = 0.13$	$\beta = 0.05$ $P = 0.15$	$\beta = -0.03$ $P = 0.32$
Lungs			$\beta = 0.27$ $P = 0.004$	$\beta = 0.32$ $P < 0.0001$	$\beta = 0.18$ $P = 0.042$	$\beta = 0.06$ $P = 0.36$	$\beta = 0.14$ $P = 0.07$	$\beta = 0.13$ $P < 0.0001$	$\beta = -0.003$ $P = 0.93$
Kidneys				$\beta = 0.45$ $P < 0.0001$	$\beta = 0.29$ $P = 0.002$	$\beta = 0.06$ $P = 0.40$	$\beta = 0.28$ $P = 0.0002$	$\beta = 0.11$ $P = 0.003$	$\beta = 0.005$ $P = 0.88$
Liver					$\beta = 0.30$ $P = 0.005$	$\beta = 0.06$ $P = 0.43$	$\beta = 0.26$ $P = 0.004$	$\beta = 0.15$ $P < 0.0001$	$\beta = -0.01$ $P = 0.74$
Digestive tract						$\beta = 0.53$ $P < 0.0001$	$\beta = 0.81$ $P < 0.0001$	$\beta = 0.05$ $P = 0.19$	$\beta = -0.07$ $P = 0.07$
<i>Stomach</i>							$\beta = 0.39$ $P < 0.0001$	$\beta = 0.04$ $P = 0.37$	$\beta = -0.03$ $P = 0.55$
<i>Intestine</i>								$\beta = 0.04$ $P = 0.39$	$\beta = -0.11$ $P = 0.013$
Spleen									$\beta = 0.11$ $P = 0.24$

Supplementary Table 4: Pair-wise phylogenetic least-squares regression (PGLS) between brain and other organ masses (N = 100 species), controlling for a) fat-free body mass, and b) whole body mass.

a)	Including <i>fat-free body mass</i> as covariate											
	PGLS						Independent contrasts (λ = 1)			Raw data (λ = 0)		
Organ	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	β	t-value	p-value	β	t-value	p-value
Mammals	N=100											
Heart	0.918	<0.0001	0.001	0.15	1.51	0.13						
Lungs	0.928	<0.0001	0.010	-0.03	-0.35	0.73						
Kidneys	0.921	<0.0001	0.004	0.01	0.10	0.92						
Liver	0.923	<0.0001	0.005	-0.02	-0.19	0.84						
Digestive tract	0.943	<0.0001	0.007	0.16	1.89	0.06						
<i>Stomach</i>	0.934	<0.0001	0.005	0.15	2.06	0.042						
<i>Intestine</i>	0.940	<0.0001	0.007	0.11	1.45	0.15						
Spleen	0.929	<0.0001	0.008	-0.02	-0.53	0.60						
Visceral organs	0.922	<0.0001	0.0020	0.05	0.46	0.64						
Adipose depots	0.938	<0.0001	0.029	-0.07	-2.42	0.017						
Primates	N=23											
Heart	0.347	1.00	0.033	0.65	2.99	0.007						
Lungs	0.703	0.13	0.25	0.44	1.93	0.07	0.44	1.97	0.06	0.51	1.97	0.06
Kidneys	0.717	0.05	0.69	0.34	1.86	0.08	0.24	1.27	0.22	0.31	1.46	0.16
Liver	0.664	0.18	0.16	0.20	1.06	0.30	0.14	0.73	0.48	0.27	1.29	0.21
Digestive tract	1.000	0.10	1.00	0.48	3.14	0.005				0.5	2.79	0.011
<i>Stomach</i>	0.857	0.07	0.57	0.17	1.35	0.19	0.22	1.71	0.10	0.14	1.03	0.31
<i>Intestine</i>	1.000	0.10	1.00	0.41	2.95	0.008				0.41	2.57	0.018
Spleen	0.838	0.036	0.29	0.15	1.56	0.13						
Visceral organs	0.663	0.17	0.31	0.50	2.35	0.029	0.51	2.43	0.025	0.57	2.46	0.023
Adipose depots	0.793	0.29	0.23	-0.01	-0.10	0.92	-0.02	-0.26	0.80	-0.09	-1.11	0.28
Carnivora	N=28											
Heart	0.000	1.00	0.0001	-0.02	-0.12	0.91						
Lungs	0.000	1.00	0.003	-0.16	-1.05	0.31						
Kidneys	0.000	1.00	0.001	0.050	0.33	0.74						
Liver	0.000	1.00	0.0003	-0.01	-0.08	0.93						
Digestive tract	0.000	1.00	0.0004	0.16	0.86	0.40						
<i>Stomach</i>	0.000	1.00	0.0002	0.08	0.55	0.59						
<i>Intestine</i>	0.000	1.00	0.0004	0.15	0.90	0.38						
Spleen	0.000	1.00	0.001	-0.03	-0.52	0.61						
Visceral organs	0.000	1.00	0.0003	-0.09	-0.41	0.69						
Adipose depots	0.000	1.00	<0.0001	-0.04	-0.90	0.38						
Rodentia	N=29											
Heart	0.762	0.0004	0.001	0.23	1.90	0.07						
Lungs	0.788	0.0002	0.003	-0.02	-0.16	0.87						
Kidneys	0.776	0.0004	0.001	-0.13	-0.78	0.44						
Liver	0.784	0.0002	0.002	-0.10	-0.70	0.49						
Digestive tract	0.774	0.0038	0.0036	-0.03	-0.18	0.86						
<i>Stomach</i>	0.805	0.0007	0.014	0.05	0.34	0.74						
<i>Intestine</i>	0.769	0.003	0.002	-0.04	-0.29	0.78						
Spleen	0.825	<0.0001	0.008	-0.08	-1.43	0.16						
Visceral organs	0.773	0.0007	0.001	-0.10	-0.48	0.63						
Adipose depots	0.821	<0.0001	0.010	-0.08	-1.97	0.06						

