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碩士班專題討論書面報告

探討白藜蘆醇、藍莓及 Metfotmin 在高脂飲食誘導的肥胖模式動物實驗其神經功能障礙及學習記憶能力的回復

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Improvements in Neuronal Dysfunction and Behavior using High-fat Diet induced Obesity Model

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Abstract

Obesity can be defined as a individual carrying too much body fat. For a person is considered obese if his Body Mass Index (BMI) is over 27. Obesity usually leads to a state of chronic inflammation that contributes to insulin resistance and T2DM, which also causes brain insulin resistance and neuronal dysfunction. 4 weeks old mice fed with high-fat diet (HFD) for 20 weeks can lead to obvious peripheral inflammation like increased serum TNF-α and leptin. Moreover, the neuronal inflammation condition would be enhanced by elevated TNF-α, microglia. More important, the insulin receptor-dependent AMPK signaling pathway would be inactivated, which can cause serious neuronal dysfunction and brain damages. Resveratrol, a nature polyphenol compound enriched in red grapes. HFD-mice fed with resveratrol for 20 weeks have a significant effect on anti-inflammation in both peripheral and neuronal systems. Then medicine (metformin) and whole food (blueberry) which have been prove to restore T2DM and decrease ROS level and oxidative stress. In metformin group, 5 weeks old rats fed with HFD for 12 weeks lead to insulin resistance condition. HFD group treat with metformin showed an obvious change in decreasing ROS level in brain mitochondrial; and in Morris water maze test, the performance was greatly improved in both acquisition and probe test. In blueberry (BB) diet, 9-month old mic were fed with HFD for 5 months, then took the Novel Object Recognition test. Results showed that the HFD mice treat with BB have better perform than the group without BB. In conclusion, obesity related cognitive impairments by neuronal insulin resistance, brain oxidative stress, and brain damages can be restored by treated with medicine, fruit extractions, or foods. We can also claim that making changes in our daily dietary

composition will be a good choice for health.

1. Introduction

肥胖是一個個體內類積過多脂肪的現象，以人來說，定義為當身體質量指標 (BMI) 超過 30 可稱之，肥胖也為已開發國家所面臨的主要問題，會導致許多併發症，如：高血壓、高血脂、心臟病、生殖障礙、痛風、第二型糖尿病及阿茲海默症等等 (Muhammad *et al*., 2009)，早先研究顯示，高脂飲食所誘導的動物實驗，除了有周邊胰島素阻抗外，也會引發神經性胰島素阻抗，此狀況在大鼠中會有導致認知和學習能力受損、增加腦內的氧化壓力引發連鎖反應等。

肥胖會導致一定程度的慢性發炎，肥胖個體脂肪組織中的 M1 巨噬細胞會增加，和脂肪組織聚合成大聚合體，分泌發炎因子如 TNF-α、IL-6 等等 (Moraes-Vieira *et al*., 2014)，使其在血液中的濃度提升，leptin 分泌量上升，adiponectin 下降，這些因素都會導致發炎反應發生，最後導致胰島素受器敏感度下降，發生胰島素阻抗，血液中胰島素濃度上升等；而腦內胰島素阻抗的成因是從周邊的脂肪組織因胰島素阻抗導致發炎反應加劇，IDE 活性下降使體內的 A-β (β-澱粉蛋白) 的濃度上升，使通道蛋白表現下降，血腦屏障功能受損，並使過量的 A-β穿入腦中，使腦中的胰島素濃度和敏感度下降，A-β亦會抑制胰島素接上腦內胰島素受器，導致腦內胰島素阻抗，此狀況又會使腦內的 A-β分泌量繼續上升，使整個情況加劇，A-β、p-Tau、GSK-3 activity、IL-6、TNF-α 等物質的量在腦內累積增加，使腦內發炎反應和氧化壓力持續提高，導致腦內神經和功能受損，進而影響到認知及學習功能，而這項路徑的也是造成阿茲海默症的主要原因，也加強了第二型糖尿病和阿茲海默症之間更深的關聯性 (Akomolafe A., *et al* 2006)。

所以本次主題的目的是要驗證在藥品、食品或是特定萃取物的飲食補充之下，進行高脂飲食下所誘導的糖尿病模式的大小鼠，是否真的會有腦內神經功能受損的狀況進而影響到其記憶和學習能力的表現，探討是否有修復的辦法；內在因子和外在表徵的綜合結果和探討其成效，用一篇動物細胞實驗和兩篇動物行為實驗來探討。

2. Resveratrol attenuates obesity-associated peripheral and central inflammation and improves memory deficit in mice fed a high-fat diet (Jeon *et al*., 2012)

白藜蘆醇 (Resveratrol, RES)，是一種天然的多酚植物素，在紅葡萄或紅酒中有很高的含量，各項實驗中證實可以改善許多慢性疾病，且用來廣泛利用在抗發炎和降低氧化壓力 (Fremont, 2000)，本篇嘗試了解白藜蘆醇除了有改善周邊發炎的效果外，是否具緩和中樞神經的發炎效果。實驗方式利用四週齡大的雄性 C67BL/6J mice 共 120 隻分成四組，分別為低脂飲食組 (LFD)、低脂飲食加白藜蘆醇組 (LFD + RES)、高脂飲食組 (HFD)、高脂飲食加白藜蘆醇組 (HFD + RES)，持續 20 週的飲食狀態，最後進行各項數據的採集及分析。

