Research Article
Volume 07:03, 2024

Journal of Biomedical and Pharmaceutical Sciences

ISSN: 2952-8100 Open Access

Fatty Acid-binding Protein 3 Knockout Mice Ameliorate High-fat Diet-induced Pain Exacerbation

Dan Tachibana, Kazuo Nakamoto, Shogo Tokuyama*

Department of Clinical Pharmacy, School of Pharmaceutical Sciences, Kobe Gakuin University, Kobe, Japan

Abstract

Objective: Obesity is associated with the exacerbation of pain. Recently, it was reported that High-Fat Diet (HFD)-induced obese mice show mechanical hypersensitivity after surgery, which causes neuroinflammation *via* microglial activation in the hypothalamus. However, the mechanism by which HFD-induced obesity exacerbates pain remains unclear. The Fatty Acid-Binding Protein (FABP) 3 belongs to a family of proteins that transports fatty acids into the cell and modulates cellular functions. We demonstrated that FABP3 was increased in the hypothalamus of postoperative pain mice, and was co-expressed in microglia. FABP3 has a high affinity for saturated Fatty Acids (FAs) and n-6 polyunsaturated FAs in HFD-fed mice, and its function is affected by different types of FAs. Here, we tested whether FABP3 is involved in the mechanism of obesity-induced pain exacerbation through microglial regulation.

Methods: C57/BL6J wild-type (WT) and FABP3 knockout (FABP3KO) mice were used in this study. These mice were fed a Control Diet (CD) or a High-Fat Diet (HFD) for eight weeks. Post-operative pain model mice were created by paw incision. Mechanical hypersensitivity was assessed using the von Frey test. Microglial expression and perimeters were analyzed using Iba-1.

Results: Wild-Type (WT) mice fed a HFD (WT/HFD) showed continuous mechanical hypersensitivity for seven days after surgery compared to WT mice fed a CD. FABP3KO mice fed a HFD (FABP3/HFD) showed a significantly reduced response time to mechanical stimuli compared to WT/HFD and recovered mechanical hypersensitivity seven days after surgery. WT/HFD mice showed increased microglial expression and morphological hypertrophy of cells with an increase in their perimeter in the median eminence of the hypothalamus seven days after surgery, whereas these changes were not observed in FABP3/HFD mice.

Conclusion: Our results showed that the deficiency of FABP3 may suppress HFD-induced pain exacerbation by regulating the hypothalamic microglia, indicating that FABP3 may be a therapeutic target for obesity-induced pain exacerbation.

Keywords: FABP3 • Obesity • Post-operative pain • Hypothalamus • Median eminence • Microglia • Iba-1

Introduction

Obesity is a health problem in modern society worldwide and causes a variety of lifestyle-associated diseases, including diabetes and cardiovascular diseases [1]. According to global data from the World Health Organization, approximately 1.9 billion adults are overweight, and approximately 650 million adults are obese. Growing evidence has suggested that obesity is a risk factor for chronic pain. For example, clinical studies have shown that obese patients with a higher body mass index show greater susceptibility to chronic pain [1,2], and obesity increases the risk of developing chronic pain after motor vehicle collision [3]. Similarly, basic research has reported that long-term High-Fat Diet (HFD)-induced obese mice show mechanical hypersensitivity and persistent pain after surgery [4,5], However, the mechanism underlying obesity-induced pain exacerbation remains unknown.

Microglia are immune cells of the central nervous system [6,7] and are known to be key players in chronic pain, which is activated after nerve injury [8]. Recently, it was reported that HFD-feeding induces microglial activation in the hypothalamus and causes neuroinflammation in the early phase of feeding [9]. Microglia is directly activated by HFD-derived saturated fatty acids, such as palmitic acid and stearic acid, and n-6 polyunsaturated fatty acids, such as linoleic acid, which is a large component of HFD [10]. Activated microglia secrete inflammatory cytokines, chemokines, and nociceptive modulatory molecules such as brain-derived neurotrophic factors, which are released in response to peripheral inflammation [11,12]. Transports of dietary-derived fatty acids from peripheral to the central nervous system are mainly mediated by Fatty Acid Binding Proteins (FABPs) or fatty acid transporter [13].