Supplementary Table 4 continued:

b)	Including <i>whole body mass</i> as covariate											
Organ	PGLS						Independent contrasts ($\lambda = 1$)			Raw data ($\lambda = 0$)		
	λ	P ($\lambda=0$)	P ($\lambda=1$)	β	t-value	p-value	β	t-value	p-value	β	t-value	p-value
Mammals	N=100											
Heart	0.905	<0.0001	0.0002	0.22	2.32	0.022						
Lungs	0.902	<0.0001	0.0005	0.07	0.67	0.50						
Kidneys	0.901	<0.0001	0.0003	0.09	0.87	0.38						
Liver	0.907	<0.0001	0.0005	0.04	0.47	0.64						
Digestive tract	0.940	<0.0001	0.003	0.22	2.70	0.008						
<i>Stomach</i>	0.928	<0.0001	0.002	0.19	2.59	0.011						
<i>Intestine</i>	0.938	<0.0001	0.003	0.16	2.31	0.023						
Spleen	0.912	<0.0001	0.0010	-0.01	-0.29	0.77						
Visceral organs	0.909	<0.0001	0.0002	0.16	1.42	0.16						
Adipose depots	0.932	<0.0001	0.024	-0.12	-4.00	0.0001						
Primates	N=23											
Heart	0.445	0.74	0.036	0.63	3.18	0.005						
Lungs	0.759	0.10	0.28	0.45	2.01	0.06	0.60	2.31	0.032	0.47	2.16	0.043
Kidneys	0.756	0.028	0.070	0.34	1.75	0.10						
Liver	0.737	0.09	0.14	0.21	1.12	0.28	0.17	0.91	0.38	0.33	1.52	0.14
Digestive tract	1.000	0.07	1.00	0.49	3.30	0.004				0.55	3.06	0.006
<i>Stomach</i>	0.887	0.033	0.53	0.17	1.28	0.21						
<i>Intestine</i>	1.000	0.06	1.00	0.42	3.22	0.004				0.45	2.92	0.009
Spleen	0.775	0.009	0.36	0.18	1.95	0.07						
Visceral organs	0.734	0.12	0.34	0.51	2.44	0.024	0.53	2.62	0.016	0.63	2.77	0.012
Adipose depots	0.781	0.26	0.21	-0.07	-0.91	0.37	-0.06	-1.01	0.32	-0.15	-1.87	0.08
Carnivora	N=28											
Heart	0.000	1.00	0.0001	0.06	0.41	0.69						
Lungs	0.000	1.00	0.001	-0.03	-0.17	0.86						
Kidneys	0.000	1.00	0.0002	0.14	0.91	0.37						
Liver	0.000	1.00	0.0002	0.07	0.63	0.53						
Digestive tract	0.000	1.00	0.0006	0.28	1.58	0.13						
<i>Stomach</i>	0.000	1.00	0.0004	0.15	0.96	0.35						
<i>Intestine</i>	0.000	1.00	0.0006	0.26	1.66	0.11						
Spleen	0.000	1.00	0.003	-0.03	-0.55	0.59						
Visceral organs	0.000	1.00	0.0001	0.13	0.63	0.53						
Adipose depots	0.000	1.00	<0.0001	-0.11	-2.09	0.047						
Rodentia	N=29											
Heart	0.732	0.001	0.0008	0.28	2.31	0.029						
Lungs	0.754	0.0006	0.0006	0.05	0.35	0.73						
Kidneys	0.754	0.0009	0.0006	-0.03	-0.17	0.86						
Liver	0.755	0.0007	0.0008	-0.07	-0.43	0.66						
Digestive tract	0.792	0.002	0.005	0.08	0.51	0.61						
<i>Stomach</i>	0.809	0.001	0.026	0.11	0.74	0.46						
<i>Intestine</i>	0.779	0.002	0.002	0.05	0.35	0.73						
Spleen	0.804	0.0001	0.003	-0.08	-1.37	0.18						
Visceral organs	0.762	0.0009	0.0006	0.03	0.12	0.90						
Adipose depots	0.800	<0.0001	0.007	-0.11	-2.67	0.013						

Supplementary Table 5: Pair-wise phylogenetic least-squares regression (PGLS) between brain and other organ masses (N = 45 species), controlling for a) fat-free body mass, and b) whole body mass.

