universität wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Effects of dietary fatty acids on juvenile development in male guinea pigs (*Cavia aperea f. porcellus*)"

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2016 / Vienna 2016

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 066 878
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Masterstudium Verhaltens-, Neuro- und Kognitionsbiologie
Betreut von / Supervisor:	Ass.-Prof. Mag. Dr. Bernard Wallner, PD

Danksagung

Für die Unterstützung bei meiner Masterarbeit möchte ich mich zu allererst bei Prof. Dr. Bernard Wallner bedanken, der die fachliche Betreuung übernommen, und mir so die Möglichkeit zur Durchführung dieser Arbeit gegeben hat. Für die konstruktiven Diskussionen während Datenaufnahme und -auswertung, sowie während dem Verfassen der Arbeit möchte ich mich herzlich bedanken. Diese haben es ermöglicht, dass meine Masterarbeit nun in der gegebenen Form vorliegt und die eine oder andere Unsinnigkeit beseitigt werden konnte.

Ein unheimlich großes Dankeschön gilt Mag. Matthias Nemeth, ohne dessen Unterstützung und wertvoller Hilfsbereitschaft diese Arbeit nicht möglich gewesen wäre. Danke für die endlosen Stunden die Du geopfert hast um mit mir die Thematik zu diskutieren, mir die nötigen Programme zur Auswertung zu erklären und mir ein neues Kapitel der Statistik näherzubringen. Tausend Dank für die Zusammenarbeit und die Möglichkeit, Teil Deines Projektes sein zu dürfen.

Vielen lieben Dank auch an Elisabeth Pschernig, MSc, die mich geduldig in die Analyse der Speichelproben unterwiesen hat und mir im Labor immer helfend zur Seite stand.

Ganz besonderer Dank gilt meinen Eltern und meiner Schwester, die mich während der gesamten Studienzeit unterstützt und immer ermutigt haben, falls etwas einmal nicht nach meinen Vorstellungen verlaufen ist. Danke, dass Ihr das hier möglich gemacht habt!

Ebenso möchte ich mich bei meinem Freund bedanken, der mir während der Masterarbeit stets den Rücken gestärkt und mich mit viel Humor aufgeheitert hat, falls es mir einmal zu viel wurde.

Ich danke auch der Universität Wien, im Speziellen dem Department für Verhaltensbiologie, für die Bereitstellung der Geräte und Softwareprogramme, die für die Aufnahme und Analyse meiner Daten nötig waren. Bei dieser Gelegenheit möchte ich auch ein Dankeschön an alle Mitarbeiter der Tierhaltung aussprechen, die sich darum gekümmert haben, dass es unseren Meerschweinchen an nichts fehlte.

Abschließend bedanke ich mich bei den vielen Hausmeerschweinchen, die die Prozedur der Datenaufnahme (fast immer) stoisch über sich ergehen haben lassen und mir so manch heiteren Moment beschert haben!

CONTENTS

INTRODUCTION

Since 1990 Barker (e. g., Barker, 1990; Barker and Martyn, 1991; Barker, 1995) has published several articles on the fetal origins hypothesis, which exemplifies the impact of environmental conditions *in utero* in relation to effects on disease expression rates in the adult organism. Such influences on the organism are called “fetal programming”. The latter alters functionally developing organs as well as the individuals’ physiology and behavior in later life time periods (Godfrey and Barker, 2001). For instance, Schöpper et al. (2012) reported a significant association between prenatal stress exposure during early- to mid-pregnancy and basal cortisol levels in domestic guinea pig offspring. They observed reduced basal cortisol concentrations during pre-pubertal phase and elevated levels in adolescent animals in contrast to offspring of undisturbed dams. Additionally, they detected a reduced hypothalamic-pituitary-adrenal (HPA) axis reactivity in prenatally stressed animals in relation to stress exposure resulting in decreased glucocorticoid secretion compared to control animals. This leads to the suggestion that the HPA axis activity in offspring is programmed by maternal stress physiology during pregnancy.

Beside physiological stress effects, nutritional condition may also influence fetal programming processes (Barker, 1997). During pregnancy alterations in maternal nutrition have serious consequences on the fetus’ endocrine status resulting in different physiological phenotypes. An example is given by the thrifty phenotype hypothesis, which states that maternal malnutrition is likely leading to poor fetal growth and therefore, to adaptions in the fetus’ metabolism for times of inadequate nutritional conditions. These adaptions result in persisting structural and functional changes of different organs and lead to an increased predisposition for the development of type 2 diabetes and metabolic syndrome (Hales et al., 1997; Hales and Barker, 2001). Therefore, the composition of the mother’s diet can have essential impact on the healthy development of an offspring because its metabolism is directly linked to blood exchange between both the mother and the fetus (e.g., Mutch and Hurley, 1973; Godfrey et al., 1996; Bayol et al., 2007; Fiset et al., 2015). Since the early ontogeny is a critical period for brain development, diet composition has fundamental impact on neuronal development and therefore on cognitive abilities and social behavior of an adult individual. The nervous tissue has the second highest lipid concentration after adipose tissue. Fatty acids are essential components of cell membranes’ phospholipids in general and in particular

participate directly in the modification of neuronal membranes and play therefore, an important role in structure and functioning of these membranes (Bourre, 2004). Due to that they are indispensable for energy production, synthesis of neuropeptides, signal transduction, and growth in the brain as well as in the whole central nervous system (Yehuda et al., 2002; Yehuda, 2003; Bhathena, 2000).

Fatty acids are classified into two categories: unsaturated fatty acids (UFAs), which can be further divided into mono- and polyunsaturated fatty acids, and saturated fatty acids (SFAs). Non-essential fatty acids, such as SFAs, are synthesized in numerous organs, even in the brain. Whereas UFAs, which are essential nutrients have to be ingested through food as they cannot be synthesized *de novo* by the organism itself or just in an insufficient way (Bourre, 2004). Depending on the carbon atom, counted from the methyl-, or "omega-" end, where the first unsaturated bond is located, omega-3 (n-3), omega-6 (n-6) polyunsaturated (PUFAs), and omega-9 (n-9) monounsaturated fatty acids (MUFAs) can be distinguished. Alpha-linolenic acid (ALA; 18:3) is known as the main fatty acid in the n-3 group because all other n-3 fatty acids derive from it. Similarly, linoleic acid (LA; 18:2) serves as dietary precursor for all other n-6 fatty acids and oleic acid (OA; 18:1) for the fatty acids in the n-9 group. It has to be mentioned that the phospholipids in the neuronal membranes of the brain aren't consisting of the precursors from the diet, but from their derived long-chained PUFAs (Bourre, 2004). The number and position of the double bonds are essential characteristics for the structure, viscosity and function of the membrane, as they influence membrane fluidity and therefore the brain physiology. Moreover, docosahexaenoic acid (DHA; 22:6; n-3), a long-chained PUFA derived from ALA has the highest concentrations in the mammalian brain and is crucial for neuronal development (Neuringer et al., 1988; Wainwright, 2007; Bourre, 2004; Fedorova and Salem, 2006; Bhatia et al., 2011). The biosynthesis site of UFA is located at the endoplasmic reticulum, where additional double bonds are introduced via desaturases and/or the fatty acid chain is elongated (de Alaniz and Marra, 2003). In contrast, SFAs might decrease the cell membranes' fluidity, impairing the cell to cell communication and nutrient transport between cells. The ingested quantity of UFAs and SFAs, as well as their ratio to each other is significant for the development and modulation of neuronal functions. In vitro studies of human adrenocortical cells including long-chained SFAs showed increased membrane microviscosity along with a decreased sensitivity to ACTH and therefore reduced cortisol

release (Whitcomb et al., 1988). Whereas higher concentrations of PUFAs incorporated into phospholipids lead to increased membrane fluidity (Yehuda et al., 2002). There is evidence that diets rich in saturated fats impair the maintenance of the body temperature in a cold environment and the sense of pain in rodents as well as the working memory and hippocampal morphology in rats (Kaplan and Greenwood, 1998; Granholm et al., 2008).

As mentioned before, fetus' metabolism is dependent on maternal nutrition state during pregnancy, and even during lactation. Therefore the composition of the diet in essential and especially long-chained PUFAs, like DHA, is vital for the adequate neuronal development of the offspring (Herrera, 2002; Wainwright, 2007; Innis, 2007; Lundqvist-Persson et al., 2010; Mennitti et al., 2015). Helland et al. (2003) was able to show a significant positive correlation between the IQ of 4 year old children and the DHA uptake of their mothers during pregnancy and lactation. Diet composition in Central Europe is characterized by a proportionate increased uptake of SFAs, trans fatty acids and decreased n-3 fatty acids. This was associated with an increase in diseases of the cardiovascular system, inflammatory processes and declining cognitive performances by numerous studies (Haast and Kiliaan, 2015; Mennitti et al., 2015; Mozaffarian et al., 2006; Solfrizzi et al., 2005). Beside the negative consequences of elevated SFA intake to health, the high n-6 to n-3 ratio adds additional health problems (Schuchardt et al., 2010). According to numerous studies, the n-6:n-3 ratio is a key factor for the neuroendocrine metabolism and seems to be more decisive than the total amount of specific PUFAs (Bhathena, 2000; Yehuda et al., 2002; Yehuda, 2003; Schuchardt et al., 2010). The adequate n-3 intake and a balanced n-6:n-3 ratio of recommended 4:1 is essential for physical (e.g., cardiovascular diseases) and neuronal health (e.g., neuropsychiatric disorders) in relation to ageing processes (Neuringer et al., 1988; Simopoulos, 2002; Bourre, 2005; Wainwright, 2007; Yehuda et al., 2002; Yehuda, 2003; Russo, 2009). Regarding postnatal growth a positive effect of DHA and arachidonic acid (AA; 20:4, n-6) supplementation in rats and guinea pigs on mineral content and mineral density of bones was observed (Weiler et al., 2012). However, after DHA + AA supplementation during pregnancy and lactation these beneficial results were only shown for female guinea pig offspring whereas in male offspring it had deleterious effects (Yin et al., 2014).

Findings also indicate a relationship between dietary fatty acid intake and the individual stress management, namely the activity of the HPA axis. Particular attention was

paid on n-3 deficiencies, like the effect of increased vulnerability to stress according to performances in cognitive tasks (Neuringer et al., 1988; Fedorova and Salem, 2006). Hennebelle et al. (2012) studied the relation between n-3 fatty acids and stress reactivity in rats. Compared to n-3 enriched individuals they found elevated corticosterone levels and stress-induced loss of weight in n-3 deficient individuals, as well as behavioral changes like reduced motor activity and more time spending self-grooming, an index of anxiety. Investigations on guinea pigs showed reduced stress-related cortisol release after the administration of n-3 and n-6 supplemented diets (Nemeth et al., 2014). Just as fatty acids have an impact on the individuals' stress reaction, e.g., secretion of cortisol which is the main glucocorticoid in guinea pigs (Malinowska and Nathanielsz, 1974), they can affect the fatty acid biosynthesis as well. The application of natural or synthetic glucocorticoids led to a decreased activity of delta-6 and delta-5 desaturases in the liver of rats, those enzymes that are responsible for the desaturation of SFAs to UFAs, whereas the delta-9 desaturase activity was increased (de Alaniz and Marra, 2003). Metabolic, glucocorticoids increase the uptake and reorganization of fatty acids in the adipose tissue as well (Macfarlane et al., 2008). Furthermore, the influence of a stressor and therefore, an increased glucocorticoid level might impair the n-6:n-3 ratio at the expense of n-3 plasma concentrations (Nemeth et al., 2014).

According behavioral effects, decreased aggressive behavior in humans was observed during the supplementation of n-3 fatty acids, whereas reduced fed n-3 levels resulted in increased disposition to aggression in rats and dogs (Bourre, 2005; Fedorova and Salem, 2006; Re et al., 2007). According to the latter results, Hilakivi-Clarke et al. (1996) found enhanced aggression in rodents after n-6 PUFA supplementation. The mentioned findings are complementary as on one side an elevated n-6 level was induced, whereas on the other side reduced n-3 levels were detected. Both is likely to result in an increased n-6:n-3 ratio, shifting the necessary balance with possible negative impacts on cognition, behavior and physiology (Bhathena, 2000; Yehuda, 2003; Bourre, 2004; Bourre, 2005). This hypothesis is supported by investigations, which show that n-3 deficiencies cannot be compensated through increased n-6 uptake according to cognitive performances (Fedorova and Salem, 2006). Even in food allergic mice the positive influence of n-3 PUFA supplementation was demonstrated, because the behavioral and physiological deficits caused through the allergy were significantly reduced (de Theije et al., 2015).

