

1 **The impact of cafeteria diet feeding on physiology and anxiety-related**
2 **behaviour in male and female Sprague-Dawley rats of different ages**

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Abstract

There is emerging experimental evidence that hyper-energetic diets not only cause obesity but also impact on behaviour in rodents. A hyper-energetic comfort diet/cafeteria diet (CD) fed during early development programmes anxiety-related behaviour in adult age, but little is known how an obesogenic CD impacts on behaviour when fed at a later age.

To this end we fed CD to Sprague-Dawley rats of both sexes at either 6 weeks or 12 months old, for a period of 6 weeks. Anxiety-related behaviour was assessed in the elevated plus maze (EPM) and the open field (OF). A glucose tolerance test was performed and metabolic indices, body weight and fat were measured.

CD-fed young adult females, but not males, had a higher energy intake, due to an overconsumption of carbohydrates and fats. Only in adult CD-fed rats of both sexes did this overconsumption led to increased weight gain. Protein intake was reduced in all CD groups. Fat mass (subcutaneous, perirenal, gonadal) increased in most CD groups, whereas brown fat increased only in adults. Triacylglycerol, free fatty acid and total cholesterol concentrations increased predominantly in adult CD-fed rats. Glucose tolerance was only impaired in adult males.

CD-fed adult males showed fewer entries into the aversive open arms and groomed more on the EPM, whereas adult females spent more time on these arms. In the OF, CD-fed females of both ages visited the inner zone more frequently and travelled a longer distance. The behavioural data suggests anxiolysis in CD-fed females and signs of increased anxiety in adult males. In conclusion, this study demonstrates that feeding CD leads to both obesity and behavioural changes in rats. Overall, these effects were more pronounced in older rats, with the behavioural effects being particularly gender dependent.

Key words:

Elevated plus maze; Open field; obesity; glucose tolerance; fat tissue; blood lipids

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1. Introduction

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One of the experimental diets that mimic highly palatable and hyperenergetic Western diets is the cafeteria or supermarket diet (Rothwell and Stock, 1979a, Sclafani and Springer, 1976). Cafeteria diets (CD) consist of multiple highly palatable human food items that are offered to rodents in addition to normal chow. Feeding animals a CD, pioneered in the 1970's and eighties by Rothwell and Stock (1988), has been one of many approaches used to study the development of obesity in laboratory rodents. It has recently been shown that feeding a cafeteria diet during early developmental stages not only promotes obesity and metabolic disturbances in later life (Akyol *et al.* , 2009, Akyol *et al.* , 2012, Bayol *et al.* , 2008), but also impacts on adult behaviour, in particular when fed during lactation. Most notably, rats exposed to lactational CD show less signs of anxiety upon exposure to the elevated plus maze (EPM), a rodent model of anxiety (Pellow *et al.* , 1985). Similar anxiolytic effects were observed when explorative behaviour and anxiety-related behaviour were investigated in the open field (Wright *et al.* , 2011a). Using a model of early postnatal overfeeding through litter size reduction, offspring showed also less anxiety upon EPM exposure (Spencer and Tilbrook, 2009).

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Apparently opposite dietary effects have been observed when rats were exposed to the hyperenergetic diets diet post weaning or in adult age. In male Wistar weanlings, one week of feeding a highly palatable, carbohydrate enriched, diet following weaning had anxiolytic effects in the open field and the EPM (Marcolin Mde *et al.* , 2012). By contrast, 6 months old male Wistar rats, previously exposed to a highly palatable sucrose enriched diet for 4 months, developed an obese phenotype, but showed increased anxiety in a light-dark exploration task (Souza *et al.* , 2007). Feeding male Fischer 344 rats a high fat diet in adulthood changed hole board exploration in a way that could be interpreted as increased anxiety (Buchenauer *et al.* , 2009). Conversely, one week feeding a high fat diet decreased a parameter of anxiety in male adult Sprague Dawley rats upon exposure to a modified version of the EPM (Prasad and Prasad, 1996).

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Although many of these studies demonstrate anxiolytic effects in response to feeding of an obesogenic diet, some revealed an anxiogenic effect of the diet. There are numerous variables that could account for the observed differences, being of both nutritional and behavioural nature. For example, most of the reported studies here

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1 used a high fat diet. High fat diets, although obesogenic, are not good proxies for the
2 Western diet consumed by humans. Feeding a cafeteria represents a sensible
3 approach to study the obesogenic (Sampey *et al.* , 2011) and behavioural effects of
4 overfeeding. In addition, both gender and age impact on emotional behaviour, but
5 also on dietary effects (Ferguson and Gray, 2005, Johnston and File, 1991, Krolow *et*
6 *al.* , 2010, Liang *et al.* , 2007, Lynn and Brown, 2010). These variables could possibly
7 contribute to the ambiguity of some results.

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10 The present study aimed at investigating the impact of feeding a cafeteria diet on
11 body weight, metabolic indices and anxiety-related behaviour considering both
12 gender and age of the subjects. To evaluate the impact of cafeteria feeding upon
13 behaviour in rodents, we started to expose Sprague-Dawley rats of both sexes either
14 at the age of 6 weeks or at an age of 12 months to a cafeteria diet. We hypothesised
15 that dietary effects on metabolism and behaviour should occur in the older rather
16 than in the younger rats.

17 18 **2. Methods**

19 20 **2.1 Animals**

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22 The investigation utilized 48 male and 48 female Sprague–Dawley rats (Shoe:SPRD,
23 Tierzucht Schönwalde GmbH, Germany) of two different ages. At the beginning of
24 the study rats were either 6 weeks or 12 months old. Rats were housed in groups of
25 4 under standard laboratory conditions (12 -h light/dark cycle, lights on at 0600 h,
26 room temperature 22 °C). All animals had ad libitum access to standard chow (ssniff
27 R/M-H, Soest, Germany) and water. Chow contained 41.2 % carbohydrates, 3.3% fat
28 and 19% protein. The study was performed according to the guidelines of the
29 German Animal Protection Law and the experimental protocol was approved by the
30 local animal protection board (LAGESO Berlin).

31 32 **2.2 Experimental design**

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34 Male and female rats of both ages (young adult = 6 weeks, adult = 12 months) were
35 randomly allocated to be fed either control chow alone (control) or chow alongside a
36 random selection of highly energetic and palatable human foods (cafeteria diet, CD).
37 Behavioural testing started after 4 weeks of feeding.

