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3 **Lactation and gestation controls on calcium isotopic compositions in a mammalian**
4 **model**

5 **Authors**

6 Auguste Hassler^{a*}, Jeremy E. Martin^a, Stéphane Ferchaud^b, Doryan Grivault^b, Samuel Le
7 Goff^a, Emmanuelle Albalat^a, Jean-Alexis Hernandez^c, Théo Tacail^d, Vincent Balter^a

8 ^a Univ Lyon, ENSL, Univ Lyon 1, CNRS, LGL-TPE, F-69007 Lyon, France

9 ^b GENESI, INRA, Rouillé, France

10 ^c Center for Earth Evolution and Dynamics, University of Oslo, N-0315 Oslo, Norway

11 ^d Bristol Isotope Group, School of Earth Sciences, University of Bristol, Bristol, BS8 1RJ, UK

12 * Corresponding author: auguste.hassler@ens-lyon.fr

13 **Abstract**

14 Lactation and gestation are among the physiological events that trigger the most intense
15 changes in body calcium (Ca) fluxes. Along with the composition of the animal diet, these
16 events are suspected to impact the Ca isotopic composition of Ca body reservoirs but their
17 dynamics are poorly understood. In this study, we monitored a group of domestic sows
18 across a full reproduction cycle. We collected tissues and fluids (blood, urine, milk,
19 colostrum, umbilical blood, adult and piglet bones) at different steps of gestation and
20 lactation, and analyzed their Ca isotopic compositions (i.e. $\delta^{44/42}\text{Ca}$) by mean of MC-ICP-MS.
21 Among other results, we report the first observations of Ca isotopic fractionation between

22 maternal and umbilical blood ($\Delta^{44/42}\text{Ca}_{\text{umbilical blood-sow blood}} = -0.18 \pm 0.11 \text{ ‰}$, $n = 3$). Our data also
23 highlight that gestation and lactation periods are characterized by small diet-bone Ca
24 isotopic offsets ($\Delta^{44/42}\text{Ca}_{\text{bone-diet}} = -0.28 \pm 0.11 \text{ ‰}$, $n = 3$), with ^{44}Ca -enriched blood
25 compositions during nursing ($\Delta^{44/42}\text{Ca}_{\text{nursing blood-gestation blood}} = +0.42^{+0.11}_{-0.12} \text{ ‰}$, $n = 3$). Under the light
26 of an up-to-date mammalian box model, we explored different scenarios of gestation and
27 lactation Ca fluxes experienced by a sow-like animal. These simulations suggest that
28 gestation changes on body $\delta^{44/42}\text{Ca}$ values result from the intensification of Ca absorption by
29 the animal, whereas the production of ^{44}Ca -depleted milk is the main driver for the ^{44}Ca
30 enrichment in blood during lactation. In addition, our results also support that bone
31 mineralization could be associated with a more restricted Ca isotopic fractionation than
32 previously envisioned. Together, these results refine the framework of Ca isotope
33 applications, notably regarding the monitoring of human bone balance and the study of
34 species and ecosystems from the present and the past.

35 1. Introduction

36 There is evidence that the Ca isotopic composition of mammal bone and teeth is controlled
37 by diet, but other physiological parameters might also be at play and the cycling of Ca and its
38 isotopic fractionation in the body is far from being fully understood¹⁻¹⁷. Among dietary
39 inputs, milk is highly depleted in heavy Ca isotopes relative to adult diet ($\Delta^{44/42}\text{Ca}_{\text{milk-diet}} = -0.6$
40 ‰)¹³, the consumption of milk affects the Ca isotopic composition of juvenile teeth, which
41 can be used to document weaning ages and nursing practices^{15,18,19}. Nevertheless, the
42 production of milk (lactation) and possibly gestation seem to affect bone and blood Ca
43 isotopic composition of breeding females^{9,14}, notably by generating male versus female
44 differences in bone Ca isotopic composition ($\Delta^{44/42}\text{Ca}_{\text{females-males}} = +0.14 \pm 0.08 \text{ ‰}$)¹⁴. Until now,

45 this phenomenon has only been described for sheep whereas there is no evidence of such
46 sexual difference in human populations^{8,14}. It may not be surprising that different mammal
47 species display different sexually driven isotopic differences due to their physiological and
48 behavioral differences. However, this observation calls for further investigations on other
49 mammal species and, more essentially, on physiological factors at play to generate these
50 sexually driven isotopic differences. Improving our knowledge about the mammalian Ca
51 isotope cycle is motivated both by the development of biomedical innovations based on Ca
52 isotope measurements^{6,20-24} and potential applications in paleoanthropology and
53 paleontology^{1,2,10,13-15,18,19,25,26}. For example, new methods for bone balance and osteoporosis
54 monitoring depend upon an accurate description of the Ca isotope cycle^{6,20-24}. Besides,
55 sexually driven Ca isotopic differences could help in detecting sex or past lactation events
56 from teeth or bones, if their causing factors could be further constrained and quantified¹⁴.
57 This would be a great opportunity for paleontologists as these are challenging to detect by
58 other means. Finally, clearly identifying the range of action of these factors seems to be a
59 necessary step to accurately reconstruct dietary preferences from Ca isotopic compositions
60 within mammalian faunas^{1,7,10-12,14}.

61 The hypothesis that sexual differences of bone Ca isotopic compositions originate from
62 gestation, lactation or both, arise from experiments and modeling, which suggest important
63 Ca isotopic fractionation during milk production (notably inferred from the $\Delta^{44/42}\text{Ca}_{\text{milk-diet}}$ of -
64 0.6 ‰)¹³ and bone mineralization^{2,5,9,13,14,17} (notably inferred from the $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$ of $-0.57 \pm$
65 0.10 ‰, n = 21; see review from²). In this scenario, milk is enriched in light Ca isotopes
66 relative to blood, and bone growth associated with gestation preferentially favors light Ca
67 isotopes during the mineralization process¹⁴. However, later studies have highlighted that
68 fractionation of Ca isotopes also occurs during urine formation through the reabsorption of

69 Ca from primary urine by kidneys ^{17,21-23,27,28}, as evidenced by differences between blood and
70 urine $\delta^{44/42}\text{Ca}$ values ($\Delta^{44/42}\text{Ca}_{\text{urine-blood}} = +1.15 \pm 0.06 \text{ ‰}$, $n = 29$)^{3,17,21,23}. In parallel, small
71 differences between blood and bone Ca isotopic compositions in humans, sheep and rats ^{3,27}
72 (i.e. $\leq 0.3 \text{ ‰}$) and new modeling integrating Ca urinary fractionation in normal conditions
73 (i.e. without gestation or lactation related Ca fluxes; see ³) also suggest a reevaluation of Ca
74 fractionation amplitude at bone mineralization ^{2,3}. It is thus necessary to integrate these
75 findings in updated models in order to unravel the underlying causes behind the observed
76 sexual differences in Ca isotopic composition, as well as to provide guidance for future
77 investigations of such gestation and lactation signals. It is also essential to widen the
78 spectrum of mammals for which such difference is documented experimentally in order to
79 test modeling predictions. The aim of this study is to address both of these aspects, by
80 documenting the effects of gestation and lactation on Ca isotopic composition in the
81 domestic pig, *Sus scrofa domesticus*, and by comparing these data with stable isotope box-
82 models that consider urinary isotopic fractionation along with other fractionation processes
83 described so far for Ca.

84 To achieve these objectives, we designed a breeding and feeding experiment consisting of a
85 close monitoring of three adult sows during a full reproduction cycle. Over the 6 months of
86 the experiment, samples including blood, urine, milk, colostrum, umbilical blood and bone
87 have been collected while maintaining a diet with a stable Ca isotopic composition. This
88 controlled environment allows us to identify the physiological drivers of Ca isotopic
89 compositions in body reservoirs at each step of the reproduction cycle. Our sampling
90 procedure allows us to compare the Ca isotopic composition of different body reservoirs at
91 rather high temporal resolution, and to monitor the evolution of their respective Ca isotopic
92 composition before, during, and after gestation and lactation periods. Attention was also

93 given to the juveniles of these three individuals, notably to their weight, in order to estimate
94 Ca transfers during gestation and milk production. Shortly after parturition, two piglets from
95 other females living in the same conditions unfortunately died. We sampled the bones of
96 these two individuals and measured their Ca isotopic composition in order to assess the
97 isotopic fractionation occurring between mother and offspring bones during gestation. Using
98 these results and literature data about pig Ca cycle during reproduction, we performed
99 different box model simulations to compare with our experimental data. Finally, we use
100 these comparisons to identify the main drivers of Ca isotopic fractionation in the body, and
101 compare our findings with previous studies from pig, deer, mice, rat, sheep and human
102 ^{3,8,9,13,14,17}.

103 **2. Material and method**

104 *2.1. Animal monitoring and sampling*

105 The use of animals for scientific purpose has been authorized in accordance with the French
106 rural and sea fishing code, notably following the articles R.214-87 and R.214-126. The ethical
107 approval was given to the project (referenced as APAFIS#13631-2018021417118920 v3) by
108 the ethics committee of animal experimentations N°084. Sampling procedures have been
109 designed to minimize animal stress and to be the least invasive possible. Moreover, this
110 study has been grafted to an already going agronomic study, therefore preventing
111 supplementary animal use.

112 The three monitored sows (C1, C2 and C3) were crossbred Landrace Français and Duroc
113 breeds, raised within the unit of Genetic, experimentation and innovative systems (GenESI)
114 of the French National Institute for Agriculture, Alimentation and Environment Research
115 (INRAE), in the facility of la gouvanière (Rouillé 86480, France, DOI:

116 10.15454/1.5572415481185847E12). Two were at their second breeding cycle (C1 and C3
117 individuals), another was at its third breeding cycle (C2). The experiment started 12 to 14
118 days after the weaning of a previous litter, a breeding cycle conducted within the same
119 environment with a similar diet made up of a mix of barley, corn, wheat, sunflower,
120 rapeseed, beets, sugar cane, calcium carbonate as well as other minor ingredients
121 constituting about 1% of the mix (e.g. sodium chloride and sodium bicarbonate). The return
122 to estrus during this 14 days period has been artificially delayed by administrating
123 Régumate® to sows for about the first 10 days after weaning. One other individual (C8) living
124 in the same conditions died from an unidentified cause between the experiment and the
125 previous breeding cycle. We collected one of its phalanxes to perform comparative bone
126 analyses. The blood, urine, colostrum, milk of sows and umbilical blood from their piglets
127 were collected at 5 key moments of their reproduction: 3 days before the insemination,
128 during the last month of gestation, shortly after parturition, during nursing and 14 days after
129 weaning (figure 1). At each step, bio-fluids were collected with the intention to minimize the
130 time lapse between each collection, in order to maximize the comparability between
131 samples from Ca reservoirs with small Ca residence time. To the same end, all sampling
132 sessions were carried in the morning, before the first meal and after at least 8 hours of
133 fasting. This procedure allows to minimize short term impacts of food intakes on Ca isotopic
134 compositions of body reservoirs. We maintained the same food supply and performed food
135 samplings during the duration of the experiment to monitor the isotopic variability of sow Ca
136 intakes.

137 Sampling sessions were conducted as follows. On sampling days, light was switched on
138 manually at the arrival of the collecting team, under the supervision of S. Ferchaud and D.
139 Grivault. Urines were preferably collected with the first urination of the day, as an attempt

140 to limit the impact of urine isotopic variability over day-time and because 24h urine
141 collections were not achievable. Urines were collected in Falcon® tubes (50 mL, REF 352070)
142 without the first milliliters of the urination, then transferred in 2 ml polypropylene (PP) tubes
143 and stored in a freezer. This step requires a close monitoring of sows to achieve the
144 collection, a success upon which subsequent samplings were initiated or not. For blood
145 collection, adult individuals were immobilized then blood was collected from the jugular vein
146 using lithium heparin tubes without gel (BD Vacutainer®, REF 367526). Blood samples were
147 centrifuged, then the plasma was collected and transferred in PP tubes prior to be store-
148 frozen. Because almost all the Ca from the blood is contained within the plasma^{3,29-31},
149 plasma and total blood Ca compositions are considered to be identical in this article. At
150 parturition, umbilical blood was collected from umbilical cords in heparin tubes without gel
151 (BD Vacutainer®, REF 367526). The umbilical plasma has been collected after centrifugation
152 and stored in the same conditions than regular plasma. At parturition and 14 days after
153 parturition, respectively 4 ml of colostrum and 2ml of milk were collected from each
154 individual. These collections were achieved in PP tubes by operating a massage of the udder.
155 No hormonal injection was carried on in that purpose. Fourteen days after the weaning of
156 their offspring and after a last round of sampling, studied animals were slaughtered to join
157 the traditional circuit of pork meat distribution. Their skulls were collected, boiled in water
158 and manually cleaned to allow bone sampling from their mandible using a handled drill
159 (8200 Dremel with tungsten steel solid carbide bit).

160 *2.2. Weight and milk production estimates*

161 We estimated the weight of the three studied sows based on the average weight recorded
162 for their congeners in the INRAE GenESI facility. At weaning of their second lactation, sows
163 from the same breed weigh 199.3 ± 17.1 kg (n = 117, \pm standard error) on average, while

164 weighing an average of 213 ± 20 kg ($n = 100$, \pm standard error) after their third lactation. This
165 is similar to what is documented in Dourmad et al. (1997)³² and Giesemann et al. (1998)³³,
166 who support that Ca reservoir sizes and Ca fluxes reported in these studies are of the same
167 order of magnitude than for the individuals of our experiment. At birth, piglets were
168 weighed to estimate the amount of Ca they received from their mother during gestation.
169 This estimation is based on the assumption that dry bone represents about 4.88% of their
170 total body mass (mean calculated from two newborns of the same breed) and that Ca
171 accounts for about 26.58% of bone mass (extrapolated from cow bone meal reference
172 material NIST SRM1486). After birth, litters were rearranged between sows from within and
173 outside the experiment, in order to equilibrate the number of piglets per sow and guarantee
174 healthy growth conditions. The piglet mass gain at weaning is thus calculated while including
175 all piglets (native and adopted) nursed per each sow, by subtracting their weight at weaning
176 by their weight at birth. This piglet daily weight gain (referred as GMQ to match the notation
177 of Etienne et al., 2000³⁴) is used to calculate the piglet daily intake of milk dry fraction
178 (referred as MS to match the notation of Etienne et al., 2000³⁴) using the following formula:
179 $MS = 0,72 (\pm 0,07) \times GMQ - 7$, where MS and GMQ are expressed in gram/piglet/day ³⁴.
180 Considering an average sow milk dry mass fraction of 18% ^{34,35} allows to estimate sow
181 average milk daily production. Five piglets died between birth and weaning with a noticeable
182 weak body condition. Their puny state at death supports that they consumed limited
183 amounts of milk and that their body mass can be neglected in the calculation of milk
184 production.

185 *2.3. Sample preparation and chromatography*

186 Complementary details regarding equipment and cleaning procedures used in this study can
187 be found in appendix A.1, the following section focus on the operations performed on

188 samples, blanks and reference materials. Prior to chromatography and concentration
189 analyses, blood, colostrum, milk and food samples have been freeze-dried, homogenized in
190 an agate mortar, weighed and placed in Perfluoroalkoxy (PFA) beakers (Savillex®). Urine
191 samples have been unfrozen and homogenized, then 500 µL of each were collected in PFA
192 beakers. Bone powders collected from mandibles were weighed and placed in PFA beakers
193 prior to digestion. Further manipulations were carried exclusively in a clean lab, under a
194 laminar flux hood (absolute filter H14) to avoid environmental contamination. After being
195 placed in PFA beakers, samples were mixed with 10 ml of distilled nitric acid (15 M, Fisher
196 Scientific®, Primar plus® – Trace analyses grade, in-house distilled) and 1 ml of 30 % suprapur
197 hydrogen peroxide (H₂O₂, Fisher Chemical®, Hampton, NH, USA) to start digestion. Samples
198 were left at ambient temperature for 1h, then placed on a hotplate at 160 °C for two days
199 and evaporated to dryness. All along this procedure, we performed periodic beaker
200 degassing to avoid critical overpressure. This procedure was repeated at least three times,
201 until complete mineralization of samples. The complete digestion of the organic matter was
202 assessed by monitoring H₂O₂ effervescence. Digested and evaporated samples were
203 dissolved in 0.5 M distilled HNO₃ (Fisher Scientific®, Primar plus® – Trace analyses grade, in-
204 house distilled), a fraction of which was kept for concentration analyses. The rest has been
205 evaporated to dryness, dissolved in 6 M distilled hydrochloric acid (Fisher Scientific®,
206 laboratory reagent grade, in-house distilled), and evaporated again prior chromatography.