Considering the current findings in the literature, the aim of the present study was to investigate the impact of pre- and postnatal dietary supplementation either with UFAs or SFAs on the postweaning development and the social behavior of male domestic guinea pigs, as the comparative impact of SFAs and UFAs on ontogenetic development seems to be so far not sufficiently examined. Domestic guinea pigs were chosen as experimental subjects since they serve as a model organism for the investigation of social behavior and stress reactions. Only male individuals were included in this study, because social hierarchy is more pronounced developed in males than in females (Sachser et al., 1998).

METHODS & MATERIAL

Ethics statement

Experiments were conducted in accordance with the Austrian laws for animal experiments and animal keeping and approved by the ethics committee of the faculty of Science and Research (BMWF66.006/0024-II/3b/2013). All efforts were made to keep pain and discomfort for the animals to a minimum.

Animal maintenance

For this study a total of 41 male domestic guinea pigs (*Cavia aperea f. porcellus*) were used, aged 31 ± 6,05 (mean ± SD) days. The animals were descendants of a heterogeneous, multicolored stock, bred at the Department of Behavioural Biology at the University of Vienna, which was newly established in 2011 with animals originating from different breeders in Austria. All animals were sexually intact, socially skilled, accustomed to daily contact with humans and could be identified by individual natural fur pattern. This study was done on the first generation of juvenile guinea pigs born to mothers (F0 generation) who had received the same experimental diet during pregnancy and lactation as their offspring did.

Until weaning animals were housed in mixed-sexed groups (F0 dams and F1 infants) according to two types of fatty acid diets and a control group (see Table 1). On weaning day at the age of approximately 30 days, the animals were transferred to three same-sexed groups referring to the different diets (Control, UFA, SFA). They lived in environmentally enriched enclosures (floor size: 3,16m²) with the floor covered with woodchip bedding material which

was renewed weekly. A light-dark cycle of 12/12h (lights on at 7 a.m.) and controlled temperature of 22 ± 2° C in the housing room were maintained throughout the study.

Experimental diets

Throughout the experiment, water and commercial guinea pig pellets (Altromin 3023, Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) were available *ad libitum*. Additionally each subject received 5 g hay per day. Food was provided daily at 08:30 a.m. The unsaturated fatty acid group (UFA group) was supplemented with walnut oil (manako Walnut Oil 153, Makana Produktion und Vertrieb GmbH, Offenbach a. d. Queich, Germany), which is rich in n-3, n-6 and n-9 fatty acids. The saturated fatty acid group (SFA group) was fed with coconut oil (manako Bio Virgin Coconut Oil 649, Makana Produktion und Vertrieb GmbH, Offenbach a. d. Queich, Germany), high in short and long-chained saturated fatty acids. Pellets and oil were mixed through shaking in freezer bags for about 10 min in order to receive a homogenous mixed feed. Percentage of oil on the weight of the guinea pig pellets was 10 % (w/w). Pellets for control group weren't further treated. Percentages of fatty acids on total food (% w/w) per experimental diet were determined by gas chromatography following the protocol of Wagner et al. (2000) (Table 1).

Table 1. *Identified fatty acids on total food (% w/w) of the three experimental diets fed to guinea pigs. (n.d. = not detectable)*

Fatty acids	Pellets (Control)	Pellets + walnut oil (UFA)	Pellets + coconut oil (SFA)
12:0	0	0	4,80
14:0	0,02	0	2,21
15:1	0	0	0,01
16:0	0,53	1,17	1,68
16:1	0,02	n. d.	n. d.
18:0	0,11	0,38	0,56
18:1n9	0,62	2,00	1,27
18:1n7 cis	0	0,13	0,05
18:2n6	1,64	7,79	2,00
18:3n6	0	0,04	0,03
18:3n3	0,33	1,40	0,27
18:2n9c12c	0	0,07	0,02
20:0	0,01	n. d.	n. d.
22:0	0	0	0,02
Total n-3	0,33	1,40	0,27
Total n-6	1,64	7,83	2,03
Total n-9	0,62	2,07	1,30
Total SFA	0,66	1,55	9,27
Total MUFA	0,62	2,12	1,34
Total PUFA	1,97	9,30	2,32
Total UFA	2,59	11,42	3,66
n-6 : n-3 ratio	4,97	5,59	7,52
M : S ratio	0,94	1,37	0,14
P : S ratio	2,98	6,01	0,25
U : S ratio	3,92	7,39	0,39

Experimental design

Birth of F1 offspring was not synchronous between all litters. Therefore, observed animals were born between April and June 2014. The study was conducted from June 2014 until October 2014. The experimental procedure started with the day of weaning of the first male individuals (Day 1 - Control: N = 9, UFA: N = 13, SFA: N = 11). On day 36 another six individuals were weaned and transferred to the same-sexed groups (Control: N = 5, UFA: N = 1). The weaning of the last individuals took place on day 71 (SFA: N = 2). Eventually, the three experimental groups comprised the following group sizes: Control N = 14, UFA N = 14, SFA N = 13. Observation time range lasted until every animal had reached an age of at least 120 days

to ensure that all subjects had reached sexual and physical maturity within the observation period.

Video recordings of the same-sexed group housing conditions were made to investigate social interactions within the three experimental diet groups, which took place in their familiar group housing enclosures. For that purpose all shelters or other environmental enrichments were removed and immediately returned after the video recordings. Recordings lasted for 30 minutes and were carried out once a month at 08:00 a.m. using digital cameras, which were located on the ceiling above the group housing enclosures. The first video recording took place on day 36, after the weaning of six individuals, and was repeated on day 65, 92 and 120. The mentioned days were chosen in order to monitor behavioral changes along with the development of the guinea pigs from juvenile phase over sexual maturity until they were full-grown. Immediately after the video recordings saliva samples were taken (see below) and body weight of every individual was measured. After the measurements animals were reintroduced into their groups. Because of the birth range and therefore, the late weaning of the last individuals from SFA group, these two subjects were only included in two out of four social interaction recordings.

Saliva sample collection and analysis

Saliva samples of every animal were taken once a month after the video recordings of social interactions during group housing in order to determine the basal salivary cortisol concentrations. Sampling always took place at the same time of day (starting at 08:30 a.m.) immediately after the video recordings before feeding in order to exclude diurnal variations. Handling lasted a maximum time of 3 minutes per animal.

Following the method of Fenske (1997) a standard cotton bud was inserted into the guinea pigs' cheek for about 30 seconds and turned around several times. Samples were centrifuged immediately after collection (14000 rpm, 10 minutes) and stored at – 20°C until further analysis. The samples were diluted 1:50 and measured in 10 µl inputs by biotin-streptavidin enzyme-linked immunoassay, following the EIA protocol by Palme and Möstl (1997) where as well cross-reactions with other steroid hormones are described. The intra- and interassay coefficients of variance were 10,29 and 4,56 %, respectively. All samples were run in duplicate.

Plasma fatty acid collection and analysis

Plasma fatty acid composition was analyzed through blood samples taken from every individual at an age of about 120 days. For collection, marginal ear veins were punctured with sterile lancets and approximately 300 µl blood was collected in heparinized capillary tubes (see Sachser and Pröve, 1984). Samples were centrifuged immediately after collection (14000 rpm, 10 minutes) and the separated plasma was stored at – 20°C until further analysis.

Quantification of plasma fatty acids was conducted through gas chromatography. Sample preparation and analysis followed the method of Wagner et al. (2000) modified by Nemeth et al. (2014). An Auto-System-Gaschromatograph (Perkin Elmer, USA) with flame ionization detector with a Rtx-2330 30 m x 0,25 mm i.d. silica column was used. Injection temperature of the prepared samples was 250°C, detection temperature was 270°C. Analysis was carried out using TotalChrome Workstation 6.3.0 (PE Nelson, Perkin Elmer, USA). For statistical analysis the determined single fatty acids were summed up to calculate the total percentages of n-3, n-6, n-9, MUFAs, PUFAs, overall UFAs and SFAs, as well as the ratios of n-6:n-3, MUFA:SFA, PUFA:SFA and overall UFA:SFA.

Behavioral analysis

Social interaction behavior in group housing condition was recorded four times with an interval of approximate 30 days on day 36, 65, 92 and 120. Video recordings were quantified with the Observer XT 10 (v10.5, Noldus, Wageningen, the Netherlands). The following behavioral variables were measured in the group housing condition: locomotion (walking, running), displacement, chasing, fighting, biting, riding, rumba-rumble, head-thrust, kick-back, marking, stand threat, following, side by side ("huddling"), nose-nose contact, naso-anal contact, social grooming. Behavioral patterns were defined according to the description of Rood (1972). All behavioral variables were recorded through continuous sampling for each individual and were measured in frequencies. Only locomotion and side by side behavior were measured in durations. For analysis, frequencies of the variables for every individual on each group housing day were summed up to "agonistic behavior" (displacement, chasing, fighting, biting, riding, rumba-rumble, head-thrust, kick-back, marking, stand threat) and "socio-positive behavior" (following, side by side, nose-nose contact, naso-anal contact, social grooming). As side by side behavior was included into the "socio-positive behavior" variable,

durations were divided into 10 sec intervals in order to convert them into frequency measurements.

For calculation of the hierarchy order within the groups, every passive pairwise interaction partner, to whom a specific agonistic behavior was directed at, was recorded. Frequency scores of every agonistic behavior an individual had to face from his conspecifics were summed up to the variable “passive agonistic behavior”. Calculation of the dominance rank on each group housing day followed the description of Singh et al. (2003). The equation was as follows: ∑ (active agonistic behavior / (active agonistic behavior + passive agonistic behavior))

Statistical analysis

Statistical analyses were carried out using IBM SPSS (v20.0, IBM Corp., Armonk, NY). Linear mixed effect models (LME) with type-I sum of squares were conducted to adjust for repeated measurements. For the analysis of behavioral variables (locomotion, active agonistic behavior, socio-positive behavior), salivary cortisol and body weight, ‘postweaning day’ (days after the weaning from dam), ‘individual age’ (in days) and ‘hierarchy’ (dominance rank on each group housing observation) were included as covariates and ‘group’ (Control, UFA, SFA) as factorial predictor. All covariates and the factorial predictor were included as fixed factors in the given order, as well as the interactions ‘group x individual age’ and ‘group x hierarchy’. Single animals were included as random factor for repeated measurements. ‘Postweaning day’ was included into the models in order to adjust the variable ‘individual age’, because on the first group housing observation (day 36) five new individuals were introduced to the control group. Residuals of all response variables (active agonistic behavior, socio-positive behavior, locomotion, body weight, cortisol concentrations) were tested for normal distribution, which was carried out by Shapiro-Wilk test and transformed (square root, third root) if necessary to ensure that the residuals were normally distributed. Results are shown as F-values with degrees of freedom and p-value. Post-hoc multiple comparisons were carried out using Bonferroni test. Results of these pairwise comparisons are shown as p-value and the estimated means + SE given from the fitted model. Control group was set as reference group in order to compare between the fatty acid groups and control group. For visualization of the results both fixed predicted values of the respective model and non-transformed actual data were used.

Data from the plasma fatty acid analysis were tested for normal distribution (Shapiro-Wilk test) and homoscedasticity (Levenes' test). One-way ANOVA with post-hoc testing (Bonferroni test) was performed in order to determine group differences. If no normal distribution or unequal variances were observed, non-parametric tests were conducted (Kruskal-Wallis test with Mann-Whitney test as post-hoc testing). Data are shown as means ± SE. Significance was set at $p \leq 0.05$.