FABPs transport fatty acids into the cytosol and contribute to the regulation of gene expressions [14]. Nine subtypes of FABPs have been identified, and it has been reported that three types of FABPs (FABP3, FABP5, and FABP7) are generally expressed in neurons and glial cells of the brain [14]. Several studies have reported a relationship between FABP level and pain. For example, intraperitoneal injections of FABP3/FABP5/FABP7 inhibitors have demonstrated antinociceptive effects in a complete Freund's adjuvant mouse model [15]. Additionally, FABP5 knockout or FABP5/FABP7 double knockout mice have demonstrated antinociceptive effects against inflammatory pain [16,17]. We also reported that FABP3 protein levels increased in the median eminence of the hypothalamus in postoperative pain model mice, and this increment improved to baseline levels as pain recovered [18]. Moreover, we found that hypothalamic FABP3 is co-expressed in microglia [18]. Thus, in this study, we tested whether FABP3 is involved in the mechanism of obesity-induced pain exacerbation through microglial regulation in FABP3 knockout (FABP3KO) mice.

***Address for Correspondence:** Shogo Tokuyamaa, Department of Clinical Pharmacy, School of Pharmaceutical Sciences, Kobe Gakuin University, Kobe, Japan, Tel: +81-78-974-1551; Fax: +81-78-974-4780, E-mail: stoku@pharm.kobegakuin.ac.jp*

Received: 22 February, 2024, Manuscript No. jbps-24-128032; **Editor assigned:** 23 February, 2024, Pre QC No. P-128032; **Reviewed:** 06 March, 2024, QC No. Q-128032; **Revised:** 12 March, 2024, Manuscript No. R-128032; **Published:** 19 March, 2024, DOI: 10.37421/2952-8100.2024.7.435

Materials and Methods

Animals

All animal experiments were conducted in accordance with the guideline (Principles for the Care and Use of Laboratory Animals adopted by the Japanese Pharmacological Society) and the ARRIVE guidelines. Animal care and use were approved by the Ethical Committee for Animal Experimentation at the Kobe Gakuin University (approval number: A23–35). C57/BL6J Wild-Type (WT) male mice (eight-weeks-old) were obtained from Japan SLC, Inc. FABP3KO mice (C57BL/6 background) were kindly provided by Dr. Yuji Owada. Mice were maintained as homozygous genotypes and were maintained in cages with a 12-hours light/dark cycle at 24 °C, and water was available ad libitum. Eight-week-old mice were fed with free access either a Control Diet (CD, 10% kcal fat, D12450J, Research Diet, Inc.) or a High-Fat Diet (HFD, 60% kcal fat, D12492, Research Diet, Inc.) for eight weeks. Body weight and food intake were measured twice a week and analyzed weekly.

Post-operative pain

Mice were deeply anesthetized using three types of mixed anesthesia, consisting of medetomidine (0.75 mg/kg, Nippon Zenyaku Kogyo, Co., Ltd.), midazolam (4 mg/kg, Maruishi Pharmaceutical Co., Ltd.), and butorphanol (5 mg/mL, Meiji Animal Health, Co., Ltd.). The plantar surface of the right hind paw was disinfected using 10% povidone-iodine. A 5 mm longitudinal incision was made through the skin and fascia of the plantar region using a number 11 blade. The plantaris muscle was elevated ten times and longitudinally incised five times. The skin was closed using single 6-0 nylon sutures. The sham group received only three types of mixed anesthesia. At the end of surgery, atipamezole (0.75 mg/kg, Nippon Zenyaku Kogyo Co., Ltd.) was administered to the animals. All drugs were dissolved in saline solution.

RNA extraction and quantitative real-time PCR

Total RNAs was extracted from the hypothalamic tissues using the RNeasy Mini Kit (Qiagen, LI, NL), following the manufacturer's instructions. Total RNA was quantified using a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Complementary DNA was synthesized from RNA using the Prime Script RT Reagent Kit (Takara Bio, Shiga, Japan) following the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was performed to evaluate the expression of the target genes using the SYBR Green PCR Master Mix (Roche Diagnostics, Basel, Swiss Confederation) and a LightCycler 96 system (Roche Diagnostics, Basel, Swiss Confederation). PCR reactions were performed under the following conditions: initial activation 95 °C for 5 minutes, followed by 40 cycles of denaturation at 95 °C for 10 seconds, annealing at 60 °C for 20 seconds and extension at 72 °C for 20 seconds. Relative mRNA expression levels were determined using the 2-△△CT method, and the results were normalized to the 18S gene. The primer sequences used are listed in Table 1.

