Effects of dietary fatty acids on the social life of male Guinea pigs from adolescence to adulthood

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ABSTRACT

Dietary intake of polyunsaturated fatty acids (PUFAs) or saturated fatty acids (SFAs) differently modulates neurophysiological and behavioral functions in response to altered hypothalamic-pituitary-adrenal (HPA)-axis activity and an individual's development. In this context, an individual's social environment, including social interactions and social hierarchies, is closely related to hormone concentrations and possibly interacts with dietary fatty acid effects. We investigated if dietary supplementation with walnut oil (high in PUFAs) and coconut fat (high in SFAs), compared to a control group, affects body mass gain, cortisol and testosterone concentrations, plasma fatty acids, and social behavior in male domestic guinea pigs from adolescence to adulthood. For analyses of cortisol and testosterone concentrations, social interactions were included as covariates in order to consider effects of social behavior on hormone concentrations. Our results revealed that SFAs increased escalated conflicts like fights and stimulated cortisol and testosterone concentrations, plasma fatty acids, and social behavior in male domestic guinea pigs from adolescence to adulthood. For analyses of cortisol and testosterone concentrations, social interactions were included as covariates in order to consider effects of social behavior on hormone concentrations. Our results revealed that SFAs increased escalated conflicts like fights and stimulated cortisol and testosterone concentrations, which limited body mass gain and first-year survival. PUFAs did not notably affect social behavior and hormone concentrations, but enabled the strongest body mass gain, which probably resulted from an energetic advantage. Neither sociopositive nor agonistic behaviors explained age-specific differences in hormone concentrations between groups. However, a high number of subdominant individuals and lower testosterone concentrations were related to increased cortisol concentrations in adult PUFAs males. Our findings demonstrate the importance of dietary fatty acids regarding behavioral and endocrine developmental processes and adaptations to the social environment by modulating HPA-axis function and body homeostasis.

1. Introduction

The pre- and postnatal dietary macronutrient content and composition shapes developmental processes by modulating body homeostasis and related metabolic, neurophysiological, and behavioral functions (Davidson et al., 2013; Gluckman et al., 2007; Reynolds et al., 2015). Homeostasis is strongly linked to hypothalamic control of energy balance (Pierce and Xu, 2010; Rui, 2013) and more specifically to the hypothalamic-pituitary-adrenal (HPA)-axis, which regulates physiological stress responses and metabolic processes by glucocorticoids such as cortisol (Sapolsky et al., 2000). Malnutrition can impair hypothalamic and HPA-axis functions, which results in disturbed homeostasis and increased risk of developing metabolic and neuroendocrine diseases and mental disorders throughout life (Chen and Su, 2013; Dearden and Ozanne, 2015; Poore et al., 2010; Sullivan et al., 2015).

The dietary fatty acid content and composition, including non-essential saturated fatty acids (SFAs) and essential omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids (PUFAs), play an important role for hypothalamic and HPA-axis functions. PUFAs are crucial components of cell membrane phospholipids (Hishikawa et al., 2017) and precursors for signal molecules such as prostaglandins and leukotrienes (Youdim et al., 2000). Dietary PUFA intake can affect neurotransmission (Chalon, 2006), counteract inflammatory responses (Song et al., 2003), or modulate gene expression (Deckelbaum et al., 2006). This is mainly mediated by the long-chain PUFAs eicosapentaenoic acid (EPA, 20:5 n-3), docosahexaenoic acid (DHA, 22:6 n-3), and arachidonic acid (AA, 20:4 n-6), which are synthesized through desaturation and elongation steps from the respective dietary essential short-chain PUFAs alpha-linolenic acid (ALA, 18:3 n-3) and linoleic acid (LA, 18:2 n-6) (Sprecher, 2000). Adequate dietary intake of n-3 and n-6 PUFAs can promote neuronal and structural development (Carlson et al., 2019; Harauma et al., 2017), whereas PUFA deficiency or increased SFA...
intake impairs neurophysiological functions and development (Granholm et al., 2008; van Elst et al., 2019).

High-fat diets, which mainly contain SFAs, not only increase the risk for obesity, but have also been shown to impair hypothalamic functions and induce depressive-like behaviors in mice (Vagena et al., 2019) and caused HPA-axis dysfunctions linked to elevated glucocorticoid concentrations in rats (Hryhorczuk et al., 2017). Similarly, n-3 PUFA deficiency also resulted in excessive glucocorticoid secretion rates and caused depressive behavior and anxiety in response to stress in rats and mice (Chen and Su, 2013; Larrieu et al., 2014). N-3 PUFA supplementation can significantly counteract these adverse physiological and behavioral influences by diminishing hypothalamic inflammation (Cintrá et al., 2012) and reducing HPA-axis responses and glucocorticoid concentrations as well as related impairments of metabolic and behavioral functions (Ferraz et al., 2011; Hennebelle et al., 2012; Nemeth et al., 2014; Song et al., 2003). Studies in humans and animal models further revealed effects of n-3 PUFAs on mental health by decreasing the risk for anxiety or depression (Ferraz et al., 2011; Su et al., 2015), reducing aggressive behavior (DeMar Jr et al., 2006; Gajos and Beaver, 2016), and restoring social deficits (De Theije et al., 2015; Fortunato et al., 2017). Interestingly, these effects were mainly observed in relation to adverse physiological conditions such as obesity, inflammation, or mental disorders, which indicate disturbed homeostasis.

Recent findings in socially housed adult guinea pigs showed that dietary SFAs at normal-caloric levels can increase cortisol concentrations even under non-stressful conditions, while PUFAs counteracted stress-related impairments of social behavior in males (Nemeth et al., 2016a). Furthermore, SFAs not only increased cortisol but also testosterone concentrations in males, which was associated with testes development but restricted body mass gain during adolescence. In PUFA supplemented males, however, hormone concentrations did not affect growth rates, resulting in highest body mass (Nemeth et al., 2019). These results suggest male-specific effects of PUFAs and SFAs on body homeostasis by modulating basal HPA-axis and hypothalamic-pituitary-gonadal (HPG)-axis activities related to altered behavior and structural development. However, long-lasting influences until adulthood and a possible impact of the social environment remain to be determined. The social environment, including social interactions and social hierarchies, is an important modulator of HPA- and HPG-axis activity (Creel et al., 2013; Hennessy et al., 2006; Sachser et al., 1998) and could interact with the effects of dietary fatty acids. This raises the question if and how far the observed and mainly opposed effects of PUFAs and SFAs on steroid hormone concentrations result from modulated social behavior and perhaps even an individual’s dominance rank.