RESULTS

Behavioral measurements

Analysis indicated a significant effect of age, hierarchy and dietary type on the expressed agonistic behavior. Individuals with higher dominance rank displayed more agonistic behavior than individuals low in the intra-group hierarchy (see Table 2). According to group-related effects animals from SFA group showed significantly more agonistic behavior towards their conspecifics than animals from UFA group ($p = 0,026$) over the whole data acquisition (Fig. 1). Additionally significant group x age interactions were detected for both fatty acid groups compared to control group (Table 2). SFA group showed increasing agonistic behavior with age, starting with fewer scores at the beginning of the observation and ending up with higher scores at an age around three to four months, whereas the expression of agonistic behavior in UFA group remained nearly constant, persistently below the scores of the control group ($F_{127} = 3,214$, $p = 0,002$, $F_{133} = 2,328$, $p = 0,021$ for SFA and UFA vs. Control respectively, Fig. 2).

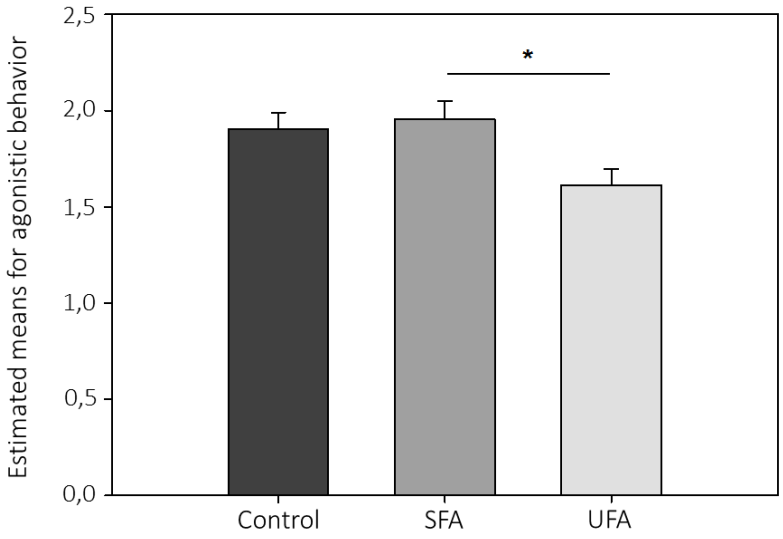

Figure 1. *Estimated means (+ SE) for displayed agonistic behavior over the four group housing observations for the three experimental groups. Significant differences between groups are labeled. * p ≤ 0,05*

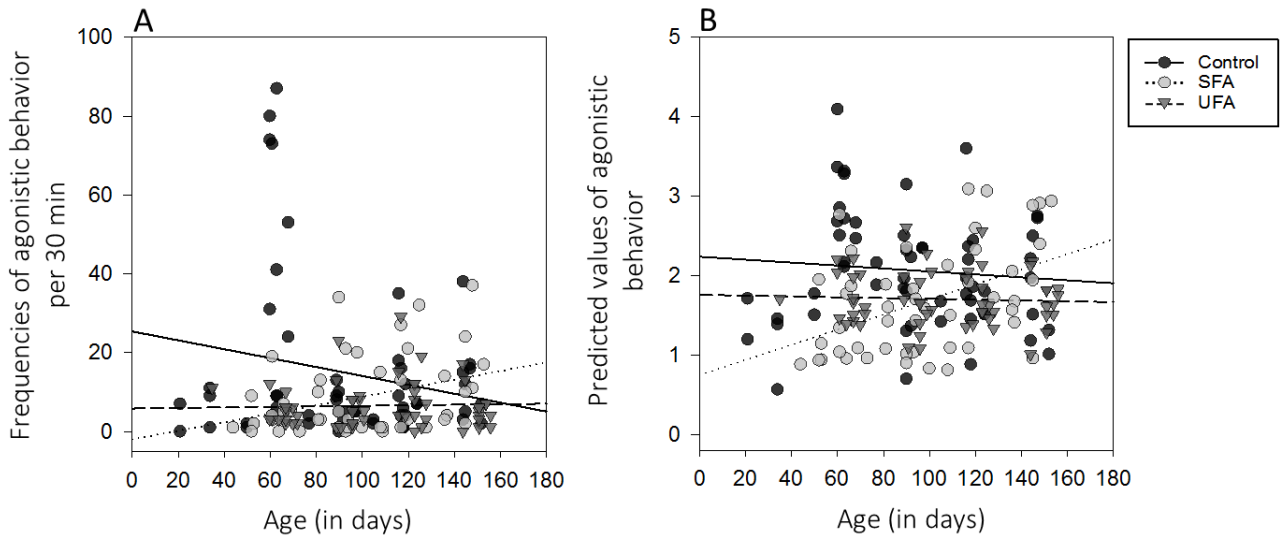

Figure 2. *Relationship between displayed agonistic behavior and age of the individuals respectively the comparison between behavioral frequencies and calculated predicted values of the LME model. A) Actual frequency data of displayed agonistic behavior (Control: r^2 = 0,037, SFA: r^2 = 0,12, UFA: r^2 = 0,001) and B) predicted values of agonistic behavior (Control: r^2 = 0,008, SFA: r^2 = 0,198, UFA: r^2 = 0,002) in relationship to the individuals' age. Significant differences were detected between Control and fatty acid groups (see text).*

Analyzed socio-positive behavior revealed increased significant effects of hierarchy and relationships to dietary types (Table 2), no age-related effects were detected. The UFA group had significantly higher frequencies compared to both SFA ($p < 0,001$) and control groups ($p = 0,001$) (Fig. 3).

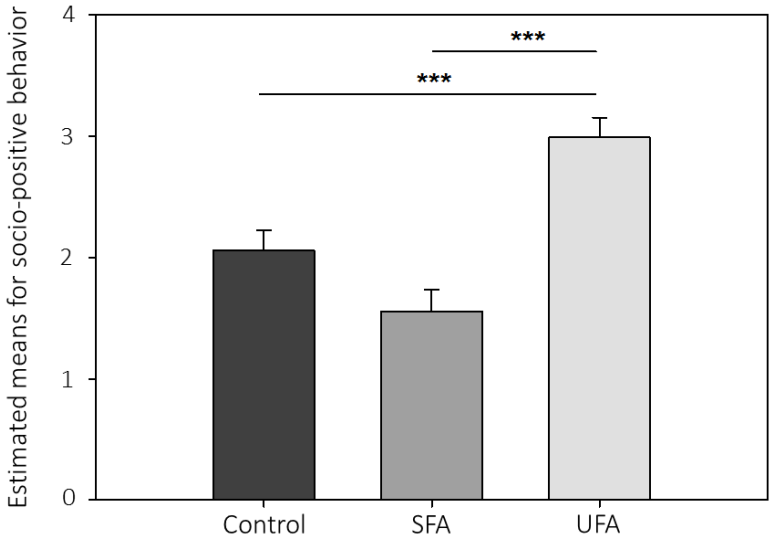

Figure 3. *Estimated means (+ SE) for displayed socio-positive behavior over the four group housing observations for the three experimental groups. Significant differences between groups are labeled. *** p ≤ 0,001*

Duration of locomotion differed significantly with age, hierarchy and between feeding groups. Dominant individuals showed increased locomotion compared to conspecifics low in the intra-group hierarchy (Table 2). Group-related effects revealed significantly increased overall locomotion of the control group compared to UFA ($p < 0,001$) and SFA group ($p = 0,002$) (Fig. 4). A significant interaction between group and age was detected as well for SFA group, showing increased locomotion with age compared to control group, but with constantly lower movement durations under three months of age ($F_{129} = 4,593$, $p < 0,001$, Fig. 5).

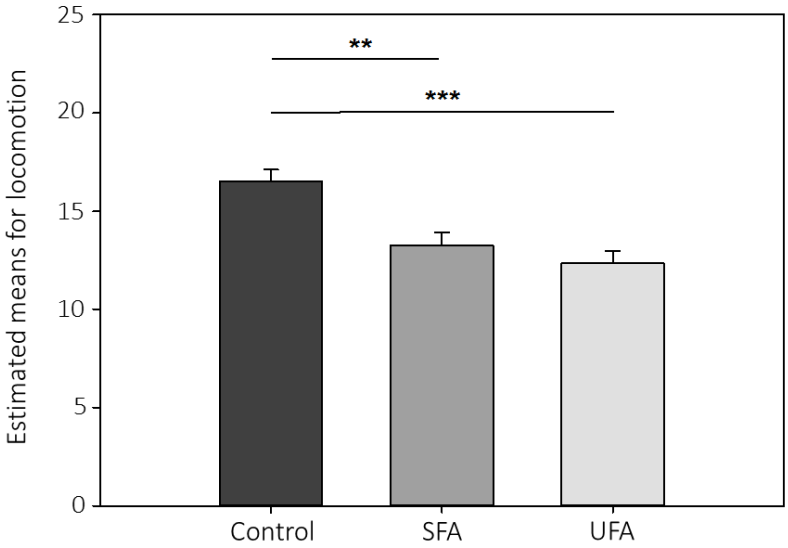

Figure 4. *Estimated means (+ SE) for the duration of locomotion over the four group housing observations for the three experimental groups. Significant differences between groups are labeled. ** p ≤ 0,01, *** p ≤ 0,001*

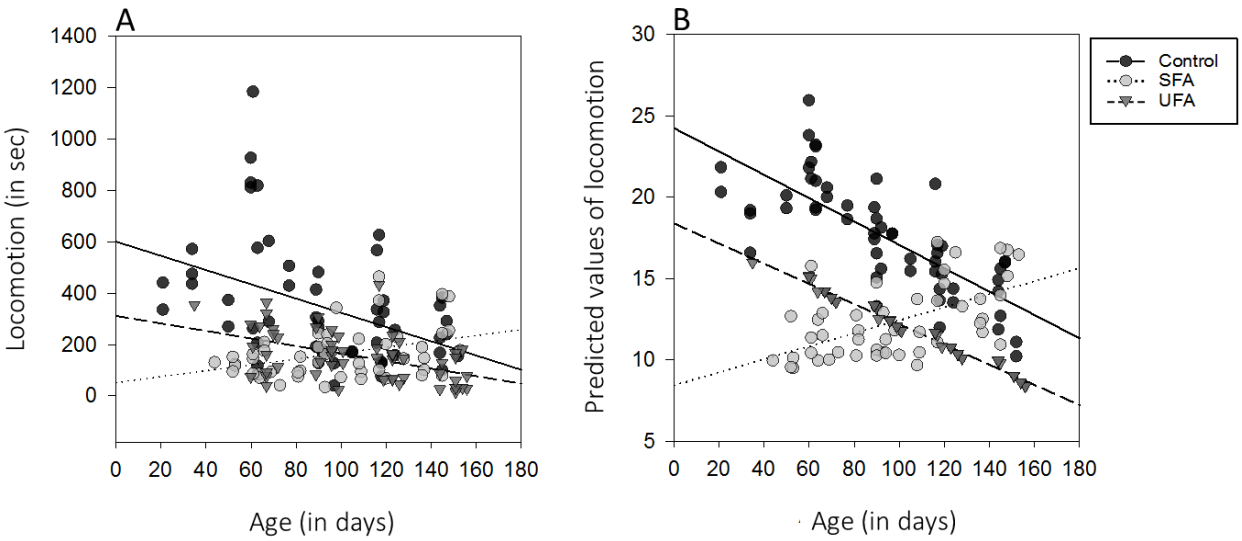

Figure 5. *Relationship between duration of locomotion and age of the individuals respectively the comparison between behavioral frequencies and calculated predicted values of the LME model. A) Actual duration of displayed locomotion (Control: $r^2 = 0{,}178$, SFA: $r^2 = 0{,}137$, UFA: $r^2 = 0{,}241$) and B) predicted values of locomotion (Control: $r^2 = 0{,}566$, SFA: $r^2 = 0{,}319$, UFA: $r^2 = 0{,}983$) in relationship to the individuals' age. Significant differences were detected between Control and SFA group (see text).*

Body weight

No group differences were detected according to the animals' body weight, but between days as animals of all three groups showed significantly increasing body weight throughout the observation period (see Table 2). UFA animals gained weight more rapidly than control animals from weaning to an age of about 4 months (Group x age interaction: $F_{126} = 3{,}354$, $p = 0{,}001$ for UFA vs. Control animals, Fig. 6).

