ELSEVIER

PII S0024-3205(96)00140-3

Life Sciences, Vol. 58, No. 19, pp. 1653-1660, 1996

0024-3205/96 $15.00 + .00

HIGH-FAT DIET INDUCES AGGRESSIVE BEHAVIOR IN MALE MICE AND RATS

Leena Hilakivi-Clarke[1,2]*, Elizabeth Cho[1], and Ighovie Onojafe[1]

[1] Lombardi Cancer Center, Georgetown University Medical Center, Reservoir Rd, NW, Washington, DC 20007 [2] Department of Psychiatry, Georgetown University Medical Center, 3800 Reservoir Rd, NW, Washington, DC 20007.

(Received in final form March 12, 1996)

Summary

The present study investigated whether dietary fat increases aggressive behavior in male mice and rats. High fat consumption may elevate circulating estrogen levels and estrogens, in turn, are associated with various non-reproductive behaviors, such as male aggression. The animals were assigned to two groups including those consuming a diet high in polyunsaturated fats (43% calories from fat) and those consuming a low-fat diet (16% calories from fat). Each male animal was housed with two females for three weeks. The male mice and rats were then confronted with an intruder kept on a medium-fat feed. The latency to first aggressive encounter was significantly shorter among the male animals kept on a high-fat diet than those males kept on a low-fat diet. Furthermore, the time spent exhibiting aggression was longer in the high-fat groups. Serum levels of estradiol (E2) were elevated by 2-fold in the male animals consuming a high-fat diet, when compared with the male animals kept on a low-fat diet. These findings suggest that dietary fat can increase aggressive behavior in male mice and rats, possibly by elevating circulating E2 levels.

Key Words: high-fat diet, aggressive behavior, diet, estradiol

Aggression and violence are major problems in many modern societies. Although socioeconomic factors are clearly involved in explaining this behavior, many biological factors also have been linked to aggression (1). In addition, it is highly probable that both factors operate together to influence aggressiveness. A potential environmental source that could affect human behavior, is diet (2). For example, altered serum glucose levels may trigger aggression (3). There also are reports linking low plasma cholesterol levels to violent and/or suicidal behavior (4,5). Low cholesterol is generally associated with the consumption of a low-fat diet. Therefore, it is important to determine whether dietary fat affects aggressive behavior.

Several clinical studies show that a high-fat intake results in increased levels of circulating free estrogens, and a low-fat diet is associated with low plasma estrogen levels (6-11). The altered estrogen levels could be due to obesity and an increased deposition of adipose tissue, resulting from a high-fat

*Correspondence should be addressed at: Leena Hilakivi-Clarke, Lombardi Cancer Center, Research Bldg, Room W40, Georgetown University Medical Center, 3970 Reservoir Rd, NW, Washington, DC 20007-2197. Telefax: (202) 687-7505

intake. Adipocytes exhibit high aromataze activity, which converts testosterone to estrogens. One third of the circulating estrogens in premenopausal women are estimated to originate from adipose tissue (12). The major component of polyunsaturated fat, linoleic acid, can reduce binding of estrogens to sex-hormone binding globulin (SHBG) and albumin, thereby increasing the circulating levels of biologically potent estrogens (13).

Estrogens are steroid hormones that are critical for gender-specific differentiation of reproductive behaviors (14). They also participate in controlling several non-reproductive behaviors (15). Many studies have demonstrated the activational effects of gonadal steroids on aggressive behavior in male mice. For example, chronic treatment with estrogens induces high levels of aggression in male mice and rats (16,17), and estrogens effectively restore fighting in castrated male mice (18,19). The present study investigated whether a high-fat diet can increase male aggression in mice and rats. We observed significantly higher levels of aggression in male rodents exposed to a high-fat diet for three weeks, when compared to aggressive behavior of males kept on a low-fat diet.

Methods

Three-month-old male C3H mice (National Cancer Institute, Frederick, MD) and male Sprague-Dawley rats (Charles Rivers, Wilmington, DE) were used. The animals were housed in temperature/humidity controlled room under a 12 hr light-dark cycle.