20 週內四組老鼠的體重變化可以看出在餵食高脂飲食下的組別體重的增加率特別明顯，然後也可以發現，在有餵食白藜蘆醇的組別 (HFD + RES) 並沒有能改善體重增加的效果，作者推測可能是劑量和餵食時間不夠等問題，但是在細胞實驗的數據上可以看出更多的差別(Fig. 1)。測量數據可分為周邊組織和腦神經組織的分析，在周邊組織分析中主要以血液內容濃度和肝細胞的分析，(Fig. 2)為使用酵素免疫分析法(ELISA)、葡萄糖耐受性測試 (GTTs) 及胰島素耐受性測試 (ITTs) 等指標在血液中的濃度變化，在 ELISA 分析中可以看出重要的發炎指標 adiponectin 的下降、leptim 的上升在 HFD 組都有和其他組明顯的差別，而在 HFD+RES 組別也都有顯著的改善現象 (Fig. 2A,2B)，然後也顯示在 HFD 組別中的血液中胰島素濃度明顯高過其他三組，代表其有顯著的胰島素阻抗現象，也證明餵食樣品組有改善的能力 (Fig. 2E)。GTTs (Fig. 2C) 是先在時間點零點時先抽血，接著利用注射方式餵食各組老鼠葡萄糖溶液，接著分別在 30 分、60 分、90 分、120 分這四個時間點幫所有老鼠抽血，最後分析這段時間內其血液中的葡萄糖濃度變化量，可了解其胰島素阻抗現象，若胰島素阻抗現象低，其對葡萄糖的利用率應該會較佳；從圖中可發現低脂飲食兩組的葡萄糖耐受性一樣良好，HFD 組的耐受性明顯的差很多，其葡萄糖濃度高，而 HFD + RES 組有明顯的下降趨勢。ITTs (Fig. 2D) 原理和 GTTs 很類似，只是注射的是胰島素，若胰島素阻抗現象高，則血液中胰島素濃度將會提升，HFD 組有明顯的濃度偏高，剩下三組雖然下降趨勢稍有差異，但最後的濃度非常的接近。接著用兩種組織染色法，分別是 H&E 和 Oil red-o，為判斷脂肪肝的重要指標 (Fig. 3A)，H&E 法能將細胞核染成深藍色，細胞質染成桃紅色，而沒被染到顏色的透明空間即為脂肪組織；Oil red-o 則是將脂肪細胞染成紅色以方便觀察，兩種染色的 HFD 組的脂肪組織呈現又大又密，在

餵食 RES 的對比之下有明顯的減少和減小的情形。(Fig. 3B) 為 4-HNE 染色法，是一種利用脂質過氧化力為標記的技術來測定脂肪含量，(Fig. 3C) 則為相對脂肪含量的比例，HFD 組的脂肪含量明顯高過其他三組，HFD＋RES 組得到非常好的改善。發炎重要因子 TNF-α在 HFD 組有明顯的上升現象，HFD ＋ RES 組得到良好的改善 (Fig. 4A)，(Fig. 4B,4C) CD-68 免疫染色法，是針對脂肪組織中遭成發炎反應的 M1 巨噬細胞的分析，這項分析可發現 HFD 組的比例明顯高過其他三組。

小鼠腦內組織分析，主要使用海馬迴的組織測量，腦內的 TNF-α和周邊組織有相同的狀況 (Fig. 5A)；Iba-1 是一個重要的發炎指標，中樞神經系統中，會和小膠質細胞結合表現，小膠質細胞是類似周邊組織的巨噬細胞，當腦內發炎因子提升時，會促使小膠質細胞的活化，而 Iba-1 就可以接上小膠質細胞，而 Iba-1 表現量 (Fig. 5B) 可看出 HFD 組的表現量高過剩下三組，也證明 RES 有趨緩小膠質細胞的活化。(Fig. 5C) 用 Iba-1 為抗體做的免疫染色， HFD 組白色箭頭所標出的為已被活化的小膠質細胞，而剩下三組的小膠質細胞則是呈現未被活化的狀態，(Fig. 5D) 4-HNE 染色法中也有和周邊組織有類似的呈現。(Fig. 6) 胰島素受器相關的 AMPK 訊息途徑，和腦內的發炎反應及 TAU 磷酸化有很大的關係，利用磷酸化和未磷酸化的比值來做對比；胰島素受器磷酸化及 adiponectin 濃度是調節 AMPK 磷酸化的重要指標，磷酸化的胰島素受器及 adiponectin 的濃度高時即能活化 AMPK 的路徑，而 HFD 組在這三項指標的比例都相對的低，在 HFD＋RES 組都有提升；未磷酸化的 GSK3 會有導致 TAU 磷酸化的功能，並使腦內神經受到損害，所以在 HFD 組可看出未磷酸化的 GSK3 的含量遠多於其他三組。(Fig. 7) Golgi's staining，一種專門染神經元和神經細胞的染色技術，HFD 組的黑三角指標指出的為神經細胞體，箭頭則為神經元樹突，可發現 HFD 組的神經細胞數量明顯低於剩下三組，也證明餵食 RES 的老鼠在神經細胞上有明顯的改善。

此實驗可得到白藜蘆醇能促進腦中的 AMPK 活化路徑，進而達到開發炎應的功效，藉由 GSK-3 的磷酸化及 Tau 的去磷酸化來避免腦袋神經的退化。

3. Effects of metformin on learning and memory behaviors and brain mitochondrial functions in high fat diet induced insulin resistant rats (Pintana *et al*., 2012)

Metformin 是被普遍利用治療第二型糖尿病的藥品，作用機制在減少肝中葡萄

醣的釋出，增強胰島素信號並調解周圍細胞受器達到減低胰島素阻抗的狀況，進而增加葡萄糖被利用率 (He *et al*., 2009; Labuzek *et al*., 2010)，並能容易且快速跨越血腦屏障，對於腦內有抗發炎和保護神經的相關作用 (Labuzek *et al*., 2010)，且在非神經細胞中 Metformin 有防止氧化壓力所造成的細胞凋亡的作用(El-Mir *et al*., 2008)。此篇目的為藉由 Metformin 的處理，在高脂飲食所誘導的胰島素阻抗大鼠，是否會有修復腦內粒線體功能和改善學習和空間記憶的能力。

實驗方法週齡雄性 WISTAR 大鼠 32 隻，將其分為正常組 (ND)、高脂飲食組 (HFD)，馴養 12 週之後進行一次的水迷宮試驗，再將 ND 組分成 NDV 餵食食鹽水組、NDM 餵食 Metformin 組，HFD 組分為 HFV 餵食食鹽水組、HFM 餵食 Metformin 組；餵食樣品前先測體重、膽固醇、血糖和 Homeostatic Model Assessment (HOMA)。食鹽水劑量 2 ml/kg/BW/day，Metformin 組為 15 mg/kg/BW twice daily，管餵 21 天，四組再進行一次水迷宮試驗，禁食後犧牲，進行膜和粒線體的分析。