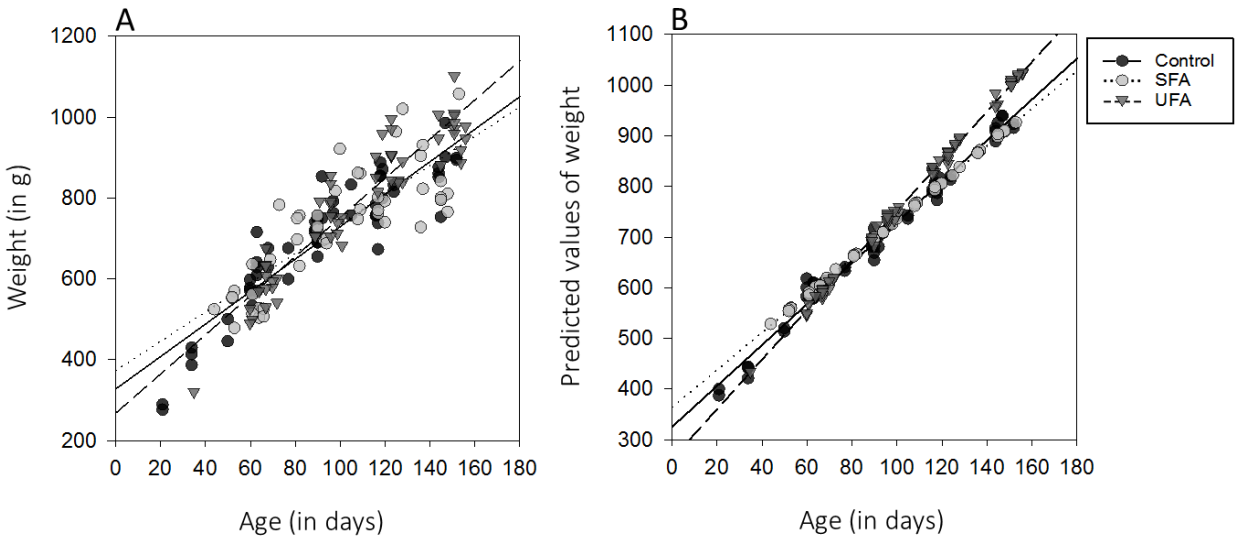

Figure 6. *Relationship between weight and age of the individuals respectively the comparison between behavioral frequencies and calculated predicted values of the LME model. A) Actual weight data (Control: $r^2 = 0,828$, SFA: $r^2 = 0,664$, UFA: $r^2 = 0,87$) and B) predicted values of weight (Control: $r^2 = 984$, SFA: $r^2 = 1,0$, UFA: $r^2 = 0,998$) in relationship to the individuals' age. Significant differences were detected between Control and UFA group (see text).*

Salivary cortisol concentrations

No differences of salivary cortisol levels in dominance rank or between diets were observed. A significant group-age interaction was detected (see Table 2) for SFA group compared to control group, which was related to increased cortisol concentrations with age. The latter started with lower concentrations at the beginning of the observation period and showing overall higher concentrations at an age around 120 days ($F_{94} = 2,581$, $p = 0,011$, Fig. 7).

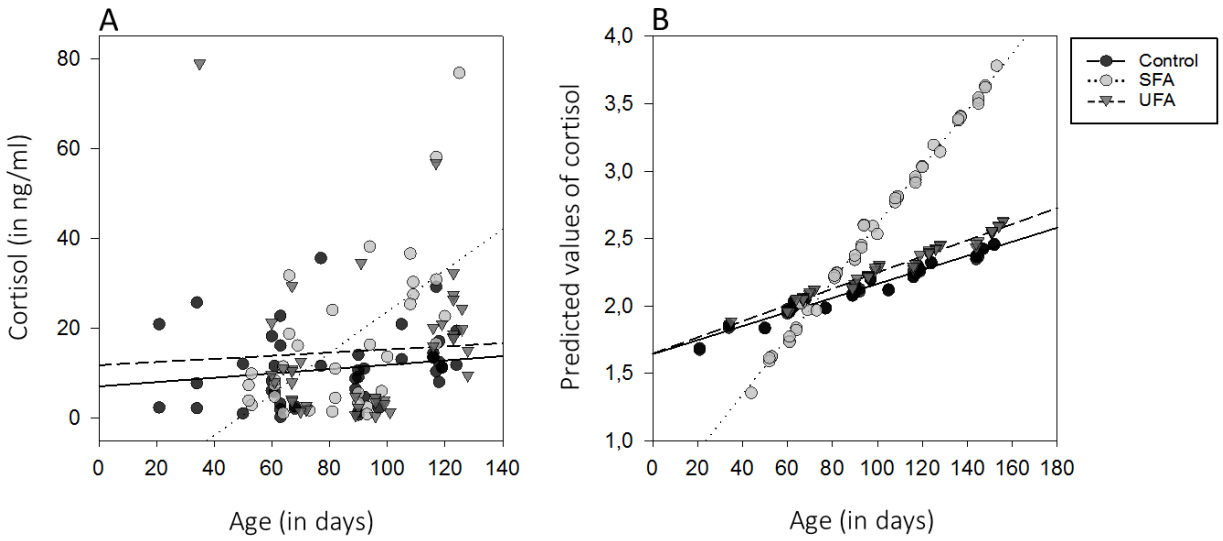

Figure 7. *Relationship between salivary cortisol concentration and age of the individuals respectively the comparison between behavioral frequencies and calculated predicted values of the LME model. A) Actual salivary cortisol data (Control: $r^2 = 0{,}032$, SFA: $r^2 = 0{,}37$, UFA: $r^2 = 0{,}003$) and B) predicted values of salivary cortisol (Control: $r^2 = 959$, SFA: $r^2 = 0{,}995$, UFA: $r^2 = 0{,}975$) in relationship to the individuals' age. Significant differences were detected between Control and SFA group (see text).*

Table 2*. ANOVA statistics for the significant terms and interactions of the fitted models. Given are the five observed response variables, the significant predictors, numerator and denominator df, F-statistic and p-value.*

Response variable	Predictor	Statistics		
		df	F-value	p-value
Active agonistic behavior	Individual age	1, 42	6,044	0,018
	Hierarchy	1, 138	176,475	< 0,001
	Group	2, 36	5,130	0,011
	Group x Age	2, 136	6,237	0,003
Socio-positive behavior	Hierarchy	1, 148	11,369	0,001
	Group	2, 38	17,906	< 0,001
Locomotion	Individual age	1, 44	21,829	< 0,001
	Hierarchy	1, 141	32,198	< 0,001
	Group	2, 39	17,014	< 0,001
	Group x Age	2, 137	12,760	< 0,001
Body weight	Individual age	1, 44	9,176	0,004
	Group x Age	2, 129	8,280	< 0,001
Cortisol	Group x Age	2, 94	3,629	0,030

Plasma fatty acid analysis

The plasma fatty acid composition in the three experimental groups are shown in Table 3. Percentages of different fatty acid types in the plasma were highly significant affected by the diet. The UFA group showed significantly higher percentages of total n-3, n-6, PUFAs and

overall UFAs than the control and SFA groups. Percentages of total n-9 and MUFAs were highest in the control group, whereas in the SFA group highest percentages of total SFAs were observed. Lowest n-6:n-3 ratio was detected in UFA group, as well as the highest MUFA:SFA (M:S), PUFA:SFA (P:S) and UFA:SFA (U:S) ratios according to the elevated PUFA and UFA and the decreased SFA percentages. No differences were observed between UFA and SFA group in total n-9 and MUFA percentages, neither between control and SFA group according to the n-6:n-3 ratio.

Table 3. *Plasma fatty acids (% of total fatty acids; mean ± SE) comparing the three experimental groups.*

Fatty acids	Control	UFA	SFA	Overall mean
Total n-3	4,38 ± 0,20 [a]	6,51 ± 0,22 [b]	3,57 ± 0,19 [c]	4,81 ± 0,23
Total n-6	46,60 ± 0,79 [a]	58,16 ± 0,40 [b]	42,08 ± 0,96 [c]	48,89 ± 1,15
Total n-9	13,64 ± 0,42 [a]	11,23 ± 0,25 [b]	10,89 ± 0,28 [b]	11,60 ± 0,27
Total MUFA	15,50 ± 0,42 [a]	11,98 ± 0,24 [b]	12,15 ±0,28 [b]	13,26 ± 0,32
Total PUFA	51,11 ± 0,84 [a]	64,77 ± 0,35 [b]	45,79 ± 0,95 [c]	53,82 ± 1,34
Total UFA	66,60 ± 0,68 [a]	76,75 ± 0,27 [b]	57,94 ± 1,09 [c]	67,08 ± 1,29
Total SFA	33,40 ± 0,68 [a]	23,25 ± 0,27 [b]	42,07 ± 1,09 [c]	32,92 ± 1,29
n-6 : n-3 ratio	10,89 ± 0,47 [a]	9,05 ± 0,32 [b]	12,21 ± 0,69 [a]	10,72 ± 0,36
M : S ratio	0,47 ± 0,02 [a]	0,52 ± 0,01 [b]	0,29 ± 0,01 [c]	0,43 ± 0,02
P : S ratio	1,54 ± 0,05 [a]	2,79 ± 0,04 [b]	1,10 ± 0,05 [c]	1,81 ± 0,12
U : S ratio	2,01 ± 0,06 [a]	3,31 ± 0,05 [b]	1,40 ± 0,06 [c]	2,23 ± 0,13

Values with different superscripts (a-c) show significant differences between groups ($p \leq 0,05$)

DISCUSSION

The present study examined the effect of pre- and postnatal dietary supplementation either with unsaturated or saturated fatty acids and their impact on behavioral and physiological parameters in weanling guinea pigs. Supplementation was focused during ontogenetic developmental phases of neuronal structures. Guinea pigs had received fatty acids prenatally, during lactation and after weaning until they were full-grown. Former studies indicated the importance of an adequate n-3 PUFA uptake, like DHA or AA, for the normal neurodevelopment (Bourre, 2004; Fedorova et al., 2009; Wainwright, 2007) as well as the benefits for neuronal development of the unborn by adequate dietary PUFA intake of their mothers (Helland et al., 2003; Innis, 2007; Mennitti et al., 2015).

Several dietary sources are available for fatty acid supplementation. In a review of Kaplan and Greenwood (1998) a few commonly used fat sources are summarized, which were applied in various studies. They vary in their absolute amount of PUFAs, MUFAs and SFAs, as well as in their n-6:n-3 ratio. For example, diets designed for a proportionate high SFA intake frequently use coconut oil, lard or palm kernel oil (e.g., Hill et al., 1993; Granholm et al., 2008). For general PUFA supplementation corn oil, soybean oil, walnut oil (all high in LA) and flaxseed oil (high in ALA) are often used (e.g., Fedorova et al., 2009; Helland et al., 2003). Cod liver and fish oil are especially applied for an increased n-3 intake, both high in total n-3 long-chained PUFAs, DHA and eicosapentaeonic acid (EPA; 20:5 n-3) (e.g., de Theije et al., 2015; Ferraz et al., 2011; Helland et al., 2003; Hennebelle et al., 2012). For the purpose of selective DHA or AA supplementation two microbial substances are available: DHASCO® and ARASCO®. These oils exhibit high contents of DHA and AA, respectively (Food Standards, 2003) and are commonly used for n-3 or n-6 PUFA supplementation (e.g., Fedorova et al., 2009; Weiler et al., 2012; Yin et al., 2014).

All three experimental groups of the present investigation received standardized food items (e.g., pellets, hay). SFA group was supplemented with coconut oil, rich in lauric acid (12:0) and myristic acid (14:0). Walnut oil, which is rich in n-6 PUFAs, was chosen for PUFA supplementation on the basis of the results of Nemeth et al. (2014) and Nemeth et al. (2015). They investigated the impact of chia seeds (high in ALA), walnuts and peanuts (high in OA) on social behavior and stress physiology in social confrontation tests, as well as sex-specific effects of UFA supplementation on spatial cognitive performances in domestic guinea pigs. Their plasma fatty acid analysis exhibited lowest percentages of total SFA along with high total PUFA percentages and an increased P:S ratio in walnut group compared to control animals. These differences between total SFA and PUFA percentages due to walnut supplementation were particularly suitable for the aim of the present investigation.