Table I

Diet formulations

Ingredients (g)	Low fat	High fat
Fat - total (corn oil)	7.0	19.4
Protein (casein+ cysteine)	20.3	20.3
Carbohydrates	62.9	34.0
Fiber	5	21.5
AIN mineral mix	1.0	1.0
AIN vitamin mix	3.5	3.5
Choline chloride	0.3	0.3
Total grams	100	100
kcal density/ g	3.7	3.7
% kcal from fat	15.6	43.1
% kcal from protein	20.4	19.2
% kcal from carbohydrates	63.8	37.6

Dietary manipulations

The animals were assigned to two groups, one of which was given a diet containing 16% calories from n-6 polyunsaturated fatty acids (low-fat diet), and the other group was given a diet containing 43% calories from fat (high-fat diet). Both rat groups contained 6 animals, and the mice groups 7 and 9 animals, respectively. The diets are within the range of fat consumed by Americans. An average American diet contains approximately 36-40% of calories from fat (20), while American vegetarians consume significantly less calories

from fat (21-23). In addition, dietary interventions designed to prevent certain cancers successfully reach fat consumption levels as low as 15-24% (7,24). The diets were provided at the time the males were put together with the females. Animals were fed *ad libitum* food and water.

The animal feed was prepared commercially by Bioserv Inc (Frenchtown, NJ) to our specifications (Table 1). The diets provided isoenergetic, semipurified diets as described by the American Institute of Nutrition (AIN) (25,26). The diets were made isocaloric by adjusting the caloric contents with fiber. The proportion of other dietary components were adjusted to ensure adequate intake of protein (casein), vitamins and trace elements. The amounts of these components per diet were approximately constant with regard to energy (27). Vitamin and mineral mixtures were balanced as described in the second report of the AIN (25).

Resident-intruder test of aggression.

A male mouse or rat becomes aggressive towards other males when housed as an "only male" among females (1). Accordingly, in the present study male animals were housed in a cage containing one male and two females for three weeks. Only males which impregnated the females were used. The special diets were offered throughout this housing period. To assess the level of aggression, females were removed and the experimental male mice and rats, kept on high- or low-fat diets, were housed individually for 24 hrs. During the individual housing, the animals continued to receive the special diets. They were then confronted in their home cage with a male intruder that had been kept on a medium-fat diet (30% calories from fat). The intruder was of the same species and continuously housed with a female. The intruder and resident had no previous contact with each other.

During a 10-min (rats) or 5-min (mice) test period, an observer, using stopwatches, monitored and recorded the latency to first aggressive encounter and the duration of various aggressive acts, including lateral threat, tail rattle, biting, attacks and fighting exhibited by the resident.

Measurement of hormone levels

Three days after the resident-intruder test, levels of 17ß-estradiol (E2) were measured from the male mice. During the 3-day interim, the animals remained singly housed and kept on the special diets. The mice were anesthetized using methoxyflurane inhalant, their blood was collected by cardiac puncture, and they were killed immediately afterward by cervical dislocation. The blood was placed in vacutainer tubes, centrifuged and extracted for plasma. Total serum concentrations of E2 were determined from the samples by using a specific double antibody kit from ICN Biomedicals, Inc. (Irvine, CA) according to the manufacturer's instructions.

Results

Food intake

There were no differences in food intake between animals kept on a low- or high-fat diet. The daily food consumption among the rats kept on a low-fat diet was 24.1±0.5 g/day and the caloric intake was 91.8±1.9 kcal/day. Among the high-fat rats the corresponding figures were 23.2±0.8 g/ day and 88.2±3.1 kcal/day.

Aggressive behavior

Among the male rats, the latency to the first aggressive encounter during a 10-min test was significantly shorter in those animals who were fed with a diet containing 43% calories from fat (range 10-80 sec, median 55 sec) than in those animals who consumed a diet containing 16% calories from fat (range 60-360 sec, median 190 sec) ($p<.01$) (Fig. 1). Due to unequal variability of the data obtained in the two groups, non-parametric Mann-Whitney U-test was used. The time spent in exhibiting aggressive behavior also was longer in the high-fat group (range 76-389 sec, median 151 sec) than in the low-fat group (range 33-203 sec, median 70 sec) ($p<.05$) (Fig. 2). Body weights did not differ between the two groups (mean±SEM; high-fat: 402.5±17.3 g; low-fat: 403.5±18.0 g).

