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**Vitamin A deficiency during the perinatal period induces changes in vitamin A metabolism in the offspring. The regulation of intestinal vitamin A metabolism via ISX occurs only in male rats severely vitamin A-deficient**

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1 **Vitamin A deficiency during the perinatal period induces changes in vitamin A**  
2 **metabolism in the offspring. *The regulation of intestinal vitamin A metabolism via ISX***  
3 ***occurs only in male rats severely vitamin A-deficient.***

4  
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26 **Abstract**

27 **Purposes:** 1) To test the hypothesis of the existence of a perinatal vitamin A (VA)  
28 programming of VA metabolism and to better understand the intestinal regulation of VA  
29 metabolism. **Methods:** Offspring from rats reared on a control (C) or a VA-deficient (D) diet  
30 from 6 weeks before mating until offspring weaning, i.e. 7 weeks after mating, were themselves  
31 reared on a C or D diet for 19 weeks, resulting in the following groups: C-C (parents fed C -  
32 offspring fed C), D-C, C-D and D-D. VA concentrations were measured in plasma and liver.  $\beta$ -  
33 carotene bioavailability and its intestinal conversion rate to VA, as well as vitamin D and E  
34 bioavailability, were assessed after gavages with these vitamins. Expression of genes involved  
35 in VA metabolism and transport was measured in intestine and liver. **Results:** C-D and D-D  
36 had no detectable retinyl esters in their liver. Retinolemia, hepatic retinol concentrations and  
37 postprandial plasma retinol response to  $\beta$ -carotene gavage were higher in D-C than in C-C.  
38 Intestinal expression of *Isx* was abolished in C-D and D-D and this was concomitant with a  
39 higher expression of *Bco1*, *Scarb1*, *Cd36* and *Lrat* in males receiving a D diet as compared to  
40 those receiving a C diet.  $\beta$ -Carotene, vitamin D and E bioavailabilities were lower in offspring  
41 receiving a D diet as compared to those receiving a C diet. **Conclusions:** A VA-deficient diet  
42 during the perinatal period modifies the metabolism of this vitamin in the offspring. *Isx*-  
43 mediated regulation of *Bco1* and *Scarb1* expression exists only in males severely deficient in  
44 this vitamin. Severe VA deficiency impairs  $\beta$ -carotene and vitamin D and E bioavailability.

45

46 **Keywords:**  $\beta$ -carotene, retinol, retinyl palmitate, bioavailability, cholecalciferol, tocopherol.

47

48 **Statements and Declarations:**

49 None of the authors reported a potential conflict of interest.

50

## 51 **1) Introduction**

52

53 Vitamin A (VA) deficiency is still a public health problem in many developing  
54 countries [1,2]. A range of strategies to fight against this deficiency are available [3], from the  
55 distribution of VA supplements to the provision, in countries where it is approved, of  
56 genetically modified organisms artificially enriched in  $\beta$ -carotene [4,5]. It has even been  
57 recently suggested that the consumption of insects enriched in proVA carotenoids, following  
58 their rearing on proVA carotenoid-containing plant by-products, could be a sustainable strategy  
59 to help fighting against this deficiency [6].

60 The pathophysiological consequences of VA deficiency are multiple and can be  
61 dramatic if not corrected [7]. Beside the many metabolic effects due in particular to the  
62 regulation by this vitamin of the expression of hundreds of genes [8], it can also cause changes  
63 in its own metabolism. As a matter of fact, it was observed in rats that VA deficiency increases  
64 intestinal  $\beta$ -carotene cleavage activity [9]. It was also observed that VA deficiency reduces  
65 intestinal  $\beta$ -carotene uptake by brush border membrane vesicles [10]. J. von Lintig and  
66 colleagues have extensively studied the factors involved in this regulation [11-13] [ENREF 13](#).  
67 They showed that when retinoic acid concentration is high, the intestinal transcription factor  
68 ISX (intestine specific homeobox) represses the expression of both *BCO1* (beta-carotene  
69 oxygenase 1), the main enzyme that cleaves  $\beta$ -carotene in the intestine [14-18], and *SCARB1*  
70 (scavenger receptor class B member 1), which encodes for SR-BI, an apical membrane protein  
71 involved in  $\beta$ -carotene uptake by enterocytes [19,20]. When VA status, and hence retinoic acid  
72 concentration, is low, it is assumed that ISX does not repress *BCO1* and *SCARB1* anymore. The  
73 consequent increase in their expression leads to a higher  $\beta$ -carotene absorption and conversion  
74 rate by the small intestine [12,21]. However, the results of two studies contradicted this  
75 mechanism [22,23]. Lemke et al. observed a decrease in  $\beta$ -carotene conversion efficiency

76 following VA supplementation together with an increase in the absorption of  $\beta$ -carotene[22]  
77 while Goswami et al. [10] observed a decrease in  $\beta$ -carotene bioavailability in case of VA  
78 deficiency. We therefore decided to reinvestigate this regulation in a recent study which aimed  
79 to assess the effect of dietary VA content on intestinal and hepatic metabolism of VA in adult  
80 rats [24]. The results of this study first allowed us to hypothesize that this regulatory  
81 mechanism may only be effective when the VA status is very low. They also allowed us to  
82 assume that this mechanism is only present in male rats. We wanted to verify these two  
83 exciting hypotheses very quickly and we therefore decided to take advantage of this study in  
84 the offspring of the rats of the previous study [24] to verify them. Moreover, the fact that VA  
85 status can regulate the intestinal expression of *SCARBI*, which encodes for the protein SR-BI,  
86 in the intestine led us to wonder whether VA status could also affect the bioavailability of other  
87 molecules whose absorption involves SR-BI, such as vitamin D and E [25]. This hypothesis is  
88 further supported by the fact that the regulation of the intestinal expression of SR-BI via ISX  
89 affected the concentrations of vitamin E in mouse tissues [13]. Moreover, supplementation of  
90 rats with retinoic acid decreased the absorption efficiency of vitamin E [26].

91 VA deficiency not only affects the health of the deficient individuals but also affects the  
92 health of their offspring. Surprisingly, although it has been suggested that VA metabolism is  
93 modified during pregnancy [27], the consequences of VA deficiency during the perinatal period  
94 on VA metabolism in the offspring has never been addressed. Given the importance of VA in  
95 many metabolic pathways, we hypothesize, by drawing a parallel with what has been suggested  
96 for energy metabolism, i.e. the thrifty phenotype hypothesis put forth by Hales and Barker [28],  
97 that VA deficiency in parents during the perinatal period could induce an adaptation of VA  
98 metabolism in the offspring, increasing its chance of survival in a VA-poor environment.

99 In summary, many questions regarding the effect of the VA content of the diet, and  
100 consequently of the VA status, on VA metabolism remain unanswered. The protocol for this

101 study was developed to determine whether there is a prenatal programming induced by VA  
102 deficiency during the perinatal period and if so, how it affects the metabolism of this vitamin in  
103 the offspring. For this, we measured the hepatic and plasma concentrations of VA, the  
104 bioavailability and the efficiency of conversion of beta-carotene into VA, and the expression of  
105 genes involved in hepatic and/or intestinal VA metabolism (**Supplemental table 1**). The data  
106 obtained also provide information on 1) the intestinal regulatory mechanism of VA metabolism  
107 mediated by ISX, 2) sex differences in VA metabolism [24,29-31], and 3) the consequences of  
108 VA deficiency on the bioavailability of other fat-soluble vitamins that share absorption  
109 pathways with  $\beta$ -carotene.