207 The chromatography procedure used for Ca chemical purification is derived from Tacail et al.
208 (2014)²⁷ and Le Goff et al. (2021)³⁶. It consists in a triple column chromatography, starting
209 with an elution on AG1 X8 resin to discard elements such as Zn and Fe, followed by an AG
210 50WX-12 resin to isolate Ca and strontium (Sr) from the matrix, and by an elution on
211 Eichrom Sr-specific resin to isolate Ca from Sr. This procedure is detailed in Table B.1. Blanks

212 have been monitored all along digestion and chromatography processes (i.e. total and
213 chromatography blanks) to control for Ca contamination levels. The use of heparin tubes for
214 blood collections (BD Vacutainer®, REF 367526) LH, is associated with additional Ca
215 contaminations. To estimate this contamination, we filled a heparin tube of MilliQ
216 (Millipore®, initial resistivity = 18 MΩ.cm at 25 °C) during one hour at ambient temperature,
217 and analyzed its Ca concentration by mean of ICP-MS.

218 *2.4. Analytical procedures*

219 Elemental concentrations have been measured on an inductively coupled plasma atomic
220 emission spectrometer (ICP-AES, model ICAP 7400 Series, Thermo Scientific®), with the
221 exception of the Ca content of heparin tubes which has been measured by mean of an
222 inductively coupled plasma mass spectrometer (ICP-MS, model ICAP Q, Thermo Scientific®).
223 The reliability of measurements has been controlled through a set of blanks and reference
224 materials, as well as by replicating measures at least twice for each sample.

225 We measured Ca isotopic ratios ($^{44}\text{Ca}/^{42}\text{Ca}$ and $^{43}\text{Ca}/^{42}\text{Ca}$) using a multi-collector ICP-MS (MC-
226 ICP-MS, Neptune Plus, Thermo Scientific®) following the method described in Tacail et al.
227 (2014)²⁷. Prior to Ca isotopic analyses, Ca purified samples were dissolved in distilled 0.05 M
228 HNO₃ in order to set the Ca concentration at 1.25 mg.L⁻¹. This concentration matches the
229 concentration of our in-house bracketing standard, a Specpure Ca plasma standard solution
230 (Alfa Aesar) named ICP Ca Lyon and described in previous studies^{1,16,18,27}. Calcium isotopic
231 composition reported in this article are all expressed as $\delta^{44/42}\text{Ca}$ values calculated based on
232 this reference material and the following formula (unless explicitly mentioned):

$$\delta^{44/42}\text{Ca} = \left(\left(\frac{^{44}\text{Ca}}{^{42}\text{Ca}} \right)_{\text{sample}} / \left(\frac{^{44}\text{Ca}}{^{42}\text{Ca}} \right)_{\text{ICP Ca Lyon}} \right) - 1 \quad [1]$$

233
234

235 With $\delta^{44/42}\text{Ca}$ values given in ‰. For more comparability with studies from other
 236 laboratories, the $\delta^{44/42}\text{Ca}_{\text{ICP Ca Lyon}}$ values are also expressed as $\delta^{44/42}\text{Ca}_{\text{SRM915a}}$ in tables and
 237 figures. Based on 71 measures synthesized in the appendix of Martin et al. (2018)¹⁰, we
 238 converted $\delta^{44/42}\text{Ca}_{\text{ICP Ca Lyon}}$ values to $\delta^{44/42}\text{Ca}_{\text{SRM915a}}$ values by adding +0.518 ‰ to $\delta^{44/42}\text{Ca}_{\text{ICP Ca}}$
 239 $_{\text{Lyon}}$ values. This conversion is associated with a wider uncertainty interval corresponding to
 240 +0.025 ‰ of the uncertainty around $\delta^{44/42}\text{Ca}_{\text{ICP Ca Lyon}}$ values (error bars within figures reflect
 241 uncertainties around $\delta^{44/42}\text{Ca}_{\text{ICP Ca Lyon}}$ values only). We used the SRM1486, a cow bone meal
 242 reference material from NIST, as a secondary standard to assess the reproducibility of the
 243 ion-exchange chromatography procedure, as well as to monitor the accuracy of MC-ICP-MS
 244 measures. Blank Ca concentrations have also been measured using the Neptune Plus MC-
 245 ICP-MS. All samples and reference material measurements have been replicated at least
 246 three times (table B.6).

247 Differences between Ca isotopic compositions are expressed as $\Delta^{44/42}\text{Ca}$, following the
 248 formula:

$$249 \quad \Delta^{44/42}\text{Ca}_{\text{X-Y}} = \delta^{44/42}\text{Ca}_{\text{X}} - \delta^{44/42}\text{Ca}_{\text{Y}} \quad [2]$$

250 Where X and Y refer to different samples or Ca isotope reservoirs. For calculating $\Delta^{44/42}\text{Ca}$
 251 values between sample types (e.g. blood, urine) at the scale of several individuals or
 252 sampling steps (e.g. the average $\Delta^{44/42}\text{Ca}$ value between blood and urine samples), the terms
 253 $\delta^{44/42}\text{Ca}_{\text{X}}$ and $\delta^{44/42}\text{Ca}_{\text{Y}}$ can simply be replaced by the mean $\delta^{44/42}\text{Ca}$ values of Ca reservoirs X
 254 and Y. Uncertainties expressed around $\Delta^{44/42}\text{Ca}$ values are the sum of the uncertainties of
 255 $\delta^{44/42}\text{Ca}_{\text{X}}$ and $\delta^{44/42}\text{Ca}_{\text{Y}}$. When a Ca flux connects two Ca reservoirs, the isotopic fractionation
 256 associated with this flux can be expressed as an isotopic fractionation factor α , calculated as
 257 follow:

258
$$\alpha_{X-Y} = \frac{\delta^{44/42} Ca_X + 1000}{\delta^{44/42} Ca_Y + 1000} [3]$$

259 Which can be approximated as:

260
$$1000 \times \ln(\alpha_{X-Y}) \approx \delta^{44/42} Ca_X - \delta^{44/42} Ca_Y [4]$$

261 Where X and Y refer to different Ca isotope reservoirs connected by the Ca flux associated to
262 the isotopic fractionation factor described by α_{X-Y} .

263 *2.5. Accuracy and precision of Ca isotopic compositions*

264 For more clarity in the following sections, the n notation refers to a number of samples or
265 specimens, whereas the n* notation specifically refers to number of replicates for a given
266 measurement. Replicating Ca isotope measurements allows us to estimate the range of
267 analytical precision of $\delta^{44/42}Ca$ values. The correlation between $\delta^{43/42}Ca$ and $\delta^{44/42}Ca$ values for
268 samples and reference materials follows the trend expected from an exponential
269 fractionation law³⁷, with a slope value of 0.502 ± 0.007 (2 s.e.), an intercept of -0.003 ± 0.005
270 (2 s.e.), a $R^2 = 0.997$ and a p-value < 0.001 (figure C.1). This demonstrates that no mass
271 independent fractionation or mass isobaric interference affect these measurements. Across
272 the six days of analytical session with the MC-ICP-MS, the reference material SRM1486
273 exhibited a mean $\delta^{44/42}Ca$ value of -1.01 ± 0.01 ‰ (2 s.e., n = 37), which is undistinguishable
274 from previously published data for this reference material^{10,18,38,39}. All these data support
275 that the Ca isotopic compositions we measure are not biased by the ion-exchange protocol
276 and MC-ICPMS setup, and that the measured Ca isotopic compositions are accurate.

277 For our bracketing standard (ICP Ca Lyon), blanks collected during the Sr purification step²⁷
278 represent about 100 ng of Ca, a negligible pollution at the scale of the 4 mg of Ca contained
279 in the solution. For samples and SRM1486, chromatography blanks contain between 52 to

280 181 ng of Ca (~100 ng of Ca in average), while blanks monitoring environmental
281 contaminations during the MC-ICP-MS session contain less than 10 ng of Ca. Heparin tubes
282 can add about 273 ± 35 ng of Ca (2 s.e., $n^* = 2$) to blood samples. For the majority of our
283 samples these blank levels are negligible compared to the amount of Ca contained in
284 samples, only 5 samples containing limited amounts of Ca could have been notably affected.
285 Uncertainty estimations and corresponding error bars have been extended accordingly
286 (table B.6). Equations behind uncertainties presented in this paper are described in details in
287 appendix (text A.2).

288 *2.6. Box model*

289 In order to identify the mechanisms behind the distribution of Ca isotopic compositions in
290 the body, we performed several simulations of a Ca box model using the Isopybox program.
291 This Python-coded program derives from a code used in previously published work ⁴⁰. It
292 iteratively calculates the evolution of isotopic compositions within interacting reservoirs of a
293 given isotopic system ³. In its current version, Isopybox allows to solve steady-state box
294 model, study the relaxation time of a system in response to a discrete perturbation, and
295 study the isotopic evolution of a system with unbalanced fluxes (providing that no box will
296 be emptied during the duration of the run). The program and its resources are accessible on
297 Github at the following address: <https://github.com/ttacail/isopybox.git>.

298 The conception of the model is described and discussed in further details in appendix (Text
299 A.3; Tables B.2, B.3, B.4). In a few words, this model is a box model designed to simulate the
300 Ca isotopic composition of a sow-like animal based on plausible Ca reservoir sizes, Ca fluxes
301 and Ca isotopic fractionation factors. We modeled different scenarios of Ca fluxes and
302 isotopic fractionation factors for the animal. First, we simulated a gestation without Ca

303 transfer to fetuses (Ca gain is transferred from extracellular fluids to the waste box with no
304 isotopic fractionation). This simulation (referred as GestFF for Gestation Fetus Free) is purely
305 conceptual but stay quite representative of the average conditions of a domestic sow, as the
306 Ca transfer to fetuses intensifies only toward the last third of the gestation period, and that
307 they generally have small nursing periods and recovery time between weaning and new
308 insemination. The second simulation is similar to the first one but includes Ca transfer to
309 fetuses (referred as GestR for Gestation Regular). This last aim to represent a sow toward
310 the end of a gestation, when Ca transfer to fetuses are intense. The third and fourth
311 simulations (LactA, LactB) represent a lactation scenario without bone loss, with relatively
312 high (LactA) and low Ca dairy excretion (LactB). Finally, we tested the influence of Ca
313 absorption (i.e. the amount of Ca transferred from the digestive tract to the blood) on body
314 Ca isotopic compositions. This test is a series of simulations which use the basis of the GestFF
315 scenario with different Ca absorption levels, respectively 75 %, 50 % and 25% of GestFF Ca
316 fluxes from digestive tract to blood. In addition to these scenarios, we tested additional
317 configurations of Ca fluxes and Ca isotopic fractionation factors in order to assess the
318 sensitivity of the model to these parameters. Monitored parameters include: the coefficient
319 of Ca isotopic fractionation at bone mineralization, the degree of bone loss, the ratio
320 between Ca absorption and excretion (i.e. by digestive secretions) and the ratio between
321 urinary and endogenous Ca losses. These last scenarios and associate results are further
322 described in appendix (Text A.3; figures C.5, C.6, C.7).

323 **3. Results**

324 *3.1. Zootechnical data*

325 Zootechnical data for sows (e.g. litter size, weaning age, quantity of milk produced) from this
326 study are reported in Table B.5. Estimations of sow body masses fall within the range of

327 body masses reported for other porcine specimens studied by Dourmad et al. (1997)³² and
328 Giesemann et al. (1998)³³. The near four-month gestation of sows multiplied by the daily flux
329 of extracellular fluids into the fetus (i.e. EF→Ft) documented by Giesemann et al. (1998)³³
330 produce estimations of total piglet Ca mass at birth which are lower but of the same order
331 than the total piglet Ca mass estimated at birth for C1 and C3 offspring (Table B.5). In this
332 experiment we estimate that sow produced an average of 10 kg of milk per day³⁴. The
333 fraction of Ca in milk samples was highly variable (3830 to 7632 ppm), which led to a wide
334 range of possible Ca dairy output, from 8.16 to 16.25 g of Ca per day. Considering that Ca
335 milk concentration is documented to be between 1700 and 2140 g/L after the second week
336 of lactation^{33,41}, the upper estimation of 16.25 g/d of Ca dairy output seems more consistent
337 and matches with Ca dairy outputs documented by Giesemann et al. (1998)³³. Overall, the
338 estimations of body masses, Ca reservoir size, placental and dairy Ca fluxes from this study
339 are consistent with those reported in Giesemann et al. (1998)³³ and the other studies used to
340 design the present model (Table B.2, B.3). This supports that these Ca flux data can be
341 reasonably used within our model to compare with experimental measurements.

342 *3.2. Experimental elemental and isotopic data*

343 Calcium isotopic compositions collected during this study (205 measures for 50 sample and 1
344 reference material) are reported as both $\delta^{44/42}\text{Ca}_{\text{ICP Ca Lyon}}$ and $\delta^{44/42}\text{Ca}_{\text{SRM915a}}$ in figure 2 and
345 table B.6. Food Ca isotopic composition remains consistently stable at $-0.23 \pm 0.06 \text{ ‰}$ ($n = 3$)
346 during the duration of the experiment (figure 3a). Bones of adult females (C1, C2, C3) display
347 $\delta^{44/42}\text{Ca}$ values distributed in a tight range between $-0.49 \pm 0.05 \text{ ‰}$ ($n^* = 4$) and -0.53 ± 0.05
348 ‰ ($n^* = 3$), with a general $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$ offset of $-0.28 \pm 0.11 \text{ ‰}$ ($n_{\text{bone}} = 3$, $n_{\text{diet}} = 3$). Bones
349 are undistinguishable from pre-insemination blood Ca isotopic compositions ($\Delta^{44/42}\text{Ca}_{\text{bone-blood}}$
350 $\approx -0.08 \pm 0.11 \text{ ‰}$, $n_{\text{bone}} = 3$, $n_{\text{blood}} = 3$). The bone $\delta^{44/42}\text{Ca}$ value of the C8 individual ($-0.51 \pm$

351 0.05 ‰, $n^* = 4$) is indistinguishable from C1, C2 and C3 individuals. Milk Ca isotopic
352 composition range between -0.70 ± 0.07 ‰ ($n^* = 3$) and -0.96 ± 0.05 ‰ ($n^* = 3$). The mean
353 difference between the food of sows and their milk ($\Delta^{44/42}\text{Ca}_{\text{milk-diet}}$) is -0.58 ± 0.12 ‰ ($n_{\text{milk}} = 3$,
354 $n_{\text{diet}} = 3$), whereas the mean difference between their blood and milk during nursing
355 ($\Delta^{44/42}\text{Ca}_{\text{milk-blood}}$) is -0.67 ± 0.12 ‰ ($n_{\text{milk}} = 3$, $n_{\text{blood}} = 3$). Colostrum samples ($n = 3$) have a wider
356 range of $\delta^{44/42}\text{Ca}$ values than milk, from -0.72 ± 0.06 ‰ ($n^* = 3$) to $-2.06^{+0.06}_{-0.05}$ ‰ ($n^* = 3$),
357 mainly because of one outlier sampled from the C3 individual. The two other colostrum
358 samples (from C1 and C2) display $\delta^{44/42}\text{Ca}$ values in the range of milk values (figure 2).
359 Umbilical blood samples ($n = 3$) show intermediate $\delta^{44/42}\text{Ca}$ values between mother blood
360 and milk, ranging between -0.30 ± 0.05 ‰ ($n^* = 3$) and -0.71 ± 0.05 ‰ ($n^* = 3$). The mean
361 $\Delta^{44/42}\text{Ca}_{\text{umbilical blood-diet}}$ offset is -0.29 ± 0.11 ‰ ($n_{\text{umbilical blood}} = 3$, $n_{\text{diet}} = 3$). Close after parturition,
362 the mean $\Delta^{44/42}\text{Ca}_{\text{umbilical blood-sow blood}}$ is -0.18 ± 0.11 ‰ ($n_{\text{umbilical blood}} = 3$, $n_{\text{sow blood}} = 3$) and $-0.14 \pm$
363 0.11 ‰ for C1 ($n_{\text{umbilical blood}} = 1$, $n_{\text{sow blood}} = 1$), the individual for which collections of blood and
364 umbilical blood were the closest in time. Thus, umbilical blood tends to be slightly depleted
365 in heavy Ca isotopes compared to sow's food and blood (figure 2). Bone samples collected
366 from the two early deceased piglets show a Ca isotopic composition undistinguishable from
367 the adult sows, with a mean $\delta^{44/42}\text{Ca}$ value of -0.50 ± 0.05 ‰ ($n = 2$). Urine Ca isotopic
368 compositions range between $+1.27^{+0.05}_{-0.09}$ ‰ ($n^* = 3$) and $+0.55 \pm 0.05$ ‰ ($n^* = 3$), with either a
369 relative stability over time (C1 and C3 individuals) or a noticeable variability (C2 individual,
370 see figure 3b).