a)	Including <i>fat-free body mass</i> as covariate					
Organ	λ	P ($\lambda=0$)	P ($\lambda=1$)	β	t-value	p-value
Mammals	N=45					
Heart	0.933	<0.0001	0.05	0.18	1.06	0.29
Lungs	0.918	<0.0001	0.024	0.003	0.02	0.99
Kidneys	0.917	<0.0001	0.02	0.05	0.30	0.77
Liver	0.919	<0.0001	0.019	0.05	0.37	0.71
Digestive tract	0.933	<0.0001	0.08	0.11	0.74	0.46
<i>Stomach</i>	0.936	<0.0001	0.10	0.16	0.99	0.33
<i>Intestine</i>	0.928	<0.0001	0.049	0.07	0.53	0.60
Spleen	0.933	<0.0001	0.06	-0.04	-0.92	0.36
Visceral organs	0.924	<0.0001	0.024	0.12	0.58	0.57
Adipose depots	0.940	<0.0001	0.14	-0.13	-3.52	0.001
Non-Rodentia	N=21					
Heart	0.987	0.001	0.86	0.04	0.14	0.89
Lungs	0.999	0.001	0.99	0.14	0.55	0.59
Kidneys	1.000	0.0006	1.00	0.32	1.12	0.28
Liver	1.000	0.0005	1.00	0.20	1.33	0.20
Digestive tract	0.981	0.002	0.80	-0.02	-0.09	0.93
<i>Stomach</i>	0.981	0.002	0.81	0.01	0.06	0.96
<i>Intestine</i>	0.981	0.002	0.80	-0.04	-0.14	0.89
Spleen	0.981	0.002	0.80	0.009	0.15	0.88
Visceral organs	1.000	0.0008	1.00	0.35	1.18	0.25
Adipose depots	0.917	0.0004	0.30	-0.18	-2.66	0.016
Rodentia	N=24					
Heart	0.646	0.07	0.001	0.53	2.65	0.015
Lungs	0.762	0.002	0.004	0.01	0.07	0.94
Kidneys	0.755	0.003	0.002	-0.12	-0.62	0.54
Liver	0.764	0.002	0.004	-0.11	-0.63	0.53
Digestive tract	0.799	0.006	0.023	0.08	0.43	0.67
<i>Stomach</i>	0.816	0.004	0.049	0.13	0.65	0.52
<i>Intestine</i>	0.783	0.005	0.01	0.04	0.25	0.80
Spleen	0.826	0.0003	0.023	-0.09	-1.47	0.16
Visceral organs	0.758	0.003	0.002	-0.05	-0.20	0.84
Adipose depots	0.829	0.0002	0.031	-0.09	-2.15	0.043

Supplementary Table 5 continued:

b) Organ	Including <i>whole body mass</i> as covariate					
	λ	P ($\lambda=0$)	P ($\lambda=1$)	β	t-value	p-value
Mammals	N=45					
Heart	0.942	<0.0001	0.09	0.33	1.98	0.054
Lungs	0.910	<0.0001	0.009	0.15	0.89	0.38
Kidneys	0.906	<0.0001	0.015	0.20	1.17	0.25
Liver	0.912	<0.0001	0.01	0.12	0.93	0.36
Digestive tract	0.945	<0.0001	0.14	0.25	1.72	0.09
<i>Stomach</i>	0.949	<0.0001	0.22	0.32	2	0.05
<i>Intestine</i>	0.935	<0.0001	0.07	0.17	1.36	0.18
Spleen	0.923	<0.0001	0.035	-0.04	-0.77	0.45
Visceral organs	0.928	<0.0001	0.022	0.33	1.64	0.11
Adipose depots	0.929	<0.0001	0.11	-0.18	-4.46	<0.0001
Non-Rodentia	N=21					
Heart	1.000	0.002	1.00	0.25	0.94	0.36
Lungs	1.000	0.001	1.00	0.34	1.26	0.22
Kidneys	1.000	0.0006	1.00	0.53	1.77	0.09
Liver	1.000	0.0006	1.00	0.26	1.70	0.11
Digestive tract	0.993	0.003	0.94	0.16	0.6	0.56
<i>Stomach</i>	0.990	0.003	0.91	0.21	0.83	0.41
<i>Intestine</i>	0.991	0.003	0.92	0.10	0.44	0.66
Spleen	0.991	0.003	0.92	0.02	0.26	0.80
Visceral organs	1.000	0.0004	1.00	0.56	1.95	0.07
Adipose depots	0.891	0.0006	0.19	-0.27	-3.68	0.002
Rodentia	N=24					
Heart	0.604	0.11	0.001	0.60	3.14	0.005
Lungs	0.712	0.007	0.001	0.12	0.59	0.56
Kidneys	0.727	0.005	0.001	-0.008	-0.04	0.97
Liver	0.725	0.005	0.002	-0.06	-0.30	0.76
Digestive tract	0.825	0.004	0.036	0.20	1.14	0.27
<i>Stomach</i>	0.845	0.004	0.12	0.27	1.26	0.22
<i>Intestine</i>	0.796	0.004	0.012	0.13	0.86	0.40
Spleen	0.800	0.001	0.011	-0.10	-1.45	0.16
Visceral organs	0.746	0.004	0.002	0.10	0.41	0.69
Adipose depots	0.810	0.0004	0.023	0.80	13.65	<0.0001

3.2. Dataset composition

This section contains a description of the composition of the dataset (Suppl. Table 6), and additional results of a procedure to control for a potential bias in the sample (Suppl. Table 7).

The composition of our dataset in relation to the total existing numbers of mammal species and families is shown in Suppl. Table 6. Although our dataset covers less than 2% of all mammal species, about a quarter of all mammal families are represented in our sample. However, Carnivora and Primates are overrepresented at the species level in our sample, whereas many small orders are not covered.

Supplementary Table 6: Composition of the organ mass sample. Numbers of mammalian species are taken from Wilson and Reeder⁴⁹.

	Species			Families		
	All	in our sample	coverage [%]	All	in our sample	coverage [%]
Marsupialia						
Didelphimorphia	87	1	1.1	1	1	100.0
Diprotodontia	143	3	2.1	11	3	27.3
other marsupials	101	0	0	9	0	0
Monotremata	5	0	0	2	0	0
Placentalia						
Artiodactyla	240	3	1.3	10	3	30.0
Carnivora	286	28	9.8	15	9	60.0
Chiroptera	1116	3	0.3	18	1	5.6
Erinaceomorpha	24	1	4.2	1	1	100.0
Lagomorpha	92	2	2.2	3	1	33.3
Primates	376	23	6.1	15	8	53.3
Rodentia	2277	29	1.3	33	11	33.3
Scandentia	20	1	5.0	2	1	50.0
Soricomorpha	428	6	1.4	4	2	50.0
other mammals	221	0	0	29	0	0
Total	5195	100	1.9	124	30	24.2

While phylogenetic methods are quite suitable to accommodate grade shifts in the data, an imbalance in the sample in combination with a different relationship within a specious taxon from that in other taxa, may still affect the results. One option to control for a possible bias is to look at large groups separately, which we did in Suppl. Tables 4 and 5. In general, the correlations are retained within the large orders, with the exception of primates (see Section 3.6).