110 **2) Material and methods**

111

112 *Chemicals*

113 Ethanol, *n*-hexane, isopropanol, trichloromethane and HPLC grade dichloromethane,  
114 methanol, acetonitrile and water were purchased from Carlo Erba reagents (Val de Reuil,  
115 France).  $\beta$ -carotene was from Carotenature GmbH (Müdingen, Switzerland). Ovolife IF 50  
116 phospholipids, which contained 50% w/w phospholipids derived from egg yolk and a  
117 maltodextrin excipient, were from Lecico, Inc. (Hamburg, Germany). *RRR*- $\alpha$ -tocopherol,  
118 retinol, retinyl palmitate, cholecalciferol, tocol, triolein, sodium chloride, sodium citrate, tris  
119 hydroxide, bovine serum albumin and protease inhibitor cocktail were from Sigma-Aldrich  
120 (Saint-Quentin Fallavier, France). Sevoflurane was from Baxter (Lublin, Poland). TRIzol  
121 reagent was from Euromedex (Souffelweyersheim, France). Dithiothreitol was from Thermo  
122 Fisher Scientific (Les Ulis, France). Phosphate buffered saline (PBS) was from Life  
123 Technologies (Illkirch, France).

124

125 *Animals*

126 Institutional guidelines for the care and use of animals were followed and all  
127 experimental procedures were approved by the local animal care and use committee (agreement  
128 number D 13-055-20). Rats were housed under standard conditions of light (12-h light/dark  
129 cycle; lights on at 8 am) and temperature (22-24°C) with free access to tap water and the  
130 different VA diet. Rats were the offspring of Sprague Dawley RjHan:SD rats from a previous  
131 study which aimed to assess the effect of dietary VA content on intestinal and hepatic  
132 metabolism of VA in adult rats [24]. Ten-week-old female and male rats were fed either a diet  
133 with usual VA content, i.e. 2300 IU/kg, thereafter called the control (C) diet, or a diet low in  
134 VA, i.e. 400 IU/kg, thereafter called the deficient (D) diet (Test Diet Limited, London, UK).

135 These diets, which differed only in their VA content, were started 6 weeks before mating, in  
136 both females and males, and were continued throughout the pregnancy and the lactation period.  
137 In order to avoid a diet-induced postnatal programming [32], each litter was culled to 12 pups  
138 (6 females and 6 males) on postnatal day 3. The offspring was weaned at postnatal day 21 and  
139 was then fed either the D diet or the C diet for 19 weeks, i.e. all along their growing period.  
140 Consequently, there were 4 groups of offspring (n=24 with 12 males and 12 females): i) C-C,  
141 i.e. rats whose parents received the C diet during the perinatal period and that received the C  
142 diet after weaning, ii) D-C, i.e. rats whose parents received the D diet during the perinatal  
143 period and that received the C diet after weaning, iii) C-D, i.e. rats whose parents received the  
144 C diet during the perinatal period and that received the D diet after weaning and iv) D-D, i.e.  
145 rats whose parents received the D diet during the perinatal period and that received the D diet  
146 after weaning.

147

#### 148 *Bioavailability measurement*

149 After 20 weeks on the diets, half of the rats in each group, i.e. 6 males and 6 females,  
150 were force-fed micronutrient-rich lipid emulsions on two occasions, separated by at least 3  
151 weeks, as previously described [24]. The first gavage was aimed at measuring vitamin D and E  
152 bioavailability while the second one was aimed at measuring  $\beta$ -carotene bioavailability and  
153 intestinal conversion into VA. Micronutrient doses were chosen in order to obtain postprandial  
154 plasma concentrations greater than our limits of quantification by HPLC analysis following a  
155 preliminary experiment. The preparation of the  $\beta$ -carotene-rich emulsion, which provided 3 mg  
156  $\beta$ -carotene/gavage, was described in a recently published paper [24]. The preparation of the  
157 emulsion rich in both cholecalciferol and  $\alpha$ -tocopherol was as follows: 0.66 mL of an aqueous  
158 solution containing 0.9% NaCl and 1.4% bovine serum albumin was first deposited on 0.33 mL  
159 triolein, into which 1 mg cholecalciferol and 10 mg  $\alpha$ -tocopherol had been incorporated. The



160 mixture was then vortexed for 6 min and sonicated for 10 min (Branson 3510 MT, 40 kHz;  
161 Branson Ultrasonics, Danbury, CT, USA) to obtain the emulsion intended for the gavage of one  
162 rat. This procedure allowed us to obtain emulsions which remained apparently, i.e. to the naked  
163 eye, stable for the duration of the gavage experiment (approximately 1 hour to force-feed an  
164 average of 6 rats).

165 Blood samples (about 500  $\mu$ L) were collected from a tail nick into tubes containing  
166 50  $\mu$ L of a 0.109 M sodium citrate solution at fast and during the postprandial period (1.5 h, 3  
167 h, 4.5 h). The last postprandial sample, taken 6 h after gavage, was obtained by cardiac  
168 puncture under deep sevoflurane anesthesia using a syringe filled with 0.109 M sodium citrate  
169 (1/10 of the blood volume to be sampled). Blood was immediately centrifuged at 4,000 g for 10  
170 min at 4°C and the resulting plasma was immediately frozen at -80°C.

171

#### 172 *Liver and intestine sample collection*

173 After 20 weeks on the diets, the remaining half of the rats, i.e. 6 females and 6  
174 males/group, were euthanized at fast to obtain tissues (liver and intestine). Liver and small  
175 intestinal samples were collected as previously described [24] and stored at -80°C.

176

#### 177 *Plasma and tissue analysis*

178 The molecules of interest were first extracted in an organic phase as previously  
179 described [24]. The organic phase was left to evaporate under nitrogen until obtaining a dried  
180 extract. All dried extracts were dissolved in 200  $\mu$ L HPLC mobile phase (see below). A volume  
181 of 50–180  $\mu$ L was used for HPLC analysis.

182 All compounds were separated using a 250 x 4.6 mm RP C18, 5  $\mu$ m Zorbax Eclipse  
183 XDB-C18 column (Agilent Technologies, Santa Clara, CA, USA) preceded by a guard column,  
184 maintained at a temperature of 35°C. The mobile phase consisted of acetonitrile-

185 dichloromethane-methanol (70:20:10; vol:vol:vol), using an isocratic elution and a flow rate of  
186 1.8 mL/min. The HPLC system comprised a separation module and a photodiode array detector  
187 (Shimadzu, Marne-la-Vallée, France). Compounds were detected at their maximum absorption  
188 wavelengths, i.e. 265 nm for cholecalciferol, 292 nm for  $\alpha$ -tocopherol and tocol (the internal  
189 standard), 325 nm for retinol and retinyl esters, and 450 nm for  $\beta$ -carotene. Retinol,  $\beta$ -carotene,  
190  $\alpha$ -tocopherol, tocol, cholecalciferol and retinyl palmitate were identified by retention times and  
191 absorption spectra coincident with authentic (>95% pure) standards. Retinyl stearate, retinyl  
192 oleate and retinyl linoleate were identified by spectral analysis and quantified by comparing  
193 peak areas with standard reference curves of retinyl palmitate, correcting by their molecular  
194 extinction coefficient relative to that of retinyl palmitate. Quantifications were performed using  
195 Chromeleon software (version 6.8).

196

#### 197 *Measurement of gene expression in liver and duodenum samples*

198 The expression levels of several genes coding for proteins known to be involved in the  
199 metabolism and transport of VA [33] were measured as previously described [24].