The aim of the present study was to analyze effects of PUFA and SFA supplementations on social behavior, cortisol, and testosterone concentrations during adolescence and adulthood of socially housed male guinea pigs. In order to detect any possible effect of these nutrients, supplementation started already at a prenatal state and continued until the end of the study, therefore, representing a permanent part of the animal’s environment. Cortisol and testosterone concentrations were also analyzed in response to initiated and received social behavior recorded directly before sample collections to consider behavioral influences on hormone secretion. SFAs were expected to increase cortisol and testosterone concentrations, which were further suggested to induce aggressive behavior and adversely affect body conditions. In contrast, PUFAs were assumed to diminish aggressiveness, decrease hormone concentrations, and, therefore, enable highest body conditions during adulthood.

2. Methods

2.1. Ethical statement

The experiment was performed in accordance with national guidelines for animal keeping and animal experiments. The study has been examined and approved by the internal board on animal ethics and experimentation of the Faculty of Life Sciences of the University of Vienna (# 2014-005) and the Austrian Federal Ministry of Science and Research (BMWF-66.006/0024-II/3b/2013).

2.2. Animals and diets

The experiments were carried out from November 2013 until January 2015. All domestic guinea pigs (Cavia aperea f. porcellus) used in this study were bred at the animal care facility of the Department of Behavioral Biology at the University of Vienna. In preparation for this study, sixty adult animals (30 males, and 30 females; age 6 to 24 months) were randomly allocated to single-sexed social groups of ten individuals, which resulted in three male and three female groups. The random allocation of individuals to social groups was carried out by persons unfamiliar with the study design. Each group’s enclosure (2 m × 1.6 m each) was equally equipped with shelters and the floor was covered with bedding material. Ambient conditions, including a temperature of 20 ± 2 °C, a humidity of 50 ± 5%, and a light-dark cycle of 12 h each (lights on at 07:00 a.m.), were maintained throughout the study.

Each male and female social group was randomly subjected to one of three diets, which differed in the fatty acid content and composition. A daily amount of 300 g guinea pig pellets (ssniff V-2233, ssniff Spezialdiäten GmbH, Soest, Germany) per social group was enriched with 10% (w/w) walnut oil (PUFA group), previously liquefied (30 °C) coconut fat (SFA group), or left untreated for the control group. Both fatty acid sources contain 100% fat; walnut oil contains 73.9% PUFAs (including n-6 and n-3 PUFAs in a ratio of 4:1) and coconut fat contains 92% SFAs. The PUFA- and SFA-enriched diets were freshly prepared every three days by mixing corresponding amounts of guinea pig pellets with walnut oil or coconut fat, respectively, in freezer bags and thoroughly shaking them for 5 min. The diets were prepared on the day before first provision to ensure a maximum absorption of oil/fat by the pellets. Regular quality checks of the diets were carried out by gas chromatographic analyses. The final fatty acid compositions of the different experimental diets are outlined in Table 1. The group-specific pellets were provided daily in several food bowls. As in previous studies (e.g. Nemeth et al., 2016a; Nemeth et al., 2018), all individuals immediately accepted the respective diets and rejections were never observed. Additionally, animals were weighed once per week, which

Table 1

| Fatty acid composition (% of total fatty acids) of the experimental diets. |
|-----------------|-----------------|-----------------|
| Fatty acids     | Control         | PUFA            | SFA              |
| C12:0           | n.d.            | n.d.            | 37.11            |
| C14:0           | 0.61            | n.d.            | 17.13            |
| C16:0           | 16.11           | 9.00            | 13.01            |
| C18:0           | 3.35            | 2.92            | 4.32             |
| C20:0           | 0.30            | n.d.            | n.d.             |
| Total SFA       | n.d.            | n.d.            | 0.13             |
| C16:1           | 0.61            | n.d.            | n.d.             |
| C18:1 n-7       | n.d.            | 0.96            | 0.40             |
| C18:1 n-9       | 18.90           | 15.41           | 9.85             |
| Total MUFA      | 19.51           | 16.38           | 10.36            |
| C18:2 n-6       | 50.00           | 60.57           | 15.66            |
| C18:3 n-6       | n.d.            | 0.30            | 0.20             |
| C18:3 n-3       | 10.06           | 10.83           | 2.08             |
| Total n-6       | 50.00           | 60.87           | 15.86            |
| Total n-3       | 10.06           | 10.83           | 2.08             |
| Total PUFA      | 60.06           | 71.70           | 17.93            |

Fatty acids were analyzed by gas chromatography. MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids. n.d.: not detectable.
helped to ensure adequate and balanced data allowing reliable within- and between groups. Although individual food intake could not be quantified, the dietary fatty acid composition for each individual within a group was the same and adequate fatty acid uptake from the diet was confirmed by measuring fatty acid patterns in plasma. Additionally, 50 g of hay per group was provided daily in a hay rack to promote abrasion of the teeth and to support intestinal activity. Water was available ad libitum in several drinking bottles.

Animals were maintained on the group-specific diet for 100 days. Male and female groups of the same dietary regime were then mated. After females were detected to be pregnant, single-sexed social groups were re-established to ensure that females remained undisturbed from male encounters during gestation. A total of 25 females gave birth to 69 pups (male and female pups per group: control 14/10, PUFA 14/9, SFA 13/9), which were housed with their mothers and the other females and pups of the same dietary group. Pups were maintained on the same diets as their mothers. Male offspring were separated from females at an age of 20–30 days and transferred to newly established single-sexed social groups for each dietary regime. Based on previous results demonstrating male-specific dietary fatty acids effects (Nemeth et al., 2016a; Nemeth et al., 2018), this study focused on male individuals. Male diet groups were maintained in their group constellation and on the respective dietary regime throughout the study until an age of 12 months.

2.3. Experimental procedures

During adolescence, at an age of 60, 90, and 120 days, as well as during adulthood, at an age of 240 days, several measurements and analyses were performed. While measurements were performed once per individual at the respective age during adolescence, measurements were conducted on three consecutive days during adulthood. Different ages during adolescence were considered to include the animals’ physical, physiological, and behavioral development in the analyses. Measurements on three consecutive days during adulthood were carried out to obtain adequate and balanced data allowing reliable within-group comparisons between adolescence and adulthood. However, this also allowed more detailed analyses during adulthood in relation to the prevalent social hierarchies.

The following procedure was carried out whenever an individual reached an age of 60, 90, 120, or 240 days, respectively. The procedure always started with video recordings at 09:00 a.m. in order to analyze an individual’s social interactions. For this, all shelters and food bowls in the enclosures were removed to ensure that all animals were permanently visible. As this was also necessary for the daily routines (e.g. cleaning of the enclosures, feeding, weekly weighing of the animals), the animals were habituated to the situation. Animals were video recorded for 30 min from the bird’s eye view with cameras fixed at the ceiling above each group’s enclosure.