Another option is to resample a better-balanced subset of the data, accepting the reduced sample size. We did this by selecting one species from each subfamily according to data quality (sample size, wild > captive, females > males, total adipose depots measured). Our final sample included 51 species. The significantly negative correlation between brain size and adipose depots mass persists in this subsample, although the p-value is slightly higher (Suppl. Table 7).

Supplementary Table 7: Pair-wise phylogenetic least-squares regression (PGLS) between brain and other organ masses (N = 51 species, one per subfamily), controlling for fat-free body mass.

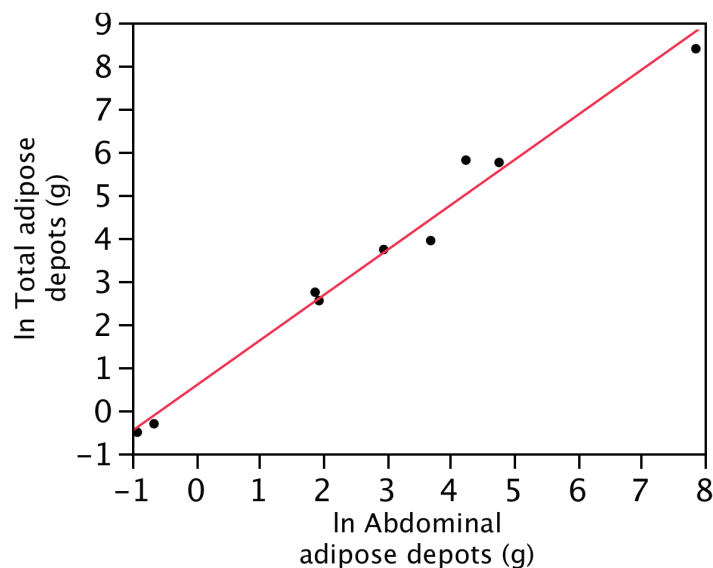
Organ	λ	P ($\lambda=0$)	P ($\lambda=1$)	β	t-value	p-value
Heart	0.936	<0.0001	0.46	0.08	0.59	0.56
Lungs	0.929	<0.0001	0.42	0.02	0.13	0.90
Kidneys	0.959	<0.0001	0.56	0.25	1.69	0.10
Liver	0.943	<0.0001	0.51	0.07	0.56	0.58
Digestive tract	0.929	<0.0001	0.42	0.01	0.06	0.95
<i>Stomach</i>	0.933	<0.0001	0.45	-0.05	-0.42	0.68
<i>Intestine</i>	0.941	<0.0001	0.51	0.05	0.44	0.66
Spleen	0.920	<0.0001	0.38	-0.03	-0.42	0.68
Visceral organs	0.941	<0.0001	0.51	0.08	0.42	0.68
Adipose depots	0.904	<0.0001	0.24	-0.10	-2.06	0.045

3.3. An alternative method to estimate total adipose depots mass

This section contains additional results on the relationship between brain size and adipose depots mass using an alternative method to estimate total adipose depots mass from abdominal depots mass (Suppl. Figure 4 and Suppl. Table 8).

As the variation in abdominal and total mass of adipose depots is rather large (cf. Suppl. Figure 1), we applied an alternative method to scale abdominal adipose depots to total adipose depots using the correlation between the two variables in 9 specimens for which we have both measurements (*Rattus norvegicus*, two *Mus musculus*, *Glis glis*, *Martes foina*, *Mephitis mephitis*, *Vulpes vulpes*, *Saimiri sciureus* and *Macaca fuscata*). A least-squares regression (Suppl. Figure 4) yields the following prediction equation:

$$\ln(\text{total adipose depots}) = 0.564 + 1.047 * \ln(\text{abdominal adipose depots})$$



Supplementary Figure 4: Regression of abdominal adipose depots vs. total adipose depots (N=9, $r^2=0.982$, $P<0.0001$).

From this, total adipose depots mass was calculated for all specimens for which only abdominal adipose depots mass was available. The resulting negative correlations between brain size and adipose depots mass are slightly stronger in all groups, most notably in all mammals combined (Suppl. Table 8). This result suggests that the found brain size – adipose depot trade-off is robust and does not rely on the details of the method used to scale the abdominal to total adipose depots mass.

Supplementary Table 8: Pair-wise phylogenetic least-squares regression (PGLS) between brain size and adipose depots mass, controlling for fat-free body mass, using an alternative method to calculate total adipose depots mass. Original results are duplicated from Suppl. Table 4a).

		N	λ	P ($\lambda=0$)	P ($\lambda=1$)	β	t-value	p-value
All mammals	Original	100	0.938	<0.0001	0.029	-0.07	-2.42	0.017
	Alternative	100	0.927	<0.0001	0.003	-0.09	-2.07	0.002
Primates	Original	23	0.793	0.29	0.23	-0.01	-0.10	0.92
	Alternative	23	0.787	0.29	0.22	-0.03	-0.41	0.69
Carnivora	Original	28	0.000	1.00	<0.0001	-0.04	-0.90	0.38
	Alternative	28	0.000	1.00	0.001	-0.07	-1.48	0.15
Rodentia	Original	29	0.821	<0.0001	0.010	-0.08	-1.97	0.06
	Alternative	29	0.822	<0.0001	0.010	-0.08	-2.02	0.054

3.4. Reducing error variation by splitting the sample into wild/captive, and male/female subsamples

This section contains additional results produced when the dataset was split into subsamples in an attempt to reduce error variation in the relationship between brain size and adipose depots mass (Suppl. Table 9).