Immunofluorescent staining

Mice were transcranially perfused with 0.9% NaCl saline solution, followed by 4% Paraformaldehyde (PFA). The brains were post-fixed with PFA, replaced with 30% sucrose/1 × Phosphate-Buffered Saline (PBS) overnight, and embedded in Optimal cutting temperature compound (Thermo Fisher Scientific Waltham, MA, USA). Using a cryostat, 30 µm coronal brain sections containing the median eminence were cut. Subsequently, sections were incubated with 4% PFA, washed with PBS containing 0.1% Tween-20 (PBST), and blocked at room temperature for 1 h in blocking buffer (3% bovine serum albumin [BSA] in PBS), followed by overnight incubation with a ionized calcium-binding adaptor molecule 1 (Iba-1) antibody (1:1000, rabbit polyclonal antibody, 019-19741, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) in 3% BSA containing PBS and Triton X-100. The following day, the sections were washed with PBST and incubated with Alexa Fluor® 488 conjugated secondary antibodies (1:200, Alexa Fluor 488 donkey anti-rabbit IgG antibody, A21206, Thermo Fisher Scientific, Waltham, MA, USA). The area and perimeter of Iba-1 positive cells in the region, defined as the median eminence, were analyzed using the ImageJ software (National Institutes of Health).

Statistical analysis

Data are presented as the mean ± SEM. The two groups were compared using unpaired two-tailed Student's t-test. For more than three groups, two-way ANOVA was used, followed by Bonferroni's post-hoc test. All analyses were performed using GraphPad Prism version 7.0 (GraphPad Software, Inc.). $p < 0.05$ was considered significant.

Results

The body weight of WT mice fed the HFD significantly increased six weeks after feeding compared with that of WT mice fed the CD diet. FABP3 mice fed the HFD showed significant weight gain at three weeks after feeding compared with WT mice fed the CD. Weight gain continued until eight weeks after feeding. In addition, the HFD of FABP3KO mice significantly increased five weeks after feeding compared to that of FABP3KO mice fed the CD. Weight gain continued until eight weeks after feedings. There were no significant differences between the WT and FABP3KO groups of mice fed the HFD (Figure 1a). The total food intake of WT and FABP3KO mice fed the HFD during the test was significantly lower than that of WT and FABP3KO mice fed the CD (Figure 1b).

The WT mice fed the CD showed significantly increased responses to mechanical stimuli of the ipsilateral hind paw one day after surgery, which lasted four days. The paw withdrawal threshold returned to baseline levels

Table 1. Sequences of primers used for quantitative real time polymerase chain reaction.

Iba-1	Forward	5′-CTTGAAGCGAATGCTGGAGAA-3′
	Reverse	5′-GGCAGCTCGGAGATAGCTTT-3′
18S	Forward	5′-GTAACCCGTTGAACCCCATT-3′
	Reverse	5′-CCATCCAATCGGTAGTAGCG-3′