Directly after video recordings at 09:30 a.m., each animal was weighed on a standard kitchen balance (accuracy ± 1 g) and a saliva sample was collected to measure saliva cortisol concentrations. Saliva cortisol concentrations were previously demonstrated to be biologically valid in guinea pigs (Fenske, 1997; Nemeth et al., 2016b) and, therefore, this minimally-invasive method was chosen. Saliva was collected using a standard cotton bud, which was inserted into the animal’s mouth for approximately one minute. The procedure of weighing and saliva sampling lasted no longer than 60 s per individual. Thereafter, an animal was immediately returned to its group and the next one was sampled. Saliva sample collection on each sampling day was carried out within a time span of 30 min between 09:30 a.m. and 10:00 a.m. Samples were centrifuged (14,000 rpm, 10 min) and pure saliva stored at −20 °C.

After weighing and saliva sampling procedures were completed in all animals, blood samples were collected between 10:00 a.m. and 10:30 a.m. to analyze plasma testosterone concentrations. Measurements of testosterone concentrations in saliva could not be implemented yet and, therefore, blood samples had to be collected. While blood samples were collected on each day during adolescence, this was only performed once during adulthood, namely on the last days of the three-day sampling period, which reduced the number of invasive sampling procedures. The whole schedule for blood samplings was also chosen because testosterone concentrations show lower fluctuation rates compared to cortisol concentrations in response to social interactions in guinea pigs (Wallner and Dittami, 2003). Additionally, plasma fatty acid patterns were analyzed in samples collected at an age of 120 days and during adulthood as indicators of the fatty acid uptake from the diet and their metabolic integration. Blood samples (300 μl) were collected by gently puncturing prominent ear veins with a sterile lancet and collecting the flowing blood with heparinized (5000 U) micropipettes. Plasma was separated by centrifugation (14,000 rpm, 10 min) and stored at −20 °C.

The idea of the whole sampling regime was to analyze differences and changes in cortisol and testosterone concentrations between groups and across time as well as in response to social interactions. This allowed, on one hand, to determine effects of social behavior on cortisol and testosterone concentrations per se and, on the other hand, to confirm any differences between groups when social behavior is considered to show possible side-effects on hormone concentrations. Moreover, adjusting hormone concentrations statistically to individual differences in initiated and received social behavior reduced dependency of hormone measurements of different individuals within a social group.

Based on results of a recent study (Nemeth et al., 2018), we included recordings of the individual’s mortality within the first 12 months of life in this study in order to analyze potential effects of dietary fatty acids and related steroid hormone concentrations during adolescence and adulthood on the first-year mortality rate.

2.4. Behavioral analyses

Videos were analyzed using The Observer XT 10 (Wageningen, the Netherlands). Frequencies of initiated (active) and received (passive) social behavior were analyzed for each individual using continuous recording (Martin and Bateson, 2007). Behaviors mainly followed the ethogram for guinea pigs by Rood (1972) and were categorized in sociopositive behavior (social grooming, nose-nose contact, ano-genital sniffing), dominant behavior (displacement [re-treat of an opponent], chase, stand threat [threat behavior in curved body posture], rumba rumble [guinea pig-specific display behavior], mounting [in terms of display behavior]), and fights (bite, fight). Although rumba rumble and mounting are male-specific sexual behaviors, these behaviors are also shown by dominant males towards subordinates in terms of display behavior (Machatschke et al., 2008). Although dominant behavior and fights both represent agonistic behavior, they were treated separately based on the intensity and the context in which they are shown. Dominant behavior is shown in order to clarify dominance relations between individuals and to prevent fights. Fights represent escalated agonistic interactions, including heavy body contact and a high risk of getting injured in such an interaction (biting and fighting).

Each individual’s hierarchy index as indicator of its social rank within its group was calculated from total initiated (active) and received (passive) dominant behaviors during adulthood (hierarchy index = active dominant behavior / (active + passive dominant behavior)). This proportion of initiated or won interactions on total interactions an individual was involved in corresponds to previous analyses of such an index in guinea pigs (e.g. Zipser et al., 2013). This calculation yielded values between 0 and 1; values closer to 0 reflect more received dominant behavior and indicate a subdominant status and values closer to 1 reflect more initiated dominant behavior and indicate a dominant status.
2.5. Steroid hormone analyses

Saliva cortisol and plasma testosterone concentrations were analyzed by biotin-strepavidin enzyme-immunoassays using cortisol- and testosterone-specific antibodies, which showed negligible cross-reactions with other steroids (Palme and Möstl, 1994; Palme and Möstl, 1997). Both assays have been demonstrated to be biologically and physiologically valid for adequate measurements in plasma of guinea pigs (Bauer et al., 2008) and also in saliva with regard to cortisol (Nemeth et al., 2016b). Saliva cortisol concentrations (ng/ml) were measured directly in 1:50 dilutions of the samples without any preceding extractions. Intra- and inter coefficients of variance were 9.75% and 4.54%, respectively. Steroids from plasma were extracted using diethylether, shaking the samples for 15 min, and freezing them overnight at −20 °C. Supernatants, which contained the steroids, were then evaporated at 30°C and diluted 1:5 for analysis of plasma testosterone concentrations (ng/ml). Intra- and inter coefficients of variance were 6.35% and 0.18%, respectively. All analyses were run in duplicates.

2.6. Fatty acid analyses

Plasma fatty acids were analyzed using gas chromatography. Preparation of fatty acids followed previously described protocols (Nemeth et al., 2014) and was performed using methanolic NaOH, containing butylated hydroxytoluene, for transesterification and boron trifluoride to obtain fatty acid methyl esters (FAMEs). FAMEs were separated by an Auto-System-Gaschromatograph (Perkin Elmer, USA) with flame ionization detector, equipped with an Rtx-2330 30 m × 0.25 mm i.d. silica column. One μl of prepared samples were injected at 250 °C and detected at 275 °C with helium acting as carrier gas. Single fatty acids were identified based on a 37 component FAME mix standard (Supelco, Bellafonte, USA) and using TotalChrome Workstation 6.3.0 (PerkinElmer, USA) for peak integration. Single fatty acids were expressed as percentage of total plasma fatty acids. For further analyses, single fatty acids were summed up to total PUFA and SFA levels in order to calculate the plasma PUFA:SFA ratio as important indicator of the fatty acid status.

2.7. Statistical analyses

Statistics were performed using R 3.5.2 (R Core Team, 2018). Linear mixed models (LMMs) were used to analyze body mass, displayed behaviors, steroid hormone concentrations, and the plasma PUFA:SFA ratio recorded during adolescence and adulthood. For each parameter, LMMs included ‘group’ (control, PUFA, SFA) and ‘age’ (60d, 90d, 120d, Adult; note: all three measurements performed during adulthood were included in one factor level; in case of plasma fatty acids only two factor levels at all: 120d and Adult), as well as their interaction as fixed effects.

