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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Abstract

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

Keywords: β-carotene, retinol, retinyl palmitate, bioavailability, cholecalciferol, tocopherol.

Statements and Declarations:

1) Introduction

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

The pathophysiological consequences of VA deficiency are multiple and can be dramatic if not corrected [7]. Beside the many metabolic effects due in particular to the regulation by this vitamin of the expression of hundreds of genes [8], it can also cause changes in its own metabolism. As a matter of fact, it was observed in rats that VA deficiency increases intestinal β-carotene cleavage activity [9]. It was also observed that VA deficiency reduces intestinal β-carotene uptake by brush border membrane vesicles [10]. J. von Lintig and colleagues have extensively studied the factors involved in this regulation [11-13] ENREF_13. They showed that when retinoic acid concentration is high, the intestinal transcription factor ISX (intestine specific homeobox) represses the expression of both *BCO1* (beta-carotene oxygenase 1), the main enzyme that cleaves β-carotene in the intestine [14-18], and *SCARB1* (scavenger receptor class B member 1), which encodes for SR-BI, an apical membrane protein involved in β-carotene uptake by enterocytes [19,20]. When VA status, and hence retinoic acid concentration, is low, it is assumed that ISX does not repress *BCO1* and *SCARB1* anymore. The consequent increase in their expression leads to a higher β-carotene absorption and conversion rate by the small intestine [12,21]. However, the results of two studies contradicted this mechanism [22,23]. Lemke et al. observed a decrease in β-carotene conversion efficiency

76   following VA supplementation together with an increase in the absorption of β-carotene[22]

77   while Goswami et al. [10] observed a decrease in β-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 *SCARB1*, 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 β-carotene.

2) Material and methods

Chemicals

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

Animals

Institutional guidelines for the care and use of animals were followed and all experimental procedures were approved by the local animal care and use committee (agreement number D 13-055-20). Rats were housed under standard conditions of light (12-h light/dark cycle; lights on at 8 am) and temperature (22-24°C) with free access to tap water and the different VA diet. Rats were the offspring of Sprague Dawley RjHan:SD rats from a previous study which aimed to assess the effect of dietary VA content on intestinal and hepatic metabolism of VA in adult rats [24]. Ten-week-old female and male rats were fed either a diet with usual VA content, i.e. 2300 IU/kg, thereafter called the control (C) diet, or a diet low in 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 β-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 β-carotene-rich emulsion, which provided 3 mg

156   β-carotene/gavage, was described in a recently published paper [24]. The preparation of the

157   emulsion rich in both cholecalciferol and α-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 α-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 µL) were collected from a tail nick into tubes containing

166   50 µL 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 µL HPLC mobile phase (see below). A volume

181   of 50–180 µL was used for HPLC analysis.

182   All compounds were separated using a 250 x 4.6 mm RP C18, 5 µm 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-

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

Measurement of gene expression in liver and duodenum samples

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

Calculations

Vitamin D and E bioavailability was estimated by measuring the areas under the curves (AUC) of their postprandial plasma concentrations (0-6 h) following force-feeding. Regarding cholecalciferol, it is well established that its fasting plasma concentration is negligible, as also observed in our study (data not shown). Moreover, when cholecalciferol is ingested, it is practically not metabolized in the upper small intestine before its absorption by intestinal cells [34]. Finally, it is assumed that most cholecalciferol absorbed by the intestinal cell is incorporated as such in chylomicrons, which are then secreted in the lymph allowing 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   β-Carotene bioavailability, β-carotene conversion rate to VA and VA status were

216   estimated as previously described [24]. Briefly, β-carotene bioavailability was estimated by

217   summing the β-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 β-carotene into VA. Concerning β-Carotene conversion rate to VA, it

220   was calculated as the percentage of bioavailable β-carotene found in the form of retinyl

221   palmitate in the plasma during the postprandial period, i.e. retinyl palmitate response / (β-

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 ± 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).

3) Results

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

Plasma and liver VA concentrations.

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

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

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

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

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

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

Postprandial plasma β-carotene and VA responses following force-feeding with β-carotene.

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286   As expected, and unlike retinol, neither β-carotene nor retinyl palmitate were detected

287   in plasma prior to gavage. **Figure 3a** shows the postprandial β-carotene response, i.e. the 0-6 h

288   AUC of its postprandial concentration, expressed in μmol/L*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 β-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 β-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, β-carotene bioavailability was always higher in females than in males

317   (+120% in females as compared to males).

318   β-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 β-carotene

323   conversion rates to VA (D-C vs C-C, +96%). Finally, β-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   *α-Tocopherol and cholecalciferol bioavailability.*

329   Results are shown in **Figures 5a and 5b**. As expected, α-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 α-tocopherol (p=0.075), but only in rats whose parents

333   were fed the D diet: offspring that received the D diet exhibited lower α-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

16

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

VA status, bioavailability of β-carotene and vitamin D and E, and β-carotene conversion rate in parents and offspring.