Plasma fatty acid status

Analysis of the plasma fatty acid status revealed the successful uptake of the supplemented fatty acids shown in increased plasma fatty acid concentrations according to the received dietary type. Referring to Tu et al. (2013) plasma fatty acid levels are correlated with the corresponding fatty acids in various organ tissues. In detail they found significant strong correlations ($r > 0,7$) for total n-3, DHA, LA and AA between brain and plasma fatty acid

concentrations. Therefore, it is assumed that detected plasma fatty acid levels serve as an indicator for the phospholipid fatty acid status in neuronal cell membranes.

The UFA group revealed the highest n-3, respectively, n-6 PUFAs and overall PUFA plasma concentrations. They exhibited the lowest SFA level and n-6:n-3 ratio, as well as the highest P:S ratio. Low SFA and high PUFA levels were already indicated by several studies to have beneficial effects on health and neuronal development (e.g., Haast and Kiliaan, 2015; Kaplan and Greenwood, 1998; Mennitti et al., 2015). Furthermore, in vitro studies revealed a stabilizing impact of n-3 PUFAs on cardiac cells and their electrical processes, whereas SFAs or MUFAs showed no such positive effects (for review see Leaf et al., 2002). The detected plasma percentages correspond with those found by Nemeth et al. (2014) and Nemeth et al. (2015) (see Table 4). Comparatively, increased percentages of total n-6, PUFA and consequently the n-6:n-3 ratio found in the current study might be explained by supplementation duration. Dietary fatty acids were supplied prenatally until early adulthood, whereas supplementation in Nemeth et al. (2014; 2015) was applied for about three weeks to adult animals only. Therefore, saturation of free plasma fatty acids of the corresponding supplementation might have been higher in the current experimental groups. On the opposite, SFA group revealed the lowest n-3, respectively, n-6 PUFAs and total PUFA concentrations, but highest total SFA plasma concentrations. Their n-6:n-3 ratio averaged out at 12,2. Control group exhibited the highest concentrations of total n-9 and MUFA plasma levels. Chronic high SFA intake is associated with metabolic diseases like hyperglycemia, hyperinsulinemia and insulin resistance which might result in severe liver damage (reviewed by Mennitti et al., 2015). Accelerated cognitive decline is linked as well with highly ingested SFAs, whereas PUFA and MUFA intake seem to be positively correlated with cognitive performances in elderly and may counteract cognitive degeneration (Solfrizzi et al., 2005). The recommended optimum n-6:n-3 ratio of about 4:1 wasn't achieved by any of the experimental groups. The possible role of this ratio during ontogeny on behavior and stress physiology is discussed below.

Table 4. *Plasma fatty acids (% of total fatty acids; mean ± SE) comparing walnut group of Nemeth et al. (2014 and 2015) and UFA group of the present investigation.*

Fatty acids	Nemeth et al. (2014 and 2015)	UFA group
Total n-3	6,79 ± 0,31	6,51 ± 0,22
Total n-6	50,92 ± 1,11	58,16 ± 0,40
Total n-9	12,93 ± 0,58	11,23 ± 0,25
Total PUFA	57,71 ± 1,10	64,77 ± 0,35
Total SFA	27,27 ± 1,12	23,25 ± 0,27
n-6 : n-3 ratio	7,87 ± 0,58	9,05 ± 0,32
P : S ratio	2,20 ± 0,13	2,79 ± 0,04

Development of body weight

According to the development of the body weight no group differences in general were detectable. Guinea pigs included in this study showed a constant gain in body weight, respective to their developmental stages. Only the UFA group exhibited a significant increased weight gain compared to control group. On the basis of this findings it is suggested that pre- and postnatal UFA supplementation leads to an accelerated postweaning development according to body weight gain. This possible circumstance comprises the potential for an earlier onset of puberty due to an increased growth rate as studied in rats (Kennedy and Mitra, 1963), which might contribute to an increased individual fitness. Along with the decreasing activity level and few escalation fights with age it is indicated that UFA animals took advantage of the high caloric supplemented food for their weight development as their energy expenditure had been comparatively low. On the contrary, no difference in weight development was detectable between SFA and control group. Similar to UFA group, SFA group had a high energy intake through fatty acid supplementation, yet their energy expenditure likely increased with age compared to those of control animals because of the elevating social escalation rate. Moreover, SFA animals showed increasing basal cortisol levels with age. Glucocorticoids exhibit lipolytic effects for the short term (Macfarlane et al., 2008), but on long term elevated cortisol levels comprise metabolic effects and presumably lead to harmful impact on health in adult individuals like increased deposition of fat and an enhanced risk for diabetes (McEwen, 2002; McEwen and Wingfield, 2003). Low cortisol levels in SFA group were detected in early adolescence with a rapid gain after an age of 80 days. This sudden increase in cortisol concentrations may have led to the mentioned lipolytic effects and serves as a

possible explanation for the lack of significant differences compared to control group. In the long term lasting high cortisol levels are expected to result in a potential increase in body weight of adult SFA individuals. This might be followed by deleterious consequences for the health like enhanced risk for insulin resistance, diseases of the cardiovascular system and even muscle and bone atrophy (reviewed by Reeder and Kramer, 2005; McEwen, 2002). These diseases are associated with high gestational SFA intake as well (reviewed by Mennitti et al., 2015). Whereas diets rich in PUFAs were associated with an enhanced affinity of insulin to its receptors due to increased membrane fluidity (Russo, 2009). Also a high P:S ratio was assumed to improve insulin response (Kaplan and Greenwood, 1998). The present results resemble those found by Pan and Storlien (1993) where diets high in n-6 PUFAs led to an increased gain in body weight in male rats. Whereas a diet supplemented with edible tallow (rich in SFAs) resulted in significantly less weight gain. Similarly, Su and Jones (1993) found higher lean body mass gains and lower fat mass gains in rats fed fish oil diets compared to beef tallow diets. In a study of Carrié et al. (2000) mice that had received fish oil during pre- and postnatal development showed accelerated weight gain and higher final body weight compared to mice fed a palm oil diet. On the contrary, Hill et al. (1993) found no different results among lard, corn oil, fish oil or triglycerides diets on body weight in adult male rats. Though, type of dietary fat influenced the animals' body fat composition and resulted in less total body fat and less insulin resistance in the fish oil group. On this basis it is assumed that body fat composition of the animals investigated in the current study most likely correlate with the type of dietary fat. As a result, animals fed the SFA diet may be more susceptible for developing insulin resistance and other pathophysiological diseases during further development and aging affecting their individual fitness. Whereas UFA animals might be less predispositioned for metabolic disorders in adulthood and therefore, most likely ending up in in an increased individual fitness due to decreased morbidity rate.

Social behavior

Less locomotion in general was observed in both FA groups in contrast to control animals. In UFA group decreasing activity levels with age were detected, still not significant as control group showed decreasing locomotor activity duration as well. However, SFA group exhibited significantly increasing locomotion with age compared to control group. Most previous studies regarding dietary fatty acids solely examined locomotor activity within spatial

cognition tasks or open field tests and concentrated on the comparison between n-3 deficient, adequate or enriched diets (for review see Fedorova and Salem, 2006). For example, young mice fed an n-3 PUFA enriched diet exhibited significantly more locomotor activity in an open field test compared to a diet enriched in SFAs. These differences weren't observable in mature and old mice fed the same diets (Carrié et al., 2000). This observation partially supports the current findings as SFA and UFA animals converged in their locomotor activity with age and exhibited comparable activity levels around early adulthood. Contradictory to the results shown here Raygada et al. (1998) found elevated locomotor activity of mice whose dams had received an n-6 PUFA enriched diet during gestation compared to a control diet. Contreras et al. (2014) found enhanced activity in locomotor activity test in rats that had received 10 µg myristic acid (14:0) subcutaneously in contrast to control animals. Additionally, these injections resulted in anxiolytic-like behavior in the elevated plus maze test comparable to the effects of diazepam. Whereas in the current investigation increasing locomotor activity in SFA group is likely explained by the simultaneously rising agonistic behavior with age. Literature findings and current results aren't quite consistent in explaining the impact of FA supplementation on locomotor activity. It is rather suggested, that different results origin from dietary effect on other behavioral variables which are differently expressed depending on the testing condition (e.g., anxiety-like behavior, aggression).

Results showed that UFA animals exhibited significantly higher frequencies of socio-positive behavior in general compared to control and SFA animals. These high scores resulted mostly from long "side by side" durations, which also serve as an indicator for the decreasing activity level over time. On the contrary, SFA group exhibited the highest frequencies of agonistic behavior in general with a constant gain of expression with age. In previous studies increased aggressive behavior in connection with fatty acids was observed in n-3 deficiency (Re et al., 2007) or, in particular, increased n-6 uptake (e.g., Hilakivi-Clarke et al., 1996). Raygada et al. (1998) found increased aggressive behavior in mice whose dams had received n-6 PUFA supplementation during gestation. In male rats pre- and postnatal n-6 PUFA supplementation led to extremely enhanced aggressive behavior towards mice introduced into the rats territory (Miachon et al., 2001). Results from UFA group contradict literature findings as they exhibited remaining low frequencies of agonistic behavior, despite supplementation high in n-6 UFAs. This observation is accompanied by significantly more

socio-positive behavior in general. In regard to the latter de Theije et al. (2015) showed food-allergy induced deficits in social behavior of mice along with reduced prefrontal cortex dopamine levels and impaired intestinal serotonin metabolism. Dietary n-3 PUFA supplementation restored reduced dopamine levels and serotonin metabolism and increased the incorporation of DHA into neuronal cell membranes. This resulted in the prevention of reduced social interactions. Also, findings from Bhatia et al. (2011) insistently indicate the necessity of an adequate PUFA intake, particularly DHA, during neurodevelopment to ensure long-term neuronal resilience and establish a significant basis for brain and behavior plasticity in the adult individual. Stated literature findings suggest a positive effect on neurochemistry, developing neuronal structures and social behavior of n-3 PUFA supplementation only. Whereas again, results from Hilakivi-Clarke et al. (1996), Raygada et al. (1998) and Miachon et al. (2001) point to a positive association between n-6 PUFAs and increased aggressive behavior. Previous results might rather illustrate the relevance of an optimal n-6:n-3 ratio and its' potential in providing neuronal cell membrane fluidity as described by Yehuda (2003). Diets which were especially designed for a disproportionate high n-6 uptake are presumably shifting the balance of n-3 and n-6 FAs with negative consequences on neuronal functioning. Although the detected n-6:n-3 ratio in UFA group is twice as high as recommended current findings regarding social behavior are a strong indicator for the necessity of an adequate total PUFA intake during gestation and early postnatal period to ensure optimal neurodevelopment. This is supported by the circumstance that during the monitored ontogenetic period no increase in agonistic behavior was observable, despite the onset of puberty which goes along with hormonal changes and inevitably alters social behavior. Guinea pigs show a relatively early sexual maturation compared to their developmental condition, as males become sexually mature shortly after weaning at an age around 40 to 80 days (Young, 1969) and adult testosterone concentration is reached about day 80 (Trillmich et al., 2006). Along with sexual maturity gonadal hormone levels rise and agonistic interactions between conspecifics naturally increase. However, together with the results of socio-positive behavior it is suggested that UFA animals are more successful in forming social bonds within their group and aggressiveness is decreased. This may lead to less experienced social stress accompanied by reduced basal cortisol levels. Social support is a decisive factor for the individual stress management. The presence of a social bonding partner is assumed to improve the physiological stress response, whereas instability within social systems is associated with an