Our organ mass sample contains specimens from a variety of living conditions, of both sexes, and from various habitats (cf. Suppl. Data), which may induce a large amount of error variation. By splitting our sample into subsets according to these groups, we can investigate whether the negative correlation between brain size and adipose depots mass is robust. We expect that the trade-off between fat storage and brain size is more pronounced in females than in males, because the former are more affected by energy constraints due to offspring production. As captivity effects are rather unpredictable (most animals gain weight in captivity, but only under good husbandry conditions), we expect that the trade-off is more pronounced in wild-caught specimens, although it may be argued that they are also more affected by seasonality effects on fat storage. Finally, storing fat may only be beneficial for survival if food availability in the habitat is seasonally variable. We therefore expect the trade-off to be more pronounced in species originating from temperate rather than from tropical habitats.

Results, shown in Suppl. Table 9, fully confirm these predictions. A significant negative correlation between brain size and adipose depots mass, controlling for fat-free body mass, is found in females, in wild-caught specimens, and in species of temperate origin, and is most pronounced in the subset of wild females. This result suggests that reducing error variation and

controlling for potentially confounding variables strengthens the evidence for a brain size-adipose depot trade-off.

Supplementary Table 9: Pair-wise phylogenetic least-squares regression (PGLS) between brain size and adipose depots mass split by habitat (temperate, tropical, both), sex (male, female), and provenience (captive, wild, no data), controlling for fat-free body mass. a) N=100 species, b) N=45 species.

a)

Group	N	λ	P ($\lambda=0$)	P ($\lambda=1$)	β	t-value	p-value
All specimens	100	0.938	<0.0001	0.029	-0.07	-2.42	0.017
Temperate	52	0.933	<0.0001	0.12	-0.08	-2.07	0.043
Tropical	43	0.934	<0.0001	0.23	-0.01	-0.24	0.81
Females	57	0.934	<0.0001	0.006	-0.09	-2.45	0.017
Males	69	0.905	<0.0001	0.006	-0.06	-1.68	0.10
Wild	39	0.964	<0.0001	0.39	-0.12	-2.74	0.010
Captive	59	0.823	<0.0001	0.019	-0.03	-0.77	0.44
Wild females	28	1.000	0.0004	1.00	-0.12	-3.42	0.002

b)

	N	λ	P ($\lambda=0$)	P ($\lambda=1$)	β	t-value	p-value
All specimens	45	0.940	<0.0001	0.14	-0.13	-3.52	0.001
Temperate	39	0.951	<0.0001	0.24	-0.1	-2.37	0.023
Tropical	4						
Females	30	1.000	<0.0001	1.00	-0.12	-4.27	0.0002
Males	33	0.876	0.0002	0.006	-0.12	-2.13	0.041
Wild	35	0.951	<0.0001	0.31	-0.14	-2.78	0.009
Captive	8	0.000	1.00	0.019	0.02	0.26	0.81
Wild females	25	1.000	0.0004	1.00	-0.13	-3.68	0.0013

3.5. Potential autocorrelation effects and compositional data analysis

This section contains results of an alternative procedure to control for the effect of body size (Suppl. Tables 10 and 11), and the results of compositional data analysis for the relationship between adipose depots mass and brain size (Suppl. Table 12).

A potential problem in the analysis of body composition data is the presence of autocorrelation effects, as the organs are part of the body mass which is used to control for size effects⁵⁰. These effects are most pronounced when the parts comprise a large proportion of the whole body, but this is not the case in most of the correlations between brain size and one of the visceral organs in our sample. Furthermore, autocorrelation assumes that the total size of a sum of variables is strictly limited and thus constant. In the case of body mass, this assumption is overly conservative, because adding the size of some organ does not need to constrain the size of other organs. Nevertheless, to validate our results we applied two procedures to control for potential autocorrelation effects.

First, if we adjust our estimate of body size by controlling for *fat-free body mass minus brain mass and the respective organ mass* instead of just fat-free body mass, p-values remain largely similar, and in no case is the level of significance affected (Suppl. Table 10). The most critical result is the negative correlation between brain size and adipose depots mass. We checked for autocorrelation effects by controlling for fat-free body mass minus brain mass (Suppl. Table 11, analogous to the procedure for visceral organs shown in Suppl. Table 10). Results remain unchanged in the N=100 species sample, but significance is lost in the subsamples of the N=45 species sample.

Second, we applied compositional data analysis to control for potential autocorrelation effects in the brain size vs. adipose depots mass correlation (Suppl. Table 12). This method was developed for mathematical geology⁵¹, and it has been applied to intraspecific body composition data before⁵². It takes into account that n parts of a whole are by necessity autocorrelated and transforms the values by projecting them onto a simplex of $n-1$ dimensions. We used the function *acom* for closed compositions in a logistic geometry, following a log-ratio approach and the isometric log-ratio transform *ilr*⁵³ from the package **compositions**⁵⁴ in R³⁵. Thus, from the raw values of brain mass, adipose depots mass and the remaining body mass without brain and adipose depots, we obtained two transformed variables, which are no longer autocorrelated. We then tested their correlation with phylogenetic regression using *pglmEstLambda* from the package **CAIC** in R. Results yield very strong negative correlations in all subsamples.