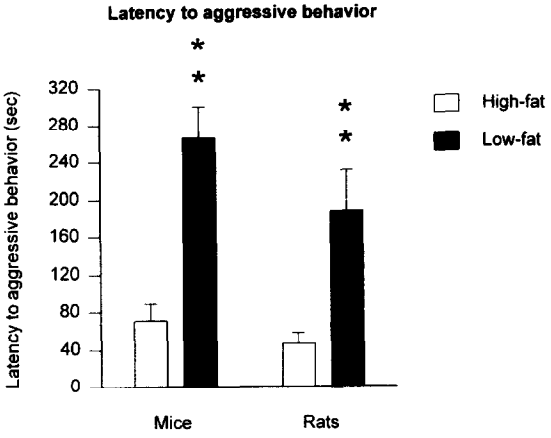

Fig. 1.
Latency to first aggressive encounter during a 10 min (rats) or 5 min (mice) resident-intruder test among male animals kept in a high-fat (43% calories from fat) or low-fat (16% calories from fat) for three weeks. The means±SEM of 6-9 animals per group are shown. ** $p<.01$ (rats: U-test; mice: t-test)

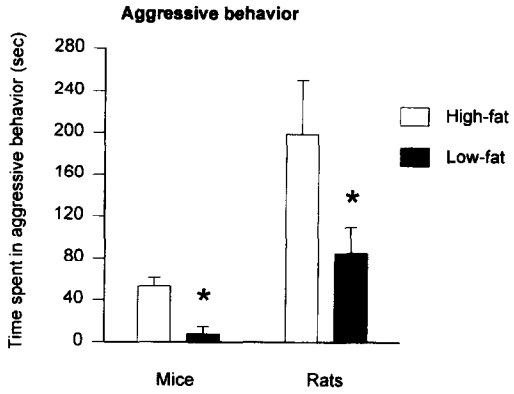

Fig. 2.
The time spent in aggressive behavior during a 10 min (rats) or 5 min (mice) resident-intruder test among male animals kept in a high-fat (43% calories from fat) or low-fat (16% calories from fat) for three weeks. The means±SEM of 6-9 animals per group are shown. * $p<.05$ (rats: U-test; mice: t-test)

The data were essentially similar among the male mice as the male rats: The latency to the first aggressive act during a 5-min test was significantly shorter among male mice who were fed with a high-fat diet (range 15-130 sec, median 100 sec), when compared with male mice who consumed a low-fat diet (range 100-300 sec, median 300 sec) (Student's t-test; t=5.5, df=10, p<.001) (Fig. 1). The time spent in aggressive encounters was significantly longer in the high-fat group (range 6-94 sec, median 52 sec) than in the low-fat group (range 0-51 sec, median 0 sec) (t=4.0, df=14, p<.001) (Fig. 2). Body weights differed significantly indicating that the high-fat male mice (33.4±2.2 g) were heavier than the low-fat male mice (26.4±0.7 g) (t=3.0, df=14, p<.01) at the time their aggressive behavior was evaluated.

Hormone levels

The mean serum E2 levels in the male mice that were fed a diet containing a high percentile of polyunsaturated fats were 2-fold higher than serum E2 levels in the low-fat group (Fig. 3). However, due to large variability in the E2 levels among the individual mice (high-fat: range 0-99 pg/ml, median 33 pg/ml; low-fat: range 0-57 pg/ml, median 13 pg/ml), this difference failed to reach statistical significance.

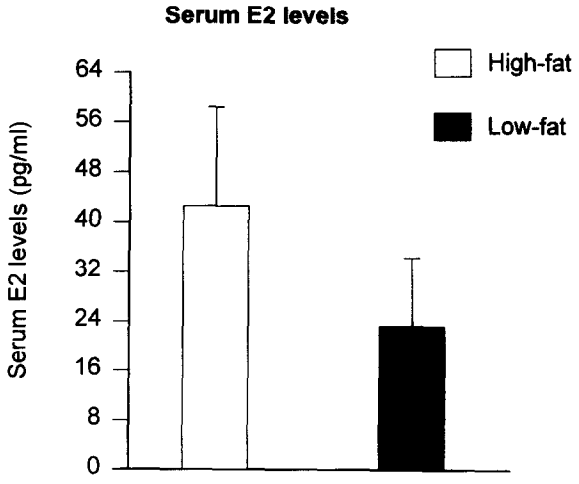

Fig. 3.
Serum E2 levels in male mice kept in a high-fat (43% calories from fat) or low-fat (16% calories from fat) for three weeks. The means±SEM of 7-9 animals per group are shown.

Discussion

The results of this study clearly show that a diet containing high quantities of polyunsaturated fats can increase aggressive behavior in both mice and rats. Since estrogens increase aggressive behavior in male rodents (16,18,19), a likely biological mechanism responsible for the behavioral effect is a fat-induced elevation in circulating estrogen levels. In humans, a consumption of a high-fat diet is associated with an increase in circulating estrogen levels (6,10). However, most experiments in animals have failed to find any difference in serum E2 levels between individuals kept on a high- and low-fat diets (28,29). The lack of difference in animals may partly reflect the fact that the diets often are non-isocaloric, and animals compensate the low caloric density of a low-fat diet by increasing feed intake over that of animals kept on a high-fat diet (Hilakivi-Clarke et al., unpublished data) . We have consistently found higher serum E2 levels in female rats kept on an isocaloric high-fat diet than in females kept on a low-fat diet (30). In the present study, the male mice kept on an isocaloric high-fat feed for 3 weeks exhibited 2-fold higher serum E2 levels than the male mice kept in a low-fat feed, but the difference was not statistically significant. Whether it is nevertheless biologically relevant, i.e.,

the non-significantly elevated serum E2 levels induce aggressiveness in the male animals consuming a high-fat diet, is not clear.