increased HPA axis reactivity (Sachser et al., 1998; Blanchard et al., 2001). On the opposite, results from SFA group indicate either a strongly unbalanced n-6:n-3 ratio affecting social behavior or a negative impact triggered by increased SFA uptake. As revealed by plasma fatty acid analysis, n-6:n-3 ratio in SFA group reached a value of about 12, three times higher than recommended. Still no difference was found compared to control group, which actually showed a decline in agonistic behavior with age. High agonistic behavior scores in control group around an age of 60 days can be ascribed to the introduction of 5 new individuals on the day of the first behavioral observation (day 36), which serves as a plausible explanation for the lack of significant difference in agonistic behavior in general compared to SFA animals. Nevertheless, the lack of significant difference in the n-6:n-3 ratio seems to be pointing at negative consequences on neuronal functioning caused by high SFA uptake. This is supported by the fact that no fatty acid deficiency had been induced in this investigation and an adequate total n-3 and n-6 PUFA intake during important ontogenetic periods of neurodevelopment was ensured. Whereas additional integration of SFAs in cell membranes was shown to reduce membrane fluidity and thus, impair cell functions (Whitcomb et al., 1988). This is of particular importance during fetal and neonatal periods, since development of neurological functions and physiological pathways might be severely perturbed by maternal diets rich in SFAs (for review see Mennitti et al., 2015). Beside the detrimental impact on health, less behavioral flexibility due to impaired neuronal cell functions and reduced neurotransmitter metabolism might be the consequence. One-time injections of palmitic acid (16:0) in mice led to distinct behavioral changes like enhanced anxiety-like behavior (Moon et al., 2014). Measurable increased levels of 5-HIAA, a serotonin metabolite, and impaired dopamine levels in the mice's brains led to the suggestion of dysregulated neurotransmitter metabolisms to be the cause of the observed changes in behavior. If even one-time injections with SFAs led to obvious short-term alterations in brain neurotransmitter systems with consequences on behavior, it is assumed that high maternal and neonatal SFA intake would likely result in even more distinct modifications of brain neurochemistry. Regarding fetal programming processes these modifications may entail impaired neurotransmitter metabolism that persists throughout life and affects the ontogenetic development of social behavior within the SFA group. As malfunctions in the serotonergic and dopaminergic system were associated with aggressive behavior by various studies (for review see Seo et al., 2008), irrationally increasing agonistic behavior in consequence of neurochemistry changes becomes inevitably maladaptive and

even life threatening. Along with the elemental hormonal and behavioral changes accompanying sexual maturity, SFA group may have been severely affected from perturbed behavioral modulation resulting in increased social escalation rate. Regarding gonadal hormones, results from Volek et al. (1997) indicate that testosterone concentrations in men are partially predicted by the ingested amount and type of dietary fat, as strong positive correlations were found for SFAs and MUFAs. High levels of ingested SFAs could have resulted in strongly enhanced testosterone secretion along with puberty in SFA group. Since high testosterone levels are associated with increased aggressiveness (Rose and Holaday, 1971; Mendonça-Furtado et al., 2014; Wallner and Dittami, 2003) an additional explanation for the increasing agonistic behavior with age is given. Additionally, in regard to aging cholesterol levels in neuronal membranes were shown to increase which leads to proceeding rigidity and impaired functioning (reviewed by Yehuda et al., 2002). This effect is even intensified through high SFA uptake as it is associated with increased LDL cholesterol and total cholesterol levels and consequently, a potentially enhanced risk for cognitive decline and dementia (Solfrizzi et al., 2005). These neuropsychological changes or disorders occurring during the aging process are linked with higher aggression levels as well (Brodaty and Low, 2003). The current findings hold the potential for increased social stress load within the SFA group due to hormonal and behavioral changes over adolescence, which might not be adequately compensated through behavior modulation in adverse situations. This instance constitutes a severe threat for survival and individual fitness of SFA animals.

Cortisol

Social interactions even in groups with established hierarchies inevitably lead to stress reactions, mainly elicited by agonistic behavior between conspecifics (Sachser et al., 1998; Blanchard et al., 2001). Guinea pigs included in this study lived in stable social environments with established hierarchies. In the present investigation no data are available on the physiological stress reaction during short-term and/or repeated stress exposure as the focus was on the basal physiological stress reactivity and its development.

Though reduced basal cortisol levels in UFA group were expected, no significant difference in secretion rates over postweaning development were detected compared to control group, which hadn’t received any fatty acid supplementations. Furthermore no mean differences in cortisol concentrations across the three experimental groups were observable.

Regarding SFA group the lack of significant results in mean cortisol levels is likely due to the low cortisol levels after weaning and the exaggerated shift around day 100. Whereas control and UFA group exhibited a constant slight increase in cortisol concentrations in time respective to the age-dependent reactivity changes of the HPA axis already shown in rats and mice (reviewed by Reeder and Kramer, 2005). Previous results suggest that the supplementation with n-3 and n-6 PUFAs positively affects the HPA axis resulting in decreased adrenocortical secretion of cortisol during a stress-inducing situation (Ferraz et al., 2011; Nemeth et al., 2014). Likewise, results from Hennebelle et al. (2012) indicate a positive impact of n-3 PUFAs on the HPA axis reactivity in male rats resulting in reduced glucocorticoid levels when faced with repeated restraint stress. On the contrary, they detected no difference in basal corticosterone levels between n-3 enriched and deficient diets. Reviewed by Yehuda (2003) the impact of n-3 and n-6 fatty acids on the immune system indirectly leads to changes in the glucocorticoid secretion as well, with n-6 FAs promoting the synthesis of IL-1 and IL-6 which facilitate the release of corticotropin-releasing-factor (CRF). Whereas n-3 fatty acids are suggested to provide counteracting effects with decreased glucocorticoid secretion. In the recent study effects of UFA supplementation on the basal HPA axis reactivity didn't seem to be pronounced during daily social interactions within their group. As already stated social support was shown to provide moderating effects on the physiological stress response. Indeed, reduced experienced social stress through social support seems to be inapplicable since UFA and control animals didn't differ in their basal cortisol secretion development nor in the mean concentrations despite significantly enhanced socio-positive behavior in UFA group. Possibly, the ontogenetic manifestation of pre- and postnatal UFA supplementation on the physiological stress response may be only visible during exposures to stressful conditions (e.g., elevated plus maze, social confrontation) or even emerge at later life stages. The delayed emergence of prenatal programming effects are apparent in results from Bhatia et al. (2011) where adequate PUFA intake during gestation, lactation and infancy was shown to be beneficial for the adult brain in terms of reduced anxiety-like behavior during adulthood. Similar results are shown by Estanislau and Morato (2006) with prenatally stressed rats exhibiting no differences in anxiety-like behavior during early and late adolescence compared to control animals. However, in early adulthood prenatally stressed animals showed a significantly increased behavioral stress response. Likewise, positive or negative consequences of an elevated n-6 plasma level and n-6:n-3 ratio during early development on

the stress response may evolve later in life of UFA animals. In regard to previous results this could be chronically elevated glucocorticoid levels in adulthood through effects of n-6 supplementation on the immune system (Yehuda, 2003) with negative long-term effects on memory (Machatschke et al., 2011), suppressed immune function and reproduction (Möstl and Palme, 2002) and other deleterious effects on health like insulin resistance, bone atrophy and hypertension (Reeder and Kramer, 2005). On the contrary, elevated total PUFA intake during aging might be of greater importance for the stress response, comparably to the results regarding social behavior. As UFA animals exhibited the highest total PUFA level and P:S ratio among all three groups it is assumed that the high PUFA supplementation holds beneficial effects in terms of a reduced HPA axis reactivity in adulthood either during stress exposure or in the familiar social environments of the animals. This positive effect of total PUFA levels on the stress response was already suggested by Nemeth et al. (2014).

SFA group exhibited a significant increase of basal cortisol levels with age compared to control group. This constant gain in cortisol secretion is most likely explained by the impaired reactivity of neuronal cell membranes in response to high SFA intake. This was already suggested regarding social behavior development. Resulting in reduced behavioral flexibility the animals might have been impeded in adequately coping with adverse social situations. According to brain neurotransmitter systems serotonergic metabolism is suggested to be increased during social stress situations, measurable in elevated 5-HIAA levels or a shifted 5-HIAA/serotonin ratio at the expense of serotonin (Blanchard et al., 2001). If this neurochemistry system was actually impaired by SFA supplementation, behavioral plasticity might be additionally aggravated resulting in increased aggression followed by enhanced cortisol secretion. Glucocorticoids mediate many metabolic processes and influence the energy requirements of an individual, as they directly affect the fatty acid biosynthesis und lead to gluconeogenesis from fat tissue in times of increased energy requirement (de Alaniz and Marra, 2003). The rising locomotion activity and agonistic behavior in SFA individuals serve as a plausible explanation for enhanced cortisol secretion rates. SFA group seemed to strongly rely on the energy mobilization by cortisol release for the increasing social escalation rate in time, because they probably suffered from the inability of adjusting their behavioral stress responses to respective stressful interactions. While no such conclusion can be drawn

for UFA and control group as they both show decreasing or remaining constant low levels of agonistic behavior, but slightly increasing cortisol levels with age.

Along with the rising stress load due to increased social interactions in puberty, prenatal stress and adaptions of the HPA axis in SFA individuals may occupy a central role for the increasing cortisol secretion. Dietary supplementation with SFAs during gestation may have led to a higher stress load in dams due to the hypothesized impaired neuronal cell reactivity. Glucocorticoid levels in mothers are passed through the placenta and lead to direct programming of the fetus' HPA axis, as well as structural and functional changes in various brain regions, e.g. the amygdala, affecting the individual's behavior (for review see Seckl, 2004). The impact of prenatal stress on the offspring's stress response changes with ontogeny as neurological structures involved in stress reactivity develop. This was tested by Estanislau and Morato (2006) who were able to show the emergence of prenatal stress effects being age-dependent. They observed increased stress-related emotional responsivity not until prenatally stressed rats had reached early adulthood, whereas no behavioral differences were detected in adolescent animals. Similar results were presented by Schöpper et al. (2011) and Schöpper et al. (2012). Increased plasma cortisol levels were found in pregnant guinea pigs after repeated stress exposure, whereas significantly decreased cortisol metabolites in feces were detected on non-stress days. Physiological data led to the conclusion of an inhibited physiological stress response due to repeated stress. When offspring was treated with the same stressor they detected decreased basal plasma cortisol and cortisol levels after stress exposure. Cortisol metabolites in feces showed decreased concentrations in pre-pubertal phase. Though, higher levels were found in post-adolescent animals. In the recent investigation a possible outcome of the assumed prenatal stress might be the down-regulation of the HPA axis in pre-pubertal SFA offspring, followed by an up-regulation in adolescent animals. This possible circumstance comprises an explanation for the low cortisol levels after weaning followed by accelerated secretion rates after sexual maturation.

Glucocorticoid levels which remain high throughout development and aging were associated with pathologies like hypertension, suppressed immune function, impaired reproduction, growth inhibition, insulin resistance and neuronal cell death (reviewed by Blanchard et al., 2001; Möstl and Palme, 2002; Reeder and Kramer, 2005), all resulting in impaired individual fitness. Beside harmful effects on health cognitive functions are

particularly afflicted from chronically elevated glucocorticoid concentrations during aging as hippocampus, hypothalamus and anterior pituitary exhibit many glucocorticoid receptors. Therefore, these brain regions are especially susceptible to permanently increased glucocorticoid levels and may lose their structural plasticity resulting in accelerated cognitive decline with age (reviewed by McEwen, 2002). In addition, possibly remaining high cortisol levels in adulthood are suggested to shift the n-6:n-3 ratio in SFA group to even higher ratio plasma levels than currently detected, since Nemeth et al. (2014) reported of impaired n-6:n-3 ratios at the expense of n-3 FAs due to increased cortisol levels after stress exposures. This may result in additional deleterious effects on cognition and memory, as a balanced n-6:n-3 ratio is assumed to protect hippocampal functions from excessively secreted glucocorticoids (Bourre, 2005).

In conclusion, both behavioral and physiological effects for the supplementation with UFAs and SFAs were found according to the postweaning development in guinea pigs. Accelerated weight gain was observed in UFA group, as well as improved social behavior within the group compared to control and SFA animals. These results were assigned to positive effects of high total PUFA supplementation on neuronal cell functions, like increased neurotransmitter release, followed by improved social behavior and less energy consumption due to the lack of escalating fights between conspecifics. In regard to further development beneficial effects on individual fitness and health are assumed. Nevertheless, pre- and early postnatal supplementation with an n-6 FA rich diet may hold the potential for detrimental effects on health and the individual's stress response, as fetal programming effects might emerge not until adulthood. Whereas SFA supplementation led to increasing agonistic behavior and locomotion activity with age, accompanied by increasing cortisol levels. Chronically elevated glucocorticoid levels comprise harmful effects on health and survival as they increase an individual's morbidity. It was concluded that high ingested amounts of SFAs have detrimental effects on behavior too, due to impaired brain neurochemistry and cell-cell communication, resulting in an impaired coping ability when faced with stress exposures and therefore, an enhanced stress load with age.

REFERENCES

Barker, D. J. P. (1990). "The fetal and infant origins of adult disease." BMJ **301**: 1111.