200

#### 201 *Calculations*

202 Vitamin D and E bioavailability was estimated by measuring the areas under the curves  
203 (AUC) of their postprandial plasma concentrations (0-6 h) following force-feeding. Regarding  
204 cholecalciferol, it is well established that its fasting plasma concentration is negligible, as also  
205 observed in our study (data not shown). Moreover, when cholecalciferol is ingested, it is  
206 practically not metabolized in the upper small intestine before its absorption by intestinal cells  
207 [34]. Finally, it is assumed that most cholecalciferol absorbed by the intestinal cell is  
208 incorporated as such in chylomicrons, which are then secreted in the lymph allowing  
209 cholecalciferol to join the general circulation during the postprandial period [35]. Thus,

210 measuring the AUC of the plasma cholecalciferol concentration during the postprandial period  
211 provides us with a good estimate of its bioavailability. Regarding vitamin E, the only difference  
212 with cholecalciferol is that its plasma concentration at fast is not null. Thus, to assess its  
213 bioavailability, it is necessary to calculate its incremental AUC, i.e. the AUC of the increase in  
214 its postprandial plasma concentration compared to its fasting plasma concentration.

215  $\beta$ -Carotene bioavailability,  $\beta$ -carotene conversion rate to VA and VA status were  
216 estimated as previously described [24]. Briefly,  $\beta$ -carotene bioavailability was estimated by  
217 summing the  $\beta$ -carotene and the retinyl palmitate responses because, under these experimental  
218 conditions, it is assumed that the postprandial plasma retinyl palmitate originates only from the  
219 intestinal conversion of  $\beta$ -carotene into VA. Concerning  $\beta$ -Carotene conversion rate to VA, it  
220 was calculated as the percentage of bioavailable  $\beta$ -carotene found in the form of retinyl  
221 palmitate in the plasma during the postprandial period, i.e. retinyl palmitate response / ( $\beta$ -  
222 carotene response + retinyl palmitate response). Concerning VA status, it was estimated using 3  
223 biomarkers that were used in the study on the parents of these animals [24]. The rationale for  
224 the use of these biomarkers, and the interpretation of variations in the values of these  
225 biomarkers, are explained in detail in the first part of the discussion of this previous study.

226

### 227 *Statistical analysis*

228 Results are expressed as means  $\pm$  SEM. Departures from normality were assessed using  
229 Q-Q plots of standardized residuals and homogeneity of variances was tested by Levene's test.  
230 In case of departure from normality or homoscedasticity, data were log-transformed. In a first  
231 approach, differences in measured variables were analyzed by 3-way ANOVA using a full  
232 factorial design with parent diet, offspring diet and sex of the animals as fixed factors. For post  
233 hoc pairwise comparisons, the Benjamini-Hochberg procedure was used, controlling the false

234 discovery rate at 0.05. Values of  $p < 0.05$  were considered significant. Statistical analyses were  
235 performed using SPSS 20 (SPSS Inc., Chicago, IL, USA).

236 **3) Results**

237

238 The protocol included 4 groups of rats to evaluate 1) the effect of the VA content of the  
239 diet given after weaning (offspring diet effect), 2) the effect of the VA content of the diet  
240 consumed by the parents (parent diet effect), and 3) the effect of the sex of the offspring (sex  
241 effect). This is summarized in **Supplemental figure 1**.

242

243 *Plasma and liver VA concentrations.*

244 First of all, it is interesting to mention that the average liver weight of male rats was  
245 significantly higher than that of female rats,  $17.4 \pm 0.5$  g vs  $10.3 \pm 0.2$  g. In addition, while  
246 female liver weights were not significantly different between the different groups, they were  
247 significantly lower in the male fed a D diet after weaning, i.e.  $16.5 \pm 0.9$  g for the C-D and  $14.7$   
248  $\pm 0.9$  g for the D-D, as compared to male fed a C diet after weaning, i.e.  $19.9 \pm 0.8$  g for the C-  
249 C and  $18.2 \pm 0.7$  g for the D-C (data not shown).

250 Plasma retinol concentrations were measured at fast in the different offspring groups  
251 (**Figure 1**). Offspring on the D diet, i.e. C-D and D-D, exhibited markedly lower retinolemia (-  
252 84% and -86%, respectively) compared to offspring on the C diet, i.e. C-C and D-C ( $p < 0.05$ ).  
253 Moreover, we also observed a significant effect of the parent diet: among offspring that  
254 received a C diet (C-C and D-C), that whose parents received a D diet, i.e. D-C, had higher  
255 retinolemia (+70% for females and +41% for males) than that whose parents received a C diet,  
256 i.e. C-C. This effect of the parent diet was also observed for female offspring that received a D  
257 diet. Indeed, females whose parents received a D diet (D-D) had higher retinolemia (+51%)  
258 than females whose parents received a C diet (C-D). Finally, there was a marked sex effect  
259 among offspring that received a C diet (C-C and D-C): males displayed significantly higher

260 retinolemia (+69% and +40%, respectively) compared to females. This sex difference was not  
261 found in the C-D and D-D groups.

262         Concerning hepatic VA concentrations, both retinol and retinyl esters were measured  
263 (**Figure 2**). We observed again a significant effect of the offspring diet: hepatic VA  
264 concentration, i.e. the sum of retinol and retinyl esters, was significantly lower in the offspring  
265 that received the D diet compared to that which received the C diet (**Figure 2a**, 80% lower in  
266 C-D vs C-C and 78% lower in D-D vs D-C). This was mostly due to the absence of retinyl  
267 esters in the offspring that received the D diet (**Figure 2b**). Indeed, although hepatic retinol  
268 concentrations were systematically lower in the offspring that received the D diet compared to  
269 that which received the C diet, this was not significant, except when comparing D-D vs D-C  
270 females (-31%) (**Figure 2c**). Concerning the different species of retinyl esters in the C-C and  
271 D-C groups, the main one was retinyl palmitate (representing at least 55% of all retinyl esters),  
272 followed by retinyl stearate (**Table 1**).

273         The effect of the parents' diet on offspring hepatic VA concentrations, i.e. D-C vs C-C  
274 and D-D vs C-D, was not significant, but the concentrations of hepatic VA, retinyl esters and  
275 retinol were systematically higher in the offspring groups from parents that received the D diet  
276 than in those from parents that received the C diet. For example, D-C females had 41% higher  
277 hepatic retinol concentration compared to C-C females, and D-C male had 31% higher hepatic  
278 retinol concentration compared to C-C males ( $p=0.09$ ).

279         Finally, VA, retinyl ester and free retinol hepatic concentrations were systematically  
280 lower in males than in females, although this was only significant in the D-C group for free  
281 retinol (30% lower in males), likely because of a lack of statistical power for this parameter.

282

283

284

285 *Postprandial plasma  $\beta$ -carotene and VA responses following force-feeding with  $\beta$ -carotene.*

286 As expected, and unlike retinol, neither  $\beta$ -carotene nor retinyl palmitate were detected  
287 in plasma prior to gavage. **Figure 3a** shows the postprandial  $\beta$ -carotene response, i.e. the 0-6 h  
288 AUC of its postprandial concentration, expressed in  $\mu\text{mol/L}\cdot\text{h}$ . There was a significant effect  
289 of the offspring diet: groups that received a D diet after weaning exhibited lower responses than  
290 those that received a C diet after weaning, i.e. C-D vs C-C (-78%) and D-D vs D-C (-59%).  
291 Concerning the effect of the parent diet, it is very interesting to note that the D-C offspring had  
292 a lower response than the C-C offspring (-64%, almost significant at  $p=0.059$ ). Finally, there  
293 was a striking sex effect, with females exhibiting a higher response than males regardless of the  
294 diet (mean of all the dietary groups: +528% in females vs males).

295 Regarding the retinyl palmitate response, **Figure 3b** shows that there was a significant  
296 effect of the offspring diet, but only in males. Indeed, males on the D diet had a significantly  
297 decreased response as compared to males on the C diet (C-D vs C-C (-78%) and D-D vs D-C (-  
298 65%)). Finally, there was a significant difference between males and females in the groups of  
299 offspring that received a D diet but not in groups that received a C diet (-69% in C-D males vs  
300 C-D females and -66% in D-D males vs D-D females).