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

4) Discussion

VA status of the different groups of rats

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

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

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

18

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

A VA-deficient diet during the perinatal period appears to improve offspring's ability to convert β-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 β-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 β-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 β-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 β-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 β-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 β-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 β-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 β-carotene

474 bioavailability increases when VA status decreases. On the contrary, the lower the VA status,

475 the lower the bioavailability of β-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 β-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 β-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 β-carotene

484   bioavailability. On the contrary, β-carotene bioavailability collapsed in these rats. Likewise, β-

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 β-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 β-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 β-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 β-carotene conversion rate (**table 2** which compiles the esults obtained in the parent

499   rats and in the offspring rats). Indeed, this supports the hypothesis that, in male rats, VA

500   deficiency increases β-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 β-carotene, but also that*

504   *of vitamins E and D.*

505   Knowing that the absorption of β-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 β-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

event of a very strong VA deficiency. In other words, there is apparently no dose-response effect of the VA content of the diet on *Isx* expression. Furthermore, the effect of *Isx* on *Bco1* and *Scarb1* expression apparently only exists in male rats. The third observation is that severe VA deficiency decreases the bioavailability of both β-carotene and vitamin D and E by an unknown mechanism, likely linked to an overall deterioration of the integrity of the intestinal mucosa due to VA deficiency. The fourth observation confirms the significant effect of the sex of the rats on vitamin A metabolism. We acknowledge we did not verify whether variations in gene expression translated into variations in protein concentrations. Nevertheless, we had shown in the study on the parents of these rats that this was the case, at least for the three key proteins measured [24].

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

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

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

554

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

555

[a]**(C-C)**, offspring whose parents received a C diet and that received a C diet; [b]**(D-C)**, offspring whose parents received a D diet and that received 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 are means ± SEM. Means with different superscript letters on the same line indicate that they are significantly different ($p<0.05$, ANOVA followed by post-hoc Tukey-Kramer test).

Table 2: Bioavailability of β-carotene and vitamin D and E, as well as β-carotene conversion rate to VA, in groups of rats with different VA status[1].

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

[1]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   [2]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   [3]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   [4]Areas under the curves (AUC) of β-carotene postprandial plasma concentrations of following gavage with β-carotene (μmol/L.h).

570   [5]Estimated according to the following formula: % conversion = AUC retinyl ester / (AUC β-carotene + AUC retinyl ester) x 100.

571   [6]AUC of cholecalciferol postprandial plasma concentrations following gavage with cholecalciferol (μmol/L.h).

572   [7]Incremental AUC of α-tocopherol postprandial plasma concentrations following gavage with α-tocopherol (μmol/L.h).

573   Values are means ± 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).

Figure legends

Fig. 1 Plasma retinol concentrations. White bars: females (n = 6/group), black bars: males (n =

6/group). **(C-C)**, offspring whose parents received a C diet and that received a C diet; **(D-C)**,

offspring whose parents received a D diet and that received a C diet; **(C-D)**, offspring whose

parents received a C diet and that received a D diet; **(D-D)**, offspring whose parents received a

D diet and that received a D diet. Bars represent means ± SEM. Gender effect p=0.007, parent

diet effect p<0.0005, offspring diet effect p<0.0005. Statistical differences between groups are

indicated in the insert. M&F indicates that there is a significant difference (p<0.05) for males

and for females. F indicates that there is a significant difference only in females. An asterisk

indicates a significant difference between males and females from a given group. * p<0.05; **

p<0.01.

Fig. 2 Hepatic VA concentrations. **a:** hepatic VA = retinyl esters + free retinol. **b:** hepatic

retinyl esters = retinyl palmitate + retinyl stearate + retinyl oleate + retinyl linoleate. **c:** hepatic

free retinol. White bars: females (n = 6/group), black bars: males (n = 6/group). **(C-C)**,

offspring whose parents received a C diet and that received a C diet; **(D-C)**, offspring whose

parents received a D diet and that received a C diet; **(C-D)**, offspring whose parents received a

C diet and that received a D diet; **(D-D)**, offspring whose parents received a D diet and that

received a D diet. Bars represent means ± SEM. **a:** Gender effect p=0.004, parent diet effect

p=0.023, offspring diet effect p<0.0005. **b:** Gender effect p=0.418, parent diet effect p=0.899,

offspring diet effect p<0.0005. **c:** Gender effect p=0.001, parent diet effect p=0.001, offspring

diet effect p=0.002. Statistical differences between groups are indicated in the insert. M&F

indicates that there is a significant difference (p<0.05) for males and for females. F indicates

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 β-

605 carotene. **a:** β-carotene responses. **b:** Retinyl palmitate responses. **c:** Retinol responses. White

606 bars: females (n = 6/group), black bars: males (n = 6/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 μmol/L*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 ± 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** β-Carotene bioavailability and conversion rate to VA in the intestine following force-

623 feeding with β-carotene. **a:** β-Carotene bioavailability, calculated by summing the β-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 β-

626 carotene. **b**: β-Carotene conversion rate to VA, calculated as the percentage of newly absorbed

627 β-carotene found in the form of retinyl palmitate, i.e. retinyl palmitate response / (β-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 ± 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:** α-Tocopherol

642 bioavailability, i.e. incremental area under the curve (AUC) of the plasma concentrations of α-

643 tocopherol measured at regular time intervals up to 6.5 hours after force-feeding with an

644 emulsion containing both α-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 ± 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 ± 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 ± 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. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

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