371 We do not identify a common temporal pattern of urine Ca isotopic composition shared by
372 the three individuals. Except for the C2 individual which shows higher urine $\delta^{44/42}\text{Ca}$ values at
373 parturition and post weaning steps, urine Ca isotopic composition seems relatively stable

374 over the experiment, although the data point of C2 is the only available for the post-weaning
375 step (figure 3b). However, all individuals share a similar temporal pattern of blood Ca
376 isotopic composition (figure 3c). Blood $\delta^{44/42}\text{Ca}$ values range between $-0.03 \pm 0.06 \text{‰}$ ($n^* = 3$)
377 and $-0.59 \pm 0.06 \text{‰}$ ($n^* = 5$). Depending on the individual, blood $\delta^{44/42}\text{Ca}$ values are either
378 stable or decrease between pre-insemination step (June) and the last month of gestation
379 (early October). The amplitude of this change is between $-0.01 \pm 0.11 \text{‰}$ ($n_{\text{pre-insemination}} = 1$,
380 $n_{\text{syn-gestation}} = 1$) and $-0.22 \pm 0.11 \text{‰}$ ($n_{\text{pre-insemination}} = 1$, $n_{\text{syn-gestation}} = 1$). However, the range of
381 blood $\delta^{44/42}\text{Ca}$ values between June and early October largely overlaps when all individuals
382 are considered together. This period is followed by a rapid increase of $+0.42^{+0.11}_{-0.12} \text{‰}$ ($n_{\text{syn-}}$
383 $n_{\text{gestation}} = 3$, $n_{\text{syn-nursing}} = 3$) in average between the last month of gestation (early October) and
384 the middle of nursing (mid-November). The onset of this change in blood Ca isotopic
385 composition is different between the individuals, with individual C3 exhibiting this offset 5
386 days after parturition whereas individual C2 still shows no sign of it 7 days after parturition
387 (figure 3c). All the individuals consistently display this change 14 days after parturition
388 (figure 3c). This phase is followed by a decrease of $-0.19^{+0.12}_{-0.13} \text{‰}$ ($n_{\text{syn-nursing}} = 1$, $n_{\text{post-weaning}} = 1$)
389 to $-0.34 \pm 0.12 \text{‰}$ ($n_{\text{syn-nursing}} = 1$, $n_{\text{post-weaning}} = 1$) after weaning (late-November) depending on
390 the individual. In the case of the C3 individual, blood $\delta^{44/42}\text{Ca}$ values are back to initial values
391 in December (respectively $-0.58 \pm 0.05 \text{‰}$ and $-0.59 \pm 0.07 \text{‰}$, $n^* = 3$ for both).

392 Calcium concentrations in urine range from 2.8 to 715.9 mg/L. At the individual level, urines
393 collected before insemination are systematically the most Ca concentrated. Ca concentration
394 then decreases during the gestation, increases in the middle of the lactation period and goes
395 back to post-birth levels after weaning (figure C.2). We found a weak linear and logarithmic
396 correlation between urine Ca concentration and isotopic composition with a R^2 of 0.37 and

397 0.35, respectively (figure C.3). We found no significant temporal pattern of blood Ca
398 concentration (figure C.2), or correlation between blood Ca concentration and isotopic
399 composition (figure C.3). All non-blood samples isotopic compositions are shown compared
400 to individual's blood compositions ($\Delta^{44/42}\text{Ca}_{\text{x-blood}}$) in figure C.4. The mean $\Delta^{44/42}\text{Ca}_{\text{urine-blood}}$ offset
401 is $+1.21^{+0.11}_{-0.13}$ ‰ ($n_{\text{urine}} = 12$, $n_{\text{blood}} = 12$), which is undistinguishable from the offset of +1.15 ‰
402 used in our model. This offset, however, changes over time, notably during the lactation
403 period when a cluster of low $\Delta^{44/42}\text{Ca}_{\text{urine-blood}}$ can be distinguished (figure 4).

404 3.3. Box model predictions

405 The structure of the box model of this study is detailed in figure 5. The evolutions of Ca
406 isotopic compositions within some of these Ca reservoirs (i.e. extracellular fluids, urine,
407 faeces, bone, milk and bulk fetus tissues) are presented in figure 6 and are the result of
408 GestFF, GestR, LactA and LactB simulations. The initial conditions of these simulations are
409 summarized in tables B.2, B.3, B.4, briefly described in section 2.6. and further detailed in
410 the appendix section (Text A.3).

411 In figure 6, three phases can be distinguished for all the four simulations. From day 2 to day
412 100, extracellular fluids, urines, milk and fetal tissues reach a transient/temporary
413 equilibrium, which depends strictly on Ca fluxes and associated Ca isotopic fractionation but
414 not on initial Ca isotopic compositions. This result is expected considering the small Ca
415 residence time of these reservoirs or their small initial size (e.g. fetal tissues). Our model
416 thus predicts a rapid reaction of these reservoirs and a relative stability of the Ca isotopic
417 composition within this time range. After 100-days and up to 10^4 days, we observe a change
418 in bone Ca isotopic composition which also results in a slight increase of $\delta^{44/42}\text{Ca}$ values of
419 extracellular fluids, urines, milk and fetal tissues. After 10^4 days, the sow system (not

420 including the fetus box) is relaxed to its steady state. We compared the four modelled
421 predictions with our experimental data, at different timings (100-days and steady state)
422 while considering different Ca isotopic fractionation factors (α_{B-EF}) at bone mineralization
423 (figure 7, see table B.4 and reference therein). At steady state, only bone Ca isotopic
424 composition is affected by α_{B-EF} . This parameter has little impact on intermediate composition
425 states at 100-day for lactation but a more pronounced impact for gestation simulations
426 (figure C.5).

427 GestFF predictions for urine and extracellular fluids fits remarkably well within the range of
428 urine and blood data obtained from pre-insemination samplings (figure 7). Ca isotopic
429 compositions recorded in bones collected from sows after the weaning of the offspring fit
430 with compositions predicted from GestFF simulation, but only for Ca isotopic fractionation
431 factor α_{B-EF} between 1 and 0.9999 (i.e. with $\Delta^{44/42}\text{Ca}_{\text{bone-blood}}$ between 0 and -0.1 ‰). Note that
432 considering the Ca residence time in bones (about 5 to 6 years for sows) and the length of
433 the experiment (less than 6 months), $\delta^{44/42}\text{Ca}$ values from sow bones collected at the end of
434 the experiment are considered to be minimally affected by gestation (3 months) and
435 lactation periods (28 days) experienced during the experiment. Instead, these bone $\delta^{44/42}\text{Ca}$
436 values are more comparable with pre-insemination body Ca isotopic compositions. This is
437 confirmed by bone data obtained from the individual C8 (the sow who died between the
438 previous breeding cycle and the monitored breeding cycle), which is identical to C1, C2 and
439 C3 bone $\delta^{44/42}\text{Ca}$ values.

440 In gestation condition (GestR), predictions for urine and fetus tissues fit well within the range
441 of experimental syn-gestation urine and syn-parturition umbilical blood compositions, for
442 steady state (i.e. post 10^4 days) as for 100-day simulations. Steady state and 100-days

443 $\delta^{44/42}\text{Ca}$ values predicted for extracellular fluids are higher than experimental blood $\delta^{44/42}\text{Ca}$
444 range of the last month of gestation, by an order of 0.1 to 0.2 ‰. None of our experimental
445 bone $\delta^{44/42}\text{Ca}$ values are comparable with 100 days or steady state gestation simulations,
446 however low $\alpha_{\text{B-EF}}$ (i.e. high enrichment in light Ca isotopes at bone mineralization) increases
447 the gap between predicted extracellular fluid $\delta^{44/42}\text{Ca}$ values at 100 days (GestR) and the
448 range of experimental syn-gestation blood $\delta^{44/42}\text{Ca}$ values (figure C.5).

449 Model predictions for urine, extracellular fluids and milk $\delta^{44/42}\text{Ca}$ values for the high dairy
450 excretion scenario (LactA) are all higher than the range of experimental data obtained during
451 nursing for these fluids, for steady state as for 100-days simulations. As these fluids keep a
452 similar Ca isotopic composition between 2 and 100 days, we can postulate that 100-days
453 simulations are comparable with experimental data at 14 days of lactation (with moderate
454 reserves regarding the overlooking of non-secreting soft tissue dynamic in the model).
455 Model predictions within the low dairy excretion scenario (LactB) for extracellular fluids and
456 milk $\delta^{44/42}\text{Ca}$ values (for steady state and for 100-days simulations) fall in the range of
457 experimental data for blood and milk obtained during nursing. For urine, only 100-days
458 simulations predict $\delta^{44/42}\text{Ca}$ values, which are in the upper range of experimental urine
459 $\delta^{44/42}\text{Ca}$ values during nursing.

460 Comparisons between lactation scenarios at 1000 days with various intensity of bone loss,
461 predict that bone loss and its intensity have a negligible impact on the state of the system at
462 1000 days (i.e. on the long term), and only strongly affects bone Ca isotopic composition
463 (figure C.6). This is the logical consequence of decreasing the bone Ca residence time by
464 decreasing the bone reservoir size through bone loss. The different configurations of Ca
465 digestive excretions we tested (i.e. $\text{K} \rightarrow \text{Ur}/\text{EF} \rightarrow \text{Fs}$ ratios ranging from 0.5 to 2) show that this

466 parameter has a restricted impact on the evolution of the system, for steady state (figure
467 C.7) as for intermediate isotopic compositions (under 10^4 days).

468 Simulations with lower Ca absorption through the digestive tract (figure 8) show that
469 decreasing Ca absorption leads to lower $\delta^{44/42}\text{Ca}$ values for urine, feces and blood, and
470 eventually bone after 100 days of simulation. By dividing Ca absorption by 4 compared to
471 gestation conditions without Ca transfer to fetuses (GestFF), blood $\delta^{44/42}\text{Ca}$ values decrease
472 by about -0.20 ‰ (figure 8). The predicted $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$ in such case is -0.46 ‰, which
473 differs from the -0.26 ‰ predicted for regular GestFF conditions, and the -0.28 ‰ we
474 observe experimentally.

475 **4. Discussion**

476 *4.1. Urine isotopic stability and Rayleigh distillation process*

477 Despite the fact that pre-insemination and post-weaning steps should be similar in terms of
478 body Ca fluxes for the animals (both take place two weeks after weaning), the range of Ca
479 concentrations reported in morning-first urines is about 2.5 wider at the pre-insemination
480 step than during all the rest of the experiment (figure C.2). This suggests that Ca
481 concentrations in these urines is quite uninformative of daily urinary Ca fluxes, probably
482 because the water balance status of the animals is too variable between samplings. Urine
483 collected with that procedure however highlights a decrease of blood to urine isotopic
484 difference during lactation ($\Delta^{44/42}\text{Ca}_{\text{urine-blood}}$ change from $+1.39_{-0.12}^{+0.11}$ ‰ during gestation to
485 $+0.90_{-0.12}^{+0.11}$ ‰ during nursing, figure 4), when sow generally increase their Ca urinary losses³³.
486 This is compatible with the effect of a Rayleigh distillation process, resulting in lower
487 $\Delta^{44/42}\text{Ca}_{\text{urine-blood}}$ for higher Ca urinary excretions^{3,22,28}. As no other factor influencing

488 $\Delta^{44/42}\text{Ca}_{\text{urine-blood}}$ has yet been described, this provides additional support that a Rayleigh
489 distillation process affects Ca isotopic fractionation within kidneys.

490 This Rayleigh distillation is also a possible explanation to why urine collected during nursing
491 displays a range of $\delta^{44/42}\text{Ca}$ values which is lower than what the model predicts (figure 7,
492 LactA and LactB). Indeed, in the model the $\Delta^{44/42}\text{Ca}_{\text{urine-blood}}$ offset is constant (equal to +1.15
493 ‰^{3,17,21,23}) and does not change with Ca urinary fluxes such as expected with a Rayleigh
494 distillation process operating at Ca renal reabsorption. Taking a Rayleigh distillation process
495 into account would mechanically decrease $\Delta^{44/42}\text{Ca}_{\text{urine-blood}}$ for high Ca urinary excretions^{3,22,28}
496 (e.g. during pig lactation³³), and thus lower blood, urine, feces and milk $\delta^{44/42}\text{Ca}$ values
497 compared to predicted values in LactA and LactB simulations (figure 7). Alternatively, taking
498 into account a lower dairy Ca flux also reduces the mismatch between experimental and
499 model data (figure 7, LactB). However, if this last scenario is consistent with the lower range
500 of milk production and Ca dairy excretion we estimate (about 8.2 g of Ca per day, table B.5),
501 it seems partially incompatible with the range of Ca concentration classically reported in milk
502 (> 1500 mg/l^{33,41} compared to a minimum of 817 mg/l for this study, table B.5). The Rayleigh
503 distillation hypothesis seems thus more parsimonious to explain the difference between
504 experimental and model data in lactation conditions.

505 Thus, our observations support that a Rayleigh distillation process operates during Ca renal
506 reabsorption, but also that comparing blood and urine Ca isotopic compositions (i.e.
507 $\Delta^{44/42}\text{Ca}_{\text{urine-blood}}$) collected by following our procedure (i.e. night of fasting, morning-first urine
508 and blood collection) can be used to detect changes in daily Ca urinary excretions without
509 having to collect 24h urines. As such, this method can help monitoring Ca retention in the

510 body and bone balance, which can be useful when 24h urine collections are difficult or
511 impossible to set up (e.g. for studying large mammals other than humans).

512 *4.2. Ca isotopic fractionation in pre-insemination conditions*

513 In pre-insemination conditions, blood is depleted in heavy Ca isotopes relative to urine

514 ($\Delta^{44/42}\text{Ca}_{\text{urine-blood}} = +1.17^{+0.11}_{-0.12} \%$, $n_{\text{urine}} = 3$, $n_{\text{blood}} = 3$; figure 3a), which is conform to the value of

515 the literature ($\Delta^{44/42}\text{Ca}_{\text{urine-blood}} \approx +1.15 \%$ ^{3,17,21,23}), an isotopic fractionation generated by the

516 preferential reabsorption of light Ca isotopes from primary urines to blood in kidneys^{17,20-24,27}.

517 The same blood samples, however, have an isotopic composition very similar to bones

518 ($\Delta^{44/42}\text{Ca}_{\text{bone-blood}} = -0.08 \pm 0.11 \%$, $n_{\text{bone}} = 3$, $n_{\text{blood}} = 3$; figure 3a), which suggest a low to null

519 amplitude of isotopic fractionation between blood and bone during mineralization. This

520 agrees with other observations of low blood-bone differences of Ca isotopic compositions in

521 sheep, rats and humans^{3,27} and predictions of quantitative modeling³, but challenges

522 previous hypotheses involving a $\Delta^{44/42}\text{Ca}_{\text{bone-blood}}$ of the order of -0.6% ^{2,5,6,14,17,21-23}.

523 Similarly, the predictions of our model are only compatible with our experimental data when

524 considering a small Ca isotopic fractionation at bone mineralization (i.e. $\Delta^{44/42}\text{Ca}_{\text{bone-}}$

525 extracellular fluids between 0 and -0.1% ; figure 7, GestFF). Along with other publications on the

526 subject^{3,27}, this finding supports that the quantitative implications of kidney-mediated Ca

527 isotopic fractionation on the body isotopic equilibrium could have been underestimated, and

528 that, conversely, Ca isotopic fractionation at bone mineralization could be less pronounced

529 than previously thought^{2,3}. This mismatch between studies is likely favored by the wide

530 diversity of Ca residence time in biological tissues and fluids that are compared. For example,

531 it takes decades for bone to be at the isotopic steady state with blood (figure 6), whereas

532 blood Ca isotopic composition can likely change in minutes to hours in response to transient

533 physiological states such as food consumption. Few hours of fasting (e.g. Heuser et al. 2016¹⁷
534 and this study) likely limit the impact of these transitory events, but also provide data which
535 are not fully representative of tissue and fluid Ca isotopic compositions over a day period.
536 Thus, accurate direct comparisons are difficult outside of long-term feeding experiments
537 (several years), whereas comparisons made in the literature are punctual in nature^{2,5,6,14,17,21-}
538 ²³.