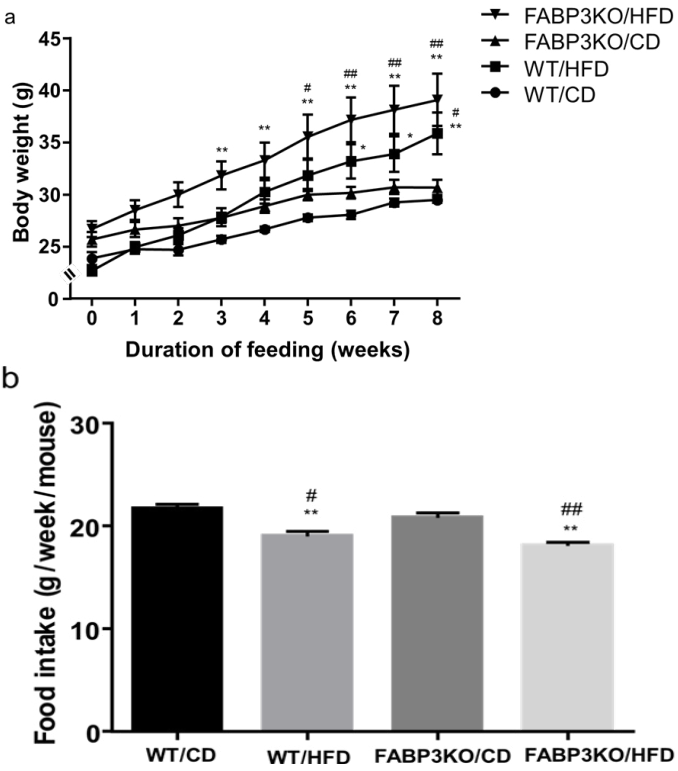

Figure 1. Effects of high-fat diet (HDF) on body weight and food intake in wild-type (WT) or FABP3 knockout (FABP3KO) mice **a.** Body weight: Body weight was measured for eight weeks in WT or FABP3KO mice fed CD or HFD. Two-way ANOVA with Bonferroni's multiple comparison post hoc test. Data are present as the mean ±standard error of the mean (SEM). WT/CD, n=5, WT/HFD mice, n=6, FABP3KO/CD, n=4, FABP3KO/HFD, n=5, **p<0.01, *p<0.05 *vs.* WT/CD, ##p<0.01, #p<0.05 *vs.* FABP3KO/CD. **b.** Food intake: The food intake was analyzed per week on either CD or HFD in WT or FABP3KO mice. One-way ANOVA with Tukey's multiple comparison post hoc test. Data are present as the mean ±standard error of the mean (SEM). WT/CD, n=8, WT/HFD mice, n=8, FABP3KO/CD, n=8, FABP3KO/HFD, n=8, **p<0.01 *vs.* WT/CD, ##p<0.01, #p<0.05 *vs.* FABP3KO/CD.

seven days after surgery. In contrast, WT mice fed the HFD showed a significantly increased number of withdrawal responses to mechanical stimuli that continued up to seven days after surgery compared to WT mice fed the CD. In sham mice fed CD or HFD, the withdrawal response time was quite low (Figure 2).

WT mice fed the HFD showed long-lasting mechanical allodynia for up to seven days compared to WT mice fed the CD, whereas FABP3KO mice fed the HFD recovered it at seven days after surgery. Response levels of WT and FABP3KO mice fed the CD showed the same low response as before surgery (Figure 3).

Iba-1 mRNA expression was significantly increased in the hypothalamus of WT mice fed the HFD compared to WT mice fed the CD before surgery and at three or seven days after surgery (Figure 4a), whereas in the median eminence of the hypothalamus, WT mice fed the HFD showed a significant increase in the area and perimeter of Iba-1 positive cells at seven days after surgery. On the other hand, the area and perimeter of Iba-1 positive cells did not change in FABP3KO mice fed HFD (Figure 4b–d).

Discussion

In this study, we examined whether mice with HFD-induced obesity had an exacerbation in postoperative pain. First, mice were fed the HFD (60% kcal fat) ad libitum to induce obesity. Mice fed the HFD gained significantly more weight until the end of the study, despite a decrease in food intake. Generally, body weight is maintained by a balance between energy intake and expenditure. Energy intake exceeds energy expenditure and causes obesity [19]. Thus, this