2.3 Feeding experiments and body weight

The cafeteria diet consisted of 7 palatable human food items with high energy content (cake, cookies, chocolate, raisin bread, cooked noodles, sausage and cheese). On average, total CD intake included $37\pm 10\%$ carbohydrates, $21\pm 5\%$ fat and $9\pm 3\%$ protein (wet weight). Each day, rats were offered a variety of four items with one item being replaced daily to maintain novelty. Items were presented in a bowl on the cage floor in excess quantities. CD presentation occurred immediately before the beginning of the dark period. During the initial four weeks of the study, food consumption and body weight were documented daily. Energy intake (kJ) and macronutrient consumption (carbohydrates including sugar, fat, and protein) were calculated from the manufacturers' data. Weight loss due to evaporation was measured in triplicate samples of each individual food item placed in empty cages. The average daily percentage change in the weight of foods ranged from 0 to 6.2 % and corresponded to an average overestimation of energy intake by 2.51 % (7.5 kJ/d), which can be considered within an acceptable error of measurement (Akyol *et al.*, 2009).

CD feeding was continued during behavioural testing to avoid any withdrawal effects that might have occurred following discontinuation (Cottone *et al.*, 2009, South *et al.*, 2012). However, to avoid any interference with behavioural testing, monitoring of food intake ceased and body weight was monitored only on a weekly basis.

2.4 Oral glucose tolerance test (OGTT)

Oral glucose tolerance test was performed after 8 weeks of feeding the CD following the behavioural experiments. After an overnight food deprivation of 16 h, a 50 % glucose solution at a dose of 2g/kg body weight was administered orally. Blood samples were obtained from the superficial tail vein of conscious animals at baseline and 10, 30, 60 and 120 min after the glucose load. Plasma glucose was assayed based on the glucose oxidase method (Trinder, 1969). Area under the curve (AUC) was determined for quantification of the OGTT.

2.5 Fat tissue and metabolic indices

At the end of the study, perirenal, subcutaneous (from both sides of the abdominal region), gonadal and interscapular brown fat tissues were dissected and weighed.

1 Blood samples were collected and centrifuged for 15 min at 4000g. The plasma was
2 frozen at -80°C until assayed. All parameters were assayed using commercially
3 available kits. Free fatty acids (Wako NEFA C Kit; Wako Chemicals, Neuss,
4 Germany), total cholesterol (Wako Free Cholesterol C Kit, Wako Chemicals, Neuss,
5 Germany) and triacylglycerol (triglyceride and free glycerol reagent, Sigma-Aldrich,
6 Munich, Germany) were analyzed in triplicate on 96-well plates.
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8 2.6 Behaviour

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10 Following 4 weeks of feeding the diet, animals were subjected to the elevated plus
11 maze (EPM) and a week later to the open field (OF). All experiments took place in a
12 sound-attenuated chamber (180 cm x 180 cm x 230 cm) between 0900 and 1200 h.
13 The chamber illumination provided 150 lx. Animals were habituated to the
14 experimental condition for one hour before starting the behavioural test. The
15 behaviour of each rat was recorded and analysed using a computer-based tracking
16 system (VideoMot 2, TSE, Germany). Testing arenas were cleaned with disinfectant
17 between each animal.
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19 Elevated plus-maze

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21 The EPM (Montgomery, 1958, Voigt *et al.*, 2005) consisted of two open arms (50 x
22 15cm), two enclosed arms (50 x 15 x 10cm) and a central platform (15 x 15cm). The
23 apparatus was raised to a height of 60 cm by a single central support. All animals
24 were exposed for 5 min to the EPM. Rats were initially placed on the centre platform
25 facing a corner, allowing equal choice of entering an open or a closed arm. The time
26 spent on the open and closed arms, the entries into the arms and the total distance
27 travelled were monitored. In addition, rearing and time spent self-grooming were
28 analysed and the ratio of open arm entries to total arm entries was calculated.
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30 Open field

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32 Rats were exposed for 5 min to an OF (Hall, 1934, Voigt *et al.*, 2005) sized 1 m² with
33 black floor and walls of 50 cm in height, virtually divided into an outer zone and an
34 inner zone (50 cm x 50 cm). Animals were placed initially into the centre (inner zone)
35 of the OF. Data were analysed for the parameters of distance travelled, latency to
36 enter the inner zone, number of entrances into the inner zone, time spent in the inner
37 zone, rearing and time spent self-grooming.
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2.7 Statistics

Significance was accepted at the $P < 0.05$ level. Data are expressed as means \pm SEM. As rats were kept in groups of four throughout the study, mean food intake of the group was used for any analysis of food consumption (energy and macronutrient intake). The corresponding results are presented as mean/d of the total consumption over the analysed period. The underlying data were analysed separately for each group. To determine an overall effect of diet, a two-way RM ANOVA (time, diet) was applied. No post hoc tests were performed. A two-way RM ANOVA (time, diet) followed by Bonferonnis's Multiple Comparison-test was applied to body weight data and the OGTT. Metabolic indices, fat mass and all behavioural data were analysed using a three-way ANOVA (diet, gender, age), with post-hoc Holm Sidak comparisons.

3. Results

3.1 Food intake and body weight

With the exception of young adult males, overall energy intake was higher in CD-fed groups (young adult females: $P < 0.05$; adult males and females: $P < 0.001$ (Table 1).

Fat intake was increased in young adult males ($P < 0.001$), females ($P < 0.01$) and in adult rats of both sexes ($P < 0.001$). Carbohydrate intake was also increased in all groups (young adult males: $P < 0.05$; young adult females: $P < 0.01$; adult rats of both sexes: $P < 0.001$). In particular sucrose intake was increased in all CD-fed groups ($P < 0.001$). Whereas chow fed controls consumed 7.5 % of their carbohydrates from sucrose, CD-fed young adult males consumed 23%, females 20%, adult males 32% and females 40% of their carbohydrates from sucrose. CD-fed animals consumed between 2.9- and 5.7-fold more fat than chow fed controls. In contrast to fat and carbohydrate intake, protein intake was reduced in all CD groups ($P < 0.001$) (Table 1).

Although energy intake was higher in young adult CD-fed females ($P < 0.01$), this did not translate into accelerated body weight development as measured over 6 weeks. Also, body weight was not different in young adult CD-fed males compared to

1 controls (Fig. 1 a,b). In contrast to young adult rats, there was a significant effect of
2 diet ($P < 0.01$) in both adult males and females with a significant interaction of diet and
3 time ($P < 0.01$) demonstrating an increasing body weight in the CD groups over time.
4 Whilst body weights of adult controls were stable across the experimental period,
5 CD-fed rats gained approximately 35 g (females) to 61 g (males), with significant gain
6 noted within 1-2 weeks (Fig.1 c,d).

3.2 Oral glucose tolerance test

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11 Fasting glucose concentrations (baseline blood sample in the glucose tolerance test)
12 were not significantly different between groups. However, as indicated by the AUC,
13 there were both effects of age ($P < 0.05$) and diet ($P < 0.001$), but not of gender. In
14 adult males, but not young adult males the AUC for the glucose tolerance curve was
15 significantly greater in males ($P < 0.01$) but not females ($P < 0.1$) fed CD (Figs 2c and
16 d). This appeared to be associated with an earlier and greater peak glucose
17 concentration and a failure to fully clear the glucose load within 120 minutes. Young
18 adult CD- fed females also exhibited a significantly higher peak glucose
19 concentration (Fig 2b) and this was delayed compared to controls. In contrast the
20 CD-fed young adult males had an early peak glucose, which was significantly greater
21 than that seen in control males (Fig 2a).

