European Journal of Neurology 2006, **13:** 1385–1388 doi:10.1111/j.1468-1331.2006.01500.x

SHORT COMMUNICATION

High-fat diet impairs hippocampal neurogenesis in male rats

A. Lindqvist[a], P. Mohapel[b,1], B. Bouter[a,1], H. Frielingsdorf[b,c], D. Pizzo[c], P. Brundin[b] and C. Erlanson-Albertsson[a]

[a]*Division for Diabetes, Metabolism and Endocrinology, Department of Experimental Medical Science, BMC F-13, Lund University, Lund, Sweden;* [b]*Neuronal Survival Unit, Department of Experimental Medical Science, Wallenberg Neuroscience Center, BMC A-10, Lund University, Lund, Sweden; and* [c]*Department for Neurosciences, University of California San Diego, San Diego, CA, USA*

Keywords:
corticosterone, fat intake, hippocampus, memory, obesity

Received 13 September 2005
Accepted 12 December 2005

High fat diets and obesity pose serious health problems, such as type II diabetes and cardiovascular disease. Impaired cognitive function is also associated with high fat intake. In this study, we show that just 4 weeks of feeding a diet rich in fat *ad libitum* decreased hippocampal neurogenesis in male, but not female, rats. There was no obesity, but male rats fed a diet rich in fat exhibited elevated serum corticosterone levels compared with those fed standard rat chow. These data indicate that high dietary fat intake can disrupt hippocampal neurogenesis, probably through an increase in serum corticosterone levels, and that males are more susceptible than females.

Introduction

The alarming trend of increases in high fat diets and obesity has lead to an escalating prevalence of type 2 diabetes and cardiovascular disease. Furthermore, several studies suggest that a diet rich in fat influences the normal development of the central nervous system and can impede cognitive performance [1–3]. New neurons are continuously produced in the dentate gyrus of the adult hippocampus. The hippocampus is important for learning and memory, and several studies have implicated a functional role of these neurons in memory formation [4]. Diets rich in fat are increasingly common. Not only have they been implicated as causative in obesity, but recently it has also become increasingly popular to eat food particularly enriched in proteins and fats in attempts to reduce weight (so called Atkins diet) [5]. Therefore we investigated the impact of high fat diet, administered at a level and duration that did not cause obesity, on hippocampal neurogenesis in young male and female rats.

Procedures and methods

Animals

Male and female Sprague–Dawley rats (140 ± 20 g) from B&K (Sollentuna, Sweden) were housed three per cage under standard conditions (12 h light/12 h dark, 22°C).

The male rats were divided into two groups: one group was fed a high-fat (HF) diet containing 42% fat (coconut butter and corn oil) by energy, as described previously [6], another group was fed standard rat chow (LF) diet (10% fat by energy, R36; Lactamin, Kimsta, Sweden). The female rats were divided in the same way. All rats had *ad libitum* access to their diets. The experiments were approved by the Local Animal Welfare Committee, Lund, Sweden.

Study design

The rats were provided with either an HF or LF diet for 4 weeks. After 2 weeks, they were injected with BrdU (50 mg/kg i.p.) every 2 h over a 6 h period. After an additional 2 weeks, they were perfused transcardially with 4% paraformaldehyde and their brains were removed for analysis. Just prior to perfusion, fat pads from the abdominal cavity were dissected out and weighed. Blood was collected from cardial puncture. Serum was obtained and stored at −20°C until analysis.

Tissue preparation and immunohistochemistry

The brains were sectioned coronally throughout the entire hippocampus into 10 series of 40μ m thick sections. Procedures for double labeling with BrdU- and NeuN-immunofluorescence have been described in detail elsewhere [7]. Briefly, free-floating sections were pretreated in 1 M HCl and incubated over two nights with rat anti-BrdU (1:100; Sigma, St Louis, MO, USA) and mouse anti-NeuN (1:100; Chemicon International, Temecula, CA, USA). Sections were then incubated with Cy3-conjugated donkey anti-rat (1:400; Jackson

Correspondence: Charlotte Erlanson-Albertsson, MD, Section for Diabetes, Metabolism and Endocrinology, Department of Experimental Medical Science, BMC, F13, SE-221 84 Lund, Sweden (tel.: 46-46-2228589; fax: 46-46-2224022; e-mail: charlotte.erlanson-albertsson@med.lu.se).