### Table 2

Model statistics (ANOVA tables) for analyses of saliva cortisol and plasma testosterone concentrations (fitted based on the AIC) during adolescence and adulthood of male guinea pigs maintained on a control, high PUFA, or high SFA diet.

| Response variable | Predictor                              | df  | F     | p     | R²  |
|-------------------|----------------------------------------|-----|-------|-------|-----|
| LOG saliva cortisol model 1 | Group                                  | 2  | 0.195 | 0.825 | 0.25 |
|                    | Age                                    | 3   | 8.352 | < 0.001 | |
|                    | Group: Age                             | 6   | 2.426 | 0.028 |   |
| LOG saliva cortisol model 2 | Group                                  | 2  | 0.834 | 0.449 | 0.45 |
|                    | Age                                    | 3   | 1.731 | 0.163 |   |
| LOG plasma testosterone model 1 | Group                                  | 2  | 0.003 | 0.955 |   |
|                    | LOG Sociopositive ACT                  | 1   | 0.003 | 0.960 |   |
| LOG Dominant ACT   | 1                                           | 6.748 | 0.010 |   |
| LOG Dominant PASS  | 1                                           | 2.824 | 0.095 |   |
| LOG Fights PASS    | 1                                           | 3.323 | 0.070 |   |
| Group: Age         | 6                                           | 2.750 | 0.014 |   |
| Group: LOG Dominant PASS | 2  | 2.924 | 0.057 |   |
| Age: LOG Sociopositive ACT | 3   | 2.052 | 0.109 |   |
| Age: LOG Sociopositive PASS | 3   | 1.871 | 0.137 |   |
| Age: LOG Dominant ACT | 3                                           | 2.650 | 0.051 |   |
| Age: LOG Dominant PASS | 3                                           | 3.418 | 0.019 |   |
| Group              | 2                                           | 3.073 | 0.072 | 0.18 |
| Age                | 3                                           | 0.132 | 0.941 |   |
| Group: Age         | 6                                           | 3.282 | 0.006 |   |
| LOG plasma testosterone model 2 | Group                                  | 2  | 4.567 | 0.023 | 0.42 |
|                    | Age                                    | 3   | 0.708 | 0.550 |   |
| LOG Sociopositive ACT | 1                                           | 23.261 | < 0.001 |   |
| LOG Dominant PASS  | 1                                           | 28.300 | < 0.001 |   |
| LOG Dominant ACT   | 1                                           | 12.582 | 0.001 |   |
| LOG Fights PASS    | 1                                           | 4.998 | 0.028 |   |
| Group: Age         | 6                                           | 3.660 | 0.003 |   |
| Group: LOG Fights PASS | 2                                           | 4.394 | 0.015 |   |
| Age: LOG Sociopositive PASS | 3                                           | 10.103 | < 0.001 |   |
| Age: LOG Dominant ACT | 3                                           | 3.125 | 0.030 |   |
| Age: LOG Dominant PASS | 3                                           | 3.124 | 0.030 |   |
| LOG saliva cortisol adult | Group                                  | 2  | 5.646 | 0.010 | 0.47 |
|                    | Testosterone                           | 1.23 | 3.488 | 0.075 |   |
|                    | Hierarchy index                        | 1.23 | 5.138 | 0.033 |   |
|                    | Group: testosteroners                   | 2.23 | 2.013 | 0.156 |   |
|                    | Group: hierarchy index                  | 2.23 | 2.687 | 0.089 |   |
|                    | Testosteroners : hierarchy index        | 1.23 | 3.605 | 0.079 |   |
|                    | Group: testosteroners : hierarchy index  | 2.23 | 1.463 | 0.252 |   |

Model 1: LMMs with group and age effects; model 2: LMMs with group and age effects including social behavior as covariates. Adult: LM with group effect including testosterone and the Hierarchy index as covariates. ACT: active/initiated behavior; LOG: log-transformed; PASS: passive/received behavior.
effects. ‘Mother ID’ and ‘individual ID’ were included as random effects to correct for relatedness and repeated measurements. LMMs were performed using package ‘lme4’ (Pinheiro et al., 2017), because the included functions enable fitting of variance structures due to heteroscedasticity of model residuals, which applied to our data. Additionally, hormonal and behavioral data had to be transformed by applying the natural logarithm in order to obtain normal distribution of the residuals. For analysis of behaviors, an additional variable (0,1) was included in order to correct for a separation event (separation of the last juveniles from their mothers and integration into the newly established male adolescent groups), which affected social interactions in the control group at an age of 60 days. In each case, this had a highly significant impact (always \( p \leq 0.001 \)) and statistically adjusting the analyses for this event normalized the behavioral data.

Additionally, a generalized linear mixed model (GLMM) using package ‘lme4’ (Bates et al., 2015) was performed including frequencies of fights and dominant behaviors as response variable with binomial distribution and logit-link, therefore analyzing the proportion of fights on total agonistic behaviors. ‘Group’ and ‘age’, as well as their interaction, were included as fixed effects, and ‘mother ID’ and ‘individual ID’ as random effects. We corrected this model also to the mentioned separation event (0,1).

In order to analyze effects of social behavior on saliva cortisol and plasma testosterone concentrations and hormone concentrations adjusted for social behavior, LMMs were performed as described above, but also including frequencies of active and passive behaviors (sociopositive, dominant, fights). All possible interactions with ‘group’ and ‘age’ were allowed in order to consider group- and age-specific effects of initiated and received social behavior on hormone concentrations. The proportion of fights (predicted values from GLMMs as described above) could not be included in these analyses, because this would have resulted in aliased coefficients due to the calculation from behavioral frequencies. However, additional LMMs for cortisol and testosterone concentrations were calculated including the active and passive proportion of fights as predictors and these were compared to the models which included single behaviors as predictors. The proportion of fights explained less of the variance (cortisol: \( R^2 = 0.37 \), testosterone: \( R^2 = 0.30 \)) compared to single types of behaviors (for \( R^2 \) values see Table 2) and generally fitted the hormonal data poorly by even increasing the variances for groups at specific ages. Therefore, considering single types of behaviors was chosen as the appropriate analysis.

For a more detailed analysis of the conditions during adulthood, relations between mean saliva cortisol concentrations, plasma testosterone concentrations, and the hierarchy index were analyzed by linear models (LMs). These included ‘group’ and the respective other measurements as predictors (for more details see result section). To analyze effects of steroid hormone concentrations on the adult plasma PUFA:SFA ratio, a LM was applied with ‘group’, the adolescent PUFA:SFA ratio, and mean adolescent and adult saliva cortisol and plasma testosterone concentrations as predictors. The first-year mortality was analyzed by applying a generalized linear model with “died” (1) or “survived” (0) as response variable with binomial distribution. Mean adolescent and adult saliva cortisol and plasma testosterone concentration were included as continuous predictors in order to determine effects of steroid hormone concentrations measured during the different ontogenetic periods on the first-year mortality rate. Finally, differences between the two groups (died, survived) in the significant predictors were determined by applying t-tests (note: all animals survived the experiments until adulthood, while cases of death occurred afterwards but still within the animals’ first year of life).