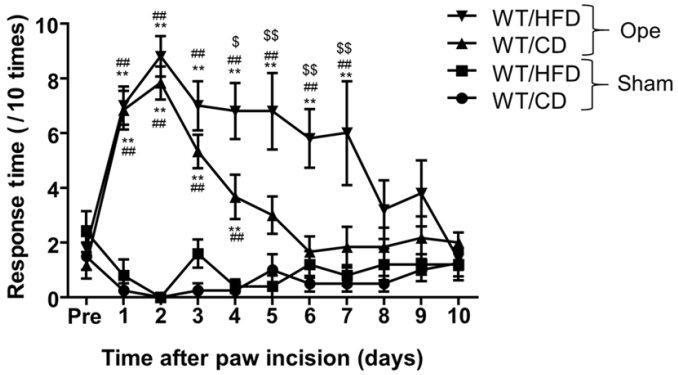

Figure 2. Time course of mechanical allodynia in the HFD-induced obesity mice. The von Frey test was conducted in four groups (WT/CD, sham, WT/HFD, sham, WT/CD, Ope, WT/HFD, and Ope) using 0.16 g filament. Two-way ANOVA with Bonferroni's multiple comparison post hoc test. Data are present as the mean ± SEM. WT/CD, sham, n=4, WT/HFD, sham, n=5, WT/CD, Ope, n=6, WT/HFD, Ope, n=5, $^{\#\#}$p<0.01, $^{\#}$p<0.05 *vs.* WT/HFD, sham.

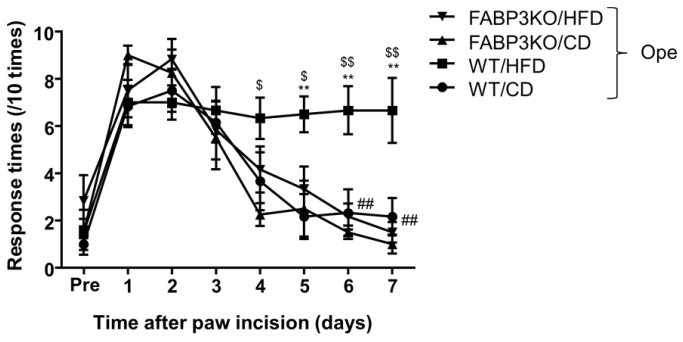

Figure 3. Time course of mechanical allodynia in the FABP3KO mice fed HFD. The von Frey test was conducted in four groups (WT/CD, Ope, WT/HFD Ope, FABP3KO/CD, Ope, FABP3KO/HFD, and Ope) using 0.16 g filament. Two-way ANOVA with Bonferroni's multiple comparison post hoc test. Data are present as the mean ± SEM. WT/CD, Ope, n=6, WT/HFD Ope, n=6, FABP3KO/CD, Ope, n= 4 FABP3KO/HFD, Ope, n=6, **p<0.01 *vs.* WT/CD Ope, $^{\#\#}$p<0.01 *vs.* WT/HFD, Ope. $^{\$\$}$p<0.01, $^{\$}$p<0.05 *vs.* FABP3KO/CD, Ope.