第二次分組之前所測量的數值 (Table. 1) 可發現 HFD 組的體重、HOMA 和膽固醇等明顯高於 ND 組，有顯著胰島素阻抗和高血脂的現象。所有實驗結束後的統計 (Table. 2)，可對照出許多明顯的差異和結果，如 HFV 組的體重、膽固醇、HOMA 值及 MDA 值等指標性的數據都顯著高於 NDV 組，顯示依然有明顯的胰島素阻抗，但在 21 天的 Metformin 處理，HFM 膽固醇、HOMA 值及 MDA 值等現象都有下降的趨勢。粒線體功能分析中，(Fig. 8A) 代表當縱軸值越高時，腦內粒線體 ROS 值升高，增加腦內氧化壓力，增加受損風險，由 HFV 組可發現此數值遠遠高過其他三組，對照出 HFM 組在 Metformin 的處理之下，腦內 ROS 有明顯的改善；(Fig. 8B) HFV 組的粒線體膜電位的變化也大於其他三組，證明在 HFV 組的腦內粒線體存在容易受損的高風險環境。在水迷宮的兩種試驗中，(Fig. 9A,9C) 在 Acquisition test (老鼠在水迷宮中找到平台所花時間) 中 HFD 組需要比 ND 組多花 20-30 秒的時間才能找到水池中的平台；經過 Metformin 處理下 HFM 組所花的時間相較於 HFV 組有明顯的進步，在 Probe test(老鼠在移除平台的情況下，在原有平台的象限停留時間) (Fig. 9B,9D) HFD 組在原有平台的象限中停留的時間也明顯少於 ND 組；HFM 組比起 HFV 組也有明顯的進步，顯示學習和記憶能力得到改善，也可以發現 ND 組在食鹽水及 Metformin 處理的情況下並沒有什麼差別。

Metformin 能降低因長期高脂飲食所引發的胰島素阻抗、腦內氧化壓力、修復

粒線體功能等作用，進而預防改善大鼠的學習記憶能力，且有潛力成為治療糖尿病患者認知障礙的藥品。

4. Blueberry supplementation improves memory in middle-aged mice fed a high-fat diet(N. Carey *et al*., 2014)

藍莓 (Blueberry,BB) 涵非常豐富的營養物質如錳、維生素 C、K 及膳食纖維等且含有很豐富的色素如類黃酮及花青素，其色素的有效成分對於逆轉老化相關的缺陷和疾病是有幫助的，且加入飲食中有減輕行為缺陷和改善老化所造成的腦內發炎和氧化壓力的問題 (Rendeiro *et al*., 2013)，此篇目標想了解在餵食藍莓，一整個食材而非藥品或萃取物的情況下，有沒有修復學習記憶能力的潛力。

實驗方法用 9 月齡中年齡 C57BL/6 小鼠 64 隻，將其分為四組，低脂飲食組(LF)、低脂飲食(10%脂肪)加 4%藍莓組 (LFBB)、高脂飲食組 (HF)、高脂飲食(60%脂肪)加 4%藍莓組(HFBB) 進行為期五個月的分組餵食，再做一項認知和記憶的實驗 Novel object recognition test，從第 2、3、4 月開始每月測一次，目的是要測試老鼠記憶力，理論上，認知和記憶較好的老鼠會花較多的時間在探索新的事物上，套用到此 recognition index (RI) 公式的話，就是指 RI 值越高認知和記憶能力越佳 (Fig. 12A)。(Fig. 10,11) 高脂組的攝入熱量較高；且不論是否有餵食藍莓，體重都有明顯的增加，應該是用食材當樣品的關係。Novel object recognition test，LF 組和 LFBB 組在三組的測試中，訓練期跟測試期有明顯差距，代表其認知能力良好，三個月內表現都維持正常，HF 組則是都是維持不良的狀況，而在 HFBB 組三次測試結果呈現明顯的改善和成長狀態 (Fig. 12B, 12C, 12D) (Fig. 13)，作者有推測對於為何 HFBB 組會在第二月後才慢慢有改善的趨勢，原因為有類似門檻或閾值的效果，BB 中的脂溶性抗氧化物在腦中累積到一定的比例才會開始有作用的機制。