Model assumptions (normal distribution and homoscedasticity of residuals) were checked by applying Shapiro-Wilk and Levene’s tests and by inspecting residual and fitted value plots. Models were fitted (removal of non-relevant interactions and fixed effects) based on the Akaike information criterion (AIC) whenever applicable. Post-hoc interaction analyses with Bonferroni corrections using package ‘phia’ (De Rosario-Martinez, 2015) were carried out on significant effects (1. group comparisons for each age, 2. within-group comparisons of whole adolescence (mean values) and adulthood, 3. within-group analysis of continuous predictor effects); note that in the case of a significant group:age interaction twelve post-hoc analyses were performed. Only significant post-hoc group comparisons and tendencies are presented in the result section; a full list of post-hoc tests is provided in Supplementary Table S1. \( R^2 \) calculations for fitted models were performed using package ‘MuMln’ (Barton, 2018). Cohen’s \( d \) for pairwise group comparisons and effects size estimates for interactions including covariates were calculated using package ‘effectsize’ (Ben-Shachar et al., 2020).

Although most of the data had to be log-transformed, only original data are presented in the result section. All model statistics are based on type 3 sum of squares. The significance level was set at \( p \leq 0.05 \) with two-sided testing.

3. Results

3.1. Body mass

Body mass was significantly affected by age interacting with group (group: \( F_{2,20} = 0.503, p = 0.612 \), age: \( F_{3,188} = 270.018, p < 0.001 \); group:age: \( F_{6,188} = 10.640, p < 0.001; R^2 = 0.72 \)). The PUFA group exhibited a higher body mass compared to the control group at an age of 120 days (\( p = 0.031 \), Cohen’s \( d = 1.14 \)) and compared to the SFA group during adulthood (\( p < 0.001 \), Cohen’s \( d = 1.57 \)) (Fig. 1). Comparing adulthood to adolescence revealed that all groups further gained body mass (Control: \( p < 0.001 \), Cohen’s \( d = 2.17 \); PUFA: \( p < 0.001 \), Cohen’s \( d = 2.10 \); SFA: \( p < 0.001 \), Cohen’s \( d = 1.62 \)).

3.2. Social behavior

Displayed sociopositive behavior was significantly affected by group, age, and their interaction (group: \( F_{2,20} = 5.766, p = 0.011 \); age: \( F_{3,190} = 3.617, p = 0.014 \); group:age: \( F_{6,190} = 2.992, p = 0.008; R^2 = 0.22 \)). Sociopositive behavior was shown more frequently in the PUFA group compared to the SFA group at an age of 60 days (\( p = 0.008 \), Cohen’s \( d = 1.28 \)). Thereafter, no differences between groups were detected (Fig. 2A). The control and PUFA groups showed a decrease in sociopositive behavior from adolescence to adulthood, while a similar pattern in the SFA group was not significant (Control: \( p = 0.019 \), Cohen’s \( d = 0.68 \); PUFA: \( p < 0.001 \), Cohen’s \( d = 0.92 \); SFA: \( p = 0.084 \), Cohen’s \( d = 0.46 \)).

No differences between groups were detected in the frequency of dominant behavior; all group-related effects were removed from the model based on the AIC. A significant effect of age (\( F_{3,196} = 3.017, p = 0.031, R^2 = 0.11 \)) revealed a general decrease in dominant

![Fig. 1. Body mass (means ± standard error) during adolescence (age 60, 90, 120 days) and adulthood of male guinea pigs maintained on a control, high PUFA, or high SFA diet. Group:age effect: p ≤ 0.001. *p ≤ 0.05 ***p ≤ 0.001.](image-url)
Significant interactions of group and age (frequency: group: PUFA and SFA group (Fig. 2B)). Behavior from adolescence to adulthood, which was shown by the PUFA, or high SFA diet. (pA) Sociopositive behavior. Group: age effect: p < 0.001. *p < 0.05, **p < 0.01. (Frequencies of social behavior were log-transformed for statistical analyses).

Fig. 2. Social behavior (means ± standard error) during adolescence (age 60, 90, 120 days) and adulthood of male guinea pigs maintained on a control, high PUFA, or high SFA diet. (A) Sociopositive behavior. Group: age effect: p < 0.01. (B) Dominant behavior. No significant group-effect. (C) Proportion of fights. Group: age effect: p ≤ 0.001. *p ≤ 0.05, **p ≤ 0.01. (Frequencies of social behavior were log-transformed for statistical analyses).

3.3. Saliva cortisol concentrations

Analysis of saliva cortisol concentrations (model 1) and adjusting for influences of initiated (active) and received (passive) social behavior (model 2) both revealed significant group:age interactions (Table 2) and revealed the same pattern (Fig. 3A). Cortisol concentrations were relatively constant when comparing an age of 60 and 90 days, but an increase in all groups thereafter resulted in significantly higher concentrations in the SFA group compared to the control group at an age of 120 days (model 1: p = 0.003, Cohen’s d = 1.41; model 2: p = 0.008, Cohen’s d = 1.34). During adulthood, cortisol concentrations were significantly higher in the PUFA (model 1: p = 0.003, Cohen’s d = 1.37; model 2: p = 0.010, Cohen’s d = 1.28) and SFA (model 1: p = 0.008, Cohen’s d = 1.30; model 2: p = 0.027, Cohen’s d = 1.15) groups compared to the control group (Fig. 3A). Only the PUFA group showed significantly increasing cortisol concentrations from adolescence to adulthood (model 1: Control: p = 1, Cohen’s d = 0.22; PUFA: p < 0.001, Cohen’s d = 0.99; SFA: p = 0.105, Cohen’s d = 0.74; model 2: Control: p = 1, Cohen’s d = 0.04; PUFA: p = 0.002, Cohen’s d = 0.93; SFA: p = 0.069, Cohen’s d = 0.68).

Regarding behavioral influences on cortisol concentrations (Tables 2, 3), only received dominant behavior tended to show group-specific effects. This was caused by a positive effect in the PUFA group compared to a negative effect in the SFA group, whereas no effect was detected in the control group. An additional interaction with age indicates that received dominant behavior in general positively affected cortisol concentrations at an age of 90 days and negatively at an age of 120 days. Initiated dominant behavior in general positively affected cortisol concentrations at an age of 60 days, with no effects afterwards (Table 3).

3.4. Plasma testosterone concentrations

Also with regard to plasma testosterone concentrations, significant interactions between group and age were detected in both analyses (Table 2). However, patterns of testosterone concentrations differed (Fig. 3B). Testosterone concentrations (model 1) were significantly elevated in the SFA group compared to the control group at an age of 90 days (p = 0.015, Cohen’s d = 1.32) and marginally lower in the PUFA group compared to the control group during adulthood (p = 0.095, Cohen’s d = 1.06). After adjusting for influences of social behavior (model 2), testosterone concentrations were higher in the SFA group compared to the control group at an age of 60 and 90 days (60 days: p < 0.001, Cohen’s d = 1.93; 90 days: p < 0.001, Cohen’s d = 1.97). The PUFA group also showed elevated testosterone concentrations compared to the control group at an age of 90 days (p = 0.041, Cohen’s d = 1.08), with a similar tendency at an age of 60 days (p = 0.061, Cohen’s d = 1.11), but concentrations remained slightly lower compared to the SFA group (60 days: p = 0.061, Cohen’s d = 1.11). Comparing adulthood to adolescence revealed that testosterone concentrations decreased in the PUFA and SFA groups (model 1: Control: p = 1, Cohen’s d = 0.01; PUFA: p = 0.001, Cohen’s d = 1.43; SFA: p = 0.001, Cohen’s d = 2.33; model 2: Control: p = 0.286, Cohen’s d = 0.46; PUFA: p = 0.052, Cohen’s d = 0.79; SFA: p < 0.001, Cohen’s d = 1.42).