301 Finally, with regard to the retinol response (**Figure 3c**), there was a significant effect of  
302 the offspring diet, but only in females. Indeed, females that received a D diet displayed a higher  
303 response compared to females that received a C diet, i.e. C-D vs C-C (+167%) and D-D vs D-C  
304 (+170%). In addition, we also observed a significant effect of the parent diet, in females on the  
305 D diet (D-D vs C-D, +100%) and in males on the C diet (D-C vs C-C, +65%). Finally, contrary  
306 to what was observed for retinyl palmitate, there was a significant difference between males  
307 and females that received a C diet (+143% in males of the C-C and D-D groups as compared to  
308 females of these groups), and not in the offspring that received a D diet.

309

310 *β-Carotene bioavailability and conversion rate to VA.*

311 With regard to  $\beta$ -carotene bioavailability (**Figure 4a**), there was a significant effect of  
312 the offspring diet: in rats from parents that received the C diet, offspring that received the D  
313 diet, i.e. C-D, exhibited lower  $\beta$ -carotene bioavailability (-64%) compared to offspring that  
314 received the C diet, i.e. C-C. In rats from parents that received the D diet, this effect was only  
315 seen in males (-63%;  $p=0.071$ ). There was no significant effect of the parent diet on this  
316 phenotype. Finally,  $\beta$ -carotene bioavailability was always higher in females than in males  
317 (+120% in females as compared to males).

318  $\beta$ -Carotene conversion rates to VA are shown in **Figure 4b**. Regarding the effect of the  
319 offspring diet, only a marginally significant increase (+78%;  $p=0.052$ ) could be seen between  
320 females that received the D diet as compared to females that received the C diet and whose  
321 parents were fed the C diet (C-D vs C-C). There was an effect of the parent diet only in females  
322 on the C diet: those whose parents had received the D diet exhibited higher  $\beta$ -carotene  
323 conversion rates to VA (D-C vs C-C, +96%). Finally,  $\beta$ -carotene conversion rates to VA were  
324 generally higher in males than in females (mean of all male groups +40% higher than the mean  
325 of all female groups), with fairly elevated values observed in males (mean rate of conversion of  
326 all male groups: 90%).

327

328  *$\alpha$ -Tocopherol and cholecalciferol bioavailability.*

329 Results are shown in **Figures 5a and 5b**. As expected,  $\alpha$ -tocopherol but not  
330 cholecalciferol was detected in plasma prior to gavage. It is remarkable to note that similar  
331 trends emerge from these two figures. There was a significant effect of the offspring diet, or  
332 marginally significant in the case of  $\alpha$ -tocopherol ( $p=0.075$ ), but only in rats whose parents  
333 were fed the D diet: offspring that received the D diet exhibited lower  $\alpha$ -tocopherol (-47% for  
334 the C-D and D-D groups as compared to the C-C and D-C groups) and cholecalciferol



335 bioavailability (+35% for the C-D and D-D groups as compared to the C-C and D-C groups)  
336 than offspring that received the C diet. There was no significant effect of the parent diet or of  
337 sex on these phenotypes.

338

339 *Expression of genes involved in VA metabolism.*

340 The expression levels of several genes coding for proteins known to be involved in the  
341 hepatic metabolism and transport of VA were measured in rats that were not force-fed (**Figure**  
342 **6**). A significant effect of the offspring diet was observed for 3 genes: *Lrat* (lecithin retinol  
343 acyltransferase), *Rbp2* (retinol binding protein 2) and *Pnpla3* (patatin like phospholipase  
344 domain containing 3). There was a complete inhibition of *Lrat* expression in the offspring fed  
345 the D diet compared to that fed the C diet, i.e. C-D and D-D vs C-C and D-C. There was also a  
346 significant decrease of the expression of *Rbp2* in males that received the D diet after weaning  
347 (C-D and D-D) compared to males that received the C diet after weaning (C-C and D-C).  
348 Finally, *Pnpla3* was not expressed in males that received a C diet after weaning (C-C and D-C)  
349 whereas it was expressed in those that had a D diet after weaning (C-D and D-D). There was no  
350 significant effect of the parent diet on gene expression levels, except for *Pnpla3*, whose  
351 expression level in females from parents on the D diet (D-C and D-D) was lower than that of  
352 females from parents on the C diet (C-C and C-D). We also observed a significant effect of sex  
353 on the expression levels of several genes, namely *Pnpla3*, *Rbp2*, *Rbp4* (retinol binding protein  
354 4), *Ttr* (transthyretin) and, to a lesser extent, *Dgat2* (diacylglycerol O-acyltransferase 2) and  
355 *Lrat*.

356 Concerning the expression levels of genes coding for proteins involved in intestinal  
357 metabolism and transport of VA, results are presented in **Figure 7**. Firstly, there was a strong  
358 effect of the offspring diet on the expression levels of *Isx*. Indeed, its expression was  
359 completely abolished in offspring fed the D diet compared to offspring fed the C diet, both in

360 females and in males. An effect of the offspring diet was also observed for 4 other genes (*Lrat*,  
361 *Scarb1*, *Cd36* (CD36 molecule) and *Bco1* (almost significant)) whose expression levels were  
362 increased in males that had a D diet after weaning compared to those that had a C diet after  
363 weaning. No such effect was observed in females. There was no significant effect of the parent  
364 diet on gene expression levels, except for *Rbp2* whose expression levels in D-C males were  
365 lower than those of C-C males. In males only, and once again with the exception of *Isx* and  
366 *Rbp1* (retinol binding protein 1), the expressions of the studied genes were always lower in the  
367 D-C groups than in the C-C groups, suggesting an effect of the parent diet, although this was  
368 only significant for *Rbp2*, likely because of an insufficient statistical power. Finally, the  
369 expression of all genes, except *Isx* and *Rbp1*, was lower in males of the D-C group than in  
370 females of the same group (significant for *Scarb1*, *Cd36* and *Lrat*, and almost significant for  
371 *Bco1*). Conversely, the expression of all genes, except again *Isx* and *Rbp1*, was higher in males  
372 of the C-D group than in females of the same group (significant for *Cd36* and *Lrat*).

373  
374 *VA status, bioavailability of  $\beta$ -carotene and vitamin D and E, and  $\beta$ -carotene conversion rate*  
375 *in parents and offspring.*

376 As the rats in this study were the offspring of rats from a previous study [24], where the  
377 dietary VA content also varied and in which we also measured the bioavailability of  $\beta$ -carotene  
378 and vitamin D and E (data not shown on these vitamins in the previous study), we gathered the  
379 results of all these groups to assess more precisely the relationships between the VA status and  
380 the studied phenotypes. The VA status for all groups is presented in **Table 2**.

381 **4) Discussion**

382

383 *VA status of the different groups of rats*

384 The main objective of this study was to assess the effect of the VA status of rats, whose  
385 parents themselves had different VA status, on VA metabolism and on  $\beta$ -carotene, vitamin D  
386 and E bioavailability. It was therefore particularly important to properly assess this status in the  
387 different groups of rats. The data collected in this study provide us with three biomarkers of  
388 VA status, namely hepatic VA concentration, fasting plasma retinol concentration, and  
389 postprandial plasma retinol response following gavage with  $\beta$ -carotene [24]. The first two  
390 (**Figures 1 and 2**) show that the offspring on a D diet, i.e. C-D and D-D, had a lower VA status  
391 than that on the C diet, i.e. C-C and D-C. Moreover, since hepatic retinyl esters constitute the  
392 main body VA reserves, we can even consider that the offspring on the D diet had no VA  
393 reserves at all. In females, the results of the third biomarker, i.e. postprandial plasma retinol  
394 response (**Figure 3c**, see [24] for detailed interpretation), are perfectly in agreement with those  
395 of the other two biomarkers. Indeed, female offspring that received a D diet had higher  
396 postprandial plasma retinol responses than female offspring that received a C diet.