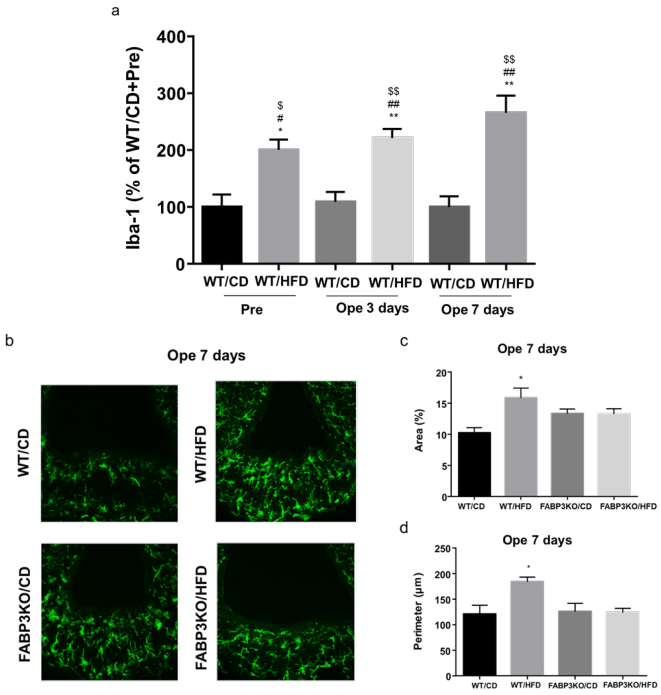

Figure 4. Analysis of microglial expression and morphology in the hypothalamus from HFD-induced obese mice. The expression levels of Iba-1 mRNA using qRT-PCR are shown **a.** Iba-1 mRNA expression in WT/CD and WT/HFD before surgery, and at three or seven days after surgery is shown in a. Data are compared by Tukey's multiple comparison test, and present as the mean ± SEM. WT/CD Pre, n=4, WT/CD Ope 3 days, n=5, WT/CD Ope 7 days, n=5, WT/CD Pre, n=5, WT/HFD Pre, n=6, WT/HFD Ope 3 day, n=6, WT/HFD Ope 7 day, n=6, *p<0.05, **p<0.01 *vs.* WT/CD Pre, $^{\#}$p<0.05, $^{\#\#}$p<0.01 *vs.* WT/CD Ope 3 days, $^{\$}$p<0.05, $^{\$\$}$p<0.01 *vs.* WT/CD Ope 7 days, Expression and morphological changes in Iba-1 positive cells in the median eminence using immunofluorescence staining are shown. **b-d.** WT/CD Ope 7 days, n=3, WT/HFD Ope 7 days, n=3, FABP3KO/CD Ope 7 days, n=3, FABP3KO/HFD Ope 7 days, n=3. *p<0.01 *vs.* WT/CD Ope 7 day, Scale bar = 50μm.

is thought to be a result of the higher caloric content in the HFD relative to the CD, such that the amount of energy intake exceeded its expenditure in mice. The early weight gain observed in FABP3KO mice fed the HFD might be due in part to the defective regulation of energy balance by FABP3. In the FABP3KO, the metabolism of fatty acids and glucose, which are energy sources in the body, is altered [20,21]. After HFD feeding for eight weeks, HFD-induced obese mice showed continuous hypersensitivity to mechanical stimuli for seven days after surgery. These results suggest that mice fed the HFD have a prolonged recovery from postoperative pain, that is, their pain is worse. Our results are consistent with previous reports that HFD-induced obesity worsens pain in a rodent pain model [22-24]. In contrast, Lian N, et al. reported that mice fed the HFD (60% kcal for fat) alone for eight weeks caused mechanical allodynia [4], but in this study, no threshold reduction was observed in mice fed the HFD alone. This discrepancy may be due to the differences in the assessment methods used in the von Frey test. However, we emphasize the importance of the observation that HFD-induced obese mice exhibit pain exacerbation.

Next, to determine whether FABP3 is involved in the exacerbation of pain in obesity, we analyzed the pain behavior in FABP3KO mice. FABP3KO mice fed the HFD had a lower number of reactions than WT mice fed the HFD; that is, postoperative pain-induced mechanical hypersensitivity was suppressed by the deletion of FABP3. These results suggest that FABP3 is partially involved in pain regulation. Finally, to elucidate the mechanism of HFD-induced pain exacerbation, we focused on the changes in microglial expression in the hypothalamus. In fact, microglia in the hypothalamus are activated by HFD [25,26]. One possible mechanism is that saturated fatty acids or n-6 polyunsaturated fatty acids in HFD bind to Toll-like receptor 4, which is expressed on microglia and induces microglial activation [27,28]. Generally, microglial activation can be assessed by the upregulation of Iba-1 expression and morphological changes [29]. In this study, an increase in Iba-1 expression and its perimeter was observed in the hypothalamus of WT mice

fed a HFD, suggesting that WT mice fed the HFD may indeed activate the hypothalamic microglia. However, HFD-induced microglial activation was not observed in FABP3KO mice fed the HFD at postoperative day seven. These results suggest that FABP3 plays a role in the regulation of the hypothalamic microglia.

More recently, Low YL, et al. reported that the microglial cell line BV2 expresses FABP3, -4, and -5 [30]. Kagawa Y, et al. reported that microglial FABP4 is involved in the regulation of neuroinflammation caused by lipopolysaccharide-induced microglial activation [31]. Therefore, we cannot exclude the possibility that other FABPs are involved in the regulation of microglial expression, resulting in pain suppression. A limitation of this study is that we used whole-body knockout mice for FABP3, as we could not determine whether microglial FABP3 is involved in the regulation of its own activation. In addition, it remains unclear whether the secondary effects caused by obesity-induced microglial activation result in pain exacerbation or how fatty acids in HFD are taken up into the brain and induce pain exacerbation.