1. Conclusion

一、肥胖所造成的發炎、氧化壓力、胰島素阻抗及糖尿病，會造成神經胰島素阻抗，導致腦內損傷並造成認知能力受損。

二、擁有改善胰島素阻抗、氧化壓力和抗發炎的物質都可能有改善腦內神經功能障礙的潛力，並預防學習和記憶方面的衰弱。

三、長期的攝食內容改變，如藥品、天然萃取物或食物都可以成為改善糖尿病及其衍生症狀的重要角色。

四、經過這三篇我們可以了解，針對飲食習慣和內容上面的改變，如增加一

先健康營養的食材或是當配著有特定效果的機能性食品來服用，都是非常好的選擇。

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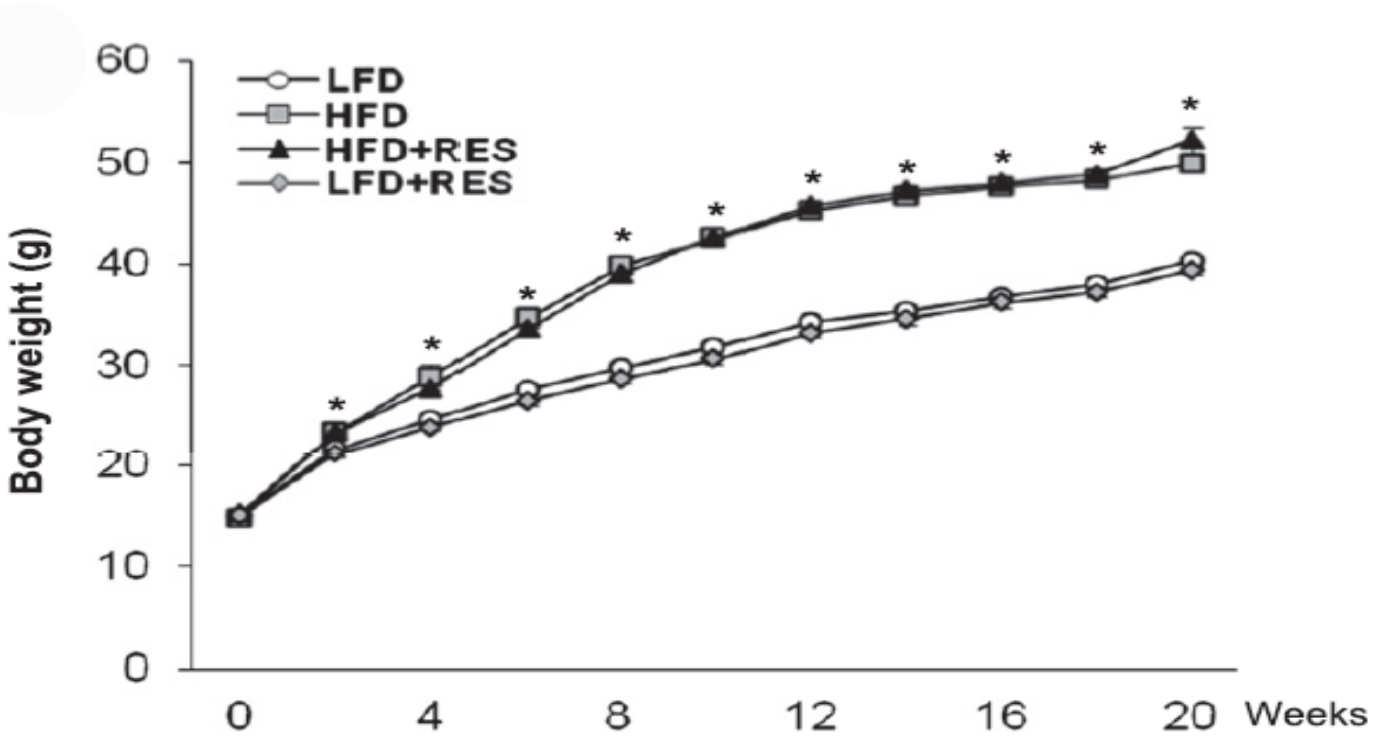

Figure 1. Effects of RES on whole body and brain weight in HFD-fed mice. Male C57BL/6J mice were fed an LFD, HFD, HFD+RES, or LFD+RES for 20 weeks (n = 20 per group). trans-RES was homogenously blended into the LFD or HFD, pelleted, and preserved in a manner to ensure the stability of RES. Graphs show change in body weight.

(Jeon *et al*., 2012)

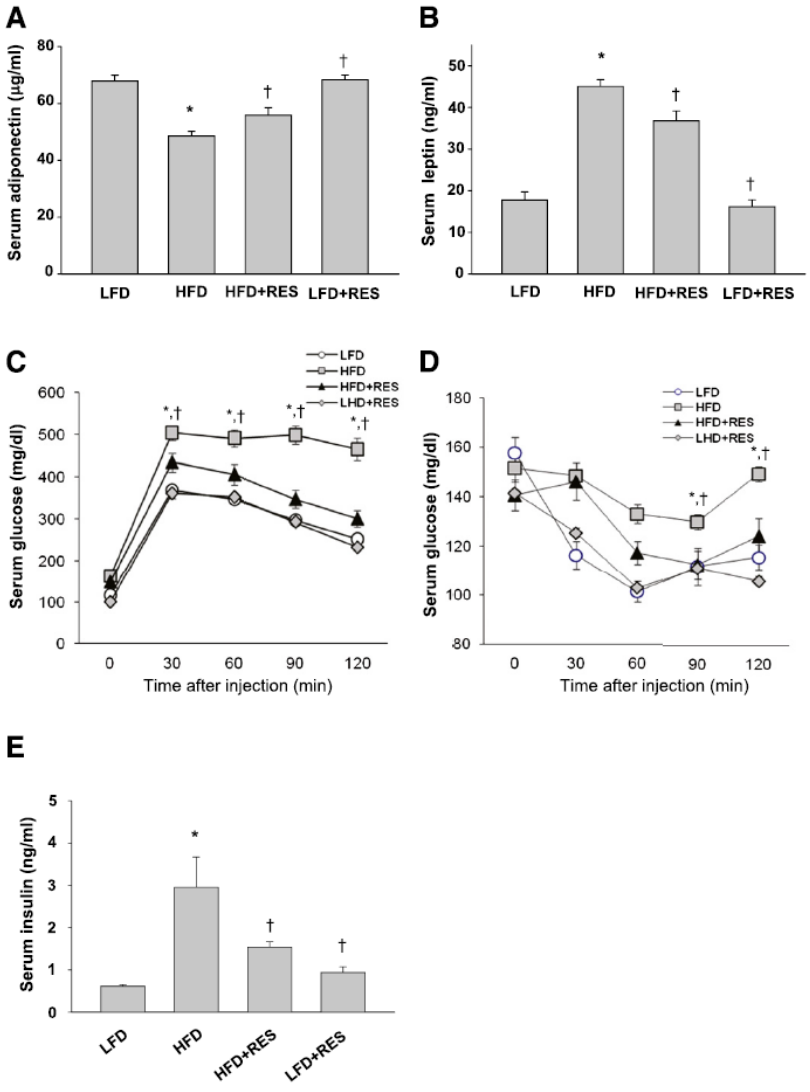

Figure 2. Effects of RES on metabolic parameters in HFD-fed mice. For ELISA analysis, mice were anesthetized with zoletil (5 mg/kg) and then blood serum was extracted transcardially through the apex of the left ventricle with a 1-mL syringe. Serum adiponectin (A) and leptin (B) levels using ELISA (n=5–7 per group). Hypoadiponectinemia and hyperleptinemia in HFD-fed mice were significantly reversed by RES treatment. C: Blood glucose levels after D-glucose (2 g/kg) injection in mice fed an LFD, HFD, HFD+RES, or LFD+RES. D: Blood glucose levels after insulin treatment (0.75 units/kg). Blood glucose levels of mice fed an HFD+RES were significantly decreased compared with HFD-fed mice. E: Serum insulin (n = 5–7 per group) levels using ELISA. RES decreased HFD-induced hyperinsulinemia. Data are mean 6 SEM. *P < 0.05 for HFD- compared with LFD-fed mice. †P < 0.05 for mice fed an LFD or HFD+RES compared with HFD-fed mice.