397

398 *A VA-deficient diet during the perinatal period appears to improve hepatic VA storage*  
399 *capacity, as well as the ability to mobilize hepatic VA stores, in the offspring.*

400 In order to assess whether VA deficiency of parents during the perinatal period may  
401 affect VA metabolism in their offspring, we first compared VA metabolism in offspring that  
402 received the same diet after weaning but that differed by the diet their parents received, i.e. D-C  
403 vs C-C and D-D vs C-D. These comparisons highlighted several differences that support the  
404 hypothesis of a prenatal programming of VA metabolism. Firstly, the offspring whose parents  
405 received a D diet exhibited a markedly higher fasting retinolaemia as compared to the offspring

406 whose parents received a C diet (except for males receiving a D diet). Secondly, concerning  
407 hepatic VA metabolism, the concentrations of free retinol were higher (close to significance) in  
408 the D-C vs the C-C groups and in the D-D vs the D-C groups. It is also worth mentioning that  
409 the concentrations of VA, i.e. the sum of retinyl esters and free retinol, and of retinyl esters  
410 alone were always higher in D-C than in C-C, although this was not significant. Taken together,  
411 these data suggest that, for the same diet after weaning, the hepatic accumulation of VA, or at  
412 least that of free retinol, was greater in rats from parents D than in rats from parents C. This  
413 may reflect a mechanism to improve the hepatic storage of VA in an environment deficient in  
414 this vitamin. Another interesting parameter of hepatic VA metabolism is the postprandial  
415 plasma retinol response following the absorption of a large amount of VA (a huge dose of  $\beta$ -  
416 carotene in this study), which reflects the ability of the liver to release retinol to peripheral  
417 organs. This parameter was also influenced by the parent diet. Indeed, although this was not  
418 always significant, this response was always higher in the offspring of parents D than in the  
419 offspring of parents C, i.e. D-C vs C-C and D-D vs C-D, and this in both sexes. This  
420 observation was consistent with the effects of the parents' diet on fasting retinolemia and on  
421 hepatic concentrations of free retinol. Indeed, the offspring from parents that received a VA-  
422 deficient diet had more free-retinol in their liver, their liver secreted more retinol in the plasma  
423 following a large dietary intake of VA, and their fasting blood concentration of retinol was  
424 higher than that of the offspring from parents that received a VA-sufficient diet. This may also  
425 reflect a mechanism to bring enough VA from its storage organ to peripheral tissues in a VA-  
426 depleted environment.

427

428 *A VA-deficient diet during the perinatal period appears to improve offspring's ability to convert*  
429  *$\beta$ -carotene to VA.*

430 In order to determine whether VA deficiency of parents during the perinatal period also  
431 affects intestinal VA metabolism in the offspring, we investigated whether the bioavailability  
432 of  $\beta$ -carotene and its conversion rate to VA were different between rats from parents with  
433 different VA diets, i.e. the D-C vs the C-C and the D-D vs the C-D. We observed a lower  
434 postprandial  $\beta$ -carotene response in the D-C vs the C-C ( $p=0.059$ , **Figure 3a**), which is  
435 consistent but only in females, with a higher observed  $\beta$ -carotene conversion rate ( $p<0.05$ ,  
436 **Figure 4b**). This suggests the female offspring from D parents had increased its ability to  
437 cleave newly absorbed  $\beta$ -carotene in an environment depleted in VA. The fact that this  
438 phenomenon was not observed in males is likely due to the fact that the conversion rate was  
439 already close to its maximum in the C-C group, i.e. more than 80%, and therefore could not  
440 increase significantly even if there was a stimulating effect due to the VA-deficient diet of the  
441 parents.

442  
443 *Regulation of intestinal VA metabolism via ISX only appears to work in cases of severe VA*  
444 *deficiency and only in males.*

445 The second main objective of this study was to explore the intestinal regulation  
446 mechanism of  $\beta$ -carotene absorption and conversion to VA as a function of the VA status. The  
447 current proposed mechanism [11] is as follows: when retinoic acid concentration decreases in  
448 the intestinal cell, which is supposed to happen in the event of a drop in dietary VA intake, the  
449 expression of *ISX* decreases. This leads to an increase in the expression of *BCO1* and *SCARB1*,  
450 which is supposed to result in an increase in  $\beta$ -carotene absorption rate and conversion to VA  
451 by the enterocyte. Our previous study in rat mothers did not allow us to confirm that the  
452 decrease in *ISX* expression led to an increase in *BCO1* and *SCARB1* expressions, and we have  
453 proposed two hypotheses to explain this apparent contradiction [24]. The first one was that the  
454 amplitude of dietary VA intakes, and consequently the variation in *Isx* expression levels, was

455 not sufficiently large to induce significant different expression levels of *Bco1* and *Scarb1*. The  
456 second one was that this regulation mechanism does not exist in females (we measured gene  
457 expression in females while all other studies were performed in male rats). In the present study,  
458 the very low VA status observed in the D offspring, i.e. C-D and D-D, was associated with an  
459 almost complete inhibition of *Isx* expression, which is in full agreement with the current  
460 paradigm [11,13]. Furthermore, in males, the inhibition of *Isx* expression was also associated  
461 with a higher expression level of *Scarb1* and *Bco1* [11,13]. Nevertheless, in females, the  
462 inhibition of *Isx* expression was not associated with the expected increase of *Bco1* and *Scarb1*  
463 expression levels, which agrees with our previous hypothesis that this regulatory mechanism  
464 only exists in males [24]. The present study does not allow us to explain why *Isx* modulates  
465 *Bco1* and *Scarb1* expression in males but not in females, and we can only speculate that it  
466 could be related to hormonal differences between females and males. Moreover, *Isx* inhibition  
467 in males was also associated with a significant increase in the expression levels of *Lrat* and  
468 *Cd36*, which suggests that these two genes may also be under the control of *Isx* in males. This  
469 is consistent with recent data showing that LRAT is involved in the ISX-mediated regulation of  
470 intestinal VA metabolism [36].

471           Unfortunately, the results from both this study and our previous study on parents, which  
472 compiled data on  $\beta$ -carotene bioavailability and conversion efficiency, in rats of both sexes  
473 with different VA status (**Table 2**), fail to confirm the current paradigm stating that  $\beta$ -carotene  
474 bioavailability increases when VA status decreases. On the contrary, the lower the VA status,  
475 the lower the bioavailability of  $\beta$ -carotene. Regarding the conversion rate, it did increase when  
476 the VA status decreased (**Table 2**), but this was only significant in females, probably because  
477 the conversion rate in males was already very high, around 83%, for the group with the highest  
478 VA status.

