Short-Term High-Fat Diet Promotes Increased Lysine Crotonylation in Cerebral Cortex

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Short Report

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Abstract

Protein lysine crotonylation is a newly discovered protein post-translational modification (PTM), which has been associated with cellular metabolism, cell cycle, gene transcription, DNA damage response. However, its potential roles related to human central nervous system diseases remain largely unknown. In the present study, we observed a significant elevated lysine crotonylation in a screening of nine lysine acylations in cortex tissues of HFD-fed mice after short-term overfeeding. On the base of previous reports and molecular weight of proteins, we also speculate that actin, ERK2 or GAPDH and CDK1 might be modified by lysine crotonylation (KCr). Taken together, our findings highlight a potential role of protein lysine crotonylation in HFD-induced brain disorders and as possible therapeutic candidates in the future.

Introduction

In living organisms and individual cells, post-translational modifications (PTMs) of proteins are crucial for regulation diverse cellular functions, such as DNA replication, transcription, tissue differentiation, apoptosis, inflammation and so on (Berdasco and Esteller, 2010; Lee, 2013). To date, more than 500 discrete types of PTMs across all 20 protein amino acids have been identified (Keenan et al., 2021). In addition to extensive studies of common PTMs, like phosphorylation and acetylation (Acetyllysine, KAc) (Verdin and Ott, 2015), several novel protein acetylation, such as, Lysine malonylation (Malonyllysine, KMal) (Xie et al., 2012), Lysine succinylation (Succinyllysine, KSu) (Xie et al., 2012), Lysine lactylation (Lactyllysine, KLa) (Qin et al., 2019), Lysine propionylation (Propionyllysine, KPr) (Chen et al., 2007), Lysine butyrylation (Butyryllysine, KBu) (Chen et al., 2007), Lysine β-hydroxybutyrylation (β-hydroxybutyryllysine, KHb) (Xie et al., 2016), Lysine glutarylation (Glutaryllysine, KGl) (Tan et al., 2014) and Lysine crotonylation (Crotonyllysine, KCr) (Tan et al., 2011) have been discovered in recent years. Moreover, these acylations have been associated with cellular metabolism (Sabari et al., 2017). Among aforementioned lysine acylations, KCr is involved in the diverse physiopathologic processes of some diseases, such as, neuropsychiatric disorder, acute kidney injury, mouse spermatogenesis, Alzheimer's disease (AD) and cancers (Liu et al., 2017; Sun et al., 2020; Wan et al., 2019; Wang et al., 2019b). Although Lysine crotonylome reveals many studies of histone crotonylation and non-histone crotonylation (Wu et al., 2017), however, whether the levels of protein KCr could be affected in HFD-induced diseases have not yet been fully addressed.

High-fat diet (HFD), known as the western diet, has been associated with neuroinflammation, apoptosis, necrosis and cognitive deficits in distinct brain regions (Keshk et al., 2020; Pistell et al., 2010). Recent studies indicates that protein acylation modifications associated with HFD and obesity in brain mainly focus on acetylation (Cai et al., 2020; Chen et al., 2015; Gonçalves et al., 2017). On the other hand, while many studies about lysine crotonylation have been reported since 2011, the influence of short-term HFD on protein KCr in cerebral cortex remains relatively under explored. In this study, our preliminary research shows that the total KCr levels of cerebral cortex among nine types of lysine acylations are significantly increased after 7 and 21 days HFD compared with chow diet. Meanwhile, current results highlight that KCr modification might become a potential intervention target to fight against HFD-related brain diseases.

Materials And Methods

Reagents

Congo red (C6767) and Coomassie Blue Fast Staining Solution (P0017) were purchased from Sigma-Aldrich and Beyotime Biotechnology respectively. All antibodies used in this study are listed in the Key Resources Table 1.