Regarding behavioral influences (Table 2, 3), the frequency of fights had a negative effect on testosterone concentrations in the control group, a positive effect in the SFA group, and no effect in the PUFA group. Initiated and received dominant behavior both interacted significantly with age: initiating dominant behavior positively affected testosterone concentrations at an age of 60 and 120 days, but strongest during adulthood; receiving dominant behavior negatively affected testosterone concentrations at an age of 120 days and during adulthood. Also sociopositive behavior significantly affected testosterone concentrations: received sociopositive behavior showed a negative effect at an age of 60 days but a positive effect during adulthood; initiated
sociopositive behavior in general positively affected testosterone concentrations (Table 3).

3.5. Conditions during adulthood

Social behavior failed to explain differences in steroid hormone concentrations between groups, especially the pronounced differences in cortisol concentrations during adulthood. Therefore, adult conditions were analyzed in more detail. In general, the hierarchy index as measurement of individual social ranks during adulthood did not differ between groups (F_{2,36} = 0.035, p = 0.966; Control: 0.44 ± 0.06, PUFA: 0.41 ± 0.09, SFA: 0.43 ± 0.08) and was positively correlated to plasma testosterone concentrations (F_{1,33} = 5.859, p = 0.021; R^2 = 0.13); group had no effect on this relation and was removed based on the AIC. Regarding adult saliva cortisol concentrations, group and the hierarchy index were the only significant predictors, but interactions of the hierarchy index with group and plasma testosterone concentrations missed the significance level marginally (Table 2). Saliva cortisol concentrations were positively affected by the hierarchy index in the control group, whereas no effects were detected in the other groups (Control: 1.78, p = 0.021; PUFA: −0.16, p = 0.779; SFA: 0.71, p = 0.269; Fig. 4A). A positive effect of the hierarchy index on saliva cortisol concentrations turned out to be linked to low plasma testosterone concentrations: individuals with a high hierarchy index but low testosterone concentrations showed highest cortisol concentrations (Fig. 4B). Adjusting for these effects diminished saliva cortisol concentrations in the PUFA group as no difference to the control group was detected anymore (Fig. 4C); the SFA group still showed increased cortisol concentrations (p = 0.006, Cohen’s d = 1.34).

3.6. Plasma fatty acids

The plasma PUFA:SFA ratio was significantly affected by a group:age interaction (group: F_{2,20} = 5.338, p = 0.014; age: F_{1,34} = 7.010, p = 0.012; group:age: F_{2,34} = 14.535, p < 0.001; R^2 = 0.67). The PUFA group exhibited the highest PUFA:SFA ratio during adolescence and the SFA group showed the lowest ratio (p < 0.001, Cohen’s d > 2 for all pairwise comparisons). The ratio strongly decreased in the PUFA group from adolescence to adulthood, which was less pronounced in the control and SFA groups (Control: p = 0.024, Cohen’s d = 1.07; PUFA: p < 0.001, Cohen’s d = 3.53; SFA: p = 0.052, Cohen’s d = 0.96). During adulthood, however, the PUFA:SFA ratio differed significantly only between the PUFA and SFA group (p = 0.021, Cohen’s d = 1.12), while the SFA group showed a slightly lower ratio compared to the control group (p = 0.073, Cohen’s d = 0.95) (Fig. 5A).

Analyzing effects of steroid hormone concentrations on this change from adolescence to adulthood revealed that mean saliva cortisol concentrations measured during adolescence group-specifically affected the adult plasma PUFA:SFA ratio (group: F_{2,32} = 6.284, p = 0.005; cortisol: F_{1,32} = 2.198, p = 0.0148; group: cortisol: F_{2,32} = 5.591, p = 0.008, R^2 = 0.47). No pronounced effects were detected in the control and SFA groups, but adolescent cortisol concentrations negatively affected the adult ratio in the PUFA group (Control: 0.61,
Adolescent testosterone as well as adult cortisol and testosterone concentrations had no significant effects and were removed from the model based on the AIC.

3.7. First year mortality

Finally, we determined possible long-term consequences of steroid hormone concentrations on the probability of dying within the first year of life. The groups showed different first-year mortality rates, with the highest probability of dying in the first year of life in the SFA group (7 out of 13; 54%), followed by the PUFA group (3 out of 14; 21%), while no control animal died in the first year (0 out of 14; 0%). Analyzing the effects of steroid hormone concentrations revealed that mean adolescent plasma testosterone concentrations and mean adult saliva cortisol concentrations were both positively associated with first-year mortality (adolescent plasma testosterone: \(z = 1.996, p = 0.046\); adult saliva cortisol: \(z = 2.143, p = 0.032\); all other effects n.s.). Both hormones showed significantly higher concentrations in animals which died within the first year (adolescent plasma testosterone: \(t = 2.668, p = 0.017\), Cohen's \(d = 0.99\); LOG adult saliva cortisol: \(t = 3.717, p = 0.003\), Cohen's \(d = 1.59\)) (Fig. 6).

4. Discussion

Recently, we showed that dietary PUFAs and SFAs differently shape the postnatal development of socially housed male guinea pigs by modulating HPA- and HPG-axis activity and the effects of cortisol and testosterone concentrations on structural growth (Nemeth et al., 2018). Male guinea pigs form clear social hierarchies (Sachser, 1986) and social interactions can affect cortisol and testosterone concentrations (Lürzel et al., 2010; Wallner and Dittami, 2003). Considering possible effects of an animal’s social interactions and social status on hormone concentrations is crucial, because this could at least be co-responsible for the detected fatty acid effects. Dietary fatty acid intake can also strongly modulate behavior through neurophysiological influences, although this has rarely been considered with regard to the social environment. N-3 PUFA deficiency or elevated SFA intake increases the
risk for mental disorders but also induces aggressive and violent behavior by changing the serotonergic or dopaminergic system (e.g. altered receptor densities or neurotransmitter release and metabolism) in relation to behaviorally important brain areas such as the amygdala, hippocampus, or frontal cortex (Chalon, 2006; De Vriese et al., 2004; Moon et al., 2014). Although the SFA group showed no pronounced differences in social behavior compared to the control group during adolescence, the increased proportion of fights during adulthood indicates frequent escalations of social conflicts in this group. Lowest plasma PUFA:SFA ratios in the SFA group suggest PUFA deficiency in neuromembrane phospholipids in these animals, because plasma fatty acids represent the pool for supplying the brain (Chen et al., 2015). Moreover, social defeat stress related to increased HPA-axis activity and glucocorticoid concentrations impairs neurogenesis and results in depressive behavior (Lehmann et al., 2013), whereas depression and aggression seemingly occur in parallel with n-3 PUFA deficiency (DeMar Jr et al., 2006). Therefore, not only elevated SFA intake or PUFA deficiency per se could have promoted aggressiveness in SFA males during adulthood, but this was perhaps even intensified by simultaneously increased cortisol concentrations (Kruk et al., 2004).