3.3 Fat

26 Perirenal fat was increased in all CD-fed groups ($P < 0.001$). This dietary effect was
27 gender dependent with smaller effects in females than in males ($P < 0.001$). Post-hoc
28 analysis revealed significant increases in perirenal fat mass in CD-fed young adult
29 males and females ($P < 0.001$), adult males ($P < 0.001$) and females ($P < 0.05$) (Table
30 2).

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32 In both CD-fed young adult males ($P < 0.001$) and females ($P < 0.01$) gonadal fat mass
33 was increased ($P < 0.001$) A significant increase of gonadal fat has been observed in
34 adult males ($P < 0.001$) but not in females where the effect of CD-feeding approached
35 significance ($P < 0.10$) (Table 2).

1 Subcutaneous fat was increased in CD-fed rats ($P<0.001$). Similar to perirenal fat, a
2 significant interaction between gender and diet occurred. The impact of diet was
3 smaller in females as compared to males ($P<0.001$). Subcutaneous fat was
4 increased in all CD groups ($P<0.001$) with the exception of young adult females
5 (Table 2).
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10 CD-fed rats had overall larger brown adipose tissue depots ($P<0.001$), but this effect
11 was not significant in young adult rats. Both CD-fed adult males and females had a
12 higher brown adipose tissue mass ($P<0.05$) (Table 2).
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16 CD feeding led to an overall increase in cholesterol ($P<0.001$), triacylglycerol
17 ($P<0.01$) and free fatty acid ($P<0.05$) concentrations. The dietary effect on free fatty
18 acids and triacylglycerol was age dependent ($P<0.01$), as triacylglycerol ($P<0.01$)
19 and free fatty acids (males: $P<0.01$, females: $P<0.05$) only increased in adult rats of
20 either sex. The increased cholesterol concentration as observed in young CD-fed
21 males ($P<0.001$) was absent in adult males. In contrast, cholesterol concentration
22 were increased in adult CD-fed females ($P<0.05$) (Table 3).
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31 3.4 Behaviour

32 3.4.1 Elevated Plus-Maze (EPM)

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35 Both the total distance travelled on the EPM and rearing were not affected by any of
36 the factors age, gender or diet. However, the anxiety-related parameters entries into
37 open arms and time spent on open arms were both increased with age and gender,
38 indicating anxiolysis. Adult rats showed a more anxiolytic profile compared to young
39 adult rats as they spent more time on the open arms ($P<0.001$) and made more
40 entries into the open arms ($P<0.001$). A similar anxiolytic profile was seen for
41 females when compared to males (entries: $P<0.05$, time: $P<0.01$).
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51 There was no overall effect of diet on EPM behaviour, although there was an effect of
52 diet via interactions with age and gender (entries: $P<0.01$; time: $P<0.05$). CD- fed
53 adult males made fewer entries into the aversive open arms (Fig. 3c). This was
54 paralleled by increased grooming in this group ($P<0.001$) (Tab. 5).
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58 CD-fed adult females spent more time on the aversive open arms ($P<0.05$). They
59 showed also tendencies towards increased entries into these arms and towards
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1 reduced grooming ($P < 0.10$) (Fig. 3, Tab. 5). Rearing and distance travelled remained
2 unchanged by diet (Tab. 4).

3.4.2 Open Field (OF)

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8 Overall, adult rats travelled a shorter distance than young adult rats ($P < 0.001$) and
9 females less than males ($P < 0.001$). The latency to enter the more aversive inner
10 zone was only affected by age ($P < 0.001$) with adult rats showing an increased
11 latency as compared to young adult rats. Adult rats made also significantly fewer
12 entries into the inner zone and spent less time there ($P < 0.001$). Overall adult rats
13 also groomed more ($P < 0.01$).

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18 In contrast to the EPM, diet had a direct significant effect on the distance travelled in
19 the open field with CD-fed rats travelling a longer distance compared to chow fed
20 controls ($P < 0.01$). Post hoc analysis revealed that both young adult and adult CD-
21 fed females but not males travelled a longer distance in the OF ($P < 0.05$) (Tab. 4).
22 Although diet had no overall effect on the latency to enter the inner zone in this
23 group, adult CD-fed females showed a reduced latency that approached significance
24 ($P < 0.10$) (data not shown). CD-fed female rats of both age groups made more
25 entries into the inner zone ($P < 0.05$), although this was not accompanied by spending
26 more time in the inner zone (Fig. 4).

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4. Discussion

Young adult and adult rats of both sexes had a very similar profile of macronutrient intake. This was characterised by higher carbohydrate and fat intake, the latter being in line with previous studies (Harris, 1993, Prats *et al.*, 1989). The reduced protein intake in the present study is in keeping with previous data (Akyol *et al.*, 2009) but would contradict other findings where protein intake was not affected by cafeteria feeding (Esteve *et al.*, 1994, Llado *et al.*, 1995). Although there is evidence for a control of protein intake (Berthoud *et al.*, 2012), a specific appetite for protein has been questioned (Galef, 2000). In the present study, rats could choose between various cafeteria food items and a simultaneously provided standard chow as provided also to the control group. The cafeteria foods ranged in protein content from

1 approximately 5g/100g to 23g/100g, with the majority below the protein requirements
2 of adult rats (9g/100g). However, the provision of chow alongside the cafeteria foods
3 ensured that sufficient protein was available to meet demand. Although this study did
4 not include preference testing, one could speculate that a preference of highly
5 palatable but low protein items could contribute to the observed low protein intake in
6 the CD-fed group. Other studies (Martire *et al.* , 2013, Prats *et al.*, 1989) show even
7 an increased protein consumption in CD-fed rats, possibly due on a to hyperphagia
8 induced by a large variety of food items presented.

9 This would be supported another study (Shafat *et al.* , 2009), where the mean protein
10 content of cafeteria items was 9.5 % compared to 9% in the present study. As Shafat
11 et al. provided a selection of 36 items, compared to 7 in the present study, novelty of
12 food relating to hypophagia could play a role in compensating for the low protein
13 content in some cafeteria items.

14 Although cafeteria diets are not strictly defined, the high sugar and fat intake as
15 observed in the present cafeteria groups is a characteristic of so called “comfort
16 foods” (Dallman *et al.* , 2005) so that this terminology could also be appropriately
17 applied to the present diet.

18
19 CD/comfort food increased energy intake in young adult females but not males.
20 Nevertheless perirenal fat increased in both groups indicating a pre-obese state
21 (Despres, 2006). Increased visceral (perirenal) fat would be detrimental to health with
22 increasing age (Huffman and Barzilai, 2009). With the only exception of total
23 cholesterol concentrations in young adult males, no other metabolic index was
24 changed by the diet.