Supplementary Table 10: Pair-wise phylogenetic least-squares regression (PGLS) between brain size and the mass of visceral organs, controlling for fat-free body mass minus brain mass and the respective organ mass. a) N=100 species, b) N=45 species.

a)	Including <i>fat-free body mass minus brain mass and organ mass</i> as covariate											
Organ	PGLS						Independent contrasts (λ = 1)			Raw data (λ = 0)		
	λ	P (λ=0)	P (λ=1)	β	t-value	p-value	β	t-value	p-value	β	t-value	p-value
Mammals	N=100											
Heart	0.918	<0.0001	0.001	0.16	1.70	0.09						
Lungs	0.926	<0.0001	0.009	-0.01	-0.08	0.93						
Kidneys	0.921	<0.0001	0.004	0.03	0.30	0.77						
Liver	0.923	<0.0001	0.004	0.02	0.30	0.77						
Digestive tract	0.945	<0.0001	0.008	0.19	2.28	0.025						
<i>Stomach</i>	0.936	<0.0001	0.006	0.16	2.27	0.026						
<i>Intestine</i>	0.942	<0.0001	0.007	0.13	1.77	0.08						
Spleen	0.929	<0.0001	0.008	-0.02	-0.44	0.66						
Visc. organs	0.923	<0.0001	0.002	0.14	1.35	0.18						
Primates	N=23											
Heart	0.325	1.00	0.032	0.68	3.12	0.005						
Lungs	0.702	0.13	0.28	0.47	2.07	0.05	0.46	2.09	0.050	0.54	2.12	0.047
Kidneys	0.719	0.0495	0.07	0.35	1.94	0.07						
Liver	0.670	0.17	0.17	0.24	1.27	0.22	0.17	0.91	0.38	0.31	1.50	0.15
Digestive tract	1.000	0.10	1.00	0.49	3.32	0.003				0.53	3.01	0.007
<i>Stomach</i>	0.882	0.07	0.66	0.18	1.47	0.16	0.23	1.80	0.09	0.15	1.14	0.27
<i>Intestine</i>	1.000	0.09	1.00	0.33	2.59	0.018				0.44	2.76	0.012
Spleen	0.845	0.034	0.31	0.16	1.61	0.12						
Visc. organs	0.674	0.16	0.35	0.54	2.80	0.011	0.54	2.79	0.011	0.61	2.91	0.009
Carnivora	N=28											
Heart	0.000	1.00	0.0005	0.01	0.04	0.96						
Lungs	0.000	1.00	0.014	-0.11	-0.76	0.45						
Kidneys	0.000	1.00	0.007	0.080	0.55	0.59						
Liver	0.000	1.00	0.0010	0.03	0.27	0.79						
Digestive tract	0.000	1.00	0.0007	0.19	1.00	0.33						
<i>Stomach</i>	0.000	1.00	0.0009	0.09	0.58	0.57						
<i>Intestine</i>	0.000	1.00	0.0006	0.17	1.02	0.32						
Spleen	0.000	1.00	0.013	-0.02	-0.44	0.67						
Visc. organs	0.000	1.00	0.002	0.04	0.20	0.85						
Rodentia	N=29											
Heart	0.760	0.0005	0.001	0.24	2.00	0.06						
Lungs	0.786	0.0002	0.003	-0.01	-0.03	0.98						
Kidneys	0.775	0.0005	0.001	-0.12	-0.66	0.51						
Liver	0.783	0.0002	0.002	-0.06	-0.44	0.66						
Digestive tract	0.779	0.0030	0.004	-0.02	0.11	0.91						
<i>Stomach</i>	0.808	0.0006	0.016	0.07	0.45	0.66						
<i>Intestine</i>	0.773	0.002	0.002	-0.01	-0.04	0.97						
Spleen	0.824	<0.0001	0.007	-0.08	-1.39	0.18						
Visc. organs	0.774	0.0006	0.001	0.01	0.05	0.96						

Supplementary Table 10 continued:

b)		Including <i>fat-free body mass minus brain mass and organ mass</i> as covariate				
Organ	λ	P ($\lambda=0$)	P ($\lambda=1$)	β	t-value	p-value
Mammals	N=45					
Heart	0.945	<0.0001	0.10	0.20	1.23	0.22
Lungs	0.930	<0.0001	0.05	0.01	0.07	0.94
Kidneys	0.930	<0.0001	0.043	0.04	0.22	0.83
Liver	0.930	<0.0001	0.033	0.09	0.82	0.41
Digestive tract	0.936	<0.0001	0.08	0.07	0.45	0.66
<i>Stomach</i>	0.949	<0.0001	0.20	0.16	0.99	0.33
<i>Intestine</i>	0.932	<0.0001	0.05	0.03	0.19	0.85
Spleen	0.939	<0.0001	0.08	-0.03	-0.62	0.54
Visceral organs	0.934	<0.0001	0.041	0.17	0.98	0.33
Non-Rodentia	N=24					
Heart	0.693	0.027	0.002	0.49	2.54	0.019
Lungs	0.781	0.002	0.009	0.01	0.50	0.96
Kidneys	0.768	0.002	0.003	-0.14	-0.72	0.48
Liver	0.78	0.001	0.007	-0.08	-0.49	0.63
Digestive tract	0.764	0.016	0.013	-0.01	-0.05	0.96
<i>Stomach</i>	0.824	0.005	0.10	0.11	0.53	0.60
<i>Intestine</i>	0.757	0.01	0.007	-0.03	-0.19	0.86
Spleen	0.827	0.0003	0.021	-0.08	-1.15	0.26
Visceral organs	0.768	0.003	0.004	-0.02	-0.10	0.92
Rodentia	N=21					
Heart	0.984	0.001	0.85	0.08	0.30	0.77
Lungs	0.995	0.001	0.95	0.15	0.57	0.57
Kidneys	1.000	0.0009	0.99	0.26	0.92	0.37
Liver	1.000	0.0004	1.00	0.25	1.77	0.09
Digestive tract	0.971	0.002	0.71	-0.03	-0.13	0.90
<i>Stomach</i>	0.974	0.001	0.74	0.002	0.006	0.99
<i>Intestine</i>	0.971	0.002	0.71	-0.04	-0.19	0.85
Spleen	0.972	0.001	0.72	0.02	0.26	0.80
Visceral organs	1.000	0.0008	1.00	0.40	1.61	0.12