Besides the serum E2 levels, the effects of a high-fat diet on male aggression could be mediated through a number of other factors. High-fat diet -induced alterations in the fatty acid content change cell membrane structure (7,8), that could then alter cell fluidity and functionality (31). These changes may, in turn, alter the release of neurotransmitters that regulate aggressive behavior and the function of their receptors. Subtle fat-induced changes in the circulating E2 levels also could affect neurotransmitters in the brain. For example, estrogens alter serotonin (5-HT) concentrations, turnover, and 5-HT receptor binding site density in some brain regions (32,33). Serotonin is closely linked to male aggression (1,34,35).

Body weight also influences aggressiveness. We have found that heavier male mice are more aggressive (36). In the present study, we used isocaloric diets to avoid changes in body weight. Our earlier study (Hilakivi-Clarke, unpublished data) as well as the results of the present study have shown that food consumption is similar among rats kept on these high- and low-fat diets. However, the male mice consuming a high-fat diet for three weeks were significantly heavier than the male mice consuming a low-fat diet. This finding may at least partly explain the difference in aggressiveness between the two groups. Dietary fat intake did not influence body weights of male rats; however, the levels of aggressiveness exhibited by rats kept on a high-fat diet were 2 times higher than those of rats kept on a low-fat diet, suggesting that the aggressiveness of male animals consuming a high-fat diet is related to the diet.

If a high-fat diet is linked to human aggression, then dietary modulations may offer new means to prevent some violent crimes. A recent study by Weidner et al. (37) indicate that a low-fat diet has beneficial effects on human behavior. These investigators found reduced incidence of aggressive hostility in men who start to consume a low-fat, high complex carbohydrate diet, when compared with the aggressivity expressed by men who ate a high-fat American diet. Thus, an appropriate diet low in fat is not only a tool in the attempts to prevent various cancers (38,39) and cardio-vascular diseases (40,41), but it also may have important beneficial influences on male aggression.

References

1. K.A. MICZEK, *The Handbook of Psychopharmacology*, L.L. Iversen, S.D. Iversen and S.H. Snyder (eds), 183-328, Plenum Press, NY (1987).
2. R.J. WURTMAN and J.J. WURTMAN, Sci Amer *260* 68-75 (1989).
3. M. VIRKKUNEN, Nutr Rev *44* 94-103 (1986).
4. C.J. GLUECK, M. TIEGER, R. KUNKEL, T. HAMER and J. SPEIRS, Am J Med Sci *308* 218-225 (1994).
5. P.F. SULLIVAN, P.R. JOYCE, C.M. BULIK, R.T. MULDER and M. OAKLEY-BROWNE, Biol Psychiatry *36* 472-477 (1994).
6. H. ADLERCREUTZ, *Nutrition, Toxicity, and Cancer*, I.R. Rowland (ed), 137-195, CRC Press, Boca Raton (1991).
7. R.T. CHLEBOWSKI, D. ROSE, I.M. BUZZARD, G.L. BLACKBURN, W.JR. INSULL, M. GROSVENOR, R. ELASHOFF and E.L. WYNDER, Breast Cancer Res Treat *20* 73-84 (1991).
8. F.C. BENNETT and D.M. INGRAM, Am J Clin Nutr *52* 808-812 (1990).
9. D.P. ROSE, J.M. CONNOLLY, R.T. CHLEBOWSKI, I.M. BUZZARD and E.L. WYNDER,