Conclusion

In conclusion, we demonstrated that HFD-induced obese mice showed delayed recovery from postoperative and worsened pain. In addition, FABP3KO mice fed the HFD showed attenuated pain exacerbation. Our findings suggest that FABP3 in the hypothalamus is involved, at least in part, in exacerbation of HFD-induced pain through microglial regulation.

Acknowledgment

We would like to thank Dr. Yuji Owada for providing the FABP3KO mice and Editage (www.editage.com) for English language editing.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Eichwald, Tuany and Sebastien Talbot. "Neuro-immunity controls obesity-induced pain." *Front Hum Neurosci* 14 (2020): 181.
2. Chin, Shao-Hua, Wei-Lin Huang, Sharmin Akter and Martin Binks. "Obesity and pain: A systematic review." *Int J Obes* 44 (2020): 969-979.
3. Mauck, Matthew C., JunMei Hu, Christopher Sefton and Robert A. Swor, et al. "Obesity increases the risk of chronic pain development after motor vehicle collision." *Pain* 160 (2019): 670-675.
4. Lian, Nan, Kaiteng Luo, Huijing Xie and Yi Kang, et al. "Obesity by high-fat diet increases pain sensitivity by reprogramming branched-chain amino acid catabolism in dorsal root ganglia." *Front Nutr* 9 (2022): 902635.
5. Guillemot-Legris, Owein, Baptiste Buisseret, Valentin Mutemberezi and Emmanuel Hermans, et al. "Post-operative pain in mice is prolonged by diet-induced obesity and rescued by dietary intervention." *Brain Behav Immun* 74 (2018): 96-105.
6. Butovsky, Oleg and Howard L. Weiner. "Microglial signatures and their role in health and disease." *Nat Rev Neurosci* 19 (2018): 622-635.
7. Vidal-Itriago, Andrés, Rowan AW Radford, Jason A. Aramideh and Cindy Maurel, et al. "Microglia morphophysiological diversity and its implications for the CNS." *Front Immunol* 13 (2022): 997786.
8. Kohno, Keita, Ryoji Shirasaka, Kohei Yoshihara and Satsuki Mikuriya, et al. "A spinal microglia population involved in remitting and relapsing neuropathic pain." *Science* 376 (2022): 86-90.
9. Jais, Alexander and Jens C. Brüning. "Hypothalamic inflammation in obesity and metabolic disease." *J Clin Invest* 127 (2017): 24-32.
10. Christ, Anette, Mario Lauterbach and Eicke Latz. "Western diet and the immune system: An inflammatory connection." *Immunity* 51 (2019): 794-811.
11. Chen, Gang, Yu-Qiu Zhang, Yawar J. Qadri and Charles N. Serhan, et al. "Microglia in pain: Detrimental and protective roles in pathogenesis and resolution of pain." *Neuron* 100 (2018): 1292-1311.
12. Valdearcos, Martin, John D. Douglass, Megan M. Robblee and Mauricio D. Dorfman, et al. "Microglial inflammatory signaling orchestrates the hypothalamic immune response to dietary excess and mediates obesity susceptibility." *Cell Metab* 26 (2017): 185-197.
13. Maekawa, Motoko, Yoshimi Iwayama, Tetsuo Ohnishi and Manabu Toyoshima, et al. "Investigation of the fatty acid transporter-encoding genes SLC27A3 and SLC27A4 in autism." *Sci Rep* 5 (2015): 16239.
14. Storch, Judith and Betina Corsico. "The multifunctional family of mammalian fatty acid–binding proteins." *Annu Rev Nutr* 43 (2023): 25-54.
15. Yan, Su, Matthew W. Elmes, Simon Tong and Kongzhen Hu, et al. "SAR studies on truxillic acid mono esters as a new class of antinociceptive agents targeting fatty acid binding proteins." *Eur J Med Chem* 154 (2018): 233-252.
16. Kaczocha, Martin, Sherrye T. Glaser, Thomas Maher and Brendan Clavin, et al. "Fatty acid binding protein deletion suppresses inflammatory pain through endocannabinoid/N-acylethanolamine-dependent mechanisms." *Mol Pain* 11 (2015): s12990-015.