Supplementary Table 11: Pair-wise phylogenetic least-squares regression (PGLS) between brain size and adipose depots mass, controlling for fat-free body mass minus brain mass. a) N=100 species, b) N=45 species.

a)		PGLS						Independent contrasts ($\lambda = 1$)			Raw data ($\lambda = 0$)		
Group	N	λ	P ($\lambda=0$)	P ($\lambda=1$)	β	t-value	p-value	β	t-value	p-value	β	t-value	p-value
Mammals	100	0.939	<0.0001	0.03	-0.07	-2.39	0.019						
Primates	23	0.805	0.28	0.25	-0.01	-0.07	0.95	-0.01	-0.24	0.81	-0.09	-1.09	0.29
Carnivora	28	0.000	1.00	0.011	-0.05	-1.02	0.32						
Rodentia	29	0.820	<0.0001	0.010	-0.08	-1.95	0.06						

b)		PGLS					
Group	N	λ	P ($\lambda=0$)	P ($\lambda=1$)	β	t-value	p-value
Mammals	45	0.948	<0.0001	0.20	-0.11	-2.84	0.007
Rodentia	24	0.826	0.0003	0.029	-0.08	-1.84	0.08
Non-Rodentia	21	0.934	0.0006	0.40	-0.15	-1.94	0.07

Supplementary Table 12: Results of a compositional data analysis between brain size, adipose depots mass and fat-free body mass. Pair-wise phylogenetic least-squares regression (PGLS) between the transformed variables. a) N=100 species, b) N=45 species.

a)		PGLS					
Group	N	λ	P ($\lambda=0$)	P ($\lambda=1$)	β	t-value	p-value
Mammals	100	0.000	1.00	<0.0001	-0.64	-8.21	<0.0001
Primates	23	0.766	0.033	0.021	-0.57	-5.78	<0.0001
Carnivora	28	0.000	1.00	0.0008	-0.66	-4.96	<0.0001
Rodentia	29	1.000	<0.0001	1.00	-0.80	-9.19	<0.0001

b)		PGLS						Raw data ($\lambda = 0$)		
Group	N	λ	P ($\lambda=0$)	P ($\lambda=1$)	β	t-value	p-value	β	t-value	p-value
Mammals	45	0.000	1.00	<0.0001	-0.80	-6.78	<0.0001			
Rodentia	24	1.000	0.21	1.00	-0.80	-9.00	<0.0001	-0.73	-4.82	<0.0001
Non-Rodentia	21	0.000	1.00	0.0001	-0.79	-4.06	<0.0001			

3.6. The special case of primates

This section contains additional results on the relationship between brain size and adipose depots mass in primates, taking data quality into account.

Our hypothesis predicted that the negative correlation between brain size and adipose depots mass should also be present in primates as a group, but it was not (cf. Suppl. Table 4a). Even if the whole primate order would be following a cognitive buffering strategy in comparison to other mammalian taxa, we still expect that the relationship would be valid within the group, as not all primate species equally rely on cognitive buffering¹⁵. There are four reasons leading us to conclude that our data of primates do not accurately reflect the trade-off between adipose depots and brain size.

First, as our primate specimens were captives from a variety of husbandry conditions, there may be a large variation in fat storage in our sample which does not reflect “true” biological variation. Seasonality should be less of a concern for captive primates, but age and cause of death may strongly affect adipose depots in unpredictable directions. Indeed, many closely related species in our sample differ considerably in their amount of adipose depots. As tip contrasts have a large impact on the results of phylogenetic methods, they may mask an underlying trend (cf.⁵⁵).

Second, Pond¹³ reported that subcutaneous fat stores are more conspicuous in primates than in other groups, and we know from qualitative comparisons that different primate species store adipose depots at different places in their body. The small-brained fat-tailed dwarf lemurs (*Cheirogaleus medius* and *C. major*) store fat in their tails, increasing their body mass up to 78% before hibernation⁵⁶. Orangutan males exhibit fatty cheek pads, and probably also store fat around the neck, in addition to abdominal fat stores⁵⁷. Even within humans, males differ in fat store distribution from females²². Therefore, our measurements and subsequent scaling of abdominal fat stores may not be accurately estimating total body fat in some primate species, and the resulting error may mask any underlying correlation.

Third, peculiarities of the gastrointestinal tract of foregut fermenters may exert an influence on the capacity to store fat in captive animals. In the order of primates, only the subfamily Colobinae belongs to this group. In contrast to hindgut fermenters (i.e. all other primates), a diet of energy-dense, low-fiber foods does not increase body mass in this group, but rather leads to a loss of body mass, diarrhea, and premature death⁵⁸. This is a well-known phenomenon in captive colobines, which are extremely sensitive to husbandry conditions and are

relatively rarely kept in zoos. Our sample contains two colobine species (*Colobus polykomos* and *Trachypithecus vetulus*). If these are excluded from the analysis, a very weak negative trend between brain size and adipose depots mass appears in the reduced sample (N=21, lambda =0.692, p-value of adipose depots on brain size controlling for body mass: 0.368, beta = -0.072).