25
26 This relatively small impact of the CD/comfort food protocol in young adult rats, and
27 the lack of effect on body weight are consistent with similar studies that have
28 considered susceptibility to obesity in rats of different ages. There are well-defined
29 compensatory mechanisms which buffer some of the dietary effects in younger rats,
30 of which the induction of thermogenesis appears to be particularly important
31 (Rothwell and Stock, 1979b, 1980). Hyperenergetic diets can also reduce the
32 expression of hypothalamic orexigenic signalling in juvenile Sprague-Dawley rats to
33 counter the obesogenic effects of the diet (Archer *et al.* , 2004). CD is more likely to
34 increase body weight in adult rats (Sclafani and Gorman, 1977) and such an age
35 dependency of the nutritional phenotype has been confirmed in the present study.
36 This observation is in-keeping with an inverse relationship between diet induced
37 thermogenesis and obesity (Rothwell and Stock, 1988, Tulp *et al.* , 1982). In the adult

1 group, the capacity for a compensatory diet-induced thermogenesis should be
2 attenuated (Rothwell and Stock, 1983). Feeding weanling rats a cafeteria diet for 30
3 days did not result in significant body weight gain when compared to chow fed
4 controls (Rothwell and Stock, 1982a). Feeding cafeteria diet to lean (+/?) Zucker rats
5 did not result in body weight gain in young but older rats (Rothwell and Stock, 1982b)
6 which would be further in keeping with the present findings. In younger rats, cafeteria
7 induced weight gain has been observed after prolonged periods of feeding (De
8 Schepper *et al.* , 1997). In a more recent study (Martire *et al.*, 2013), CD-fed rats
9 were significantly heavier by week four of feeding, but energy intake compared to
10 chow fed rats was higher than in the present study.

11 The observed increase in energy intake led to significant weight gain in adults on
12 comfort food. This went along with impaired glucose tolerance in CD-fed adult males,
13 but less so in females. In adult rats, all fat depots were increased, including brown fat
14 tissue. The latter confirms earlier reports demonstrating a stimulatory effect of
15 cafeteria diet on brown adipose tissue growth (Himms-Hagen *et al.* , 1981, Rothwell
16 and Stock, 1982a) . Altogether the changes in body weight, fat tissue and metabolic
17 indices suggest a stronger metabolic and obesogenic effect of the diet in adult rats.

18
19 The main focus of the present study was on the evaluation of whether well-
20 established obesogenic effects of comfort foods translate into behavioural changes. If
21 so, the overall more severe impact of diet on metabolism in adult rats should lead to
22 a higher incidence of behavioural effects in that group. Indeed, and in line with a
23 previous study (Liang *et al.* , 2008), we noted an overall anxiolytic effect of the diet in
24 adult females, seen both during EPM and OF exposure. However, a more
25 differentiated pattern emerged upon closer inspection. Plus maze behaviour was
26 influenced by both gender and age, and the effect of diet was mediated via an
27 interaction with these factors. In fact, comfort food only had a behavioural effect in
28 adult rats of both sexes and was less influential in young adult females. It is unlikely
29 that the lack of behavioural effects in young adult males was due to the length of the
30 feeding period as behavioural effects have been shown after a feeding period as
31 short as one week in the past (Prasad and Prasad, 1996).

32
33 In adult rats, the dietary effects on behaviour were different between males and
34 females. The only effects in adult males were increased grooming and less entries
35 into the aversive open arms upon exposure to the test. If anything this would suggest
36 a potential anxiogenic effect in males. The anxiolytic effect in females was expressed
37 by the increased time spent on the aversive open arms (Pellow *et al.*, 1985). In

1 addition, the decreased grooming in females would be in line with an anxiolytic profile
2 in adult females. Grooming is a self-directed behaviour. When shown upon exposure
3 to aversive situations, as for example the plus maze or the open field, grooming can
4 be interpreted as a de-arousing activity. Anxious rats groom more often and
5 anxiolytic drugs reduce grooming behaviour (Dunn *et al.* , 1981, Voigt *et al.*, 2005).
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10 Rats on comfort food travelled a longer distance in the open field, indicating that
11 increased body weight did not translate into reduced locomotor activity. Under very
12 similar experimental conditions we have previously demonstrated an inverse
13 relationship between anxiety and the distance travelled in the open field (Voigt *et al.*,
14 2005). This would suggest that the increased distance travelled in females on
15 comfort food could be a sign of anxiolysis. Further support for the interpretation that
16 the effect on distance is not just an unspecific locomotor effect comes from the
17 observation that females on comfort food visited the aversive inner zone of the open
18 field more frequently. The tendency to visit the inner zone sooner would also support
19 this interpretation.
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28 The data clearly demonstrated an impact of diet on behaviour, although the anxiolytic
29 effect occurred only in females. This could be due to lower baseline anxiety in
30 females (Johnston and File, 1991, Lynn and Brown, 2009, 2010), rendering females
31 more prone to anxiolytic effects of diet. This interpretation would also be in-keeping
32 with the present observation that the dietary effects were more pronounced in the
33 adult group since adult rats had an overall more anxiolytic EPM profile than young
34 adult rats as shown previously (Lynn and Brown, 2009), although not consistently
35 across studies (Chepulis *et al.* , 2009, Ferguson and Gray, 2005).
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44 However, this would not explain why adult male rats showed some indication of an
45 anxiogenic dietary effect, opposite to the one observed in females in the present
46 study. Such an anxiogenic effect, however, would be in line with previous reports
47 showing an anxiogenic effect of highly palatable, carbohydrate enriched diets in
48 males (Buchenauer *et al.*, 2009, Souza *et al.*, 2007). Although these studies did not
49 investigate females, one could speculate that the anxiogenic effect in males could be
50 attributed to the enriched carbohydrate component in the diet. Rats of both age
51 groups had a higher carbohydrate intake, but anxiogenic effects were only seen in
52 older males. This was the only group with an indication of significantly impaired
53 glucose metabolism. Interestingly, both human and rodent studies provide evidence
54 for increased anxiety in hyperglycaemic states (Rajashree *et al.* , 2011, Ramanathan
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1 *et al.* , 2000, Sommerfield *et al.* , 2004), although findings in humans are
2 contradictory (Bouwman *et al.* , 2010). Males had also an increased fat intake in the
3 present study and a high fat diet would possibly cause anxiolysis in Sprague-Dawley
4 males (Prasad and Prasad, 1996). Prasad and Prasad (1996) did not find
5 behavioural effects of carbohydrates. Therefore, dietary fat could be a candidate for
6 the observed behavioural effects in the present study, but despite an increased
7 intake, no behavioural change occurred in young adult males. In adults, however, an
8 increased fat intake went along with increased anxiety in males and anxiolysis in
9 females. Feeding low protein diet during lactation and beyond also causes anxiolysis
10 (Francolin-Silva *et al.* , 2006, Hernandes and Almeida, 2003), but diet manipulation
11 during lactation can programme adult behaviour (Wright *et al.*, 2011a) and dietary
12 manipulations during adulthood as in the present study could involve different
13 mechanisms. Nevertheless the anxiolysis in females on comfort food was paralleled
14 with reduced protein intake, but similarly in all the other groups, where low protein
15 intake had no anxiolytic effect. As this observation would appear to contradict any
16 assertion that protein is a single factor responsible for the behavioural effects seen in
17 females, we would suggest that CD/comfort food as models of a Western diet have a
18 more complex effect of behaviour that cannot be attributed to a single macronutrient
19 alone.