Barker, D. J. P. (1995). "Fetal origins of coronary heart disease." BMJ **311**: 171-174.

Barker, D. J. P. (1997). "Maternal nutrition, fetal nutrition, and diseases in later life." Nutrition **13**(9): 807-813.

Barker, D. J. P. and C. N. Martyn (1991). Maternal and fetal origins of cardiovascular disease. Epidemiology of Peripheral Vascular Disease. London, Springer**:** 247-254.

Bayol, S. A., S. J. Farrington and N. C. Stickland (2007). "A maternal 'junk food' diet in pregnancy and lactation promotes an exacerbated taste for 'junk food' and a greater propensity for obesity in rat offspring." British Journal of Nutrition **98**(04): 843-851.

Bhathena, S. J. (2000). "Relationship between fatty acids and the endocrine system." BioFactors **13**: 35-39.

Bhatia, H. S., R. Agrawal, S. Sharma, Y.-X. Huo, Z. Ying and F. Gomez-Pinilla (2011). "Omega-3 Fatty Acid Deficiency during Brain Maturation Reduces Neuronal and Behavioral Plasticity in Adulthood." PLoS ONE **6**(12): e28451.

Blanchard, R. J., C. R. McKittrick and D. C. Blanchard (2001). "Animal models of social stress: Effects on behavior and brain neurochemical systems." Physiology & Behavior **73**: 261-271.

Bourre, J. M. (2004). "Roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during ageing." The Journal of Nutrition, Health & Aging **8**(3): 163-174.

Bourre, J. M. (2005). "Dietary omega-3 fatty acid and psychiatry: mood, behaviour, stress, depression, dementia and aging." The Journal of Nutrition, Health & Aging **9**: 31-38.

Brodaty, H. and L.-F. Low (2003). "Aggression in the elderly." The Journal of clinical psychiatry **64**(4): 36-43.

Carrié, I., P. Guesnet, J. M. Bourre and H. Francès (2000). "Diets containing long-chain n-3 polyunsaturated fatty acids affect behavior differently during development than ageing in mice." British Journal of Nutrition **83**: 439-447.

Contreras, C. M., J. F. Rodríguez-Landa, R. I. García-Ríos, J. Cueto-Escobedo, G. Guillen-Ruiz and B. Bernal-Morales (2014). "Myristic Acid Produces Anxiolytic-Like Effects in Wistar Rats in the Elevated Plus Maze." BioMed Research International **2014**: 1-8.

de Alaniz, M. J. T. and C. A. Marra (2003). "Steroid hormones and fatty acid desaturases." Prostaglandins, Leukotrienes and Essential Fatty Acids **68**: 163-170.

de Theije, C. G. M., L. W. J. van den Elsen, L. E. M. Willemsen, V. Milosevic, G. A. H. Korte-Bouws, S. Lopes da Silva, L. M. Broersen, S. M. Korte, B. Olivier, J. Garssen and A. D. Kraneveld (2015). "Dietary long chain n-3 polyunsaturated fatty acids prevent impaired social behaviour and normalize brain dopamine levels in food allergic mice." Neuropharmacology **90**: 15-22.

Estanislau, C. and S. Morato (2006). "Behavior ontogeny in the elevated plus-maze: prenatal stress effects." International Journal of Developmental Neuroscience **24**(4): 255-262.

Fedorova, I., N. Hussein, M. H. Baumann, C. Di Martino and N. Salem (2009). "An n-3 fatty acid deficiency impairs rat spatial learning in the Barnes maze." Behavioral Neuroscience **123**(1): 196-205.

Fedorova, I. and N. Salem (2006). "Omega-3 fatty acids and rodent behavior." Prostaglandins, Leukotrienes and Essential Fatty Acids **75**(4-5): 271-289.

Fenske, M. (1997). "The use of salivary cortisol measurements for the non-invasive assessment of adrenal cortical function in guinea pigs." Experimental and Clinical Endocrinology & Diabetes **105**: 163-168.

Ferraz, A. C., A. M. Delattre, R. G. Almendra, M. Sonagli, C. Borges, P. Araujo, M. L. Andersen, S. Tufik and M. M. S. Lima (2011). "Chronic ω-3 fatty acids supplementation promotes beneficial effects on anxiety, cognitive and depressive-like behaviors in rats subjected to a restraint stress protocol." Behavioural Brain Research **219**(1): 116-122.

Fiset, C., F. M. Rioux, M. E. Surette and S. Fiset (2015). "Prenatal Iron Deficiency in Guinea Pigs Increases Locomotor Activity but Does Not Influence Learning and Memory." PLoS ONE **10**(7): e0133168.

Food Standards, A. N. Z. (2003). "DHASCO and ARASCO oils as sources of long-chain polyunsaturated fatty acids in infant formula - A Safety Assessment." Technical Report Series **22**.

Godfrey, K. M. and D. J. P. Barker (2001). "Fetal programming and adult health." Public Health Nutrition **4**(2B): 611-624.

Godfrey, K. M., D. J. P. Barker, C. Osmond and V. Cox (1996). "Maternal nutrition in early and late gregnancy in relation to placental and fetal growth." BMJ **312**: 410-414.

Granholm, A.-C., H. A. Bimonte-Nelson, A. B. Moore, M. E. Nelson, L. R. Freeman and K. Sambamurti (2008). "Effects of a saturatated fat and high cholesterol diet on memory and hippocampal morphology in the middle-aged rat." Journal of Alzheimers Disease **14**(2): 133-145.

Haast, R. A. M. and A. J. Kiliaan (2015). "Impact of fatty acids on brain circulation, structure and function." Prostaglandins, Leukotrienes and Essential Fatty Acids **92**: 3-14.

Hales, C. N. and D. J. P. Barker (2001). "The thrifty phenotype hypothesis." British Medical Bulletin **60**: 5-20.

Hales, C. N., M. Desai and S. E. Ozanne (1997). "The Thrifty Phenotype Hypothesis: How Does It Look After 5 Years?" Diabetic Medicine **14**: 189-195.

Helland, I. B., L. Smith, K. Saarem, O. D. Saugstad and C. A. Drevon (2003). "Maternal Supplementation With Very-Long-Chain n-3 Fatty Acids During Pregnancy and Lactation Augments Children's IQ at 4 Years of Age." Pediatrics **111**(1): e39-e44.

Hennebelle, M., L. Balasse, A. Latour, G. Champeil-Potokar, S. Denis, M. Lavialle, P. Gisquet-Verrier, I. Denis and S. Vancassel (2012). "Influence of omega-3 fatty acid status on the way rats adapt to chronic restraint stress." PLoS ONE **7**(7): e42142.

Herrera, E. (2002). "Implications of Dietary Fatty Acids During Pregnancy on Placental, Fetal and Postnatal Development — A Review." Placenta **23**: 9-19.

Hilakivi-Clarke, L., E. Cho and I. Onojafe (1996). "High-fat diet induces aggressive behavior in male mice and rats." Life Sciences **58**(19): 1653-1660.

Hill, J. O., J. C. Peters, D. I. N. G. Lin, F. Yakubu, H. Greene and L. Swift (1993). "Lipid accumulation and body fat distribution is influenced by type of dietary fat fed to rats." International Journal of Obesity and related metabolic disorders: Journal of the International Association for the Study of Obesity **17**(4): 223-236.

Innis, S. M. (2007). "Fatty acids and early human development." Early Human Development **83**(12): 761-766.

Kaplan, R. J. and C. E. Greenwood (1998). "Dietary saturated fatty acids and brain function." Neurochemical Research **23**(5): 615-626.

Kennedy, G. C. and J. Mitra (1963). "Body weight and food intake as initiating factors for puberty in the rat." The Journal of Physiology **166**(2): 408-418.

Leaf, A., Y.-F. Xiao and J. X. Kang (2002). "Interactions of n-3 fatty acids with ion channels in excitable tissues." Prostaglandins, Leukotrienes and Essential Fatty Acids **67**(2-3): 113-120.

Lundqvist-Persson, C., G. Lau, P. Nordin, B. Strandvik and K.-G. Sabel (2010). "Early behaviour and development in breast-fed premature infants are influenced by omega-6 and omega-3 fatty acid status." Early Human Development **86**(7): 407-412.

Macfarlane, D. P., S. Forbes and B. R. Walker (2008). "Glucocorticoids and fatty acid metabolism in humans: fuelling fat redistribution in the metabolic syndrome." Journal of Endocrinology **197**(2): 189-204.

Machatschke, I. H., B. Bauer, L. M. Glenk, E. Millesi and B. Wallner (2011). "Spatial learning and memory differs between single and cohabitated guinea pigs." Physiology & Behavior **102**(3-4): 311-316.

Malinowska, K. W. and P. W. Nathanielsz (1974). "Plasma aldosterone, cortisol and corticosterone concentrations in the new-born guinea-pig." The Journal of Physiology **236**(1): 83-93.

McEwen, B. S. (2002). "Sex, stress and the hippocampus: allostasis, allostatic load and the aging process." Neurobiology of Aging **23**: 921-939.
McEwen, B. S. and J. C. Wingfield (2003). "The concept of allostasis in biology and biomedicine." Hormones and Behavior **43**(1): 2-15.
Mendonça-Furtado, O., M. Edaes, R. Palme, A. Rodrigues, J. Siqueira and P. Izar (2014). "Does hierarchy stability influence testosterone and cortisol levels of bearded capuchin monkeys (Sapajus libidinosus) adult males? A comparison between two wild groups." Behavioural Processes **109**: 79-88.
Mennitti, L. V., J. L. Oliveira, C. A. Morais, D. Estadella, L. M. Oyama, C. M. Oller do Nascimento and L. P. Pisani (2015). "Type of fatty acids in maternal diets during pregnancy and/or lactation and metabolic consequences of the offspring." The Journal of Nutritional Biochemistry **26**(2): 99-111.
Miachon, S., S. Augier, M. Jouvenet, P. Boucher and J. J. Vallon (2001). "Nutritional parameters modify muricidal behavior of male Wistar rats - Preventive effects of amino acids and 4' Cl diazepam." Life Sciences **69**: 2745-2757.
Moon, M. L., J. J. Joesting, M. A. Lawson, G. S. Chiu, N. A. Blevins, K. A. Kwakwa and G. G. Freund (2014). "The saturated fatty acid, palmitic acid, induces anxiety-like behavior in mice." Metabolism **63**(9): 1131-1140.
Möstl, E. and R. Palme (2002). "Hormones as indicators of stress." Domestic Animal Endocrinology **23**: 67-74.
Mozaffarian, D., M. B. Katan, A. Ascherio, M. J. Stampfer and W. C. Willett (2006). "Trans fatty acids and cardiovascular diseases." The New England Journal of Medicine **354**: 1601-1613.
Mutch, P. B. and L. S. Hurley (1973). "Effect of zinc deficiency during lactation on postnatal growth and development of rats." Journal of Nutrition **104**: 828-842.
Nemeth, M., E. Millesi, K.-H. Wagner and B. Wallner (2014). "Effects of diets high in unsaturated fatty acids on socially induced stress responses in guinea pigs." PLoS ONE **9**(12): e116292.
Nemeth, M., E. Millesi, K.-H. Wagner and B. Wallner (2015). "Sex-Specific Effects of Diets High in Unsaturated Fatty Acids on Spatial Learning and Memory in Guinea Pigs." PLoS ONE **10**(10): e0140485.
Neuringer, M., G. J. Anderson and W. E. Connor (1988). "The essentiality of n-3 fatty acids for the development and function of the retina and brain." Annual Review of Nutrition **8**: 517-541.
Palme, R. and E. Möstl (1997). "Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood." International Journal of Mammal Biology **62**(Suppl. II): 192-197.
Pan, D. A. and L. H. Storlien (1993). "Dietary lipid profile is a determinant of tissue phospholipid fatty acid composition and rate of weight gain in rats." The Journal of Nutrition **123**(3): 512-519.
Raygada, M., E. Cho and L. Hilakivi-Clarke (1998). "High maternal intake of polyunsaturated fatty acids during pregnancy in mice alters offsprings' aggressive behavior, immobility in the swim test, locomotor activity and brain protein kinase C activity." The Journal of Nutrition **128**(12): 2505-2511.
Re, S., M. Zanoletti and E. Emanuele (2007). "Aggressive dogs are characterized by low omega-3 polyunsaturated fatty acid status." Veterinary Research Communications **32**(3): 225-230.
Reeder, D. M. and K. M. Kramer (2005). "Stress in free-ranging mammals: integrating physiology, ecology, and natural history." Journal of Mammalogy **86**(2): 225-235.
Rood, J. P. (1972). "Ecological and Behavioural Comparisons of Three Genera of Argentine Cavies." Animal Behaviour Monographs **5**: 1-IN4.
Rose, R. M. and J. W. Holaday (1971). "Plasma testosterone, dominance rank and aggressive behaviour in male rhesus monkeys." Nature **231**: 366-368.
Russo, G. L. (2009). "Dietary n−6 and n−3 polyunsaturated fatty acids: From biochemistry to clinical implications in cardiovascular prevention." Biochemical Pharmacology **77**(6): 937-946.
Sachser, N., M. Dürschlag and D. Hirzel (1998). "Social relationships and management of stress." Psychoneuroendocrinology **23**(8): 891-904.