Fourth, some of the primate specimens in our sample were rather light-weight in comparison to the wild adult body mass of the respective sex (<75%, *Cebus apella*, *Macaca nigra*, *Symphalangus syndactylus*, *Theropithecus gelada*, and *Mandrillus sphinx*). If we exclude these specimens from the analysis (in addition to the two colobines, see point 3), the negative trend between adipose depots and brain size, controlling for body mass, actually becomes significant (N=16, lambda =0.018, p =0.022, beta = -0.180). The same result is obtained by excluding only one single data point, *Cebus apella* (N=20, lambda =0, p-value of adipose depots on brain size controlling for body mass: 0.022, beta = -0.181). The latter species is represented by a single female individual of only 70% of the normal female body mass of this species. However, its adipose depots mass is, contrary to the overall low body mass, very high in comparison to other primates (51.4g abdominal fat mass, 1750g body mass). This leads to an extremely large contrast with its closest relative in our sample, a male *Saimiri boliviensis*, which exhibits a very low value of adipose depots mass (4.85g abdominal fat mass, 1003g body mass). But how can we know which of the two individuals is an outlier? For *Saimiri*, we have dissected two other specimens of a closely related species, *Saimiri sciureus*, which were not included in the final sample because their skull was damaged. All three *Saimiri* individuals exhibit a similarly low amount of abdominal adipose depots relative to body mass (less than 1% abdominal fat in all three specimens, as compared to 3% in the *Cebus apella*). It is therefore likely that the *Cebus apella* data point is an outlier, but we cannot say whether this is also found in other individuals of the species or genus or whether it is an abnormality of this specimen.

In conclusion, all four points taken together confirm that there is good reason to maintain that the negative correlation between adipose depots and brain size would also be found in primates, if more and more accurate data could be obtained. Obviously, we need more and more accurate data to settle this issue.

3.7. Costs of transporting adipose depots

This section contains a discussion of the energetic cost of transporting adipose depots in mammals, and a calculation of these costs in humans vs. chimpanzees.

Interspecifically, the metabolic cost of transport in animals increases with body mass, as demonstrated by Taylor et al.⁵⁹ for a wide range of animals. The cost of transport per gram body mass decreases with body mass^{-0.33}, and thus the cost of transport of the whole body increases with body mass^{0.66}. However, the relationship varies between orders or taxa groups, and there seems to be a steeper increase in rodents (N=15 species) and primates (N=10, including *Tupaia* and *Homo sapiens*) than in artiodactyls (N=10), carnivores (N=11) or marsupials (N=11)⁵⁹. In any case, this interspecific relationship is likely to yield an underestimate of the metabolic cost of transport of additional adipose depots, as the musculoskeletal system does not grow in concert with additional body mass from fat storage.

Overall, the energy spent on locomotion varies widely between individuals of a species, depending on season, reproductive state, food availability and other variables. It can be estimated from day ranges and time spent on locomotion (cf.^{16,60}), although day range is usually underestimating actual path length, which has a fractal dimension, and time spent on locomotion is confounded by the fact that some locomotion is also needed for other activities such as foraging or social life. Small animals of less than 500g body mass spend only about 1% of their total energy on locomotion, whereas this value increases to 5-15% in larger animals¹⁶.

These values may seem to be rather small. However, the actual cost of carrying adipose depots may not only consist of an increase of the direct costs of locomotion, but in addition of an indirect cost of being less swift to escape predators. This cost depends on the lifestyle and may be higher in terrestrial and/or small species. Jumping distance is impaired in fatter cats⁶¹, and in small monkeys forced to carry additional weights on their trunk⁶². Maximum running speed does not generally increase with body mass within taxa⁶³, and it even decreases in artiodactyls. In human athletes, the fastest and most enduring sportsmen are usually those with the lowest percentage of body fat, if training levels are also taken into account⁶⁴. Therefore, it seems justified to assume that an increased percentage of adipose depots, without a parallel increase in the size of the musculoskeletal system, significantly decreases maximum running speeds also in animals.

Extant human foragers spend between 22% and 18% of their daily energy expenditure on locomotion (estimates of Leonard and Robertson⁶⁰, and of Isler and van Schaik⁷ following a model of Pontzer and Wrangham⁶⁵), and chimpanzees between 16% and 30% (same sources). Using the same equations, 10% additional fat stores would increase the percentage of energy used for locomotion about 1% in humans, but 2-3% in chimpanzees (the exact chimpanzee value differs according to whether we use the Taylor and Rowntree⁶⁶ equation for treadmill running of a juvenile chimpanzee (body mass 17kg), or the Taylor *et al.*⁵⁹ equation derived from 10 primate species).

Recently, Hanna and colleagues⁶⁷ have shown that vertical climbing efficiency increases only very slightly with body mass in primates (exponent of 0.11, not significantly different from zero), i.e. the cost of travel during climbing is almost directly proportional to body mass. We therefore expect that animals that include a fair amount of vertical travel are affected more strongly than predominantly terrestrial species by an increase in the size of adipose depots.

In conclusion, direct and indirect costs of adipose depots through their effect on locomotor efficiency are clearly evident, although they probably vary quite considerably between lineages or even more closely related species. Furthermore, available data and models confirm that the costs of quadrupedal locomotion and vertical climbing in nonhuman apes show a steeper increase with body mass than human walking or running. Efficient bipedalism similar to that of modern humans probably evolved in the first members of the genus *Homo* about 2 Ma ago²⁷. Therefore, we conclude that storing fat would be less costly for an efficient biped such as early *Homo* than for their ancestors.

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