20
21 It is tempting to speculate that obesity is causative factor for the observed
22 behavioural effects. However, genetically obese rats do not show changes in anxiety-
23 related behaviour (Chaouloff, 1994). Also, despite an overall similar pattern of obesity
24 in adult male and female rats, opposite behavioural effects were observed. Within
25 females, both groups showed anxiolytic effects upon OF exposure, but only the older
26 females could be deemed obese. This observation suggests that the behavioural
27 effects of hyper-energetic diets can also, at least in part, occur in non-obese subjects
28 (Stice *et al.* , 2012). In addition, the observed gender differences accompanied with
29 otherwise very similar nutritional profiles, at least in the adult groups, could possibly
30 be due to hormonal effects. Increased anxiety goes along with an activated
31 hypothalamic-pituitary-adrenal (Landgraf, 2005, Pego *et al.* , 2010) and
32 corticosterone levels increase following exposure to anxiety tests in rodents (File *et al.* , 1994). Obesity and the metabolic syndrome are also associated with increased
33 glucocorticoid activity (Alemany, 2012, Pasquali *et al.* , 2008) and differential
34 interactions of sexual hormones with the HPA axis in males and females (Handa *et al.* , 1994, Walf and Frye, 2006) could possibly account for the gender differences in
35 behaviour as observed in cafeteria fed rats. However, the effects of sex hormones
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1 can be bidirectional, depending on hormone concentration (Walf and Frye, 2006),
2 and in genetically obese Zucker rats the abnormalities of the HPA axis (Duclos *et al.*
3 , 2005, Fletcher *et al.* , 1986) (but see (Shargill *et al.* , 1987) do not directly translate
4 into increased anxiety (Chaouloff, 1994). Female rats, obese following unspecific
5 postnatal overfeeding, show anxiolysis upon exposure to the EPM, but no change in
6 baseline corticosterone concentration (Spencer and Tilbrook, 2009). Cafeteria
7 feeding can even abolish a stress-induced increase in corticosterone (Zeeni *et al.* ,
8 2013). The impact of obesogenic high fat diets on corticosterone concentrations
9 remains inconclusive (Auvinen *et al.* , 2011). In summary, the latter findings indicate
10 that more research would be needed to fully understand the complex interactions
11 between sex hormones, diet and behaviour.

12
13 Previous studies demonstrated anti-stress effects of hyper-energetic diets (Buwalda
14 *et al.* , 2001, Krolow *et al.*, 2010, Maniam and Morris, 2010). Therefore, and in line
15 with the present findings, one could conclude that Western diets could have
16 beneficial behavioural effects, at least in rodents. While this could be true in the short
17 term, increased glucose tolerance and the emerging signs of obesity illustrate the
18 inconsistency of this argument.

19
20 CD studies showed both prenatal and early postnatal (lactational) programming
21 effects on behaviour suggesting vulnerability of behavioural traits to effects of CD
22 largely lie in pre-weaning phase (Wright *et al.*, 2011a, Wright *et al.* , 2011b).The
23 present study adds evidence that older adulthood is another vulnerable phase,
24 although some behavioural effects occurred also in younger rats. The early
25 developmental effects of CD feeding could be seen as programmed (tissue
26 remodelling, endocrine programming, epigenetic regulation of gene expression)
27 (Langley-Evans, 2009, McGowan *et al.* , 2008, Tarry-Adkins and Ozanne, 2011), as
28 they were observed after discontinuation of the hyperenergetic diet and following
29 several months of subsequent chow feeding. Relatively little is known how
30 hyperenergetic diets impact on the physiology of anxiety. High fat diets can
31 programme glucocorticoid signalling and the expression of glucocorticoid receptors in
32 the amygdala (Sasaki *et al.* , 2013), although the glucocorticoid response was not
33 affected in another study (Shalev *et al.* , 2010). Stress-induced increase in
34 corticotropin releasing hormone expression was reduced by a palatable high fat diet.
35 This went along with ameliorated anxiety-related behaviour (Maniam and Morris,
36 2010). Structural effects of high energy diets have also been reported, demonstrating
37 a compromised blood brain barrier (Kanoski *et al.* , 2010). Future studies should

1 cover both the early developmental periods and higher age to take the dynamic
2 nature of diet-brain interactions into account.

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5 4 In conclusion the present study demonstrates that feeding a cafeteria diet/comfort
6 diet, mimicking a Western diet, leads to both obesity and behavioural changes in
7 5
8 6 rats. Overall, these effects were more pronounced in older rats, with the behavioural
9 effects being gender dependent.
10 7

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Legends

Figure 1. Body weight gain. Control (white symbols), CD (black symbols). a: young adult males, b: young adult females, c: adult males, d: adult females. Values are means \pm SEM. $n=12$ /group. Two-way ANOVA on RM followed by Bonferroni's Multiple Comparison-Test. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs. control.

Figure 2. Oral glucose tolerance test. Blood concentration [mmol]. Control (white symbols), CD (black symbols). a: young adult males, b: young adult females, c: adult males, d: adult females. Values are means \pm SE, $n=6$ /group. Two-way ANOVA on RM followed by Bonferroni's Multiple Comparison-Test. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Figure 3: Elevated plus-maze behaviour. Entries into [% of total] and time [s] spent on open arms. Control (white columns), CD (black columns). a: young adult males, b: young adult females, c: adult males, d: adult females, e: young adult males, f: young adult females, g: adult males, h: adult females. There was no overall effect of diet on EPM behaviour, although there was an effect of diet via interactions by age and gender (entries: $P<0.01$; time: $P<0.05$). Values are means \pm SEM. $n= 6-11$ /group. Three-way ANOVA followed by Holm Sidak-Test. * $P<0.05$ vs. control.