In contrast to the SFA group, social behavior was modulated by PUFAs already during adolescence. At an age of 60 days, soon after weaning, PUFAs showed high frequencies of sociopositive behavior. N-3 PUFA supplementation in domestic pigs similarly resulted in more play behavior and social activities after weaning (Clouard et al., 2015). This points to a better coping with the situation, which includes separation from the mother, transfer into a new enclosure, and becoming a new member of a social group. Soon afterwards, however, PUFA animals in our study also showed high levels of aggressiveness at an age of 90 days, which is in strong contrast to the findings on decreased aggression after PUFA supplementation (e.g. Gajos and Beaver, 2016). A possible explanation for this is the first attempt of PUFA males to establish a social hierarchy at this age, which would usually be related to initially increased aggressive interactions (Nemeth et al., 2014; Wallner and Dittami, 2003). Interestingly, cortisol concentrations were lowest at this age in PUFA males, which suggests a stress buffering effect of PUFAs in the context of aggressiveness. However, it has to be noted that behavioral observations in our study were mainly carried out to consider effects of initiated and received social behavior directly before sample collections on steroid hormone concentrations. The single-point behavioral analyses during adolescence may, therefore, not be representative for a longer period. In contrast, behavioral analyses on three consecutive days during adulthood revealed that PUFA animals showed lowest frequencies of dominant behavior and fights at this age, which was in strong contrast to the high aggressiveness of SFA animals. These results indicate that dietary PUFAs and SFAs differently modulate social behavior and especially aggressiveness during adolescence and adulthood.

Analyzing saliva cortisol and plasma testosterone concentrations in response to initiated and received social behavior observed directly before sample collections increased the overall explained variance in steroid hormone concentrations but did not explain group and age differences. Analyses mainly revealed effects of dominant behavior. Initiating and receiving more dominant behavior both resulted in higher cortisol concentrations. Additionally, initiating dominant behavior resulted in higher testosterone concentrations and receiving dominant behavior had a negative effect. This definitely reflects the social system and corresponds to the general knowledge on hormone-behavior relations in guinea pigs: social dominance or a high social status is usually linked to elevated testosterone concentrations, while establishing and maintaining a social system in general is energetically demanding and considered as “stressful” (Lürzel et al., 2010; Nemeth et al., 2014; Sachser et al., 1998; Wallner and Dittami, 2003). Sociopositive behavior was also positively related to testosterone concentrations, which indicates a general higher social activity in dominant males (Nemeth et al., 2016a; Wallner and Dittami, 2003). It is therefore not surprising that including behavioral expression rates in the analyses of steroid hormone concentrations resulted in higher percentages of explained variance. The fact that even the relatively short single-point behavioral analyses yielded these results, which highly correspond to the well-known hormone-behavior-relations found in guinea pigs, underlines the validity of our data and results. However, the SFA group tended to show divergent hormone-behavior-relations compared to the other groups: receiving more dominant behavior was linked to lower cortisol concentrations and fights were related to higher testosterone concentrations. Adjusting testosterone concentrations to these behavioral influences resulted in even higher predicted concentrations in SFA animals. This strongly indicates an altered endocrine regulation and overproduction of testosterone in relation to social behavior in SFA males during adolescence. This might be simply related to an investment in testicular development (Nemeth et al., 2018), but perhaps even to impaired neurophysiological functions or an increased steroidogenesis in general. Nevertheless, increased testosterone concentrations have previously been shown to be associated with dietary SFA intake in humans (Volek et al., 1997), but the underlying mechanisms remain to be determined.

A variety of studies revealed pronounced molecular, neurophysiological, and metabolic influences of dietary fatty acids on steroid

![Fig. 6. Steroid hormone concentrations of male guinea pigs that survived or died in the first year of life. (A) Plasma testosterone concentrations measured during adolescence. (B) Saliva cortisol concentrations measured during adulthood. * p ≤ 0.05. ** p ≤ 0.01.](image-url)
hormone secretion rates and HPA- and HPG-axis activities. PUFA deficiency has been shown to result in excessive HPA-axis activity, for instance by modulating the gamma-aminobutyric acid (GABA) system, which usually inhibits HPA-axis responses (Chen and Su, 2013). PUFAs also modulate the expression of StaR, the steroidogenic acute regulatory protein, which controls cholesterol transport within mitochondria for steroid hormone biosynthesis (Wathes et al., 2007). Furthermore, PUFAs and SFAs differently regulate lipoprotein metabolism and the related cholesterol and lipid transport in the body. In this context, PUFAs reduce low-density-lipoprotein (LDL)-cholesterol and also the risk of atherosclerosis (Fernandez and West, 2005). Specifically in adrenal tissues, PUFAs can diminish the activity of key enzymes involved in steroidogenesis and ultimately reduce cortisol production (Xie et al., 2016). PUFA deficiency or elevated SFA intake can impair a cascade involved in the synthesis and regulation of steroid hormones and perhaps result in increased HPA- and HPG-axis activities and associated steroid hormone concentrations as found here. However, it remains unclear if elevated steroid hormone concentrations were recorded due to dietary SFAs apply to elevated intake of these nutrients itself or rather to a PUFA deficiency induced by elevated SFA intake.

A common result of long-term high-SFA intake is increased abdominal fat storage and elevated risk for obesity and neurophysiological impairments, but this is not necessarily related to a higher body mass (Sanz et al., 2000b; Vagena et al., 2019). Nevertheless, SFAs have repeatedly been shown to promote steroidogenesis in adipose tissue through expression of StaR (Moliae et al., 2019) or 11β-HSD1, the 11β-hydroxysteroid-dehydrogenase type1, which catalyzes the conversion of inactive cortisone to cortisol (Petrus et al., 2015; Vara Prasad et al., 2010). These findings highly correspond to the SFA group in our study. SFA males exhibited the lowest body mass, although they were fed with 10% (w/w) fatty acids on total food, and showed the highest saliva cortisol concentrations, which could not be explained by their behavior. Cortisol production by adipose tissue is a reasonable explanation for HPA-axis dysfunctions and impaired negative feedback as found previously for diets high in SFAs (Lomax et al., 2013; Nemeth et al., 2016a). Adipose cortisol production is probably independent of the HPA-axis and its control of cortisol synthesis and release in response to stress, resulting in an overproduction of this hormone.