Animals, High-fat diet

C57BL/6J wild type male mice (8-weeks old) were provided by Gem Phar ma tech Co., Ltd (Nanjing, China). Low fat and no sugar Chow diet (TP23100) and High fat diet (HFD, TP23103, ~45% of energy) were provided by Trophic Animal Feed High-tech Co., Ltd (Nantong, China). After 7 days adaptation, mice were randomly divided into three groups (n=3 mice/group). Chow diet groups were served as controls. Other two groups were fed with HFD for 7 days and 21days respectively. Mice were subsequently humanely sacrificed under sodium pentobarbital anesthesia. Cerebral cortex was collected and homogenized in RIPA lysis buffer (P0013B). The homogenized tissues were centrifuged at 13,000 rpm for 30min and the supernatants were kept for later steps. Mouse studies were reviewed and approved by the Committee Guide of Wenzhou Medical University (ethical number 2019-75; Wenzhou, China.). All mice were maintained on a 12 hours light-dark cycle and temperature 24°C–25°C with free access to water and food.

Western blot analysis, Coomassie Blue and Congo red Staining

Western blot, Coomassie blue and Congo red analysis were carried out as described by us (Wang et al., 2021; Wang et al., 2020; Xu et al., 2019). Briefly, proteins (15μg) were separated on 5~10% Tris–glycine SDS–PAGE gels and transferred onto nitrocellulose membranes. The membranes were then used to perform Congo red staining. Meanwhile, the gels were stained by Coomassie blue. In addition, membranes were probed with primary acyllysine-specific antibodies directed against KMal, KSu, KLa, KPr, KBu, KHb, KGl, KAc and KCr. Following 3 washes of 10 min each with TBST, a HRP conjugated secondary antibody was used in immunoassays, and then membranes were automated imaged. Subsequently, the membranes were stripped with Beyotime Stripping Buffer (P0025). Then anti-vinculin antibody were run on the Nitrocellulose western blot.

Statistical analysis

All data were reported as Mean ± SD (standard deviation). All statistical analyses were performed using GraphPad software (GraphPad Prism version 8.00, San Diego, CA). Differences between *two groups* were tested by unpaired two-tailed Student's t-test. The significance level were set when *P < 0.05.

Results

Short-term HFD feeding increased crotonyllysine levels in mouse cerebral cortex

To investigate the changes of protein lysine acylations in brain after 21 days short-term HFD, nine types of lysine acylations were detected by western blotting in mouse cerebral cortex lysates. We routinely loaded 15μg total protein onto each line in chow diet and HFD groups, then equal loading was verified by Congo red Nitrocellulose staining and Coomassie blue gels staining (Fig. 1A, B). Subsequently, we observed no changes of KMal, KSu, KLa, KPr, KBu, KAc, KHb and KGl in HFD groups compared to chow diet (Fig. 1C-J). Furthermore, although KLa in one group of HFD decreased remarkably, but no significant changes were observed in cerebral cortex of HFD compared to chow diet mice (Fig. 1F). Together, our results suggest that 21 days HFD does not regulate the aforementioned eight modifications of lysine acylations in mouse cerebral cortex. However, we found occasionally that significant elevation of KCr was observed in HFD groups after 21 days overfeeding compared to chow diet ($P < 0.01$, Fig. 1K-L). In addition, to explore whether the increase of KCr levels also could occur at day 7 after HFD, we detected protein KCr and observed significant elevation of KCr expressions in HFD groups compared to chow diet ($P < 0.05$, Fig. 1M-N). In conclusion, our research indicate that KCr elevation might play major roles in multiple biological processes in mouse cerebral cortex exposed to short-term HFD.

Discussion

In the current study, we analyzed the nine types of acylation patterns in chow diet and HFD mice after short-term overfeeding. We observed an elevation of protein KCr levels in cerebral cortex tissues of chow diet and HFD animal models. Yet other eight acylation categories, such as, KMal, KSu, KLa, KPr, KBu, KAc, KHb and KGl did not demonstrate significantly changes in HFD groups compare with chow diet. Finally, we hypothesize that brain cortex-specific elevation of KCr might be common in short-term HFD.