539 Nevertheless, apart from blood to bone differences, these two models of Ca isotopic
540 fractionation (i.e. small versus large Ca isotopic fractionation at bone mineralization) can
541 generate similar body Ca isotopic compositions despite their conceptual differences. For
542 example, as long as bone resorption is associated with increased Ca urinary losses and bone
543 accretion with decreased Ca urinary losses, both models stay compatible with the decrease
544 of blood and urine $\delta^{44/42}\text{Ca}$ values documented during bone loss events^{6,20-23,28}. However,
545 considering a smaller Ca isotopic fractionation at bone mineralization also provides new
546 perspectives about some observations of the literature. For example, induced bone loss in
547 Göttingen minipigs¹⁷ is not associated with higher Ca urinary excretions or with a decrease of
548 blood and urine $\delta^{44/42}\text{Ca}$ values^{17,42}. However, blood and urine $\delta^{44/42}\text{Ca}$ values do decrease for
549 humans with bone loss^{20,24,28}, while there are clues that bone loss comes with higher Ca
550 urinary excretions for them (e.g. higher Ca concentrations in urines: Eisenhauer et al., 2019;
551 Heuser et al., 2019)^{20,28}. This suggests that bone losses can be efficiently monitored with Ca
552 isotopes only when associated to increased urinary excretions, which is precisely the kind of
553 difference predicted by the model we support in this study (i.e. $\Delta^{44/42}\text{Ca}_{\text{bone-blood}} < 0.3 \text{ ‰}$,
554 $\Delta^{44/42}\text{Ca}_{\text{urine-blood}} \approx +1.15 \text{ ‰}$). Overall these results thus specify the range of physiological and
555 pathological contexts where Ca isotopes can be used to monitor bone balance.

556 4.3. Ca isotopic fractionation from diet to bone during gestation

557 The difference between diet and bone Ca isotopic composition reported in this study
558 ($\Delta^{44/42}\text{Ca}_{\text{bone-diet}} = -0.28 \pm 0.11 \text{ ‰}$, $n_{\text{bone}} = 3$, $n_{\text{diet}} = 3$) is at odds with the relatively constant value
559 generally reported among mammals (mean $\Delta^{44/42}\text{Ca}_{\text{bone-diet}} = -0.54 \pm 0.08 \text{ ‰}$ ^{2,3,5,9,13,17,27}). This
560 study is among the first to report such a different and smaller $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$ value. To our
561 knowledge, only Heuser et al. (2016)¹⁷ previously described a similarly low $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$
562 value in Göttingen minipigs with glucocorticosteroid induced osteoporosis ($\Delta^{44/42}\text{Ca}_{\text{bone-diet}} = -$
563 $0.32 \pm 0.15 \text{ ‰}$)¹⁷, although this result falls in the range of uncertainty reported in the
564 literature^{2,3,5,9,13,17,27}. This raises questions about the cause behind these differences.
565 Simulations of gestation without Ca transfer to fetal tissues (GestFF) predicts the same
566 isotopic compositions than what we observe experimentally and similarly generate a small
567 $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$ (figure 7). This suggests that apparently no exotic parameter is needed to
568 generate this small $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$. An important point is that the GestFF simulation involves
569 Ca fluxes documented during gestation with the exception of fetal Ca transfer (table B.3).
570 This is relevant as a general condition of the sows only because these domestic animals
571 underwent repetitive gestations, representing more than two-third of their life time after
572 their sexual maturity. In the wild, medium and large sized mammals generally experience
573 bigger time gaps between gestations, with $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$ documented so far arising largely
574 from wild animals, non-breeding animals or primiparous females^{5,9,13,17}. This suggests that
575 changes in Ca fluxes during gestation can change the bone-diet isotopic offset (i.e.
576 $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$).

577 The study of sow Ca balance in normal adult condition (i.e. outside of the gestation and
578 lactation periods) attracted little attention so far. However, we can reasonably assume that
579 Ca absorption in the digestive track increases during gestation compared to normal

580 conditions, with gestation involving more Ca dietary intakes in order to adapt to higher Ca
581 demands (see example for humans: Kovacs and Fuleihan, 2006)⁴³. Dividing Ca absorptions by
582 4 (compared to GestFF conditions) changes predicted $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$ values from -0.26 ‰ to -
583 0.46 ‰ (figure 8), which then falls within the range of the general $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$ documented
584 for mammals (mean $\Delta^{44/42}\text{Ca}_{\text{bone-diet}} = -0.54 \pm 0.08 \text{ ‰}$ ^{2,3,5,9,13,17,27}). This demonstration is purely
585 conceptual as only Ca absorption, fecal Ca losses and the blood→waste Ca flux are modified
586 from GestFF conditions in figure 8, whereas changing Ca absorption in a real organism would
587 likely trigger other changes in Ca fluxes (e.g. urinary). Moreover, any modification of the
588 ratio between Ca absorption and Ca urinary excretion fluxes can modify the $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$ ³.
589 Monitoring an animal population with various Ca absorption levels (e.g. with different Ca
590 content in the diet), would thus be necessary to precisely describe the effect of Ca
591 absorption on body isotopic compositions. Nevertheless, there remains a strong suspicion
592 that the small $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$ reported in this study could be the consequence of prolonged
593 higher food Ca absorptions (with a lower Ca absorption/Ca urinary excretion ratio), notably
594 caused by consecutive gestation periods. Furthermore, the reduction of the $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$
595 can also be further amplified by the repeated export of light Ca isotopes through placental
596 and milk transfers (see sections 4.4. and 4.5.).

597 This consequence of frequent reproductions is important to consider for Ca isotope studies
598 involving livestock animals, but likely plays a minor role in animal populations reproducing
599 less intensively. Thus, we can reasonably assume that this gestation effect is only a minor
600 issue for trophic studies of wild faunas involving Ca isotopes. First, because gestation
601 decreases the $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$ values but does not cancel entirely the trophic level effect (TLE).
602 Second, because this phenomenon will be active only for females with gestation periods

603 constituting the majority of their lifetime, a condition which is relevant only for a fraction of
604 females in specific mammal species and populations⁴⁴.

605 4.4. Gestation effects on body $\delta^{44/42}\text{Ca}$ values

606 As discussed in the previous section, gestation periods appear to be associated with smaller
607 $\Delta^{44/42}\text{Ca}_{\text{blood-diet}}$ and $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$ offsets, resulting in overall higher body $\delta^{44/42}\text{Ca}$ values than
608 what could be expected in normal conditions (figures 7 and 8). However, there is no major
609 change of urine and blood Ca isotopic compositions between pre-insemination and end-
610 gestation periods. We do observe a decrease of blood $\delta^{44/42}\text{Ca}$ values of about -0.2 ‰ for two
611 individuals (C1 and C2, figure 3c), but we suspect that this difference is a stochastic
612 underestimation of blood $\delta^{44/42}\text{Ca}$ inter-individual variability during the last month of
613 gestation. This phenomenon is likely because only 3 individuals have been monitored and
614 that blood $\delta^{44/42}\text{Ca}$ values from this period are by far the most homogenous of all sampling
615 steps (figure 3c). Alternatively, this could be explained by the fact that blood compositions
616 are not entirely free of nursing influence for C1 and C2 individuals at the pre-insemination
617 step, as it follows a previous weaning by only 12 to 14 days (similarly as the monitored post-
618 weaning step).

619 The fact that urines and blood $\delta^{44/42}\text{Ca}$ values remain stable, or decrease between pre-
620 insemination and end-gestation periods (figure 3), is at odds with modeling predictions
621 suggesting an increase of +0.10 to +0.15 ‰ (figure 7, difference between GestFF and GestR).
622 A possibility is that Ca isotopic fractionations between sow blood and fetal tissues are in
623 reality less pronounced than what is considered in GestR simulation (i.e. a $\Delta^{44/42}\text{Ca}_{\text{umbilical blood-}}$
624 sow blood closer to 0 ‰ than -0.18 ‰). At the moment, the data we obtained from sow blood
625 and umbilical blood at birth are the first direct data to document potential isotopic

626 fractionations associated with this flux. However, it is yet to be confirmed if the average
627 difference of -0.18 ± 0.11 ‰ we measure between these fluids (or -0.14 ± 0.11 ‰ for the
628 best temporal match) is representative of a full gestation period or only of birth. Several
629 independent clues suggest that the general $\Delta^{44/42}\text{Ca}_{\text{umbilical blood-sow blood}}$ offset could be closer to 0
630 ‰. For example, the individual C2 carried only 2 piglets against 16 and 18 piglets for C1 and
631 C3, which should result in Ca placental transfers that would be 5 to 6 times smaller for C2
632 (see total piglet Ca mass in Table B.5). However, despite this huge difference and the fact
633 that these Ca transfers should peak around the sampling period ^{45,46}, body Ca isotopic
634 compositions of C2 do not exhibit an exotic pattern between pre-insemination and late
635 gestation periods compared to C1 and C3 (figure 3). Additionally, the two juveniles who died
636 at and closely after birth have bone Ca isotopic compositions undistinguishable from adults
637 (figure 2). A similar observation has been documented between human young infants and
638 adults who display low to absent difference between their bone Ca isotopic compositions ⁸.
639 This seems partly incompatible with the low $\delta^{44/42}\text{Ca}$ values recorded in human enamel
640 growing in utero ^{18,19}, but could be explained if umbilical blood Ca is only significantly ⁴⁴Ca-
641 depleted during a relatively short period before birth, such as suggested by other human
642 enamel data ¹⁵. If further investigations confirm that the general Ca isotopic fractionation
643 between gestating females and their fetuses is small, this would make gestation model
644 predictions (GestR) converge with gestation predictions without Ca placental transfer
645 predictions (GestFF), with the bulk fetus Ca isotopic composition being equal to sow
646 extracellular fluids in average (figure 7, GestFF). Nevertheless, the fact that Ca isotope body
647 compositions predicted for 100-days gestation simulations (GestR) remain in the range of
648 pre-insemination experimental $\delta^{44/42}\text{Ca}$ values (figure 7), emphasizes that even a Ca isotopic
649 fractionation factor of 0.99982 at Ca placental transfers from sow to fetuses (representative

650 of a $\Delta^{44/42}\text{Ca}_{\text{umbilical blood-sow blood}} = -0.18 \text{ ‰}$) would have a restricted effect on sow body Ca
651 isotopic compositions. Finally, contrary to what we consider in the model of this study
652 (figure 5), Ca transfer does occur in reality from the umbilical reservoir to the sow blood
653 reservoir ⁴⁷. We can suppose that this flux is associated with an isotopic fractionation factor
654 equal to 1 or less (because this is generally what is observed for trans-membrane
655 transport³). As such, this flux likely attenuates the effect of placental Ca transfers on sow
656 body composition and further explains why we do not detect $\delta^{44/42}\text{Ca}$ differences between
657 bones from newborn and adults, or between C2 $\delta^{44/42}\text{Ca}$ patterns and those of the other
658 specimens.

659 These results also provide new insights about the higher bone $\delta^{44/42}\text{Ca}$ values ($+0.14 \pm$
660 0.08 ‰) documented for ewes when compared to male sheep from the same herd ¹⁴. With a
661 model which is very different from that of this study, as it notably involves important Ca
662 isotopic fractionation at bone mineralization and no renal fractionation, the authors pointed
663 to bone accretion during gestation as one of the potential drivers of the sexual isotopic
664 difference. Our data do not support this hypothesis, because we do not observe any marked
665 increase in blood $\delta^{44/42}\text{Ca}$ values during gestation, while blood is expected to experience an
666 even greater increase of $\delta^{44/42}\text{Ca}$ values than bone in such a scenario. This can be caused by
667 the fact that sows did not experience bone accretion over the course of their gestation, but
668 can be explained more generally because bone mineralization seems associated with a Ca
669 isotopic fractionation factor closer to 1 than previously thought (see section 4.2. and ^{2,3}).
670 Nevertheless, we demonstrated that gestation periods could increase body $\delta^{44/42}\text{Ca}$ values
671 for other reasons than bone accretion (figure 8 and section 4.3.). To some extent, gestation
672 periods are thus likely contributing to the higher bone $\delta^{44/42}\text{Ca}$ values documented in female
673 sheep ¹⁴.

674 4.5. Control of lactation on body $\delta^{44/42}\text{Ca}$ values

675 Our experiment highlights a significant increase of adult blood $\delta^{44/42}\text{Ca}$ values during nursing
676 compared to pre-parturition values ($\Delta^{44/42}\text{Ca}_{\text{nursing blood-gestation blood}} = +0.42^{+0.11}_{-0.12}\text{‰}$, figure 3c),
677 joining similar observations done in mice⁹. Giesemann et al. (1998)³³ showed that besides
678 milk production, lactation in sows was associated with bone resorption as well as higher Ca
679 dietary absorption, digestibility and urinary excretions. Having higher Ca urinary losses
680 during lactation is however not a constant among mammals. This is notably the opposite of
681 what was documented for humans⁴³, and emphasizes why changes in $\Delta^{44/42}\text{Ca}_{\text{urine-blood}}$ (figure
682 4, discussed in section 4.1.) cannot be used as a universal lactation signal. Simulations
683 highlight that higher Ca dietary absorption, urinary excretion and milk production conjointly
684 increase blood $\delta^{44/42}\text{Ca}$ values during lactation (figure 7, 8, C.6, C.7), with milk production
685 being the dominant factor of this isotopic change. As a flux, Ca dairy excretion exceeds Ca
686 urinary excretions by two orders of magnitude (table B.5 and³³) and constitutes by far the
687 biggest change from normal or gestation conditions to lactation conditions. This flux comes
688 with important Ca isotopic fractionation at milk production, as suggested by $\Delta^{44/42}\text{Ca}_{\text{milk-diet}}$
689 data (-0.6 ‰¹³; $-0.58 \pm 0.12 \text{‰}$, this study) and $\Delta^{44/42}\text{Ca}_{\text{milk-blood}}$ data ($-0.67 \pm 0.12 \text{‰}$, this
690 study), and therefore strongly affects blood and the whole body Ca isotopic compositions.
691 This is particularly clear when comparing low versus high milk production simulations (LactA
692 and LactB, figure 7). A decrease of about half the Ca dairy excretion (between LactA and
693 LactB) results in a decrease of $\delta^{44/42}\text{Ca}$ values of the order of 0.25 ‰ in extracellular fluids
694 (i.e. blood), urine and milk. The increase of $\delta^{44/42}\text{Ca}$ values in the maternal blood during
695 nursing can therefore be described as a lactation signal which exceeds the effects of
696 gestation. Nevertheless, the amount of milk produced per day and Ca dietary intakes also
697 change a lot between species. This heterogeneity between mammal species could thus be an

698 important modulatory factor for the expression of this lactation signal among mammal
699 species.

700 *4.6. Lactation signal record within mineralized tissues*

701 In terms of rate, body Ca reservoirs are expected to react very rapidly after the onset of
702 lactation Ca fluxes, with the exception of bone (figure 6). In our experiment however, these
703 changes seem to appear only after a few days from parturition (e.g. 5 days for C3, figure 3c).
704 This minor inconsistency can be explained partly by the fact that our model neglects the Ca
705 storage in non-secreting organs and soft tissues, but also by the lower dairy Ca excretion
706 through colostrum and milk during the first days following parturition ⁴⁸. As for its onset, the
707 blood lactation signal we describe also attenuates rapidly (in few weeks) as the sow comes
708 back to normal physiological conditions. It seems to be the case 14 days after weaning, with
709 blood post-weaning $\delta^{44/42}\text{Ca}$ values matching pre-insemination values (this is particularly
710 clear for the C3 individual, figure 3c), although we cannot guarantee that the pre-
711 insemination step was completely free of nursing influence (only 12 to 14 days separate this
712 step from the weaning of the previous litter). Preserving such a lactation signal within
713 mineralized tissues (i.e. bones and teeth) is thus only possible if the lactation is sustained for
714 enough time. For bones, the model predicts about 100 days before a significant change can
715 be recorded (figure 6) and a shorter time if bone loss is involved (figure C.6). A similar animal
716 with longer nursing period than sows would thus likely preserve a lactation signal within
717 bone, providing that it dies close enough from a lactation period to not attenuate the signal
718 too much with bone remodeling (in the model 50% of the Ca of bones is renewed after
719 about 3.8 years). Nevertheless, preserving such signal in teeth that would mineralize during
720 a lactation period is also possible for certain mammal species and would likely constitute an
721 even better record considering how enamel grows and preserves isotopic compositions ⁴⁹.