(Jeon *et al*., 2012)

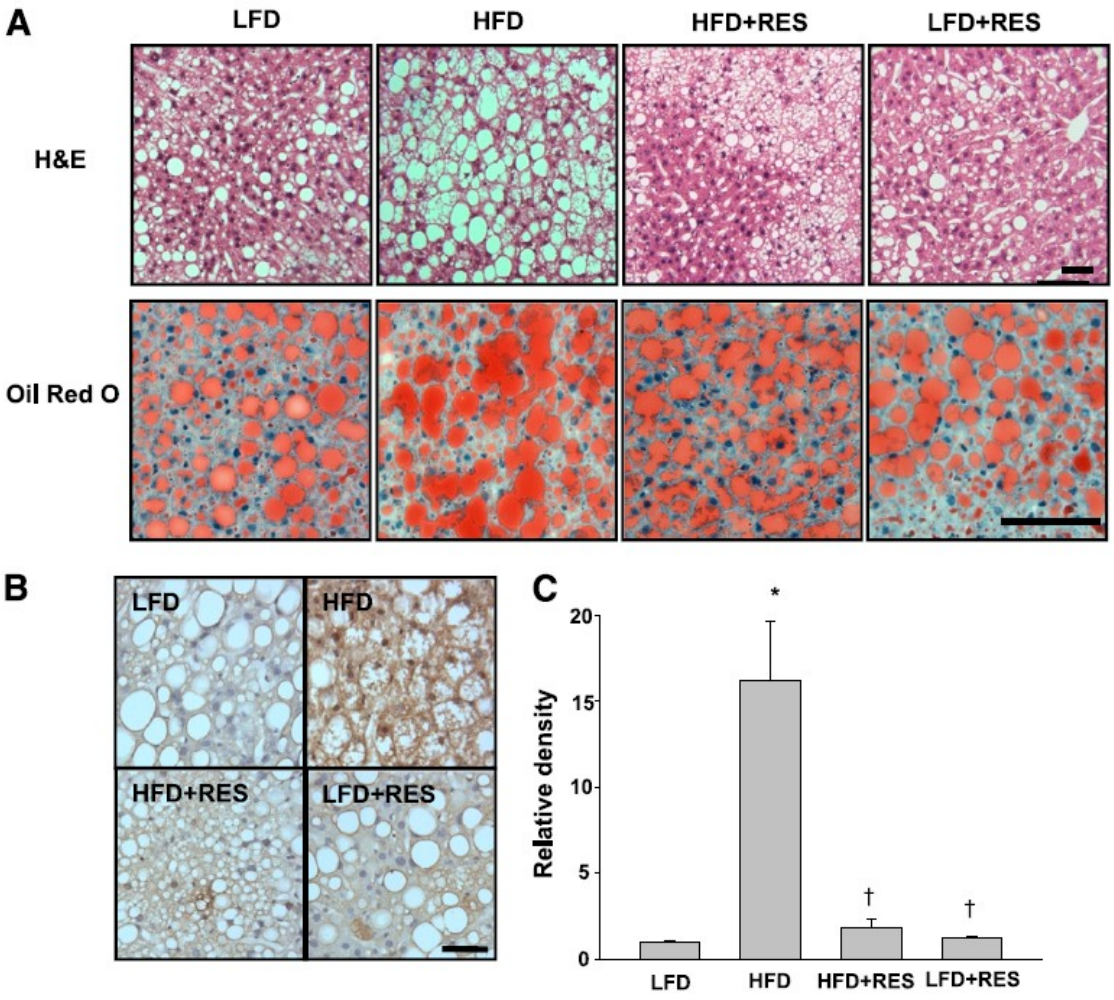

Figure 3. Effects of RES on hepatic steatosis, oxidative stress, and macrophage infiltration in HFD-fed mice. A: Representative microphotographs of hematoxylin and eosin (H&E)- and Oil Red O–stained liver section from mice fed an LFD, HFD, HFD+RES, or LFD+RES. B: Representative microphotographs of immune-stained 4-HNE in liver sections from each group. C: Quantitative expression of 4-HNE is shown as relative density. Data are mean 6 SEM. *P < 0.05 for HFD- compared with LFD-fed mice. †P < 0.05 for mice fed an LFD or HFD+RES compared with HFD-fed mice. Scale bar = 50 μm.

(Jeon *et al*., 2012)