Sachser, N. and E. Pröve (1984). "Short-term effects of residence on the testosterone responses to fighting in alpha male guinea pigs." Aggressive Behavior **10**(4): 285-292.
Schöpper, H., R. Palme, T. Ruf and S. Huber (2011). "Chronic stress in pregnant guinea pigs (Cavia aperea f. porcellus) attenuates long-term stress hormone levels and body weight gain, but not reproductive output." Journal of Comparative Physiology B **181**(8): 1089-1100.
Schöpper, H., R. Palme, T. Ruf and S. Huber (2012). "Effects of prenatal stress on hypothalamic–pituitary–adrenal (HPA) axis function over two generations of guinea pigs (Cavia aperea f. porcellus)." General and Comparative Endocrinology **176**(1): 18-27.
Schuchardt, J. P., M. Huss, M. Stauss-Grabo and A. Hahn (2010). "Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and behaviour of children." European Journal of Pediatrics **169**(2): 149-164.
Seckl, J. R. (2004). "Prenatal glucocorticoids and long-term programming." European Journal of Endocrinology **151**: U49-U62.
Seo, D., C. J. Patrick and P. J. Kennealy (2008). "Role of serotonin and dopamine system interactions in the neurobiology of impulsive aggression and its comorbidity with other clinical disorders." Aggression and Violent Behavior **13**(5): 383-395.
Simopoulos, A. P. (2002). "The importance of the ratio of omega-6/omega-3 essential fatty acids." Biomedicine & Pharmacotherapy **56**: 365-379.
Singh, M., M. Singh, A. K. Sharma and B. A. Krishna (2003). "Methodological considerations in measurement of dominance in primates." Current Science **84**(5): 709-713.
Solfrizzi, V., A. Dintrono, A. Colacicco, C. Capurso, A. Delparigi, S. Capurso, A. Gadaleta, A. Capurso and F. Panza (2005). "Dietary fatty acids intake: possible role in cognitive decline and dementia." Experimental Gerontology **40**(4): 257-270.
Su, W. and P. J. Jones (1993). "Dietary fatty acid composition influences energy accretion in rats." The Journal of Nutrition **123**(12): 2109-2114.
Trillmich, F., C. Laurien-Kehnen, A. Adrian and S. Linke (2006). "Age at maturity in cavies and guinea-pigs (Cavia aperea and Cavia aperea f. porcellus): influence of social factors." Journal of Zoology **268**(3): 285-294.
Tu, W. C., B. S. Mühlhäusler, L. N. Yelland and R. A. Gibson (2013). "Correlations between blood and tissue omega-3 LCPUFA status following dietary ALA intervention in rats." Prostaglandins, Leukotrienes and Essential Fatty Acids **88**(1): 53-60.
Volek, J. S., W. J. Kraemer, J. A. Bush, T. Incledon and M. Boetes (1997). "Testosterone and cortisol in relationship to dietary nutrients and resistance exercise." Journal of Applied Physiology **82**(1): 49-54.
Wagner, K.-H., E. Auer and I. Elmadfa (2000). "Content of trans fatty acids in margarines, plant oils, fried products and chocolate spreads in Austria." European Food Research and Technology **210**: 237-241.
Wainwright, P. E. (2007). "Dietary essential fatty acids and brain function: a developmental perspective on mechanisms." Proceedings of the Nutrition Society **61**(01): 61-69.
Wallner, B. and J. Dittami (2003). "Behavioural and physiological consequences of home advantage resource holding in male guinea pigs." Acta ethologica **5**(2): 101-105.
Weiler, H. A., J. Zhao, L. Park, J. R. Kohut, L. L. Burr and S. C. Fitzpatrick-Wong (2012). "Maternal supplementation with dietary arachidonic and docosahexaenoic acids during lactation elevates bone mass in weanling rat and guinea pig offspring even if born small sized." Prostaglandins, Leukotrienes and Essential Fatty Acids **86**(1-2): 61-70.
Whitcomb, R. W., W. M. Linehan and R. A. Knazek (1988). "Effects of long-chain, saturated fatty acids on membrane microviscosity and adrenocorticotropin responsiveness of human adrenocortical cells in vitro." Journal of Clinical Investigation **81**: 185-188.
Yehuda, S. (2003). Omega-6/omega-3 ratio and brain-related functions. Omega-6/Omega-3 Essential Fatty Acid Ratio: The Scientific Evidence. A. P. Simopoulos and L. G. Cleland. Basel, Karger. **92:** 37-56.

Yehuda, S., S. Rabinovitz, R. Lcarasso and D. Imostofsky (2002). "The role of polyunsaturated fatty acids in restoring the aging neuronal membrane." Neurobiology of Aging **23**(5): 843-853.
Yin, Z., S. Agellon, P. Lavery and H. Weiler (2014). "Dietary supplementation with long chain polyunsaturated fatty acids in pregnant guinea pigs has sex-dependent effects on growth and bone outcomes in offspring." Prostaglandins, Leukotrienes and Essential Fatty Acids **91**(1-2): 31-38.
Young, W. C. (1969). "Psychobiology of sexual behavior in the guinea pig." Advances in the Study of Behavior **2**: 1-110.

ANHANG

Abstract

Fatty acids are essential components of cell membranes' phospholipids in general and in particular participate directly in the modification of neuronal membranes. During gestation and lactation the composition of the mother's diet especially in fatty acids has essential impact on the healthy development of an offspring. Dietary polyunsaturated fatty acids (PUFAs), like n-3 or n-6 fatty acids, were associated with positive effects on neurodevelopment, social behavior and the physiological stress response. Whereas, an elevated intake of saturated fatty acids (SFAs) was associated with negative consequences on health like an increased risk for cardiovascular diseases and impaired cognitive performances. Aim of the present investigation was to directly compare the impact of pre- and postnatal fatty acid supplementation either with UFAs or SFAs on the physiological and behavioral development of juvenile domestic guinea pigs. In the recent study 41 male domestic guinea pigs from a F1 generation were used. Their dams had been assigned to three experimental groups throughout gestation with different diets: one group was supplemented with walnut oil (high in UFAs), one with coconut oil (high in SFAs). The third group remained on a control diet. After parturition male offspring remained on the same diets their dams had received. After weaning video recordings of the three same-sexed groups were made 4 times in an interval of 30 days in order to investigate daily social interactions within the three experimental groups. Immediately after video recordings saliva samples were taken and body weight was measured to monitor the development of cortisol secretion and weight gain. Animals supplemented with UFAs exhibited a significant accelerated gain in body weight compared to control group, as well as highest frequencies of socio-positive behavior over the whole observation period compared to both control and SFA animals. Increasing locomotion activity and rising agonistic behavior with age were observed in animals with SFA supplementation. They expressed the highest frequencies of agonistic behavior in general as well. Additionally, strongly increasing cortisol concentrations with age were found in SFA group. In conclusion, beneficial effects on development and individual fitness are assumed due to pre- and postnatal UFA supplementation. It is supposed that UFAs promote the successful forming of social bonds due to improved neuronal cell functions and decrease aggressiveness. These results indicate the necessity of an adequate UFA intake during important ontogenetic periods of neurodevelopment. Whereas on the contrary, high ingested amounts of SFAs may lead to increased aggressiveness caused by impaired neuronal cell functioning resulting in an enhanced stress load. Thus, permanently increased cortisol levels comprise harmful effects on health and survival of the individuals with aging.

Deutsche Zusammenfassung

Fettsäuren sind essentielle Bestandteile der Phospholipide einer jeden Zellmembran und im Besonderen an der Modifikation neuronaler Membrane beteiligt. Während Schwangerschaft und Laktation hat die Zusammensetzung der mütterlichen Ernährung, vor allem in deren Fettsäuregehalt, grundlegenden Einfluss auf die gesunde Entwicklung des Nachwuchses. Mit der Nahrung aufgenommene ungesättigte Fettsäuren (UFS), wie n-3 oder n-6 Fettsäuren, wurden mit positiven Effekten auf die neuronale Entwicklung, das Sozialverhalten und die physiologische Stressreaktion in Verbindung gebracht. Während hingegen eine erhöhte Aufnahme von gesättigten Fettsäuren (GFS) mit negativen Folgen für die Gesundheit, wie ein erhöhtes Risiko für Herz-Kreislauf-Erkrankungen und beeinträchtigter kognitiver Leistung assoziiert wurde. Ziel der gegenwärtigen Untersuchung war der direkte Vergleich von prä- und postnataler Fettsäureergänzung mittels gesättigter oder ungesättigter Fettsäuren auf die physiologische Entwicklung und das Verhalten von juvenilen Hausmeerschweinchen. Für die Studie wurden 41 männliche Hausmeerschweinchen einer F1 Generation herangezogen. Deren Muttertiere waren während der Schwangerschaft drei experimentellen Gruppen zugeordnet worden, welche verschiedene Nahrungsergänzungen erhielten: einer Gruppe wurde ergänzend Walnussöl verabreicht (reich an UFS), einer anderen Kokosnussöl (reich an GFS). Die dritte Gruppe wurde mit einer Kontrolldiät gefüttert. Nach der Geburt erhielten die männlichen Nachkommen die gleiche Nahrung wie deren Muttertiere zuvor. Nach Entwöhnung von den Müttern wurden Videoaufnahmen, insgesamt 4-mal im Abstand von 30 Tagen, von den drei gleichgeschlechtlichen Gruppen durchgeführt, um die täglichen sozialen Interaktionen innerhalb der Gruppen aufzuzeichnen. Unmittelbar nach den Videoaufnahmen wurden Speichelproben entnommen, sowie das Körpergewicht der Tiere gemessen, um Entwicklung der Cortisolausschüttung und Gewichtszunahme zu überwachen. Jene Tiere, die eine mit UFS angereicherte Nahrung erhalten hatten zeigten eine signifikant beschleunigte Zunahme an Körpergewicht im Vergleich zur Kontrollgruppe, sowie das meiste sozio-positive Verhalten über den gesamten Zeitraum verglichen mit Kontroll- und GFS Tieren. Steigende Bewegungsaktivität und zunehmendes agonistisches Verhalten mit dem Alter wurde bei jenen Tieren mit einer GFS Ergänzung beobachtet. Ebenso zeigten diese das meiste agonistische Verhalten über den gesamten Beobachtungszeitraum. Zusätzlich wurden in der GFS Gruppe stark ansteigende Cortisolkonzentrationen mit dem Alter gemessen. Folgernd wird angenommen, dass die prä- und postnatale Ergänzung mittels UFS Vorteile für Entwicklung und individuelle Fitness mit sich bringt. Es wird vermutet, dass UFS aufgrund verbesserter neuronaler Zellfunktionen den Aufbau von sozialen Bindungen begünstigen und Aggressivität vermindern. Die Ergebnisse weisen auf die Notwendigkeit einer adäquaten UFS Aufnahme während wichtiger ontogenetischer Phasen der neuronalen Entwicklung hin. Wohingegen die gesteigerte Aufnahme an GFS womöglich in erhöhter Aggressivität resultiert, hervorgerufen durch beeinträchtigte neuronale Zellfunktionen, was wiederrum zu einer gesteigerten Stressbelastung führt. Folglich permanent erhöhte Cortisolwerte können schädliche Effekte auf Gesundheit und Überleben der Individuen mit fortschreitendem Alter haben.