722 As previously mentioned, it has been shown that modern ewes have higher bone $\delta^{44/42}\text{Ca}$
723 values than males within the same herd¹⁴. Besides bone accretion during gestation, the
724 authors pointed to milk excretion as an alternative driver of this sexual isotopic difference.
725 Our study supports that such signals can result from Ca flux changes during lactation, mainly
726 because of the milk excretion, but also to a lesser extent because of the increase of dietary
727 Ca intakes and urinary excretion during lactation and preceding gestation. The average
728 $\Delta^{44/42}\text{Ca}_{\text{females-males}}$ reported in bones of ewes compared to male sheep ($+0.14 \pm 0.08 \text{‰}$)¹⁴, falls
729 within the range of what could be reasonably expected for pigs if they sustained a lactation
730 over 1 year without undergoing bone loss (figure 6). Although this is longer than what ewes
731 usually do and very far from sow typical nursing duration⁵⁰, lactation induced bone loss can
732 increase the bone reactivity to lactation isotopic changes and likely helps recording such a
733 lactation signal (figure C.6). Consecutive gestations and lactations, such as classically
734 experienced by livestock animals, and a death relatively close from lactation, are also
735 favorable factors to preserve such signals. This makes lactation a very suitable candidate to
736 explain differences in bone Ca isotopic composition between sexes of adult mammal
737 populations having a similar diet, such as observed for modern ewes¹⁴. Besides, this
738 provides an additional explanation to why human populations studied so far do not exhibit
739 such differences^{8,14}. Obviously the predictions of our model cannot be directly applied to
740 sheep, as many Ca fluxes and reservoir sizes change between sow and sheep in addition to
741 the length of lactation of these animals. However, our model and experimental data provide
742 the basics and order of magnitude of what could be expected in other medium to large
743 mammal species. Considering all of that, preserving this lactation signature within bones or
744 teeth of other mammal species, including extinct ones, seems plausible. This would allow
745 studying past lactation habits using bone or teeth Ca isotopic compositions, but could

746 concurrently complicate the use of Ca isotopes as a trophic indicator. However, our study
747 suggests the timings and lengths of lactation periods necessary to preserve such a lactation
748 signal likely concern a fraction of mammal species only⁴⁴. Additionally, the sheep population
749 studied by Reynard et al. (2010)¹⁴ suggests that this signature of the lactation tends to stay
750 of small amplitude ($+0.14 \pm 0.08 \text{ ‰}$)¹⁴, despite the fact that females from this population
751 went through 3 consecutive gestations and lactations in that study. Thus, the effects of
752 lactation we describe here do not seem to be a major issue for using Ca isotopes as a trophic
753 indicator, especially when studying wild faunas.

754 **5. Conclusion**

755 Through modeling and a longitudinal monitoring of a sow population, this study provides
756 new insights about the mammalian isotopic Ca cycle. Our data support a model of Ca
757 isotopic fractionation with a less pronounced fractionation during bone mineralization than
758 previously proposed ($\Delta^{44/42}\text{Ca}_{\text{bone-blood}}$ below -0.3 ‰ instead of about -0.6 ‰), a change which
759 specifies the range of physiological and pathological contexts where Ca isotopes can be used
760 to monitor bone balance. Although of small amplitude and apparently not representative of
761 the full length of the gestation, we detected that umbilical blood is ⁴⁴Ca-depleted compared
762 to maternal blood ($\Delta^{44/42}\text{Ca}_{\text{umbilical blood-sow blood}} = -0.18 \pm 0.11 \text{ ‰}$, $n = 3$; or $-0.14 \pm 0.11 \text{ ‰}$ for the
763 best temporal match). Apart from this fractionation, gestation seems to be associated with
764 overall higher body $\delta^{44/42}\text{Ca}$ values and lower isotopic differences between diet and bones
765 than for other mammals in normal physiological conditions ($\Delta^{44/42}\text{Ca}_{\text{bone-diet}} = -0.28 \pm 0.11 \text{ ‰}$),
766 possibly because of higher Ca absorptions by the digestive track. Lactation periods are
767 associated with even higher blood $\delta^{44/42}\text{Ca}$ values ($\delta^{44/42}\text{Ca}_{\text{blood}}$ change of $+0.42^{+0.11}_{-0.12} \text{ ‰}$ during
768 nursing) and with small isotopic differences between urine and blood ($\Delta^{44/42}\text{Ca}_{\text{urine-blood}} =$

769 $+0.90^{+0.11}_{-0.12}\text{‰}$), although this last observation is likely a less universal lactation marker. The
770 high blood $\delta^{44/42}\text{Ca}$ values are mainly caused by milk production and excretion, and are likely
771 the main cause of male versus female differences in bone Ca isotopic composition
772 documented in certain mammal populations. According to our model, the preservation of
773 such lactation events in bone is favored by greater Ca dairy excretions, by longer lactation
774 periods, by smaller time gaps between nursing periods, as well as by a death or bone
775 sampling close to the lactation period.

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786 **8. Data Availability Statements**

787 The data underlying this article are available in the article and in its online supplementary
788 material. The Isopybox program used for building box model simulations and its resources
789 are accessible on Github at the following address: <https://github.com/ttacail/isopybox.git>.

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895

896 **10. Figures and tables**

897 *Figure 1. Sampling time chart*

898 Key steps and periods within the reproduction cycle of monitored specimens are
899 represented at the top of the figure. Sampling operations have been essentially conducted
900 during five key periods represented as dashed red area in the time chart and delimited by
901 dashed lines. Starting and ending dates of these periods are indicated at the bottom of the
902 time chart. From left to right these periods are representing: pre-insemination conditions,
903 the last month of gestation, the post-parturition period, nursing and post-weaning
904 conditions. Each sampling which lead to a $\delta^{44/42}\text{Ca}$ measure is represented by a colored bar.

905 *Figure 2. Fluid and tissue Ca isotopic compositions*

906 Ca isotopic compositions of all tissues and fluids collected from C1, C2, C3 and juvenile
907 individuals, expressed in ‰ as $\delta^{44/42}\text{Ca}_{\text{ICP Ca Lyon}}$ and $\delta^{44/42}\text{Ca}_{\text{SRM915a}}$ values. In these boxplots, the
908 central line represents the median, boxes are limited by their 1st and 3rd quartiles, and
909 whiskers extend to maximum and minimum values \pm the uncertainties. The light green
910 shaded area represent the range of Ca isotopic composition of the diet with the mean
911 identified by the dashed line.

912 *Figure 3. Ca isotopic compositions over time*

913 Ca isotopic compositions of all samples collected from C1, C2 and C3, expressed in ‰ as
914 $\delta^{44/42}\text{Ca}_{\text{ICP Ca Lyon}}$ and $\delta^{44/42}\text{Ca}_{\text{SRM915a}}$ values over time. (a) All tissue and fluid Ca isotopic

915 compositions, (b) urine focus, (c) blood focus. Arrows, dashed black lines and associated P
916 and W marks on the x temporal axis respectively highlight parturition and weaning dates.
917 The size of the C2 colostrum data point is increased to make it visible despite the
918 superposition with the C2 umbilical blood data point. Error bars represent the range of
919 uncertainty around each value.

920 *Figure 4. Temporal evolution of $\Delta^{44/42}\text{Ca}_{\text{blood}}$ values*

921 Summary of $\Delta^{44/42}\text{Ca}_{\text{X-blood}}$ values (i.e. the difference between blood and other sample $\delta^{44/42}\text{Ca}$
922 values). For each individual, sample Ca isotopic compositions have been compared with
923 blood Ca isotopic compositions of the same sampling step, with the exception of bones
924 which are compared with pre-insemination blood $\delta^{44/42}\text{Ca}$ values. The shaded orange area
925 covers the range of urine $\Delta^{44/42}\text{Ca}_{\text{X-blood}}$ values \pm the error bars. The orange line represents the
926 moving average of $\Delta^{44/42}\text{Ca}_{\text{X-blood}}$ values calculated for each sampling step. The size of the C2
927 colostrum data point is increased to make it visible despite the superposition with the C2
928 umbilical blood data point.

929 *Figure 5. Model of Ca cycle*

930 Box model of Ca body cycle adapted from the human model proposed by Tacail, 2017³. The
931 purple arrow and box are implemented to the model for gestation runs. The blue arrow and
932 box are implemented to the model for lactation runs. Yellow arrows represent Ca fluxes
933 going to the waste box, a theoretical reservoir of virtually infinite size which prevents Ca
934 output fluxes to interact with the rest of the system. The dashed yellow arrow is a
935 conceptual flux associated with no isotopic fractionation, notably used to nullify the growth
936 of the sow-like animal (see section 2.6.). The $\Delta^{44/42}\text{Ca}$ values considered in the model are
937 specified along their flux (hypotheses behind these values are specified in table B.4)

938 *Figure 6. Model evolution to steady state*

939 Evolution of Ca isotopic composition of sow Ca reservoirs in general conditions (GestFF),
940 gestating conditions (GestR), and lactating condition with higher or lower dairy Ca excretion
941 (LactA and LactB, respectively). The x axis represents the time in days. These graphs
942 represent scenarios with no Ca isotopic fractionation at bone mineralization (i.e. $\alpha_{B-EF} = 1$),
943 however only bone Ca isotopic composition is notably affected by this fractionation factor.
944 Initial conditions of these simulations are summarized in tables B.2, B.3, B.4 and further
945 detailed in section 3.3.

946 *Figure 7. Model and experimental data comparison*

947 Comparison of modeling and experimental data in general condition (GestFF), gestating
948 condition (GestR), and lactating conditions with higher or lower dairy Ca excretion (LactA
949 and LactB, respectively). Points represent simulation results whereas colored zones
950 represent the range of $\delta^{44/42}\text{Ca}$ values measured during our experiment at comparable stage
951 of the reproductive cycle. Simulation results are presented at day 100 and after 1000 days
952 when the sow system is in steady state (noted std*). Steady state simulations presented
953 here consider different Ca isotopic fractionation factor at bone mineralization (i.e. α_{B-EF}
954 between 1 and 0.9994), representing a $\Delta^{44/42}\text{Ca}_{\text{bone-blood}}$ offset between 0 and 0.6 ‰ (only
955 bone is affected by this value). Bone data points tend toward red colors for $\alpha_{B-EF} = 1$ (i.e. no
956 Ca isotopic fractionation at bone mineralization) and light blue colors for $\alpha_{B-EF} = 0.9994$, with
957 0.0001 of difference between each neighbor color level. GestFF results are compared with
958 pre-inseminations $\delta^{44/42}\text{Ca}$ values for urine and blood and with post-weaning $\delta^{44/42}\text{Ca}$ values
959 for bones (see section 3.3.). GestR $\delta^{44/42}\text{Ca}$ values are compared with late gestation urine,
960 sow blood and umbilical blood $\delta^{44/42}\text{Ca}$ values at parturition. LactA and LactB are compared
961 with urine, blood and milk $\delta^{44/42}\text{Ca}$ values from the nursing period. Bone $\delta^{44/42}\text{Ca}$ values are

962 not displayed for 100 day simulations, because at this time bone are not yet significantly
963 affected by changes in body Ca isotopic composition (see figure 6).

964 *Figure 8. Ca absorption effect on body $\delta^{44/42}\text{Ca}$ values*

965 Body Ca isotopic compositions predicted by our model at steady state in general condition
966 (GestFF), with various Ca isotopic fractionation factor during bone mineralization (i.e. $\alpha_{\text{B-EF}}$),
967 and various Ca absorption levels in the digestive track. Bone $\delta^{44/42}\text{Ca}$ predicted values are
968 confounded with extra cellular fluids values for $\alpha_{\text{B-EF}} = 1$ (i.e. no Ca isotopic fractionation at
969 bone mineralization), and tend toward light blue colors for $\alpha_{\text{B-EF}} = 0.9994$, with 0.0001 of
970 difference between each neighbor color level. Absorption levels presented on the x axis
971 range from 100% (left) to 25% (right) of Ca absorptions described by Giesemann et al. (1998)
972 during gestation (table B.3). The grey shaded area represents the range of food $\delta^{44/42}\text{Ca}$
973 values from our experiment (uncertainties included), while the black line represents the
974 average food $\delta^{44/42}\text{Ca}$ value. The green shaded area represents the range of bone $\delta^{44/42}\text{Ca}$
975 values expected with a mean $\Delta^{44/42}\text{Ca}_{\text{bone-diet}}$ of $-0.54 \pm 0.08 \text{‰}$ ^{2,3,5,9,13,17,27}.

976 **11. Appendix A - Supplementary text**

977 *Text A.1 - Labware, consumables and cleaning procedures*

978 This section provides additional details about the equipment used for this study as well as
979 cleaning procedures performed before its use.

980 *Text A.2 – Uncertainty of Ca isotopic compositions*

981 This section describes in details how uncertainties presented in this study are calculated.

982 *Text A.3 - Box model conception*

983 This section describes with more details the conception of box-model simulations discussed
984 in this paper.

985 **12. Appendix B - Supplementary tables**

986 *Table B.1 - Chromatography*

987 Three column chromatography procedure. This procedure is adapted from the methods of
988 Tacail et al. (2014)²⁷ and Le Goff et al. (2021)³⁶.

989 *Table B.2 - Model input (Initial reservoir sizes)*

990 Reservoir sizes are expressed in mg of Ca and estimated from the following references
991 ^{3,27,32,59-61}.

992 *Table B.3 - Model input (Ca fluxes)*

993 Ca fluxes are described for the following simulations: GestFF, GestR, LactA, LactB, LactL,
994 LactH and for GestFF with 75 % less Ca absorption in the digestive track. The bulk of these
995 estimation are retrieved from Giesemann et al. (1998)³³, completed and compared with data
996 from the present study and the following references ^{3,32,34,35,58,62-64}.

997 *Table B.4 - Model input (Ca isotopic fractionation)*

998 We considered 6 Ca isotopic fractionation process in our model, based on data from this
999 study and the literature ^{2,3,5,13,14,17,21-23,27}.

1000 *Table B.5 - Zootechnical data*

1001 This table outline important zootechnical data collected from our experiment, such as the
1002 number of piglet per sow, weight and milk production estimations.

1003 *Table B.6 - Isotopic and elemental concentration data*

1004 This table synthetize all the data of Ca isotopic composition and elemental concentrations
1005 collected in this study, respectively expressed in ‰ and mg/kg of lyophilized sample (or mg/l
1006 for urine samples). In accordance with the data reported in appendix of Martin et al. (2018)¹⁰
1007 the uncertainties around $\delta^{44/42}\text{Ca}_{\text{SRM } 915a}$ values reported in this table are equal to the sum

1008 between $\delta^{44/42}\text{Ca}_{\text{ICP Ca Lyon}}$ uncertainties and 0.025 ‰, the uncertainty around the $\delta^{44/42}\text{Ca}$ value
1009 of SRM915a measured against the ICP Ca Lyon. Sampling step labels correspond to periods
1010 at pre-insemination (AvIA), last month of gestation (G3), parturition (MB), nursing (M14) and
1011 post-weaning (S14).

1012 **13. Appendix C - Supplementary figures**

1013 *Figure C.0 – SRM bootstrapping and baseline uncertainties*

1014 This figure synthetizes the results of different random samplings with replacement
1015 performed in the dataset of SRM1486 $\delta^{44/42}\text{Ca}$ values collected for this study. For each
1016 graphs, this random sampling collected a number n^* of values (3, 4, 6 or 10) within the
1017 SRM1486 dataset constituted by 37 original values. A mean is calculated for each sampling
1018 of n^* values and the procedure is repeated 100 000 times for each n^* level. The difference
1019 between these means and the true mean of the dataset is calculated and the distribution of
1020 the results is presented within the graphs (in absolute value). Green zones represent 95 % of
1021 the means from the bootstrapping that were the closest from the true mean, red zones
1022 represent the 0.1 % of the means from the bootstrapping that were the most different from
1023 the true mean, blue zones represent intermediate values. For $n^* = 3$ (i.e. three sampling with
1024 replacement), a number quite representative of the number of replicates for the $\delta^{44/42}\text{Ca}$
1025 measures of samples from this study, 95 % of means from the bootstrapping fall within a \pm
1026 0.05 ‰ range around the true mean of the SRM1486 dataset. We designated this value as a
1027 minimum uncertainty around sample $\delta^{44/42}\text{Ca}$ measures.

1028 *Figure C.1 - Three isotopes plot*

1029 In this figure $\delta^{43/42}\text{Ca}$ values are plotted as a function of $\delta^{44/42}\text{Ca}$ values (‰, ICP Ca Lyon) for all
1030 samples and reference materials of this study, in black and green respectively. Ca isotopic

1031 compositions fall on a line with a y-axis intercept of -0.003 ± 0.005 ‰ (2 standard errors),
1032 indistinguishable from theoretical 0 ‰ intercept. The slope value of this line is 0.502 ± 0.007
1033 (2 standard errors), virtually identical to the 0.507 slope predicted by the exponential mass-
1034 dependent fractionation law. The two most external blue lines delimit the prediction
1035 interval, whereas the two red lines correspond to the 95 % confidence interval of the
1036 regression line. The regression line is represented in blue and is assimilated with the black
1037 dashed line representing the theoretical function (visible at the bottom left of the graph).
1038 The average 2 standard errors for $\delta^{43/42}\text{Ca}$ and $\delta^{44/42}\text{Ca}$ values is represented as a blue cross at
1039 the bottom right of the graph.