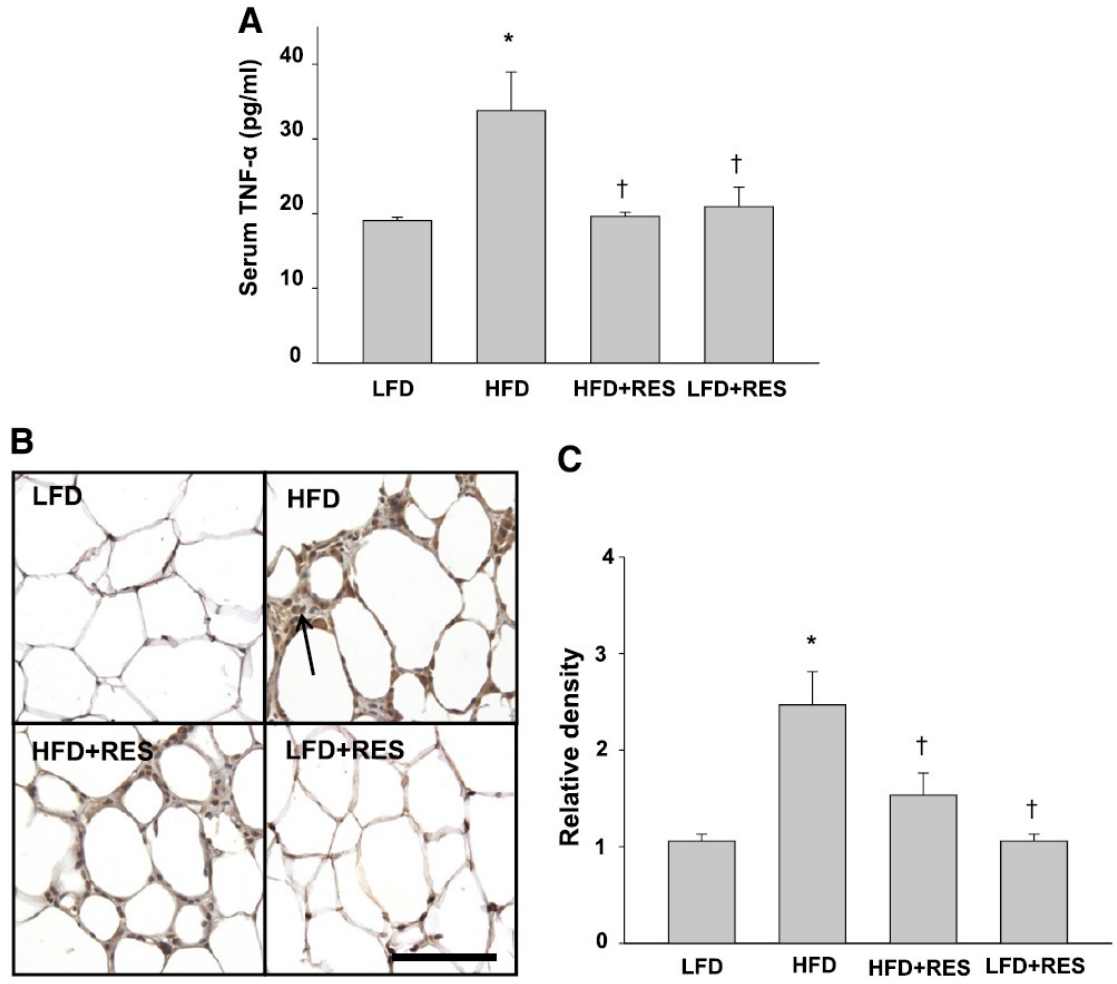

Figure 4. Effects of RES on serum TNF-α and macrophage infiltration in adipose tissue in HFD-fed mice. A: Concentration of TNF- α (n = 5–7 per group) from serum of mice fed an LFD, HFD, HFD+RES, or LFD+RES using ELISA. RES significantly inhibited the HFD-induced increase of TNF- α production. B: Representative microphotographs of immune-stained CD68 in epidermal fat pads from each group. CD68-expressing cells were deposited in HFD-treated adipose tissue. Arrow indicates macrophage. Scale bar = 50 μm. C: Quantitative expression of CD68 is shown as relative density. Data are mean 6 SEM. *P < 0.05 for HFD- compared with LFD-fed mice. †P < 0.05 for mice fed an LFD or HFD+RES compared with HFD-fed mice.

(Jeon *et al*., 2012)