17. Bogdan, Diane M., Keith Studholme, Adriana DiBua and Chris Gordon, et al. "FABP5 deletion in nociceptors augments endocannabinoid signaling and suppresses TRPV1 sensitization and inflammatory pain." *Sci Rep* 12 (2022): 9241.
18. Tachibana, Dan, Kazuo Nakamoto and Shogo Tokuyama. "Changes in median eminence of fatty acid–binding protein 3 in a mouse model of pain." *Neuropsychopharmacol Rep* 42 (2022): 52-58.
19. Binder, Elke, Francisco J. Bermúdez-Silva, Caroline André and Melissa Elie, et al. "Leucine supplementation protects from insulin resistance by regulating adiposity levels." *PloS one* 8 (2013): e74705.
20. Binas, B. E. R. T., Heike Danneberg, J. I. M. McWhir and Linda Mullins, et al. "Requirement for the heart-type fatty acid binding protein in cardiac fatty acid utilization." *FASEB J* 13 (1999): 805-812.
21. Shearer, Jane, Patrick T. Fueger, Jeffrey N. Rottman and Deanna P. Bracy, et al. "Heart-type fatty acid-binding protein reciprocally regulates glucose and fatty acid utilization during exercise." *Am J Physiol Endocrinol Metab* 288 (2005): E292-E297.
22. Loredo-Pérez, Aleyda A., Carlos E. Montalvo-Blanco, Luis I. Hernández-González and Maricruz Anaya-Reyes, et al. "High-fat diet exacerbates pain-like behaviors and periarticular bone loss in mice with CFA-induced knee arthritis." *Obesity* 24 (2016): 1106-1115.
23. Song, Zongbin, Wenrui Xie, Sisi Chen and Judith A. Strong, et al. "High-fat diet increases pain behaviors in rats with or without obesity." *Sci Rep* 7 (2017): 10350.
24. Song, Zongbin, Wenrui Xie, Judith A. Strong and Temugin Berta, et al. "High-fat diet exacerbates postoperative pain and inflammation in a sex-dependent manner." *Pain* 159 (2018): 1731-1741.
25. Valdearcos, Martin, Megan M. Robblee, Daniel I. Benjamin and Daniel K. Nomura, et al. "Microglia dictate the impact of saturated fat consumption on hypothalamic inflammation and neuronal function." *Cell Rep* 9 (2014): 2124-2138.
26. Baufeld, Caroline, Anja Osterloh, Stefan Prokop and Kelly R. Miller, et al. "High-fat diet-induced brain region-specific phenotypic spectrum of CNS resident microglia." *Acta Neuropathol* 132 (2016): 361-375.
27. Wang, Zhen, Dexiang Liu, Fuwu Wang and Shangming Liu, et al. "Saturated fatty acids activate microglia *via* Toll-like receptor 4/NF-κB signalling." *Br J Nut* 107 (2012): 229-241.
28. Milanski, Marciane, Giovanna Degasperi, Andressa Coope and Joseane Morari, et al. "Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: Implications for the pathogenesis of obesity." *J Neurosci* 29 (2009): 359-370.
29. Yi, Min-Hee, Yong U. Liu, Anthony D. Umpierre and Tingjun Chen, et al. "Optogenetic activation of spinal microglia triggers chronic pain in mice." *PLoS Biol* 19 (2021): e3001154.
30. Low, Y. L., Y. Pan, J. L. Short and J. A. Nicolazzo. "Profiling the expression of fatty acid-binding proteins and fatty acid transporters in mouse microglia and assessing their role in docosahexaenoic acid-d5 uptake." *Prostaglandins Leukot Essent Fatty Acids* 171 (2021): 102303.

31. Kagawa, Yoshiteru, Yi Ling Low, Jae Pyun and Umberto Doglione, et al. "Fatty acid-binding protein 4 is essential for the inflammatory and metabolic response of microglia to lipopolysaccharide." *J Neuroimmune Pharmacol* 18 (2023): 448-461.

How to cite this article: Tachibanaa, Dan, Kazuo Nakamotoa and Shogo Tokuyamaa. "Fatty Acid-binding Protein 3 Knockout Mice Ameliorate High-fat Diet-induced Pain Exacerbation." *J Biomed Pharm Sci* 07 (2024): 435.