1040 *Figure C.2 - Ca concentration variability over time*

1041 (a) Evolution of Ca concentrations in morning urines over the experiment period. (b)
1042 Evolution of Ca concentrations in blood dry fraction over the experiment period. These
1043 concentrations are expressed in mg of Ca per kg of urine and lyophilized blood, respectively.

1044 *Figure C.3 - Correlation between Ca concentration and isotopic composition*

1045 (a) Logarithmic correlation between urine Ca isotopic compositions and urine Ca
1046 concentrations ($R^2 = 0.37$, p-value = 0.04). (b) Linear correlation between urine Ca isotopic
1047 compositions and urine Ca concentrations ($R^2 = 0.35$, p-value = 0.04). (c) Linear correlation
1048 between Ca isotopic compositions and Ca concentrations in blood dry fraction ($R^2 < 0.01$, p-
1049 value = 0.89).

1050 *Figure C.4 - $\Delta^{44/42}\text{Ca}_{\text{x-blood}}$ summary*

1051 The graph summarizes the data $\Delta^{44/42}\text{Ca}_{\text{x-blood}}$ collected in this study. For each individual,
1052 sample Ca isotopic compositions have been compared with blood Ca isotopic compositions

1053 of the same sampling step, with the exception of bones which are compared with pre-
1054 insemination blood $\delta^{44/42}\text{Ca}$ values.

1055 *Figure C.5 - 100-day gestation and lactation simulations with variable α_{B-EF}*

1056 Body Ca isotopic compositions predicted by our model at 100 days in gestation condition
1057 (GestR), and lactation conditions with higher or lower milk Ca output (LactA and LactB
1058 respectively). For each of these conditions, we simulated various degree of Ca isotopic
1059 fractionation at bone mineralization (i.e. α_{B-EF} include between 1 and 0.9994), representing
1060 $\Delta^{44/42}\text{Ca}_{\text{bone-blood}}$ offsets between 0 and 0.6 ‰. Colored areas represent the range of
1061 experimental data for urine (yellow), blood (red), fetal tissues (purple) and milk (blue). GestR
1062 $\delta^{44/42}\text{Ca}$ values are compared with late gestation urine, sow blood and syn-parturition
1063 umbilical blood $\delta^{44/42}\text{Ca}$ values. LactA and LactB are compared with urine, blood and milk
1064 $\delta^{44/42}\text{Ca}$ values from the nursing period.

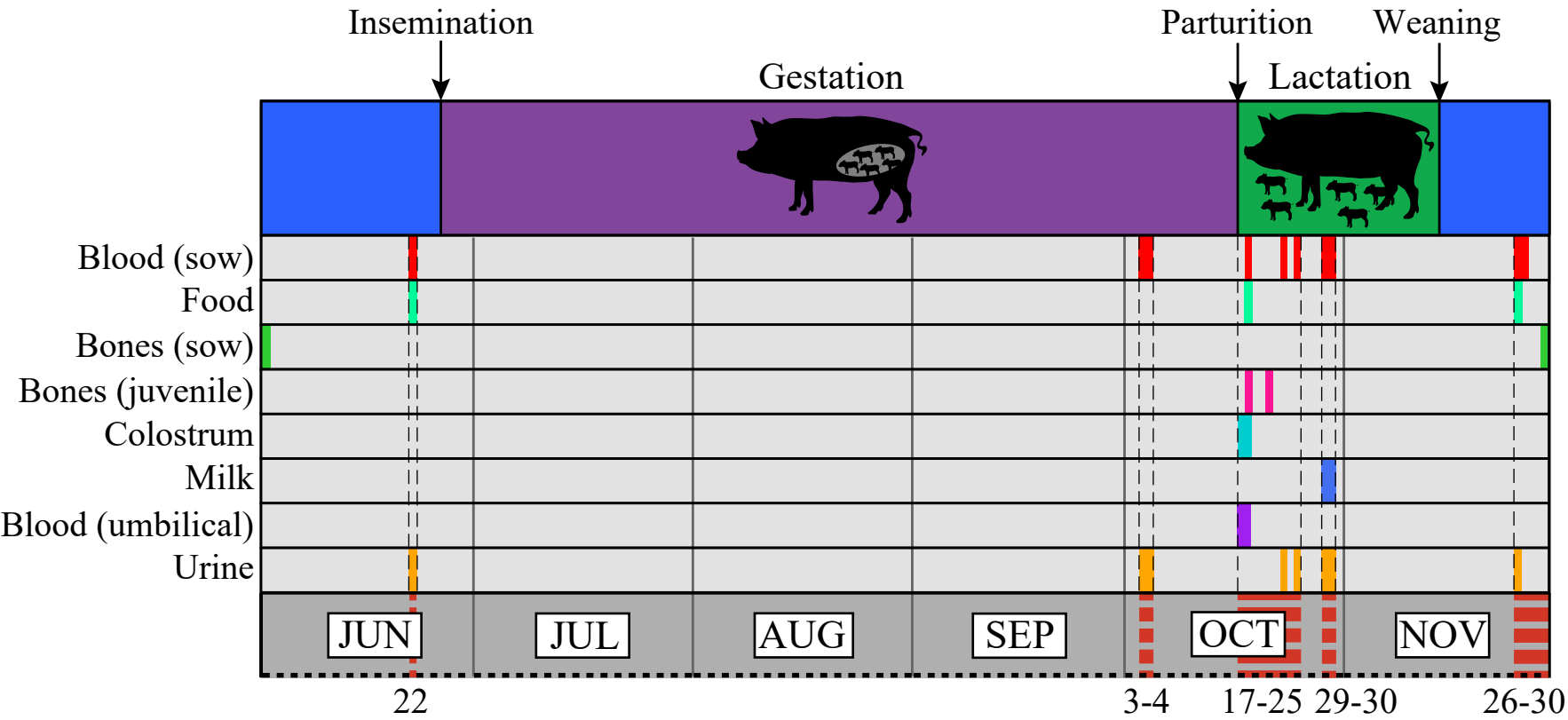
1065 *Figure C.6 - 1000-day lactation simulations with variable α_{B-EF} and degree of bone loss*

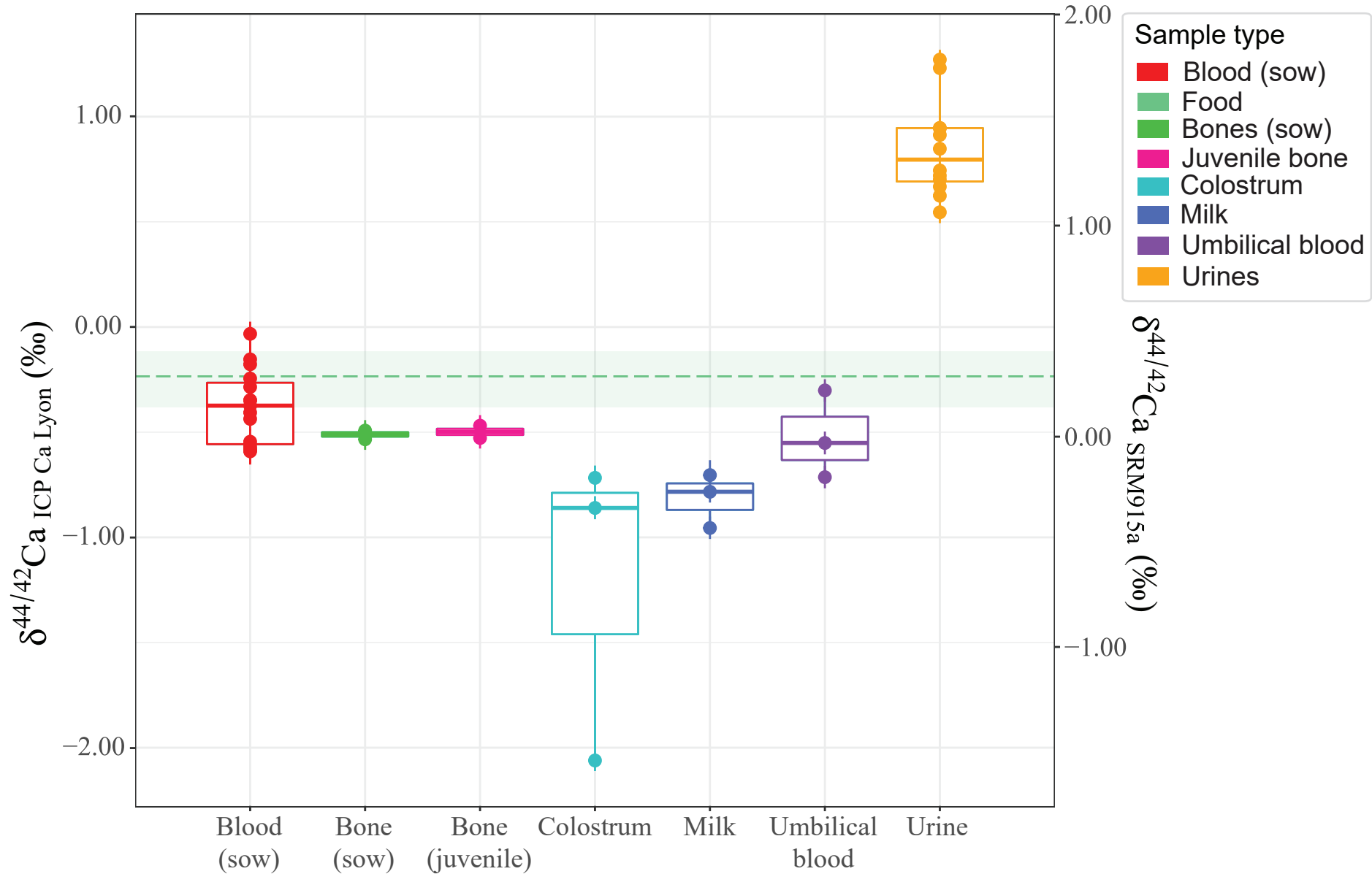
1066 Body Ca isotopic compositions predicted by our model at 1000 days in lactation conditions
1067 without bone loss (LactA), with moderate bone loss (LactL) or important bone loss (LactH).
1068 For each of these situations, we simulated various degree of Ca isotopic fractionation at
1069 bone mineralization (i.e. α_{B-EF} include between 1 and 0.9994), representing $\Delta^{44/42}\text{Ca}_{\text{bone-blood}}$
1070 offsets between 0 and 0.6 ‰.

1071 *Figure C.7 - steady state simulations with variable $K \rightarrow \text{Ur}/EF \rightarrow Fs$ ratio*

1072 Body Ca isotopic compositions predicted by our model at steady state (for the sow) in
1073 normal condition (GestFF), gestation condition (GestR) and lactation condition (LactA). These
1074 simulations are done with a α_{B-EF} of 1 and variable ratio of urinary versus endogenous Ca

1075 losses (i.e. K_{ur} / EF_{Fs}), from 0.5 to 2. We changed this ratio by modifying Ca endogenous
1076 losses while keeping Ca urinary losses constant.



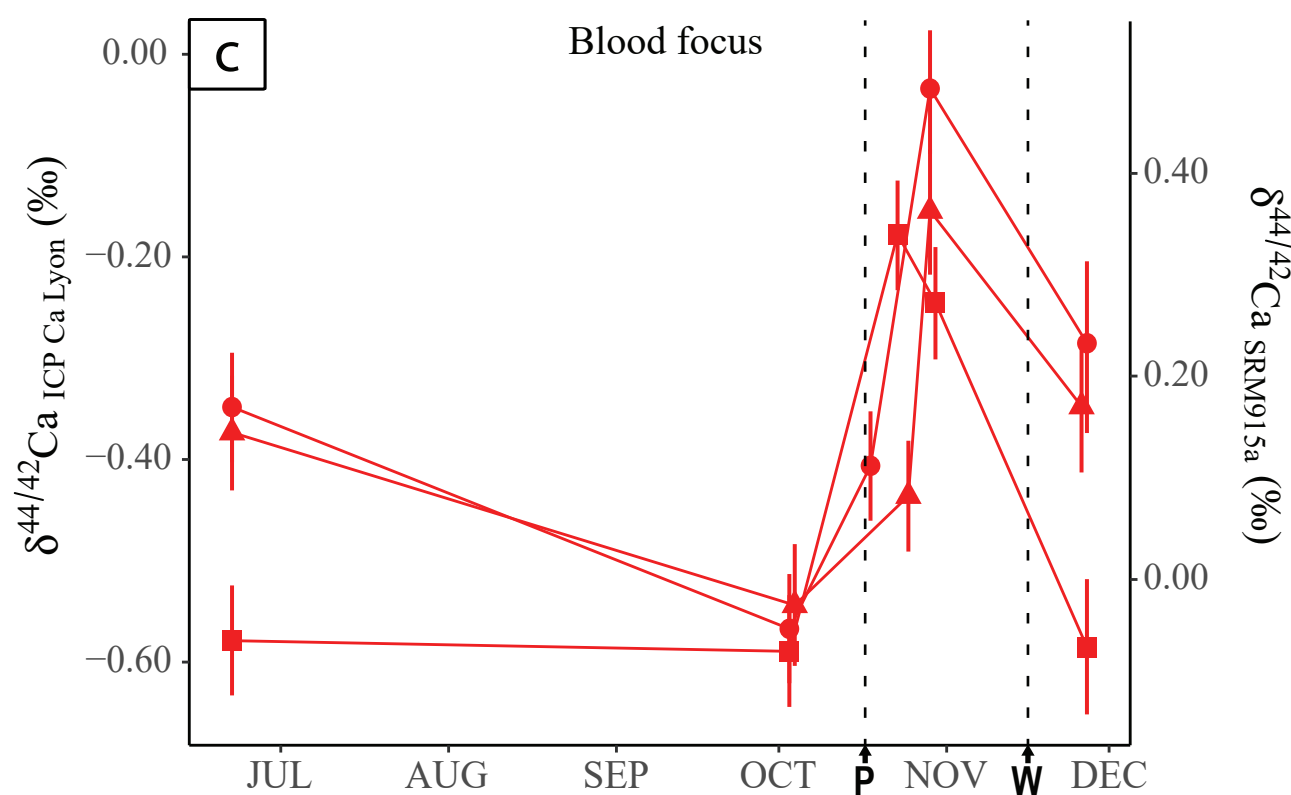
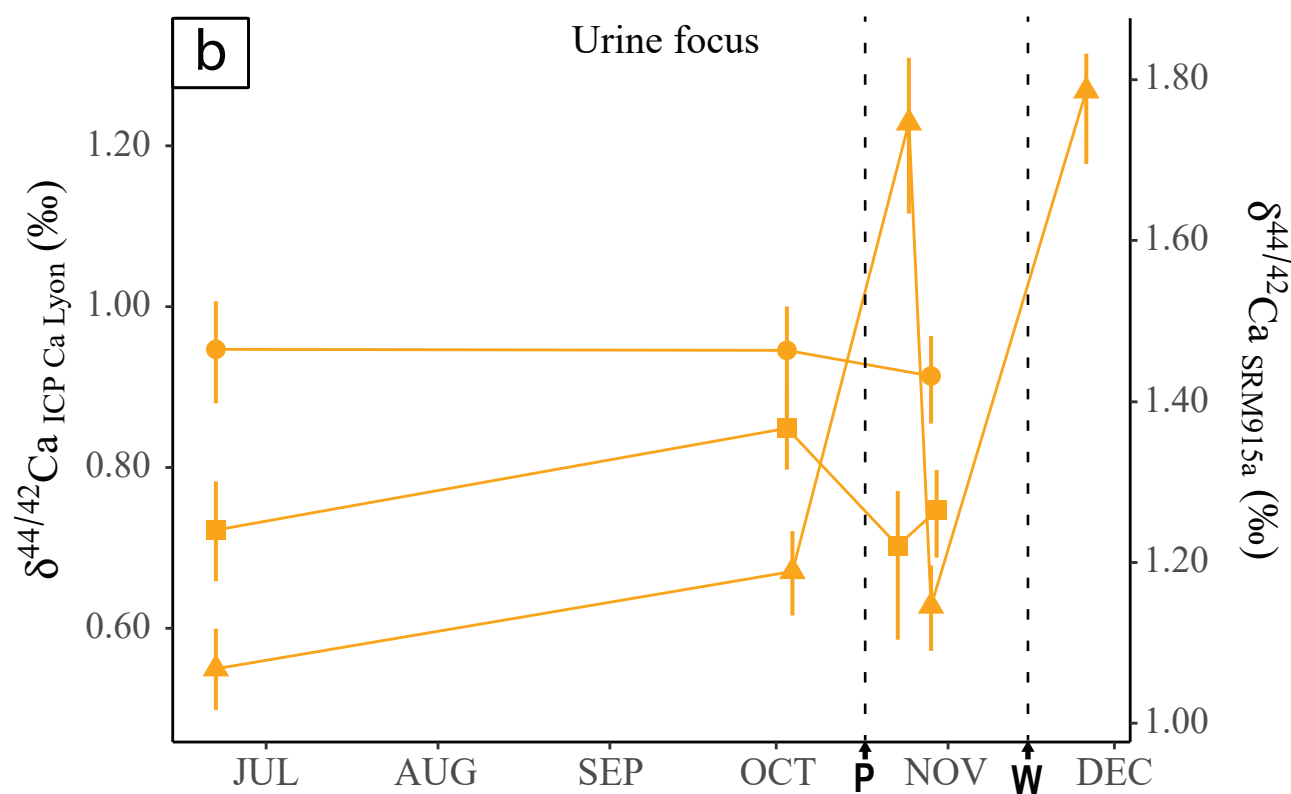
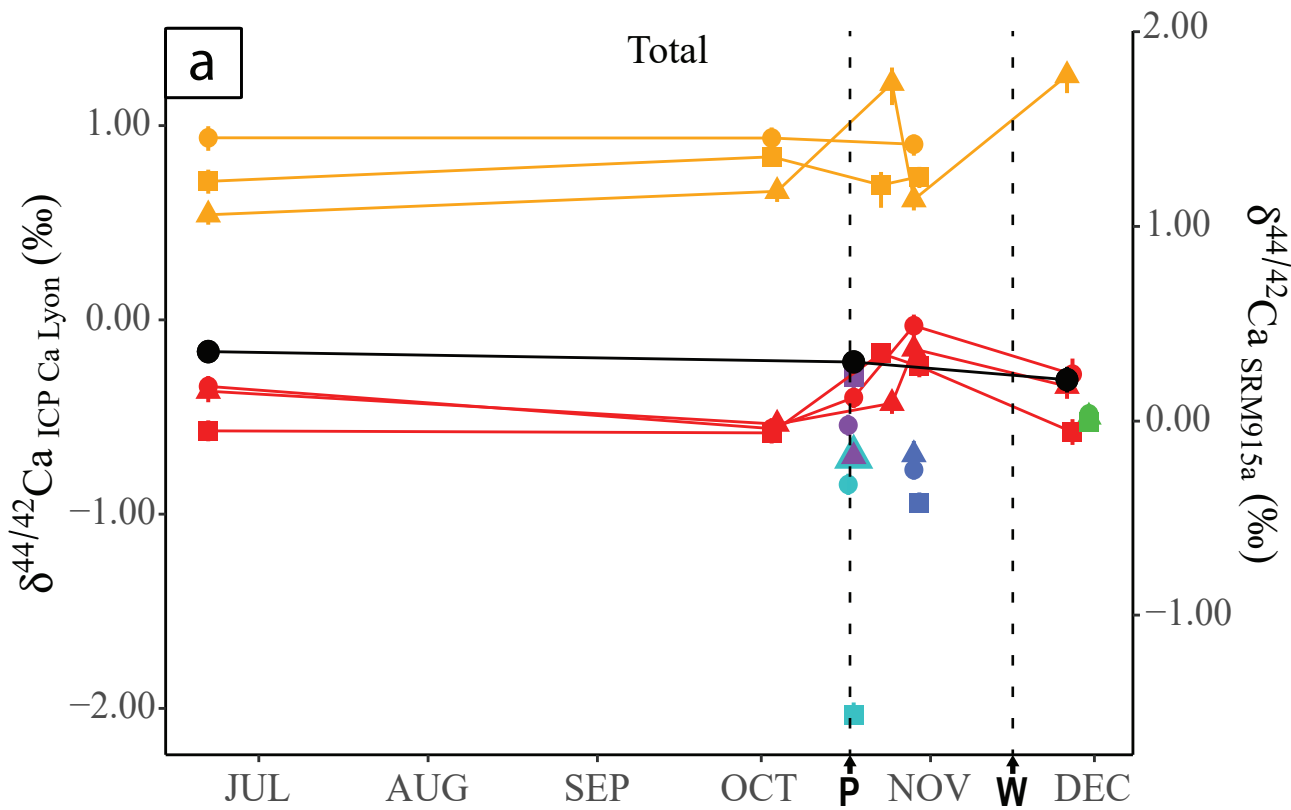