[1]PM and BB contributed equally.

ImmunoResearch Inc., West Grove, PA, USA) and biotinylated horse anti-mouse antibody (1:200; Vector Laboratories, Burlingame, CA, USA) followed by Streptavidin Alexa Fluor 488 (1:200; Molecular Probes, Eugene, OR, USA).

Quantification of BrdU and NeuN immunoreactive cells

The numbers of BrdU immunoreactive (IR) cells were quantified in 6 equally spaced sections per rat, located between 3.0 and 4.5 mm caudal to bregma (encompassing the dorsal hippocampal region). All positive cells were counted in the dentate gyrus granule cell layer including the subgranular zone and expressed as mean number of cells per section. Fifty BrdU cells per rat were analyzed for co-localization with NeuN in three rats from each group using a confocal laser-scanning microscope (Zeiss, Thornwood, NY, USA, LSM510) equipped with an argon/krypton laser. Z stacks from sections scanned at 1 μm thickness were reconstructed using LSM 510 software.

Serum corticosterone analysis

Serum corticosterone was analyzed using a radioimmunoassay and protocol from MP Biomedicals (Orangeburg, NY, USA). Intra- and inter-assay variations were 4.4% and 6.5%, respectively.

Statistical analysis

All data are expressed as mean ± SEM. Weights of abdominal fat pads were corrected for differences in body weight by dividing the weight of the fat by the body weight. The data were analyzed using Student's *t*-test or two-way analysis of variance (ANOVA). The differences were considered significant when $P < 0.05$.

Results

Weight gain and adiposity

No differences in body weight gain and fat accumulation were observed in male rats receiving LF or HF diets. The females, however, exhibited a significant weight gain and mesenteric fat accumulation when given HF diet compared with LF diet ($P < 0.05$ for weight gain and $P < 0.005$ for fat accumulation, respectively, Fig. 1a and b).

Amount of BrdU IR cells

In general, female rats displayed lower numbers of newborn cells in the dentate gyrus (main effect of gender, $F(1,17) = 15{,}8$, $P < 0.005$; Fig. 1c). Importantly, the number of newly born cells in the dentate gyrus was reduced by high-fat diet (main effect of diet, $F(1,17) = 24.8$, $P < 0.005$; Fig. 1c). Males and females responded differently to the HF diet, such that the effect of HF was only apparent in males (diet × gender interaction, $F(1,17) = 8.05$, $P < 0.05$). Thus, whilst male rats fed HF decreased their neurogenesis by approximately 40%, there was no effect of HF diet in female rats. To determine whether HF diet influenced neurogenesis specifically, BrdU-immunostained cells were examined for co-expression of the mature neuron-specific marker, NeuN (Fig. 1e). We found on average 88 ± 2% of BrdU+ cells to be neurons, with no differences in the ratio of new neurons between the different diets or between males and females. This indicates that the vast majority of the observed reduction in hippocampal cell genesis in males given HF diet was due to a reduction in neurogenesis.

Serum corticosterone analysis

Females and males responded differently to the diet regarding serum corticosterone levels (diet × gender interaction, $F(1,12) = 9.09$, $P < 0.05$, Fig. 1d). Thus, corticosterone levels were nearly threefold higher in male rats fed HF diet compared with males fed LF diet, whereas in female rats the corticosterone levels were not different in rats fed HF or LF diet.