Figure 4: Open-field behaviour. Entries [number of events] into and time [s] spent in inner zone. Control (white columns), CD (black columns). a: young adult males, b: young adult females, c: adult males, d: adult females, e: young adult males, f: young adult females, g: adult males, h: adult females. The latency to enter the more aversive inner zone was only affected by age ($P<0.001$) with adult rats showing an increased latency as compared to young adult rats. Adult rats made also significantly fewer entries into the inner zone and spent less time there ($P<0.001$). Values are means \pm SEM. $n= 12$ /group. Three-way ANOVA followed by Holm Sidak-Test. * $P<0.05$ vs. control.

Table 1

Energy and macronutrient intake in controls and CD-fed rats.

Diet	Age	Gender	Energy intake (kJ/d)		Carbohydrate Sucrose (g/d)		Fat (g/d)		Protein (g/d)	
			Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Chow	Young Adult	Male	258.10	2.54	9.14 0.69	0.09 0.01	0.73	0.01	4.21	0.04
		Female	202.70	1.70	7.18 0.54	0.06 0.01	0.57	0.00	3.31	0.03
	Adult	Male	227.20	2.03	8.04 0.60	0.07 0.01	0.64	0.00	3.71	0.03
		Female	158.50	2.72	5.61 0.42	0.10 0.01	0.45	0.01	2.59	0.04
Cafeteria	Young Adult	Male	304.90	9.85	12.06* 2.72***	0.77 0.18	2.09 ***	0.16	0.93 ***	0.03
		Female	252.40 **	4.62	9.41** 1.85***	0.39 0.09	1.68 **	0.10	1.47 ***	0.08
	Adult	Male	362.90 ***	8.63	11.76*** 3.74***	0.58 0.17	3.65 ***	0.15	1.08 ***	0.04
		Female	258.70 ***	7.53	8.77*** 2.55***	0.44 0.14	2.42 ***	0.15	0.79 ***	0.04

n=12/group. Two-way ANOVA on RM. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs. control.

Table 2

Fat mass in controls and CD-fed rats.

Diet	Age	Gender	Perirenal fat (g)		Gonadal fat (g)		Subcutaneous fat (g)		Brown fat (g)	
			Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Chow	Young Adult	Male	1.86	0.15	3.35	0.16	3.56	0.17	0.34	0.02
		Female	3.16	0.22	1.53	0.12	2.62	0.09	0.36	0.03
	Adult	Male	6.12	0.44	7.97	0.49	7.61	0.55	0.30	0.03
		Female	2.82	0.32	2.52	0.21	3.76	0.12	0.35	0.02
Cafeteria	Young Adult	Male	4.81 ***	0.34	5.91 ***	0.30	6.11 ***	0.33	0.41	0.03
		Female	4.75 ***	0.34	2.36 **	0.17	2.90	0.12	0.45	0.04
	Adult	Male	11.52 ***	0.75	12.45 ***	0.70	13.37 ***	0.55	0.40 *	0.03
		Female	4.02 *	0.29	3.18	0.27	5.33 ***	0.33	0.44 *	0.03

Perirenal and subcutaneous fat were increased in all CD-fed groups ($P < 0.001$). ANOVA revealed a significant interaction between gender and diet with smaller dietary effects in females ($P < 0.001$). ANOVA further indicated that subcutaneous fat was influenced by interactions of diet and age with larger increases in adult animals ($P < 0.05$). Values are means and SEM. $n = 11-12$ /group. Three-way ANOVA followed by Holm Sidak-Test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control.

Table 3

Plasma lipid concentrations (mM) in controls and CD-fed rats.

Diet	Age	Gender	Cholesterol		Triacylglycerol		Free fatty acids	
			Mean	SEM	Mean	SEM	Mean	SEM
Chow	Young adult	Male	1.68	0.048	0.76	0.06	0.11	0.01
		Female	2.08	0.069	0.85	0.07	0.18	0.03
	Adult	Male	3.48	0.14	1.02	0.06	0.11	0.01
		Female	2.16	0.19	1.22	0.09	0.16	0.02
Cafeteria	Young adult	Male	2.08 ***	0.06	0.97	0.09	0.11	0.11
		Female	2.22	0.05	0.69	0.10	0.16	0.02
	Adult	Male	3.67	0.17	1.36**	0.10	0.19 **	0.02
		Female	2.96 *	0.29	1.97 **	0.25	0.22 *	0.02

ANOVA indicated that CD feeding led to an overall increase in cholesterol ($P < 0.001$), triacylglycerol ($P < 0.01$) and free fatty acid ($P < 0.05$) concentrations. The dietary effect on free fatty acids and triacylglycerol was influenced by interactions of age ($P < 0.01$), as triacylglycerol ($P < 0.01$) and free fatty acids (males: $P < 0.01$, females: $P < 0.05$) only increased in adult rats of either sex. Values are means and SEM. $n = 11-12/\text{group}$. Three-way ANOVA followed by Holm Sidak-Test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control.

Table 4

Distance travelled and rearing upon EPM and OF exposure

			Elevated Plus Maze				Open Field			
			Distance (m)		Rearing (number)		Distance (m)		Rearing (number)	
Diet	Age	Gender	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Chow	Young Adult	Male	22.87	0.61	22.70	1.43	32.95	0.76	27.91	1.88
		Female	21.86	1.23	21.36	1.98	31.38	1.23	26.25	1.48
	Adult	Male	21.47	0.63	20.13	1.66	24.31	1.20	23.58	2.33
		Female	20.51	1.04	20.63	2.43	30.03	1.52	29.17	2.54
Cafeteria	Young Adult	Male	21.45	0.75	19.25	1.55	34.51	1.08	27.58	2.28
		Female	24.03	1.02	22.25	1.41	35.84	1.66	34.33	3.20
	Adult	Male	22.96	0.51	23.82	1.52	24.94	1.51	24.50	1.48
		Female	20.46	0.56	15.00	3.80	35.80	1.87	28.83	1.79

In contrast to the EPM, ANOVA revealed a direct significant effect on the distance travelled in the open field with CD-fed rats travelling a longer distance compared to chow fed controls ($P < 0.01$). $n = 6-11$ /group. Three-way ANOVA followed by Holm Sidak-Test. * $P < 0.05$ vs. control.

Table 5

Grooming time (s) upon EPM and OF exposure

			Elevated Plus Maze		Open Field		
Diet	Age	Gender	Mean	SEM	Mean	SEM	
Chow	Young Adult	Male	2.2	1.2	3.3	1.3	
		Female	7.6	2.9	7.2	2.1	
	Adult	Male	0.2	0.2	13.7	4.6	
		Female	8.3	3.6	10.6	1.8	
	Cafeteria	Young Adult	Male	5.8	3.0	6.9	2.7
			Female	4.2	1.8	8.1	2.4
		Adult	Male	7.5 ***	2.0	13.7	3.1
			Female	0.3	0.3	11.4	2.5

n= 6-11/group. Three-way ANOVA followed by Holm Sidak-Test. * $P < 0.05$ vs. control.