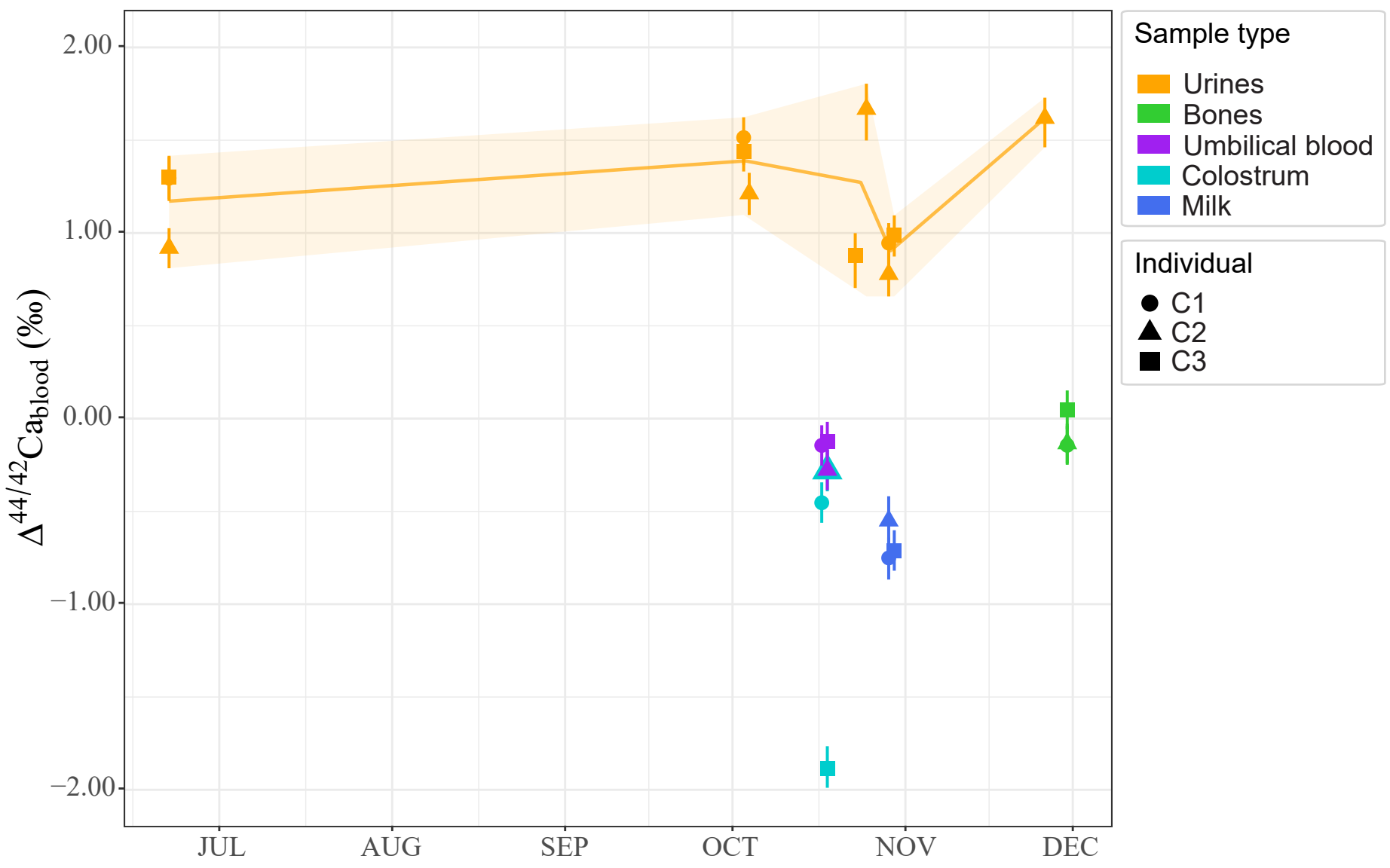
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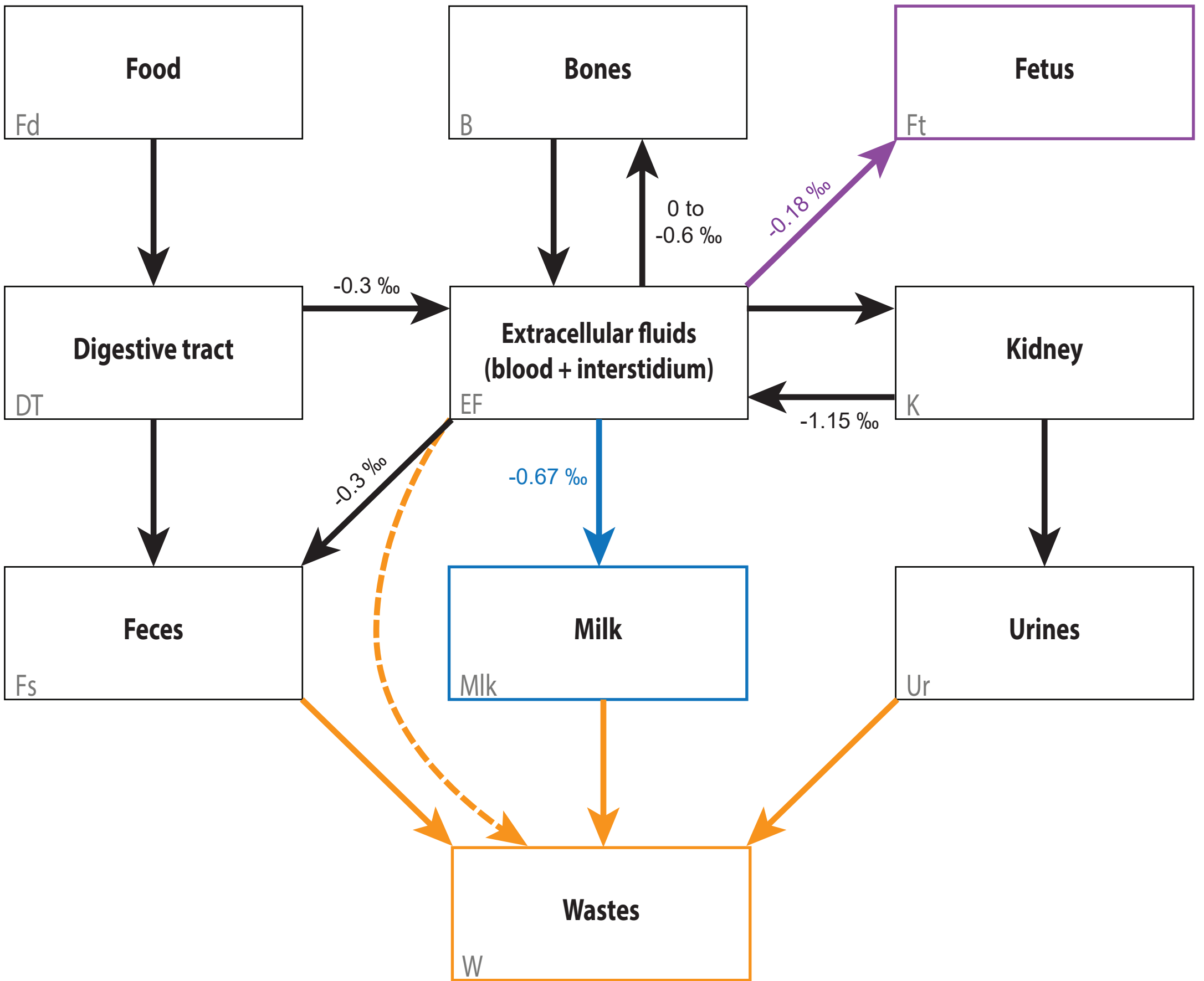
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■ Urine ■ Bone ■ Colostrum