Discussion

Male rats fed a high-fat diet exhibited a reduction in hippocampal neurogenesis. As these animals did not exhibit increased fat accumulation compared with male rats fed a low-fat diet, the reduction in hippocampal neurogenesis was independent of adipose tissue accumulation. Rather the effect was related to the actual ingestion of dietary fat.

There was lower neurogenesis in female compared with male rats, regardless of diet. A possible explanation could be differences in serum corticosterone levels between males and females. Thus, on low-fat diet we found that female rats had higher corticosterone levels than males (Fig. 1d). With a high-fat diet there was no change in corticosterone levels in females, whereas males displayed a significant increase. We cannot rule out that the observed elevation in serum corticosterone levels in male rats during high-fat diet are stress effects occurring independent of diet.

Increased levels of corticosterone are well known to inhibit hippocampal neurogenesis [8,9] and adrenalectomy increases the number of surviving newborn neurons [10], supporting a role for corticosterone in regulating hippocampal neurogenesis. Diets rich in fat

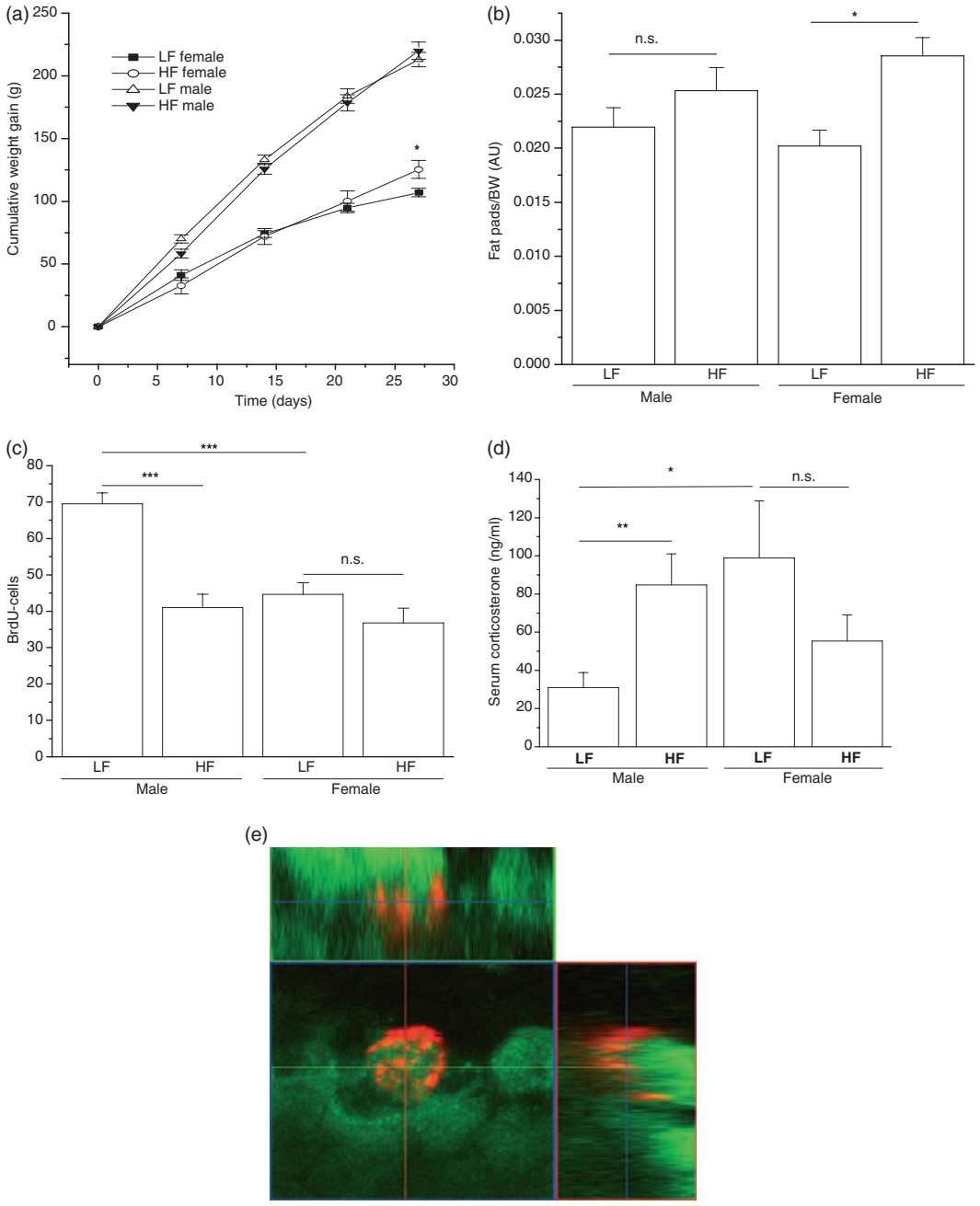

Figure 1 (a) Body weight gain, (b) adipose tissue accumulation, (c) number of neuronal cells in the dentate gyrus of hippocampus and (d) serum corticosterone levels in male and female Sprague–Dawley rats ($n = 6$ in each group) fed a low-fat diet (10% fat) (LF) and a high-fat diet (42% fat) (HF) *ad libitum* for 4 weeks. (e) Representative confocal orthogonal reconstruction of a cell co-expressing BrdU (red) and NeuN (green) in the granule cell layer of a male rat, scale bar representing 5μ m.

have previously been shown to increase serum levels of corticosterone in male rats as early as 7 days after initiation of the diet, and corticosterone levels have remained elevated for up to 5 months of high-fat feeding [11,12]. Notably, corticosterone levels have previously not been measured in female rats fed a high-fat diet. The fact that high-fat diet did not reduce hippocampal neurogenesis in females could be related to effects of estrogen, which is known to promote neurogenesis in the dentate gyrus of female rats through stimulation of insulin-like growth factor-1 [13].