Regular mechanisms of KCr modification are involved in Lysine crotonyltranferase (KCTs, writer), decrotonylase (KDCRs, eraser) and reader. Currently, KCTs are grouped into three major families: P300/CBP (p300/CREB-binding protein), MYST (Moz, Ybf2, Sas2, Tip60), and GNAT (GCN5-related N-acetyltransferase) in metazoans (Qin et al., 2019). In addition, KDCRs can be classfied into two major categories: HDAC I and HDAC III (Qin et al., 2019). Crotonate can not only be generated by colon microbial fermentation, but also is the short-chain fatty acid (SCFA) precursor of crotonyl-CoA (Stilling et al., 2016). Convertion of SCFA crotonate into crotonyl-CoA is mediated by Acyl-CoA synthetase short chain family member 2 (ACSS2) or other metabolic pathways, such as fatty acid β-oxidation pathway and the lysine degradation pathway (Sabari et al., 2015) (Lin et al., 2012). Currently, the data featured in the literatures show that KCr modifications are involved in the aberrant neuroanatomical structures of BTBR mice (Wang et al., 2019a), depressive disorder of male mice (Liu et al., 2019) in the central nervous system. At the same time, we also observed two increased KCr modifications bands adjacent to 40 and 35kDa at day 7 and 21 after HFD. Because of no available KCr antibody for immunoprecipitation, we cannot confirm the exact proteins modified by KCr in HFD groups. However, we speculated that KCr proteins

might be actin, ERK2 or GAPDH and CDK1, according to molecular weight of these proteins and a report from high-resolution liquid chromatography-tandem MS (LC-MS/MS) (Xu et al., 2017).

In summary, elevated KCr modifications in cerebral cortex may exert direct influence over regulating metabolism and other unknown functions in short-term HFD. Future work in this field should focus on the identification of specific proteins related to KCr modifications, and evaluate their roles whether these are protective or pathogenic responses in HFD-induced brain diseases.

Abbreviations

Malonyllysine: KMal, Succinyllysine: KSu, Lactyllysine: KLa, Propionyllysine: KPr, Butyryllysine: KBu, β-hydroxybutyryllysine: KHb, Glutaryllysine:KGl, Acetyllysine: KAc, Crotonyllysine: KCr, High-fat diet: HFD, Post-translational modifications: PTMs, short-chain fatty acid :SCFA, Acyl-CoA synthetase short chain family member 2 :ACSS2.

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

Wang Jun-Ling, Jia-Jia Zhang, Xiao-Lou Wang and Xu Chao-Jin analyzed the data; Xu Chao-Jin, and Wang Jun-Ling wrote the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