Fig. 1

Growth curves

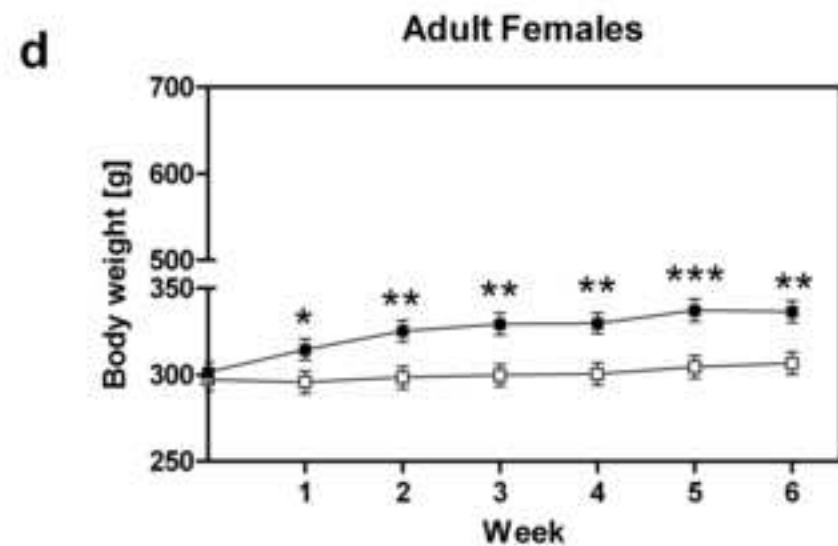
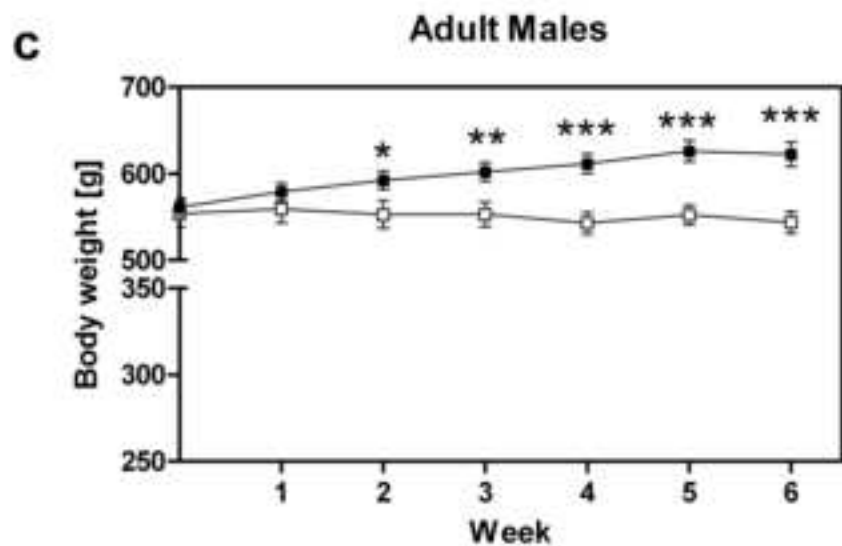
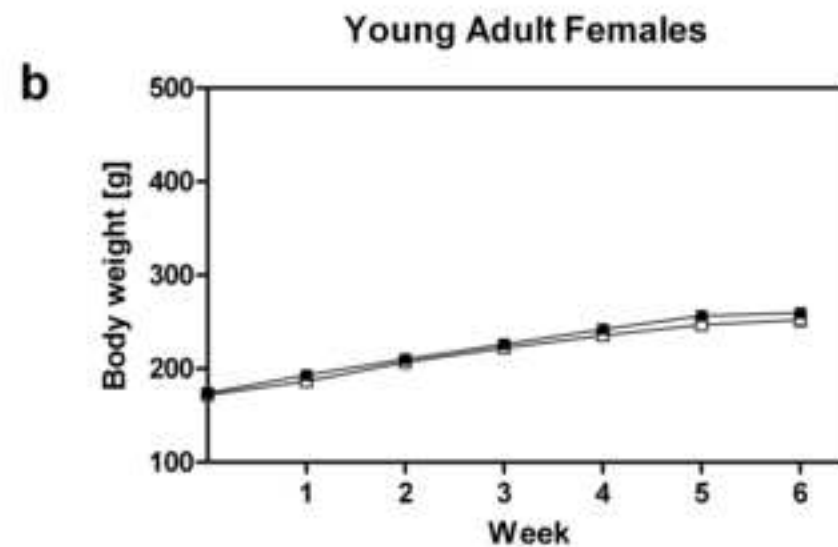
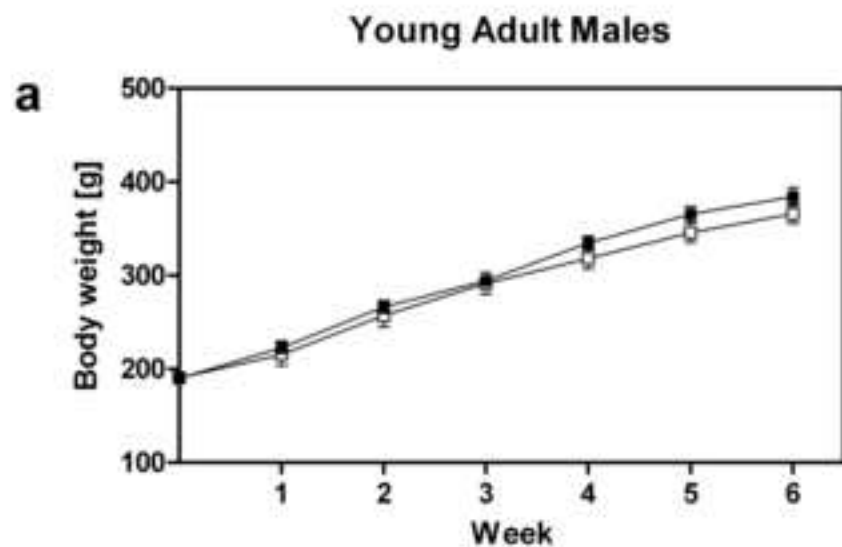