Feeding high-fat diets have been shown to impair spatial learning in normal rodents and rats subjected to traumatic brain injury [3,14,15]. These reported be-

havioral impairments may be mediated by a reduction in hippocampal neurogenesis. Several lines of evidence suggest that newborn hippocampal neurons contribute to learning and memory. First, these new hippocampal neurons adopt morphological and physiological properties of mature granule neurons [16]. Second, factors that up- or down-regulate neurogenesis have the same directional effect on hippocampus-mediated learning [17,18]. Third, a learning experience can promote hippocampal neurogenesis [19]. Finally, impeding hippocampal neurogenesis blocks some forms of hippocampus-dependent learning [20]. Interestingly, our data may be directly relevant to humans. Clinical studies show that diets rich in saturated fats cause cognitive decline in aged individuals and people afflicted with dementia [1,2].

In conclusion, our study provides the first compelling evidence that a high intake of dietary fat *per se* has a negative influence on hippocampal neurogenesis. Importantly, dietary fat can reduce neurogenesis even in the absence of increased body weight and adipose tissue accumulation in male rats. We suggest that this decrease in neurogenesis is mediated through increased serum corticosterone levels. Our results underscore the potential detrimental effects that dietary choices can have on normal brain function. As high-fat diets have been shown to increase serum cortisol levels in human subjects [21], our findings highlight the possibility that such diets can negatively affect brain function.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council to C E-A and PB, and Dr A. Påhlssons Foundation.