479           The variations in  $\beta$ -carotene bioavailability that were measured in this study, together  
480 with the variations in *Scarb1* and *Cd36* expression levels, raise questions about the importance  
481 of proteins encoded by these genes in  $\beta$ -carotene bioavailability. Indeed, the increase in the  
482 expression of *Scarb1* and *Cd36* in the males that received a D diet (C-D and D-D) as compared  
483 to those that received a C diet (C-C and D-C), did not translate in an increase in  $\beta$ -carotene  
484 bioavailability. On the contrary,  $\beta$ -carotene bioavailability collapsed in these rats. Likewise,  $\beta$ -  
485 carotene bioavailability was also decreased in the females that received a D diet (C-D and D-  
486 D), in comparison to females that received a C diet (C-C and D-C), while *Scarb1* and *Cd36*  
487 expression levels were not significantly different between these four groups. The observed  
488 decrease in  $\beta$ -carotene absorption efficiency in the event of VA deficiency fully disagrees with  
489 the paradigm but is perfectly in agreement with the results of Boileau et al. [10]. Our first  
490 hypothesis is that a major mechanism involved in  $\beta$ -carotene bioavailability other than that  
491 going through SR-BI and CD36 [20,25] was strongly impaired by VA deficiency. A second  
492 hypothesis is that VA deficiency affected the integrity of the intestinal mucosa and its normal  
493 functioning. It is indeed well established that VA is involved in the development of epithelia.  
494 Further experiments are needed to address these hypotheses. Concerning the effect of VA status  
495 on  $\beta$ -carotene conversion rate to VA, the results which showed that VA deficiency completely  
496 inhibited the expression of *Isx* and increased the expression of *Bco1* in males (**Figure 7**), are  
497 very consistent with the results that showed that the lowest VA status was associated with the  
498 highest  $\beta$ -carotene conversion rate (**table 2** which compiles the results obtained in the parent  
499 rats and in the offspring rats). Indeed, this supports the hypothesis that, in male rats, VA  
500 deficiency increases  $\beta$ -carotene conversion rate to VA by increasing the expression of *Bco1* via  
501 inhibition of *Isx* expression.

502

503 *Severe VA deficiency seems to decrease not only the bioavailability of  $\beta$ -carotene, but also that*  
504 *of vitamins E and D.*

505         Knowing that the absorption of  $\beta$ -carotene and vitamin D and E is carried out by  
506 mechanisms that are partly common, e.g. implication of common apical membrane proteins  
507 such as SR-BI and CD36 [37,38], we hypothesized that variations in VA status, which are  
508 assumed to modulate the expression of some of these proteins, could also influence the  
509 bioavailability of these vitamins. The data obtained in this study, combined with those of the  
510 previous study on the parents of these rats (**Table 2**), show that only a very deficient VA status,  
511 such as that observed in the offspring fed a D diet (C-D and D-D), significantly impaired the  
512 bioavailability of these two vitamins, in both males and females. The available data do not  
513 allow us to identify the mechanism but, as aforementioned, it is possible that this deficiency  
514 profoundly altered the integrity of the intestinal mucosa and therefore its ability to absorb these  
515 two vitamins as well as other nutrients and micronutrients.

516

517 *Four key observations about VA metabolism that emerge from this study*

518         On the whole, the results of this study highlighted four new observations on the effect  
519 of the VA content of the parent and offspring diets on the hepatic and intestinal metabolism of  
520 this vitamin in the offspring. The first one is that there may be a prenatal programming of VA  
521 metabolism in the offspring when the diet of the parents is deficient in VA. Indeed, the  
522 offspring of VA-deficient parents had higher retinolemia, higher hepatic accumulation of VA  
523 and higher ability to cleave  $\beta$ -carotene in the intestine. Based on Barker's hypothesis [28], we  
524 suggest that this perinatal programming of VA metabolism allows the offspring to increase  
525 their chance of survival in an environment that does not provide sufficient VA sources. This  
526 perinatal programming is probably due to epigenetic mechanisms which remain to be  
527 identified. The second observation is that *Isx* expression is significantly modulated only in the



528 event of a very strong VA deficiency. In other words, there is apparently no dose-response  
529 effect of the VA content of the diet on *Isx* expression. Furthermore, the effect of *Isx* on *Bco1*  
530 and *Scarbl* expression apparently only exists in male rats. The third observation is that severe  
531 VA deficiency decreases the bioavailability of both  $\beta$ -carotene and vitamin D and E by an  
532 unknown mechanism, likely linked to an overall deterioration of the integrity of the intestinal  
533 mucosa due to VA deficiency. The fourth observation confirms the significant effect of the sex  
534 of the rats on vitamin A metabolism. We acknowledge we did not verify whether variations in  
535 gene expression translated into variations in protein concentrations. Nevertheless, we had  
536 shown in the study on the parents of these rats that this was the case, at least for the three key  
537 proteins measured [24].

538

539 *Reflections on the consequences that this new knowledge on the metabolism of VA could have*  
540 *within the framework of the strategies of fight against this deficiency.*

541 All these new observations on VA metabolism, if they are confirmed by other studies,  
542 in particular clinical ones, could be considered in the fight against VA deficiency. Indeed, if the  
543 perinatal programming of VA metabolism is confirmed, it should be ensured that individuals  
544 from VA-deficient parents, and that could therefore be adapted to absorb and store VA more  
545 efficiently, would not be intoxicated by the high VA doses given as supplements to fight  
546 against this deficiency. Also, our results also suggest that individuals severely VA-deficient  
547 could display lower absorption efficiency of vitamin D and E and therefore have an insufficient  
548 status in these vitamins. The status of these vitamins must therefore be checked in populations  
549 very deficient in VA and corrected if necessary. Finally, the effect of sex on vitamin A  
550 metabolism, which had already been observed in humans, and which was investigated further  
551 in this study and in the previous one on the parents of these rats, suggests that it would be  
552 desirable to adapt VA supplementation according to the sex of the individuals.

553

**Table 1: Retinyl ester concentrations (nmol/g tissue) in the liver of the different groups of rats.**

554

	Females		Males	
	C-C <sup>a</sup>	D-C <sup>b</sup>	C-C	D-C
<b>Retinyl palmitate</b>	1.49 ± 0.37 <sup>a</sup>	1.56 ± 0.30 <sup>a</sup>	1.19 ± 0.30 <sup>a</sup>	1.33 ± 0.32 <sup>a</sup>
<b>Retinyl stearate</b>	0.74 ± 0.15 <sup>a</sup>	0.82 ± 0.10 <sup>a</sup>	0.32 ± 0.08 <sup>b</sup>	0.48 ± 0.14 <sup>b</sup>
<b>Retinyl oleate</b>	0.15 ± 0.04 <sup>a</sup>	0.16 ± 0.03 <sup>a</sup>	0.09 ± 0.02 <sup>a</sup>	0.15 ± 0.05 <sup>a</sup>
<b>Retinyl linoleate</b>	0.15 ± 0.03 <sup>a</sup>	0.29 ± 0.06 <sup>b</sup>	0.13 ± 0.04 <sup>a</sup>	0.15 ± 0.03 <sup>a</sup>

555

556 <sup>a</sup>(C-C), offspring whose parents received a C diet and that received a C diet; <sup>b</sup>(D-C), offspring whose parents received a D diet and that received  
557 a C diet. Note that the C-D and D-D groups are not shown because, as shown in Figure 2b, no retinyl ester was detected in these groups. Values  
558 are means ± SEM. Means with different superscript letters on the same line indicate that they are significantly different (p<0.05, ANOVA  
559 followed by post-hoc Tukey-Kramer test).

560 **Table 2: Bioavailability of  $\beta$ -carotene and vitamin D and E, as well as  $\beta$ -carotene conversion rate to VA, in groups of rats with different**  
 561 **VA status<sup>1</sup>.**