Although all types of fats can be stored as triglycerides in fat depots and play an important role for energy balance, PUFAs are mobilized and oxidized faster than SFAs (Raclot, 2003; Sanz et al., 2000a). This might entail less pronounced fat stores and perhaps even a more direct involvement of PUFAs in energy balance during energetically demanding and stressful situations (Buckley and Howe, 2009). Increasing cortisol concentrations during adolescence are a common pattern in guinea pigs (Schöpper et al., 2012), which is probably related to structural development, sexual maturity, and establishment of their social system. Although cortisol concentrations increased in all groups with age, only the PUFA group showed a decrease in the plasma PUFA:SFA ratio in relation to that hormonal pattern, which was mainly caused by a decrease in plasma PUFAs (data not shown). This probably reflects increased energy mobilization in response to elevated cortisol concentrations, which would have enabled a better coping with this energetically demanding period and yielded a higher body mass in PUFA animals. No such effects were detected in the SFA group, whose body mass gain and development was presumably limited by their increased steroid hormone concentrations (Nemeth et al., 2018). PUFAs may therefore support developmental processes not only by neurophysiological influences, but perhaps even by simply providing energy for structural growth and maintenance of body conditions.

Increased cortisol concentrations in the PUFA group during adulthood represents an unexpected result. This could not be explained by their social behavior, but by the conditions during adulthood. Adult testosterone concentrations were in general positively related to social hierarchy ranks. Lower testosterone concentrations in the PUFA group were explained by less agonistic interactions and a high number of subdominant individuals. In contrast to the control group, the hierarchy index in the PUFA group was not related to saliva cortisol concentrations: subdominant and dominant individuals showed similar cortisol concentrations. However, highest cortisol concentrations turned out to be associated with low testosterone concentrations in dominant individuals. This effect might be explained by other findings in guinea pigs, where elevated testosterone concentrations caused by social interactions seemingly diminished cortisol responses (Lürzel et al., 2010; Lürzel et al., 2011). Individuals with low testosterone concentrations can then be assumed to exhibit high cortisol concentrations. This testosterone-cortisol interaction combined with a high number of subdominant individuals in the PUFA group explained their increased cortisol concentrations compared to the control group during adulthood. In contrast, cortisol concentrations in control individuals were directly linked to social hierarchy ranks during adulthood and this was also paralleled by a high frequency of dominant behavior at this age. Only in SFA animals, cortisol concentrations remain unexplained by social behavior and the social hierarchy during adulthood. Similar to increased plasma testosterone concentrations during adolescence, we conclude that elevated saliva cortisol concentrations during adulthood also represent an overproduction in relation to the animals’ social system and were most likely caused by elevated SFA intake.

Adequate HPA-axis functions, including responses to stress and a negative feedback of glucocorticoids, are important to maintain homeostasis and enable an unbiased development (McEwen and Wingfield, 2003). Homeostatic perturbations linked to long-term or chronically increased cortisol concentrations represents a serious threat to an individual due to lower physiological and HPA-axis responsiveness (Rich and Romero, 2005; Romero et al., 2009). A chronic stress state can impair wound healing (DuRant et al., 2016) or lipid synthesis (Chuang et al., 2010) and may even result in glucocorticoid receptor resistance and increased disease risk (Cohen et al., 2012). The ultimate and most dramatic result of long-term increased glucocorticoid but also testosterone concentrations is indicated in our study: both were related to an early death. Although a higher proportion of SFA animals died within their first year of life, this hormonal effect was not diet-specific and would apply to all animals under long-term increased steroid hormone concentrations. Especially HPA-axis dysfunctions and chronically increased glucocorticoid concentrations are related to serious pathologies, such as an impaired immune system (Chrousos, 2009). Increased testosterone concentrations linked to social dominance also imply a high energetic investment (Goymann and Wingfield, 2004; Sapolsky, 2004) and may obviously have detrimental effects. Cortisol and testosterone concentrations in SFA animals were not explained by social interactions, but a higher proportion of fights usually results in wounds. An impaired HPA-axis or generally long-term increased cortisol concentrations may have also delayed wound healing in these animals or even increased the risk for infections due to an impaired immune system. HPA-axis hyperactivity or dysregulation is also related to tumor development, a probably faster ageing, and earlier death in rats (Cavigelli and McClintock, 2003). Taken together, long-term increased steroid hormone concentrations in SFA males were definitely a major threat to homeostasis. After the end of this study, at an animals’ age of 12 months, further SFA males died, which corroborates the mentioned negative effects. However, a low but not significant proportion of PUFA animals also died in the first year due to slightly elevated adolescent testosterone and adult cortisol concentrations. Although we explain the latter by decreased testosterone concentrations during adulthood, these obvious stress responses were not diminished by PUFAs. PUFAs may indeed provide additional energy and promote neurophysiological and behavioral functions, but exceedingly high omega-3 PUFa levels have also been shown to compromise homeostasis because of increased oxidative stress (Feillet-Coudray et al., 2013). Adequate and balanced dietary PUFA intake should definitely be carried out at normal caloric levels in order to maintain homeostasis and health.
These results provide a first insight into potential effects of high fatty acid nutritional conditions as part of an animal’s environment. However, different ontogenetic states, including pre- and postnatal periods as well as adolescence and adulthood, must be considered in future studies with regard to ontogeny-specific effects of PUFAs and SFAs on social aspects. Moreover, in this respect, and with regard to studying dietary fatty acid effects in a social environment, the unit of interest (e.g. individual or social group) plays an important role in the study design. This may represent a limitation to the results presented here. Our statistical analyses should have reduced the dependency in steroid hormone concentrations within a social group by adjusting measurements to individual differences in the frequencies of social behavior. Nonetheless, each social group can be considered as a single unit, and independency of measurements within a group cannot be fully assured. Future studies should therefore consider appropriate numbers of replicates for each group in order to exclude effects, which are related to the social group structure rather than to dietary fatty acid effects.

The findings presented here demonstrate the importance of adequate and balanced dietary fatty acid intake the structural, physiological, and behavioral development. The endocrine system apparently plays an essential role in this context, whereas effects of dietary fatty acids on social behavior turned out to be circumstantial with regard to altered steroid hormone concentrations. Nonetheless, the social system must not be neglected, because effects of dietary fatty acids differ between individual physiological states and stress responses (Hellhammer et al., 2012) and are related to differences in social hierarchy ranks as presented here. Although we were able to explain some of the variance in steroid hormone concentrations by the animals’ social behavior and social system, we conclude that dietary PUFAs and SFAs can ultimately and directly affect the endocrine system and related effects on metabolic processes and health.

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Data availability

The datasets generated and/or analyzed during this study are available from the corresponding author on request.

Declaration of competing interest

The authors declare that they have no competing interests.

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