References

1. Solfrizzi V, Panza F, Capurso A. The role of diet in cognitive decline. *Journal of Neural Transmission* 2003; **110:** 95–110.
2. Kalmijn S. Fatty acid intake and the risk of dementia and cognitive decline: a review of clinical and epidemiological studies. *The Journal of Nutrition, Health & Aging* 2000; **4:** 202–207.
3. Molteni R, Barnard RJ, Ying Z, Roberts CK, Gomez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience* 2002; **112:** 803–814.
4. Kempermann G. Why new neurons? Possible functions for adult hippocampal neurogenesis. *Journal of Neuroscience* 2002; **22:** 635–638.
5. Brehm BJ, Seeley RJ, Daniels SR, D'Alessio DA. A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. *Journal of Clinical Endocrinology and Metabolism* 2003; **88:** 1617–1623.
6. Lindqvist A, de la Cour CD, Stegmark A, Hakanson R, Erlanson-Albertsson C. Overeating of palatable food is associated with blunted leptin and ghrelin responses. *Regulatory Peptides* 2005; **130:** 123–132.
7. Mohapel P, Ekdahl CT, Lindvall O. Status epilepticus severity influences the long-term outcome of neurogenesis in the adult dentate gyrus. *Neurobiology of Disease* 2004; **15:** 196–205.
8. Ambrogini P, Orsini L, Mancini C, Ferri P, Barbanti I, Cuppini R. Persistently high corticosterone levels but not normal circadian fluctuations of the hormone affect cell proliferation in the adult rat dentate gyrus. *Neuroendocrinology* 2002; **76:** 366–372.
9. Heine VM, Maslam S, Joels M, Lucassen PJ. Prominent decline of newborn cell proliferation, differentiation, and apoptosis in the aging dentate gyrus, in absence of an age-related hypothalamus-pituitary-adrenal axis activation. *Neurobiology of Aging* 2004; **25:** 361–375.
10. Montaron MF, Petry KG, Rodriguez JJ, *et al.* Adrenalectomy increases neurogenesis but not PSA-NCAM expression in aged dentate gyrus. *European Journal of Neuroscience* 1999; **11:** 1479–1485.
11. Tannenbaum BM, Brindley DN, Tannenbaum GS, Dallman MF, McArthur MD, Meaney MJ. High-fat feeding alters both basal and stress-induced hypothalamic-pituitary-adrenal activity in the rat. *American Journal of Physiology* 1997; **273:** E1168–E1177.
12. De Schepper J, Zhou X, De Bock S, *et al.* Study of serum leptin in cafeteria-diet-overfed rats. Influence of diet, insulin and corticosterone. *Hormone Research* 1998; **50:** 271–275.
13. Perez-Martin M, Azcoitia I, Trejo JL, Sierra A, Garcia-Segura LM. An antagonist of estrogen receptors blocks the induction of adult neurogenesis by insulin-like growth factor-I in the dentate gyrus of adult female rat. *European Journal of Neuroscience* 2003; **18:** 923–930.
14. Granholm AC, Moore AB, Bimonte-Nelson HA, *et al.* Abstract 323.4. A high-fat, high-cholesterol diet impairs working memory in middle-aged rats. Society for Neuroscience 34th Annual Meeting, San Diego, CA, USA.
15. Wu A, Molteni R, Ying Z, Gomez-Pinilla F. A saturated-fat diet aggravates the outcome of traumatic brain injury on hippocampal plasticity and cognitive function by reducing brain-derived neurotrophic factor. *Neuroscience* 2003; **119:** 365–375.
16. van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. *Nature* 2002; **415:** 1030–1034.
17. Gould E, Tanapat P. Stress and hippocampal neurogenesis. *Biological Psychiatry* 1999; **46:** 1472–1479.
18. Nilsson M, Perfilieva E, Johansson U, Orwar O, Eriksson PS. Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. *Journal of Neurobiology* 1999; **39:** 569–578.
19. Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ. Learning enhances adult neurogenesis in the hippocampal formation. *Nature Neuroscience* 1999; **2:** 260–265.
20. Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E. Neurogenesis in the adult is involved in the formation of trace memories. *Nature* 2001; **410:** 372–376.
21. Asensio C, Muzzin P, Rohner-Jeanrenaud F. Role of glucocorticoids in the physiopathology of excessive fat deposition and insulin resistance. *International Journal of Obesity and Related Metabolic Disorders* 2004; **28**(Suppl. 4)**:** 45–52.