562

Group <sup>2</sup>	Vitamin A status <sup>3</sup> (nmol/g)		$\beta$ -Carotene bioavailability <sup>4</sup> ( $\mu$ mol/L.h)		$\beta$ -Carotene conversion rate <sup>5</sup> (%)		Vitamin D response <sup>6</sup> ( $\mu$ mol/L.h)		Vitamin E response <sup>7</sup> ( $\mu$ mol/L.h)	
	females	males	females	males	females	males	females	males	females	males
<b>HVA p</b>	1849 $\pm$ 173 <sup>a</sup>	1195 $\pm$ 181 <sup>a</sup>	0.537 $\pm$ 0.120 <sup>a</sup>	0.047 $\pm$ 0.022 <sup>a*</sup>	26 $\pm$ 6 <sup>b</sup>	83 $\pm$ 4 <sup>b*</sup>	19 $\pm$ 3 <sup>a</sup>	16 $\pm$ 3 <sup>a</sup>	144 $\pm$ 53 <sup>a</sup>	55 $\pm$ 24 <sup>a*</sup>
<b>MVA p</b>	506 $\pm$ 45 <sup>b</sup>	506 $\pm$ 77 <sup>b</sup>	0.210 $\pm$ 0.069 <sup>b</sup>	0.034 $\pm$ 0.014 <sup>a*</sup>	64 $\pm$ 9 <sup>a</sup>	86 $\pm$ 5 <sup>b</sup>	21 $\pm$ 2 <sup>a</sup>	17 $\pm$ 4 <sup>a</sup>	122 $\pm$ 21 <sup>a</sup>	76 $\pm$ 26 <sup>a*</sup>
<b>LVA p</b>	55 $\pm$ 20 <sup>c</sup>	124 $\pm$ 37 <sup>c*</sup>	0.366 $\pm$ 0.092 <sup>b</sup>	0.028 $\pm$ 0.008 <sup>a*</sup>	43 $\pm$ 6 <sup>b</sup>	88 $\pm$ 5 <sup>b*</sup>	21 $\pm$ 3 <sup>a</sup>	17 $\pm$ 4 <sup>a</sup>	114 $\pm$ 39 <sup>a</sup>	75 $\pm$ 26 <sup>a*</sup>
<b>C-C o</b>	2.5 $\pm$ 0.6 <sup>d</sup>	1.7 $\pm$ 0.4 <sup>d</sup>	0.183 $\pm$ 0.047 <sup>b</sup>	0.032 $\pm$ 0.008 <sup>a*</sup>	37 $\pm$ 8 <sup>b</sup>	82 $\pm$ 3 <sup>b*</sup>	14 $\pm$ 3 <sup>a</sup>	14 $\pm$ 2 <sup>ab</sup>	42 $\pm$ 7 <sup>b</sup>	50 $\pm$ 12 <sup>a</sup>
<b>D-C o</b>	2.9 $\pm$ 0.4 <sup>d</sup>	2.1 $\pm$ 0.5 <sup>d</sup>	0.053 $\pm$ 0.010 <sup>c</sup>	0.023 $\pm$ 0.003 <sup>a*</sup>	72 $\pm$ 4 <sup>a</sup>	87 $\pm$ 4 <sup>b*</sup>	16 $\pm$ 1 <sup>a</sup>	18 $\pm$ 2 <sup>a</sup>	54 $\pm$ 7 <sup>b</sup>	52 $\pm$ 7 <sup>a</sup>
<b>C-D o</b>	0.0 $\pm$ 0.0 <sup>e</sup>	0.0 $\pm$ 0.0 <sup>e</sup>	0.046 $\pm$ 0.011 <sup>c</sup>	0.002 $\pm$ 0.001 <sup>b*</sup>	66 $\pm$ 6 <sup>a</sup>	93 $\pm$ 6 <sup>ab*</sup>	9 $\pm$ 2 <sup>b</sup>	11 $\pm$ 1 <sup>b</sup>	30 $\pm$ 5 <sup>b</sup>	24 $\pm$ 4 <sup>b</sup>
<b>D-D o</b>	0.0 $\pm$ 0.0 <sup>e</sup>	0.0 $\pm$ 0.0 <sup>e</sup>	0.030 $\pm$ 0.010 <sup>c</sup>	0.002 $\pm$ 0.001 <sup>b*</sup>	80 $\pm$ 7 <sup>a</sup>	97 $\pm$ 2 <sup>a*</sup>	10 $\pm$ 1 <sup>b</sup>	10 $\pm$ 2 <sup>b</sup>	23 $\pm$ 3 <sup>c</sup>	29 $\pm$ 6 <sup>b</sup>

563 <sup>1</sup>Pooling of the data obtained in this study and in a previous study dedicated to study VA metabolism in the parents of these rats [24].

564 <sup>2</sup>The MVA and LVA groups were the parents of the four others. (HVA p): parents that were fed a high VA diet (9858 IU/kg diet); (MVA p):  
565 parents that were fed a medium VA diet (2300 IU/kg); (LVA p): parents that were fed a low VA diet (400 IU/kg). Note that the MVA and LVA  
566 diets fed to the parents (p) contained the same amount of VA than the control diet (C) and the deficient diet (D) fed to the offspring (o),  
567 respectively.

568 <sup>3</sup>The VA status was estimated by measuring retinyl ester concentrations in the liver, which is assumed to be the best biomarker of VA status [39].

569 <sup>4</sup>Areas under the curves (AUC) of  $\beta$ -carotene postprandial plasma concentrations of following gavage with  $\beta$ -carotene ( $\mu\text{mol/L.h}$ ).

570 <sup>5</sup>Estimated according to the following formula: % conversion =  $\text{AUC retinyl ester} / (\text{AUC } \beta\text{-carotene} + \text{AUC retinyl ester}) \times 100$ .

571 <sup>6</sup>AUC of cholecalciferol postprandial plasma concentrations following gavage with cholecalciferol ( $\mu\text{mol/L.h}$ ).

572 <sup>7</sup>Incremental AUC of  $\alpha$ -tocopherol postprandial plasma concentrations following gavage with  $\alpha$ -tocopherol ( $\mu\text{mol/L.h}$ ).

573 Values are means  $\pm$  SEM (n=4-6 depending on the group). Different letters in the same column indicate significant differences ( $p < 0.05$ ) between  
574 groups. ANOVA followed by post-hoc Tukey-Kramer test. An asterisk associated with a mean in a group of males indicates that this mean is  
575 significantly different ( $p < 0.05$ ) from that of the corresponding group of females (Student t-test for unpaired values).

576 **Figure legends**

577

578 **Fig. 1** Plasma retinol concentrations. White bars: females (n = 6/group), black bars: males (n =  
579 6/group). **(C-C)**, offspring whose parents received a C diet and that received a C diet; **(D-C)**,  
580 offspring whose parents received a D diet and that received a C diet; **(C-D)**, offspring whose  
581 parents received a C diet and that received a D diet; **(D-D)**, offspring whose parents received a  
582 D diet and that received a D diet. Bars represent means  $\pm$  SEM. Gender effect  $p=0.007$ , parent  
583 diet effect  $p<0.0005$ , offspring diet effect  $p<0.0005$ . Statistical differences between groups are  
584 indicated in the insert. M&F indicates that there is a significant difference ( $p<0.05$ ) for males  
585 and for females. F indicates that there is a significant difference only in females. An asterisk  
586 indicates a significant difference between males and females from a given group. \*  $p<0.05$ ; \*\*  
587  $p<0.01$ .

588

589 **Fig. 2** Hepatic VA concentrations. **a:** hepatic VA = retinyl esters + free retinol. **b:** hepatic  
590 retinyl esters = retinyl palmitate + retinyl stearate + retinyl oleate + retinyl linoleate. **c:** hepatic  
591 free retinol. White bars: females (n = 6/group), black bars: males (n = 6/group). **(C-C)**,  
592 offspring whose parents received a C diet and that received a C diet; **(D-C)**, offspring whose  
593 parents received a D diet and that received a C diet; **(C-D)**, offspring whose parents received a  
594 C diet and that received a D diet; **(D-D)**, offspring whose parents received a D diet and that  
595 received a D diet. Bars represent means  $\pm$  SEM. **a:** Gender effect  $p=0.004$ , parent diet effect  
596  $p=0.023$ , offspring diet effect  $p<0.0005$ . **b:** Gender effect  $p=0.418$ , parent diet effect  $p=0.899$ ,  
597 offspring diet effect  $p<0.0005$ . **c:** Gender effect  $p=0.001$ , parent diet effect  $p=0.001$ , offspring  
598 diet effect  $p=0.002$ . Statistical differences between groups are indicated in the insert. M&F  
599 indicates that there is a significant difference ( $p<0.05$ ) for males and for females. F indicates  
600 that there is a significant difference only in females. ns: not significant. For p-values comprised

601 between 0.05 and 0.10, the p-value is given. The three asterisks indicate a significant difference  
602 ( $p < 0.001$ ) between males and females from this group.