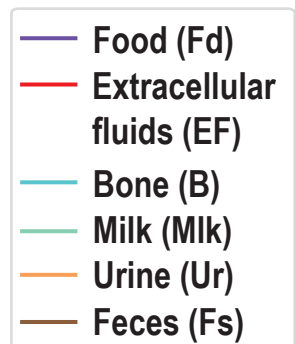
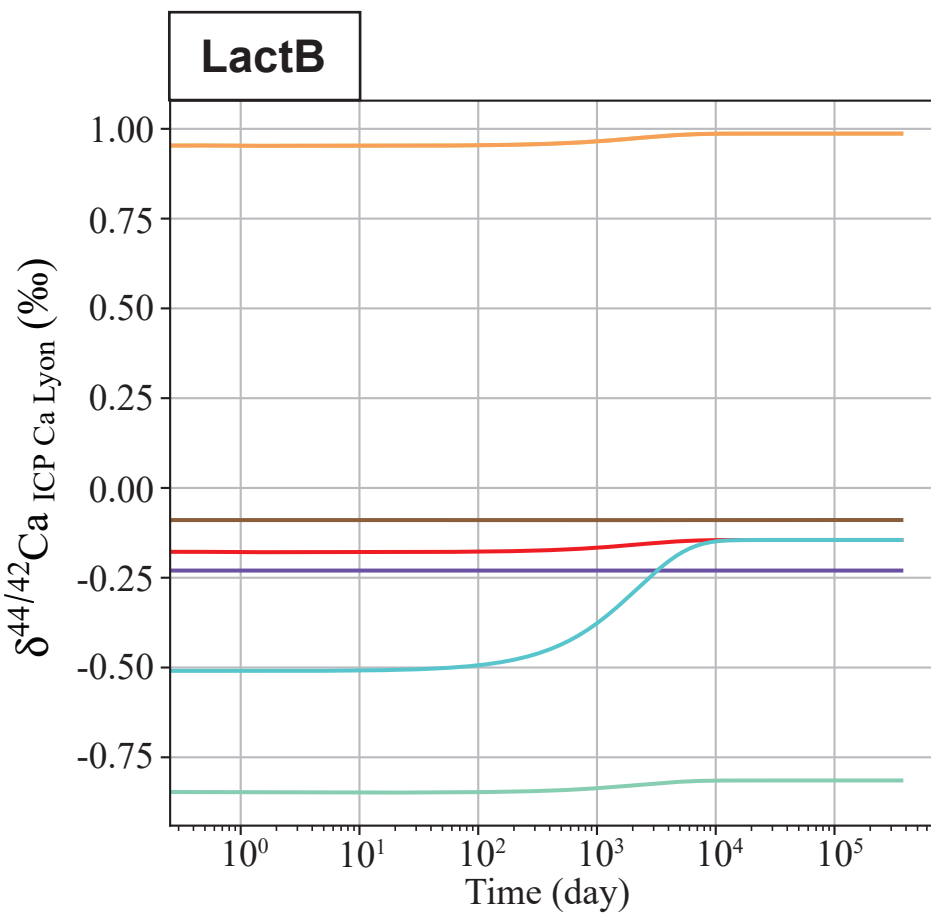
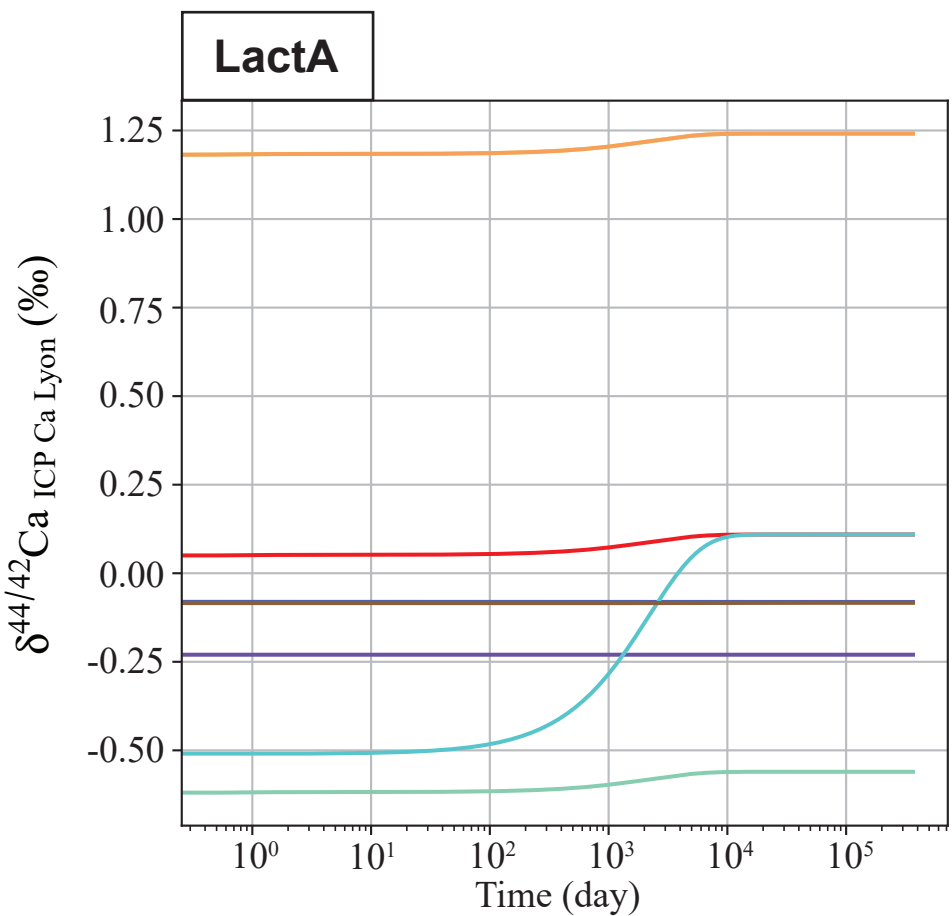
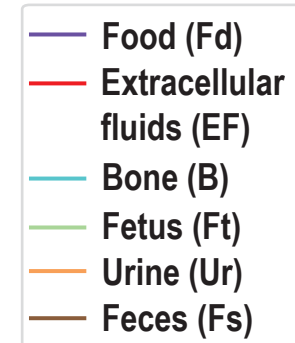
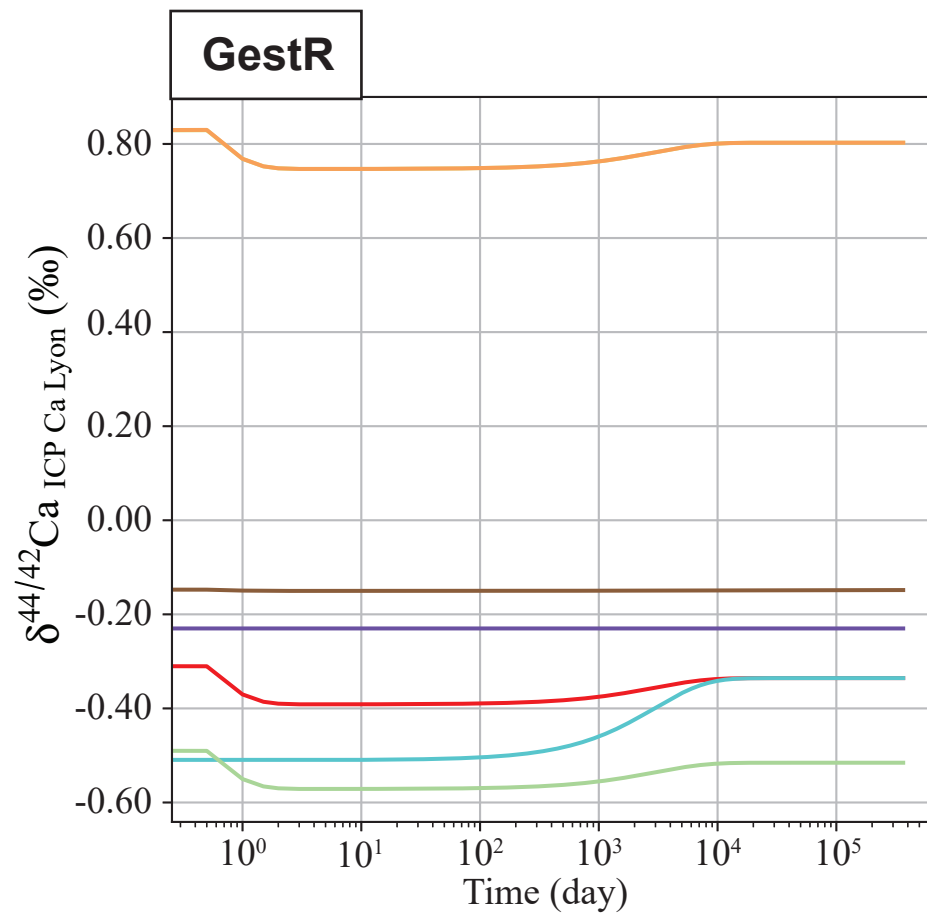
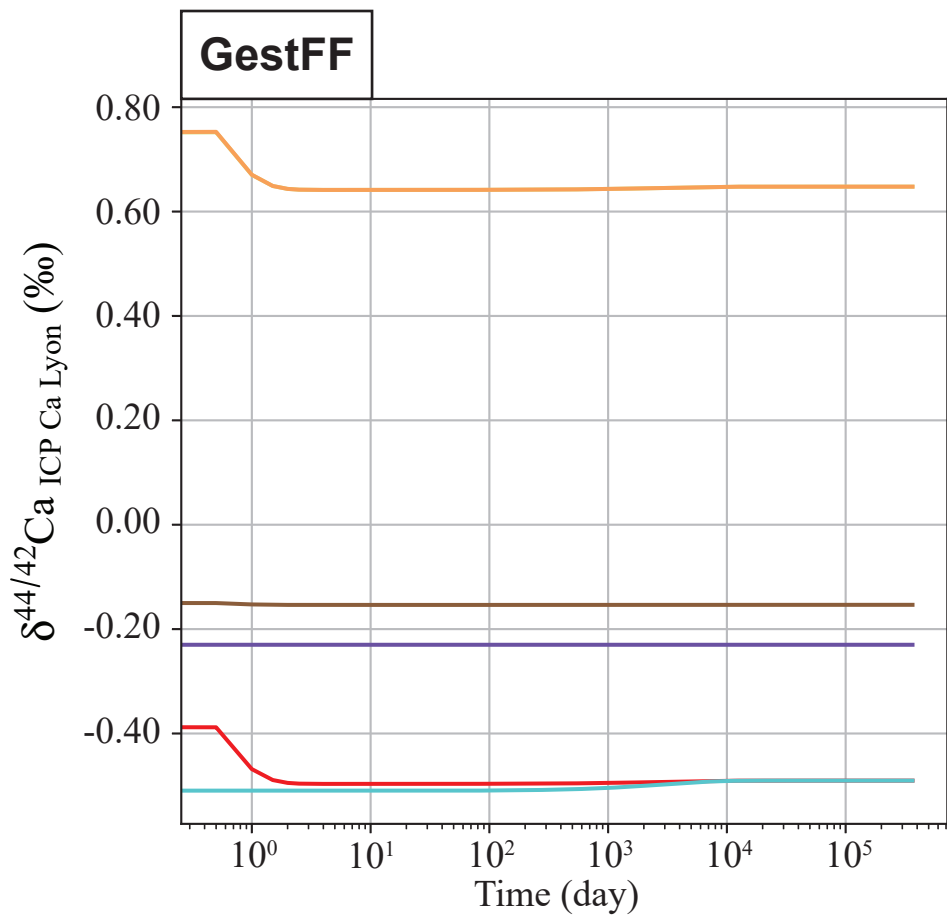
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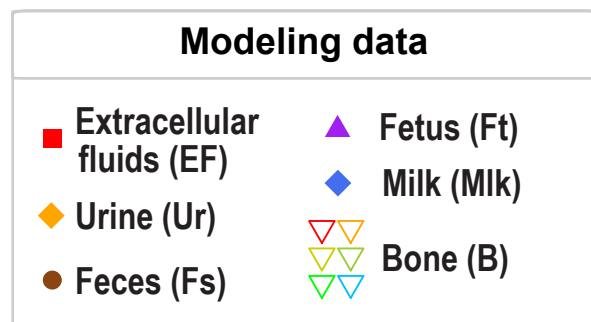
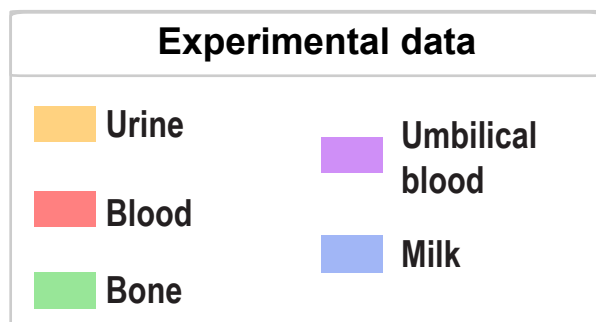
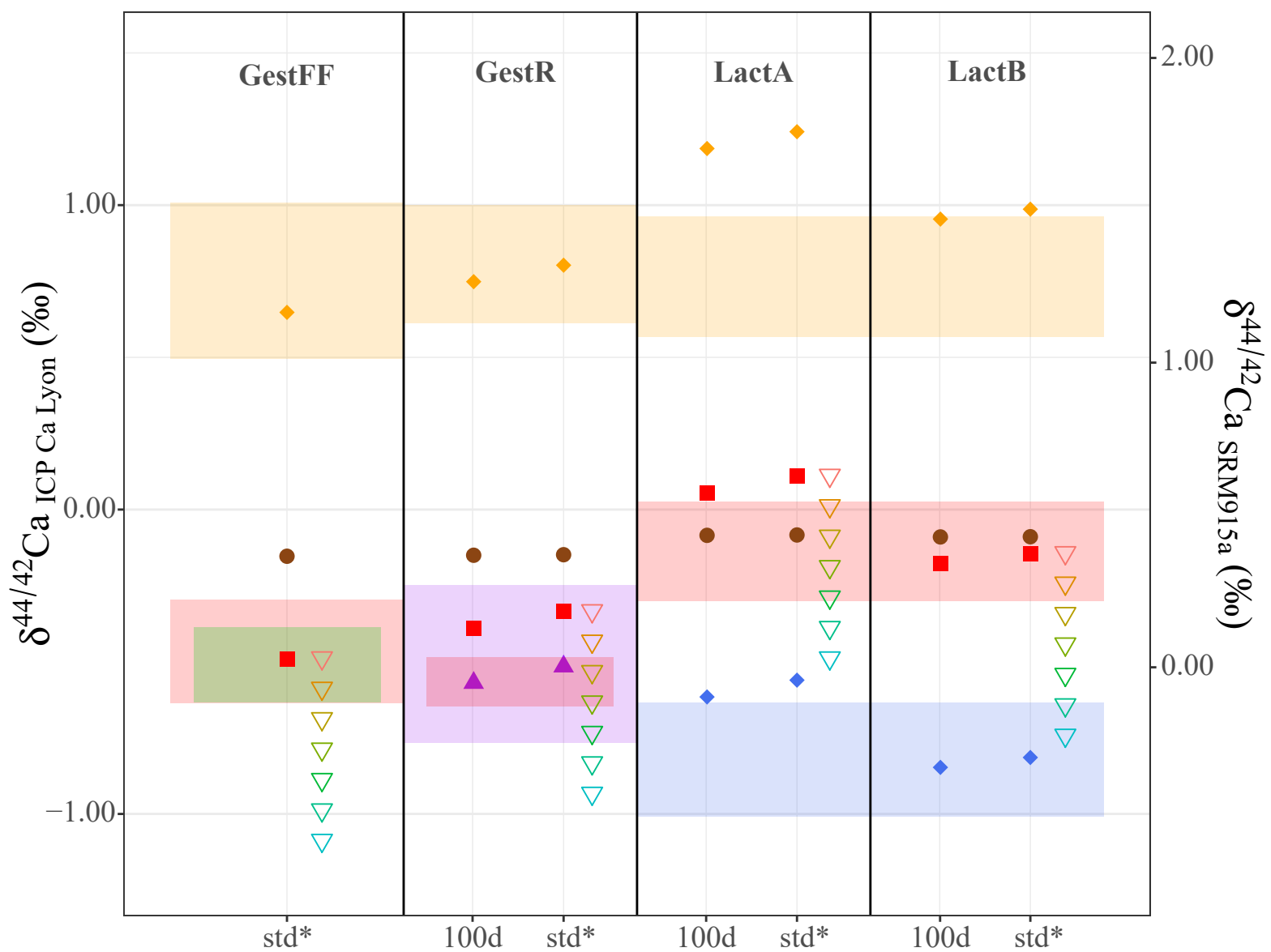
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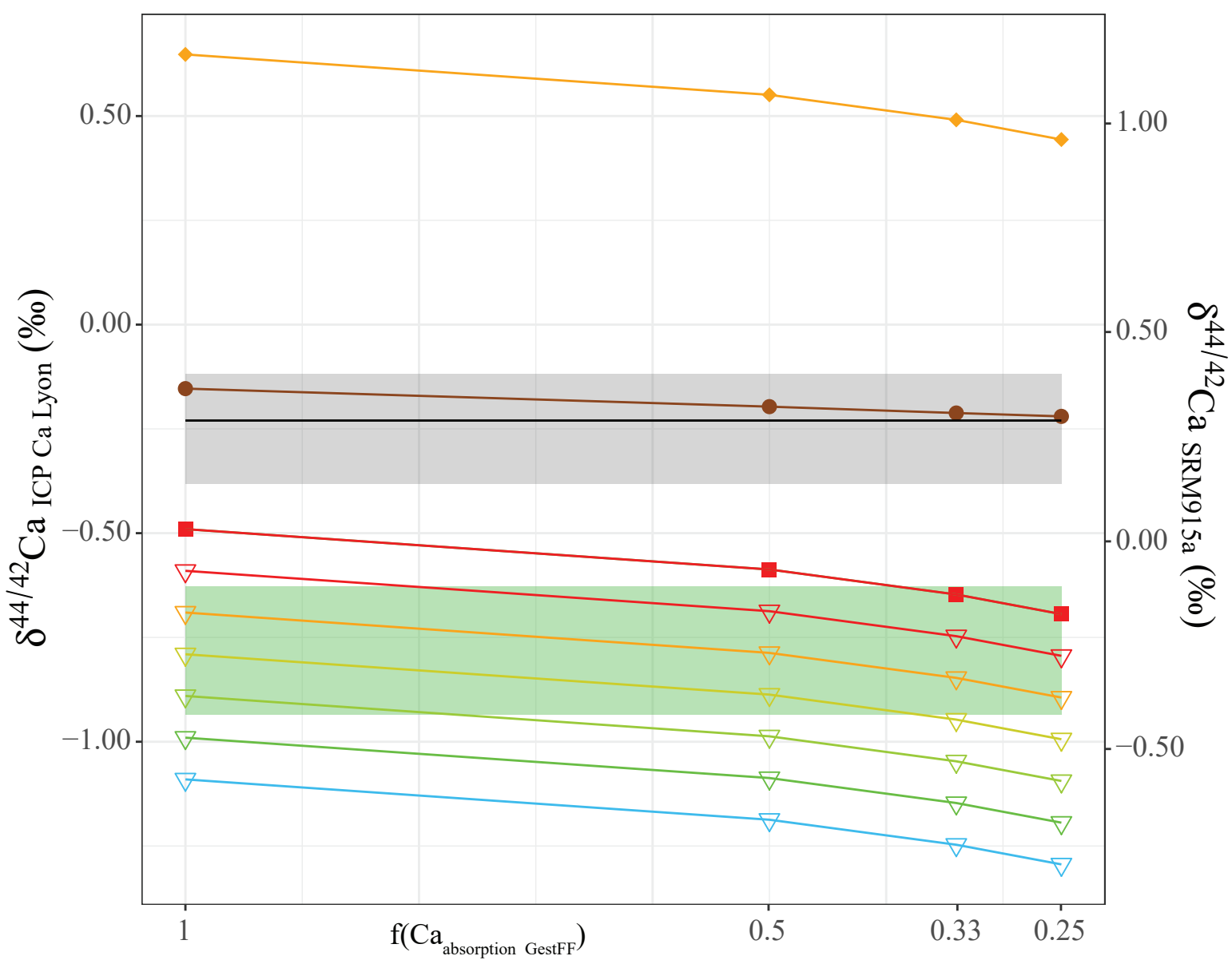












Experimental data

- Diet $\delta^{44/42}\text{Ca}$ values (this study)
- Expected bone $\delta^{44/42}\text{Ca}$ values with $\Delta^{44/42}\text{Ca}_{\text{bone-diet}} = -0.54 \pm 0.08$ ‰

Modeling data

- ◆ Urine (Ur)
- Feces (Fs)
- Food (Fd)
- Extracellular fluids (EF)
- ▽ Bone (B)