Breast Cancer Res Treat *27* 253-262 (1993).
10. H. ADLERCREUTZ, S.L. GORBACH, B.R. GOLDIN, M.N. WOODS, J.T. DWYER and E. HAMALAINEN, J Natl Cancer Inst *86* 1076-1082 (1994).
11. B.R. GOLDIN, M.N. WOODS, D.L. SPIEGELMAN, C. LONGCOPE, A. MORRILL-LA BRODE, J.T. DWYER, L.J. GUALTIERI, E. HERTZMARK and S.L. GORBACH, Cancer *74* 1125-1131 (1994).
12. R.E. FRISCH. *Adipose Tissue and Reproduction*, p.23, Karger, Basel (1990).
13. P.F. BRUNING and J.M.G. BONFRER, Ann N Y Acad Sci 257-264 (1990).
14. R.W. GOY and B.S. MCEWEN. *Sexual differentiation of the brain*, p.23, MIT Press, Cambridge, MA (1980).
15. W.W. BEATTY, *Handbook of Behavioral Neurobiology. Sexual Differentation*, A.A. Gerall, H. Moltz and I.L. Ward (eds), 85-128, Plenum Press, New York (1992).
16. N.E. VAN DE POLL, N.J. BOWDEN, H.G. VAN OYEN, F.H. DE JONGE and H.H. SWANSON, *Psychopharmacology of Sexual Disorders*, M. Segal (ed), 63-67, Libbey, London (1985).
17. L. HILAKIVI-CLARKE, J Stud Alcohol *In press* (1995).
18. N.G. SIMON and R.E. WHALEN, Agg Behav *12* 255-266 (1986).
19. L. HILAKIVI-CLARKE and R. GOLDBERG, Alcohol Clin Exp Res *19* 708-713 (1995).
20. N.F. BOYD, M. COUSINS, G. LOCKWOOD and D. TRITCHLER, Br J Cancer *62* 878-881 (1990).
21. K. RESNICOW, J. BARONE, A. ENGLE, S. MILLER, N.J. HALEY, D. FELMING and E. WYNDER, J Am Diet Assoc *91* 447-453 (1991).
22. V. SPAWAN, P. PONGPAEW, R. TUNGTRONGCHITR, S. TAWPRASERT, S. CHANGBUMRUNG, P. MIGASENA and F.P. SCHELP, Int J Vitam Nutr Res *62* 324-329 (1992).
23. J.C. BARBOSA, T.D. SCHULTZ, S.J. FILLEY and D.C. NIEMAN, Am J Clin Nutr *51* 798-803 (1990).
24. N.F. BOYD, M. COUSINS and V. KRIUKOV, J Clin Epidemiol *45* 31-38 (1992).
25. AIN REPORT, J Nutr *110* 1726 (1980).
26. AIN REPORT, J Nutr *107* 1340-1348 (1977).
27. E.A. JACOBSON, K.A. JAMES, H.L. MEWMARK and K.K. CARROLL, Cancer Res *49* 6300-6303 (1989).
28. L.A. COHEN, M.E. KENDALL, E. ZANG, C. MESCHTER and D.P. ROSE, J Natl Cancer Inst *83* 496-501 (1991).
29. S.K. CLINTON, P.S. LI, A.L. MULLOY, P.B. IMREY, S. NANDKUMAR and W.J. VISEK, J Nutr *125* 1192-1204 (1995).
30. L. HILAKIVI-CLARKE, R. CLARKE, I. ONOJAFE, M. RAYGADA, E. CHO and M.E. LIPPMAN, Ann New Acad Sci *768* 327-330 (1995).
31. J.J. JURKOWSKI and W.T. CAVE, J Natl Cancer Inst *74* 1145-1150 (1985).
32. S.G. BECK, W.P. CLARKE and J. GOLDFARB, Neurosci Lett *106* 181-187 (1989).
33. C.T. FISCHETTE, A. BIEGNON and B.S. MCEWEN, Science *222* 333-335 (1983).
34. L. VALZELLI, S. BERNASCONI and M. DALESSANDRO, Pharm Res Comm *13* 891-897 (1986).
35. L. VALZELLI and S. BERNASCONI, Neuropsychopharmacology *5* 129-135 (1979).
36. L.A. HILAKIVI-CLARKE and R.G. LISTER, Agg Behav *18* 281-287 (1992).
37. G. WEIDNER, S.L. CONNOR, J.F. HOLLIS and W.E. CONNOR, Ann Intern Med *117* 820-823 (1992).
38. N.F. BOYD, L.J. MARTIN, M. NOFFEL, G.A. LOCKWOOD and D.L. TRITCHLER, Br J Cancer *68* 627-636 (1993).
39. W.C. WILLETT, M.J. STAMPFER, G.A. COLDITZ, B.A. ROSNER and F.E. SPEIZER, N

Eng J Med *323* 1664-1672 (1990).
40. J. TUOMILEHTO, J.T. SALONEN and B. MARTI, BMJ *295* 623-627 (1987).
41. J.E. MANSON, G.A. COLDITZ and M.J. STAMPFER, N End J Med *322* 882-889 (1990).