603

604 **Fig. 3** Postprandial plasma responses of different VA species following force-feeding with  $\beta$ -  
605 carotene. **a:**  $\beta$ -carotene responses. **b:** Retinyl palmitate responses. **c:** Retinol responses. White  
606 bars: females ( $n = 6/\text{group}$ ), black bars: males ( $n = 6/\text{group}$ ). **(C-C)**, offspring whose parents  
607 received a C diet and that received a C diet; **(D-C)**, offspring whose parents received a D diet  
608 and that received a C diet; **(C-D)**, offspring whose parents received a C diet and that received a  
609 D diet; **(D-D)**, offspring whose parents received a D diet and that received a D diet.  
610 Postprandial plasma response means incremental area under the curve (AUC, expressed in  
611  $\mu\text{mol/L}\cdot\text{h}$ ) of the plasma concentrations of the molecule of interest measured at regular time  
612 intervals up to 6.5 hours after force-feeding. Bars represent means  $\pm$  SEM. **a:** Gender effect  
613  $p < 0.0005$ , parent diet effect  $p = 0.004$ , offspring diet effect  $p = 0.253$ . **b:** Gender effect  $p = 0.02$ ,  
614 parent diet effect  $p = 0.007$ , offspring diet effect  $p < 0.0005$ . **c:** Parent diet effect  $p = 0.022$ ,  
615 offspring diet effect  $p = 0.203$ . Statistical differences between groups are indicated in the insert.  
616 M&F indicates that there is a significant difference ( $p < 0.05$ ) for males and for females. M or F  
617 alone indicates that there is a significant difference only in males or in females. ns: not  
618 significant. For p-values comprised between 0.05 and 0.10, the p-value is given. An asterisk  
619 indicates a significant difference between males and females from a given group. \*  $p < 0.05$ ; \*\*  
620  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

621

622 **Fig. 4**  $\beta$ -Carotene bioavailability and conversion rate to VA in the intestine following force-  
623 feeding with  $\beta$ -carotene. **a:**  $\beta$ -Carotene bioavailability, calculated by summing the  $\beta$ -carotene  
624 and the retinyl palmitate responses because, under these experimental conditions, we assumed  
625 that postprandial plasma retinyl palmitate originated only from the intestinal metabolism of  $\beta$ -

626 carotene. **b**:  $\beta$ -Carotene conversion rate to VA, calculated as the percentage of newly absorbed  
627  $\beta$ -carotene found in the form of retinyl palmitate, i.e. retinyl palmitate response / ( $\beta$ -carotene  
628 response + retinyl palmitate response). White bars: females (n = 6/group), black bars: males (n  
629 = 6/group). (**C-C**), offspring whose parents received a C diet and that received a C diet; (**D-C**),  
630 offspring whose parents received a D diet and that received a C diet; (**C-D**), offspring whose  
631 parents received a C diet and that received a D diet; (**D-D**), offspring whose parents received a  
632 D diet and that received a D diet. Bars represent means  $\pm$  SEM. **a**: Gender effect  $p < 0.0005$ ,  
633 parent diet effect  $p = 0.99$ , offspring diet effect  $p < 0.0005$ . **b**: Gender effect  $p < 0.0005$ , parent diet  
634 effect  $p = 0.001$ , offspring diet effect  $p = 0.001$ . Statistical differences between groups are  
635 indicated in the insert. M&F indicates that there is a significant difference ( $p < 0.05$ ) for males  
636 and females. M or F alone indicates that there is a significant difference only in males or in  
637 females. ns: not significant. For p-values comprised between 0.05 and 0.10, the p-value is  
638 given. An asterisk indicates a significant difference between males and females from a given  
639 group. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

640

641 **Fig. 5** Vitamin E and D bioavailability following force-feeding. **a**:  $\alpha$ -Tocopherol  
642 bioavailability, i.e. incremental area under the curve (AUC) of the plasma concentrations of  $\alpha$ -  
643 tocopherol measured at regular time intervals up to 6.5 hours after force-feeding with an  
644 emulsion containing both  $\alpha$ -tocopherol and cholecalciferol. **b**: Cholecalciferol bioavailability,  
645 i.e. AUC of the plasma concentrations of cholecalciferol measured at the same time intervals  
646 after force-feeding with the same emulsion. White bars: females (n = 6/group), black bars:  
647 males (n = 6/group). (**C-C**), offspring whose parents received a C diet and that received a C  
648 diet; (**D-C**), offspring whose parents received a D diet and that received a C diet; (**C-D**),  
649 offspring whose parents received a C diet and that received a D diet; (**D-D**), offspring whose  
650 parents received a D diet and that received a D diet. Bars represent means  $\pm$  SEM. **a**: Offspring

651 diet effect  $p < 0.0005$ . **b**: Offspring diet effect  $p < 0.0005$ . Statistical differences between groups  
652 are indicated in the insert. M&F indicates that there is a significant difference ( $p < 0.05$ ) for  
653 males and for females. ns: not significant. For p-values comprised between 0.05 and 0.10, the  
654 p-value is given.

655  
656 **Fig. 6** Expression levels of hepatic genes involved in VA metabolism. White bars: females (n =  
657 6/group), black bars: males (n = 6/group). **(C-C)**, offspring whose parents received a C diet and  
658 that received a C diet; **(D-C)**, offspring whose parents received a D diet and that received a C  
659 diet; **(C-D)**, offspring whose parents received a C diet and that received a D diet; **(D-D)**,  
660 offspring whose parents received a D diet and that received a D diet. The full names of the  
661 genes not commented in the text are: *Ces1e* (carboxylesterase 1E), *Bco2* (beta-carotene  
662 oxygenase 2) and *Dgat1* (diacylglycerol O-acyltransferase 1). P-values for the effect of each  
663 tested factor, e.g. gender, parent diet and offspring diet, on the expression of each gene are  
664 shown in supplemental Table 2. Bars represent means of fold changes  $\pm$  SEM. Statistical  
665 differences between groups are indicated in the insert. M&F indicates that there is a significant  
666 difference ( $p < 0.05$ ) for males and for females. M or F alone indicates that there is a significant  
667 difference only in males or in females. ns: not significant. For p-values comprised between 0.05  
668 and 0.10, the p-value is given. An asterisk indicates a significant difference between males and  
669 females from a given group. \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

670  
671 **Fig. 7** Expression levels of intestinal genes involved in VA metabolism. White bars: females (n  
672 = 6/group), black bars: males (n = 6/group). **(C-C)**, offspring whose parents received a C diet  
673 and that received a C diet; **(D-C)**, offspring whose parents received a D diet and that received a  
674 C diet; **(C-D)**, offspring whose parents received a C diet and that received a D diet; **(D-D)**,  
675 offspring whose parents received a D diet and that received a D diet. The full names of the



676 genes are either in the text at their first occurrence or in the legend of figure 6. Bars represent  
677 means of fold changes  $\pm$  SEM. P-values for the effect of each tested factor, e.g. gender, parent  
678 diet and offspring diet, on the expression of each gene are shown in supplemental Table 2.  
679 Statistical differences between groups are indicated in the insert. M&F indicates that there is a  
680 significant difference ( $p < 0.05$ ) for males and for females. M or F alone indicates that there is a  
681 significant difference only in males or in females. For p-values comprised between 0.05 and  
682 0.10, the p-value is given. An asterisk indicates a significant difference between males and  
683 females from a given group. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

684 **Credit author statement:**

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696 Original Draft.

697 **5) References**

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