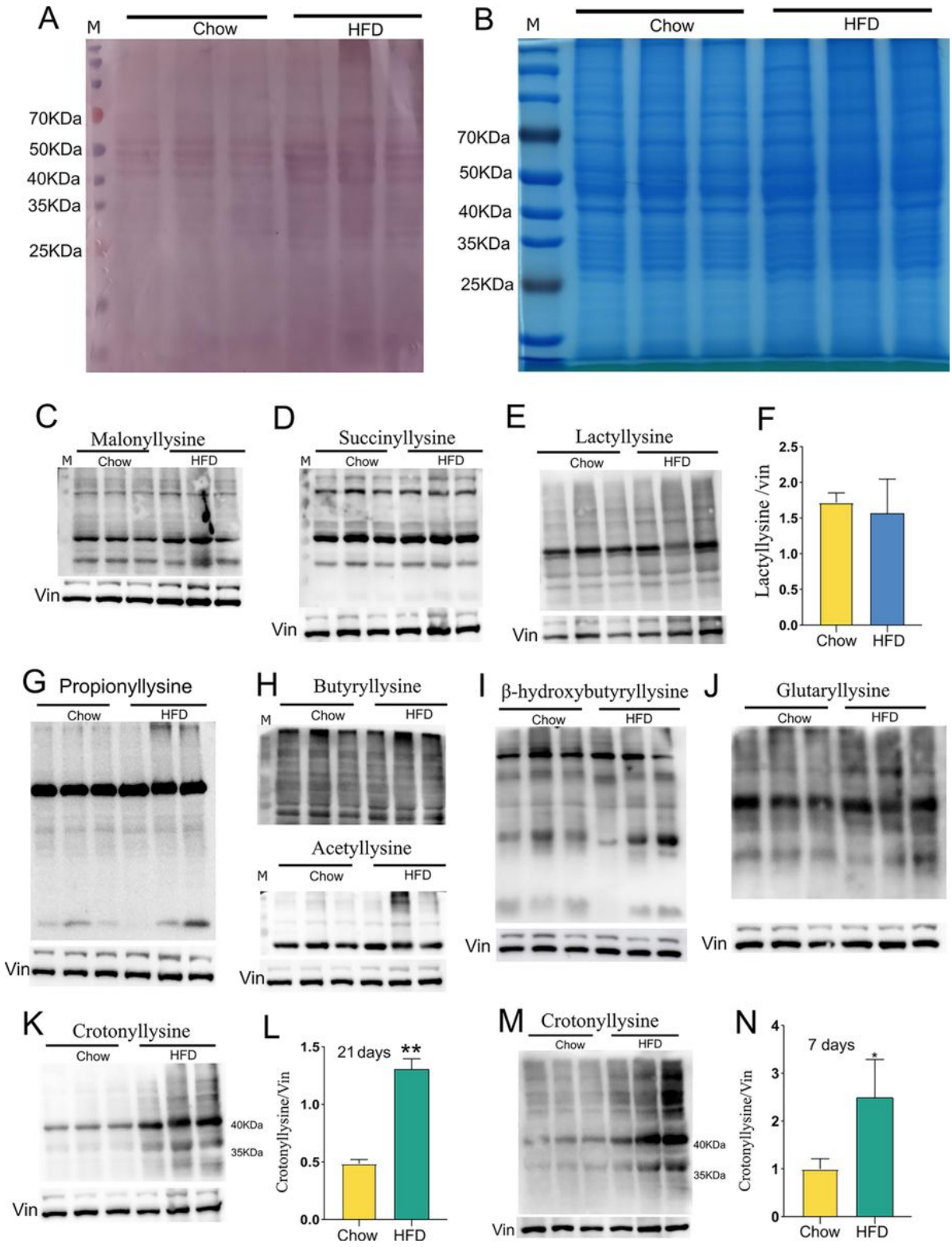

Figure 1

Nine lysine acylation changes in cerebral cortex under short-term chow diet and HFD feeding. Supernatants were collected from mice brain cortex fed by a chow diet (n=3 mice) and HFD for 7 days (n=3 mice) or 21 days (n=3 mice), which were followed by staining and blots. (A) Congo red Staining and (B) Coomassie blue staining serve as a loading control. (C-L) Western blots detecting of cortex protein acetylation with different acyllysine-specific antibodies after 21 days overnutrition (C) KMal, (D) KSu, and

(E) KLa. (F) Quantification of KLa in (E). (G) KPr, (H) KBu and KAc. (I) KHb, (J) KGl and (K) KCr. (L) Quantification of KCr in (K). (M) KCr at day 7. (N) Quantification of KCr in (M). Protein molecular weight markers are indicated on the left-hand side. The levels of acyllysine-specific related proteins were normalized to the levels of vinculin. Values are presented as means ±SD. *P < 0.05 and **P < 0.01 versus chow diet; Two-tailed Student's t test. M: Marker, Chow: Chow diet, HFD: High-fat diet, Vin: vinculin.

Supplementary Files

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- Table.1KeyResources.tif