Fig. 2

Glucose tolerance test

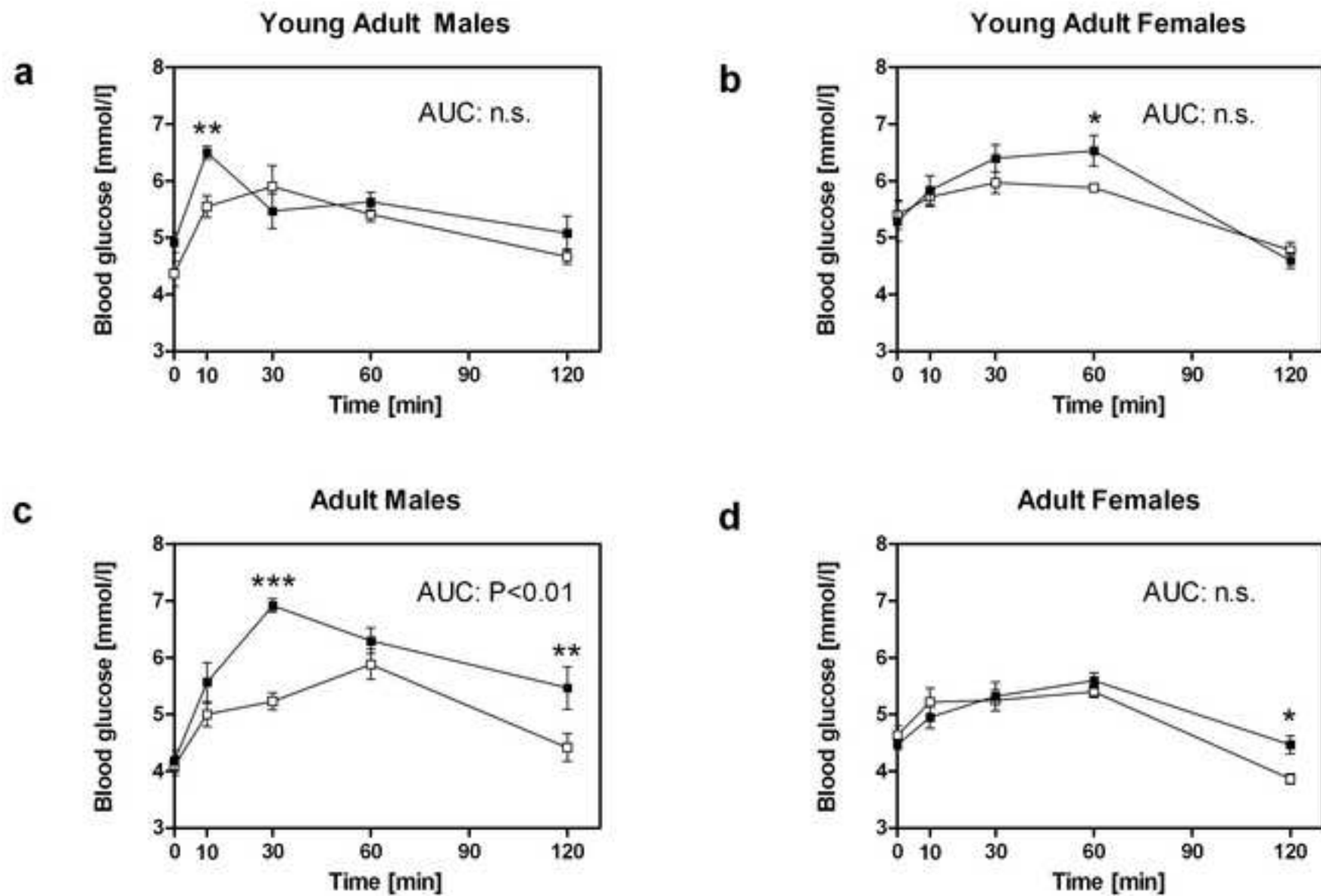


Fig. 3

Elevated plus maze: Percentage of open arm entries and time on open arms

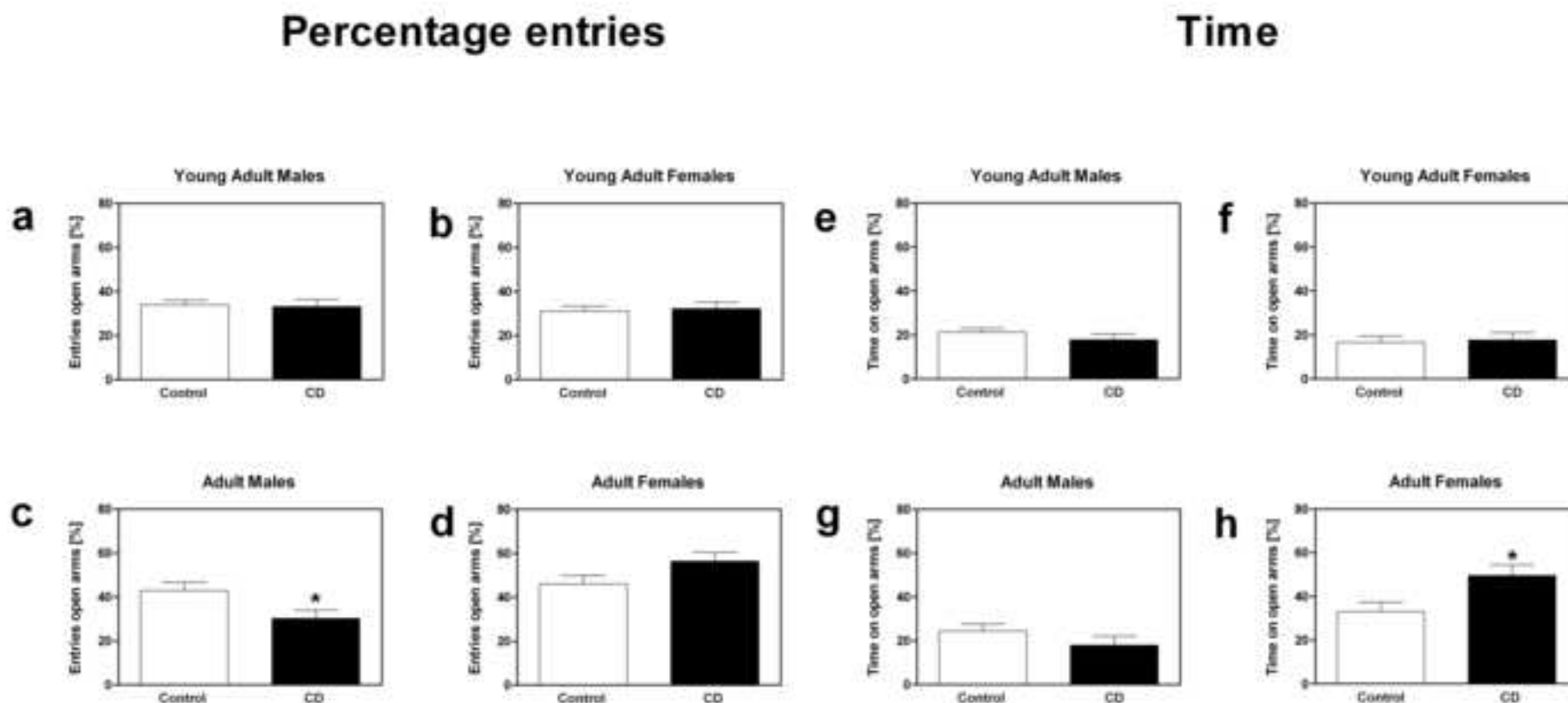


Fig. 4

Open field: Entries and time inner zone