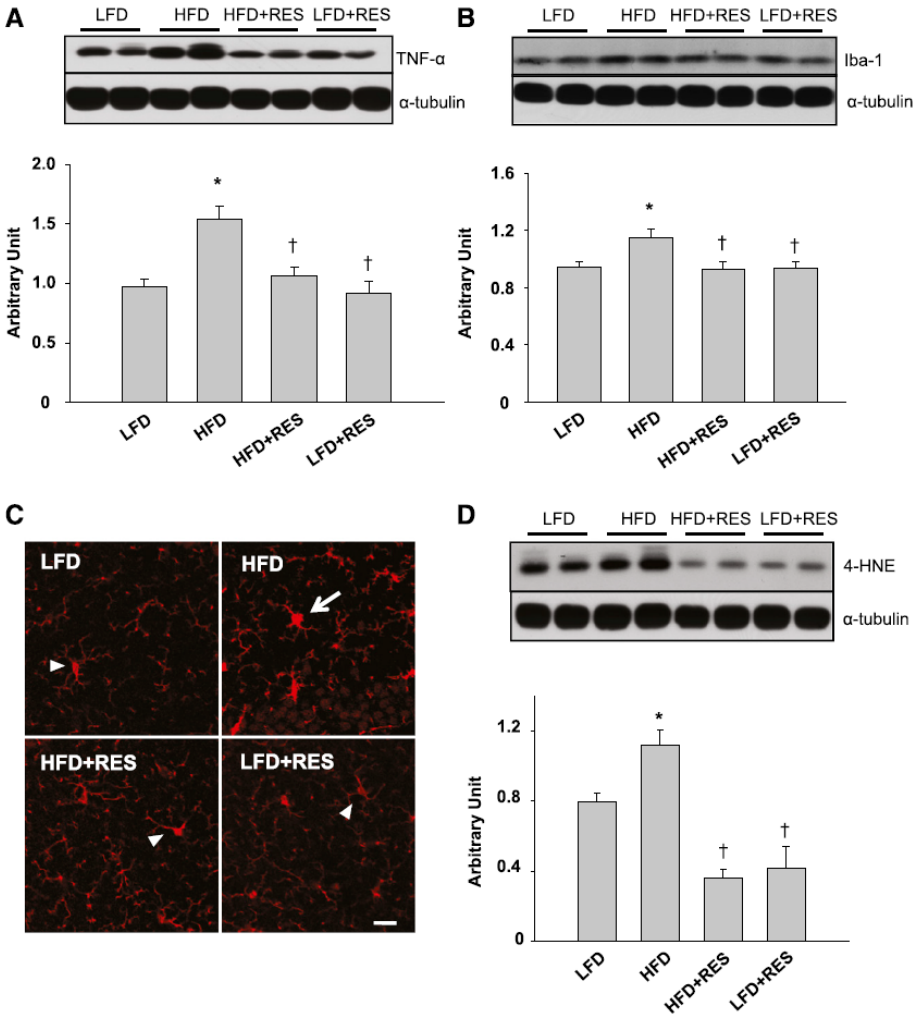

Figure 5. Effects of RES on neuronal inflammation in the hippocampus of HFD-fed mice. A: Western blot showing hippocampal TNF- α in mice fed an LFD, HFD, HFD+RES, or LFD+RES. Quantification of hippocampal TNF- α from Western blot analysis. Densitometry values for TNF- α were normalized to a-tubulin and are represented as arbitrary units (AUs). B: Western blot showing hippocampal Iba-1 in each group of mice. Quantification of hippocampal Iba-1 from Western blot analysis. Densitometry values for Iba-1 were normalized to a-tubulin and are represented as AUs. C: Representative microphotographs of immune-stained Iba-1 in CA1 region of the hippocampus from each group. Ramified microglia are present in the hippocampus of mice fed an LFD, HFD+RES, or LFD+RES, whereas activated microglia are present in HFD-fed mice. Arrow or arrowheads indicate activated microglia or ramified microglia, respectively. Scale bar = 20 μm. D: Western blot showing hippocampal 4-HNE in each group of mice. Quantification of hippocampal 4-HNE from Western blot analysis. Densitometry values for 4-HNE were normalized to a-tubulin and are represented as AUs. Data are mean 6 SEM. *P < 0.05 for HFD-compared with LFD-fed mice. †P < 0.05 for mice fed an LFD or HFD+RES compared with HFD-fed mice.

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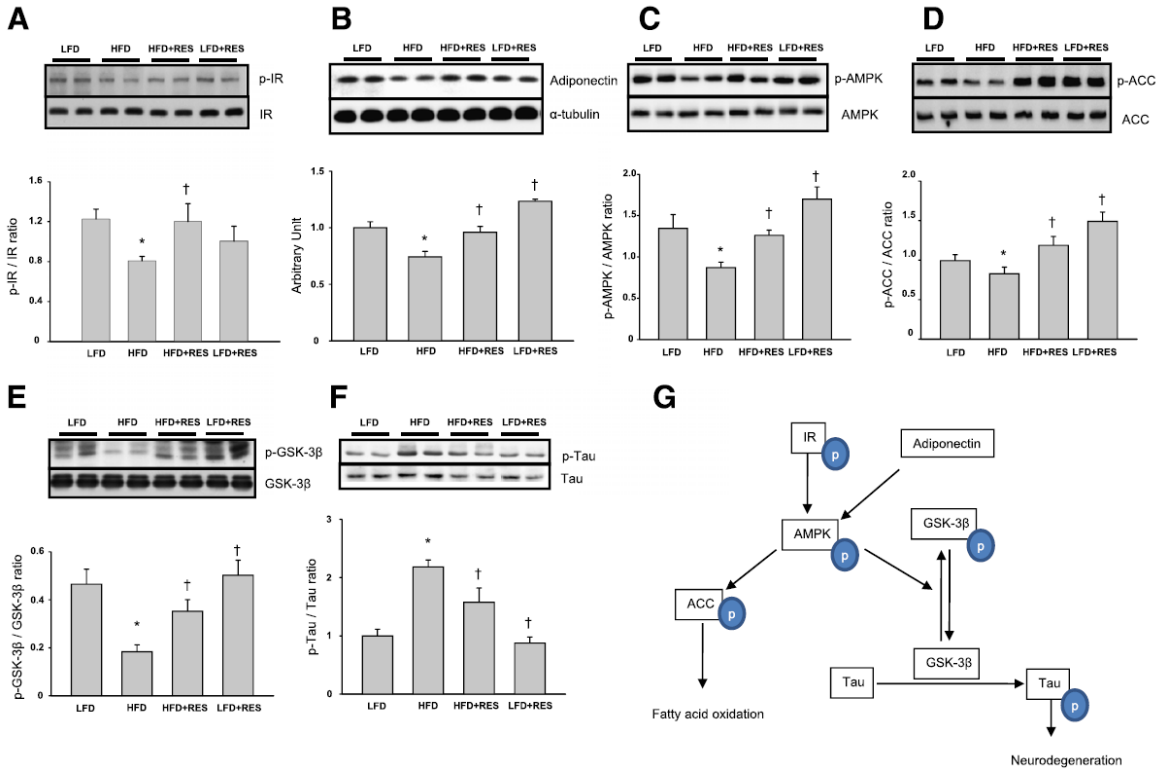

Figure 6. Effects of RES on IR-mediated AMPK signaling pathway in the hippocampus of HFD-fed mice. A: Western blot showing p-IR and IR in the hippocampus of mice fed an LFD, HFD, HFD+RES, or LFD+RES. Densitometry values of p-IR were normalized to IR and represented as arbitrary units (AUs). B: Western blot showing adiponectin in the hippocampus. Densitometry values of adiponectin were normalized to a-tubulin and represented as AUs. Western blot showing phosphorylation of AMPK (C), ACC (D), GSK-3b (E), and tau (F) in the hippocampus from each group. Quantification of the phosphorylation of each protein from Western blot analysis. Densitometry values for each p-protein were normalized to total protein and are represented as AUs. Data are mean 6 SEM. *P < 0.05 for HFD-compared with LFD-fed mice. †P < 0.05 for mice fed an LFD or HFD+RES compared with HFD-fed mice. G: Proposed model of the phosphorylated regulation of IR-mediated AMPK signaling pathway in the hippocampus.

(Jeon *et al*., 2012)

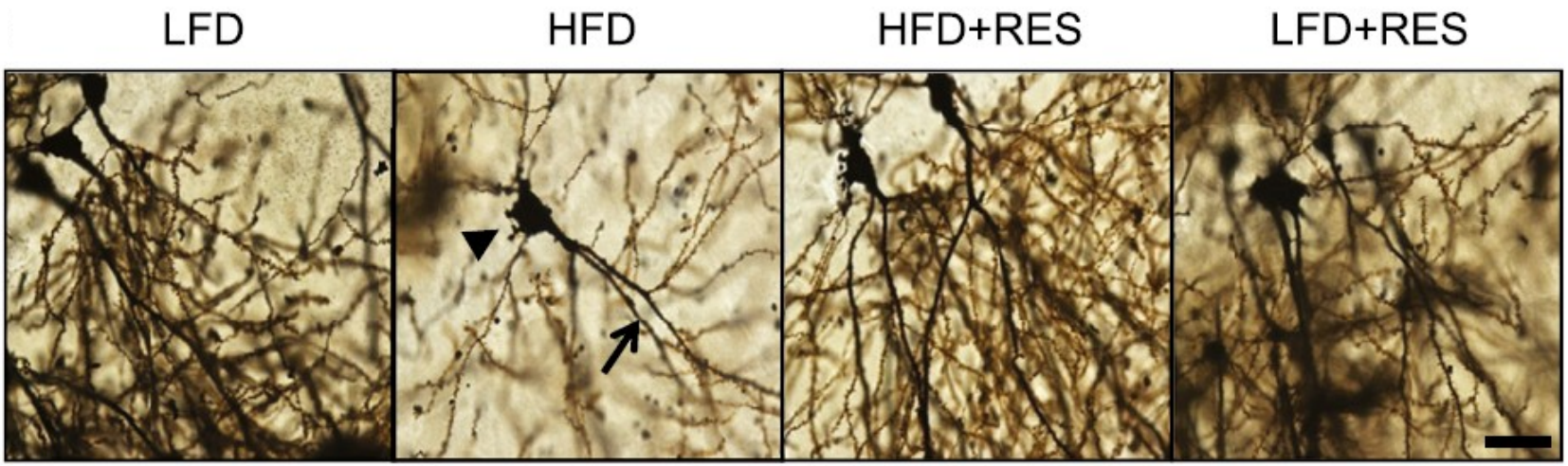

Figure 7. Effects of RES on neuronal degeneration in the hippocampus of HFD-fed mice. Representative, higher resolution microphotographs of Golgi-stained neurons in the hippocampus of mice fed an LFD, HFD, HFD+RES, or LFD+RES. Arrowhead and arrow indicate neuronal soma and dendrite, respectively. Scale bar =50 μm.

(Jeon *et al*., 2012)

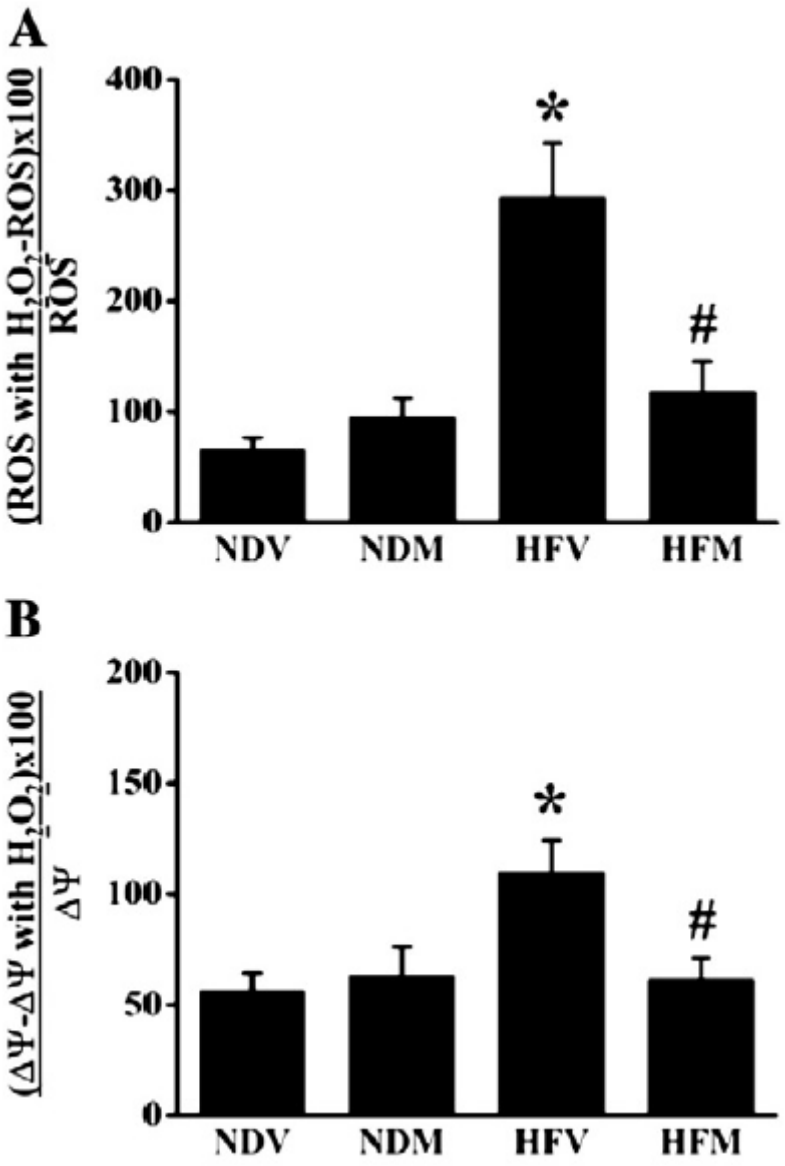

Figure 8. The effect of metformin treatment for 21 days on the brain mitochondrial dysfunction of HFD-fed rats. Metformin administration reduced the brain ROS production following H2O2 application caused by HFD consumption compared with HFV (A). Metformin significantly decreased brain mitochondrial membrane potential change following H2O2 application compared with HFV. $p < 0.05$; NDV vs. HFV, #$p < 0.05$; HFV vs. HFM. NDV: normal diet-fed rats with vehicle treatment, NDM: normal diet-fed rats with metformin treatment, HFV: high fat diet-fed rats with vehicle treatment and HFM: high fat diet-fed rats with metformin treatment.

(Pintana *et al*., 2012)

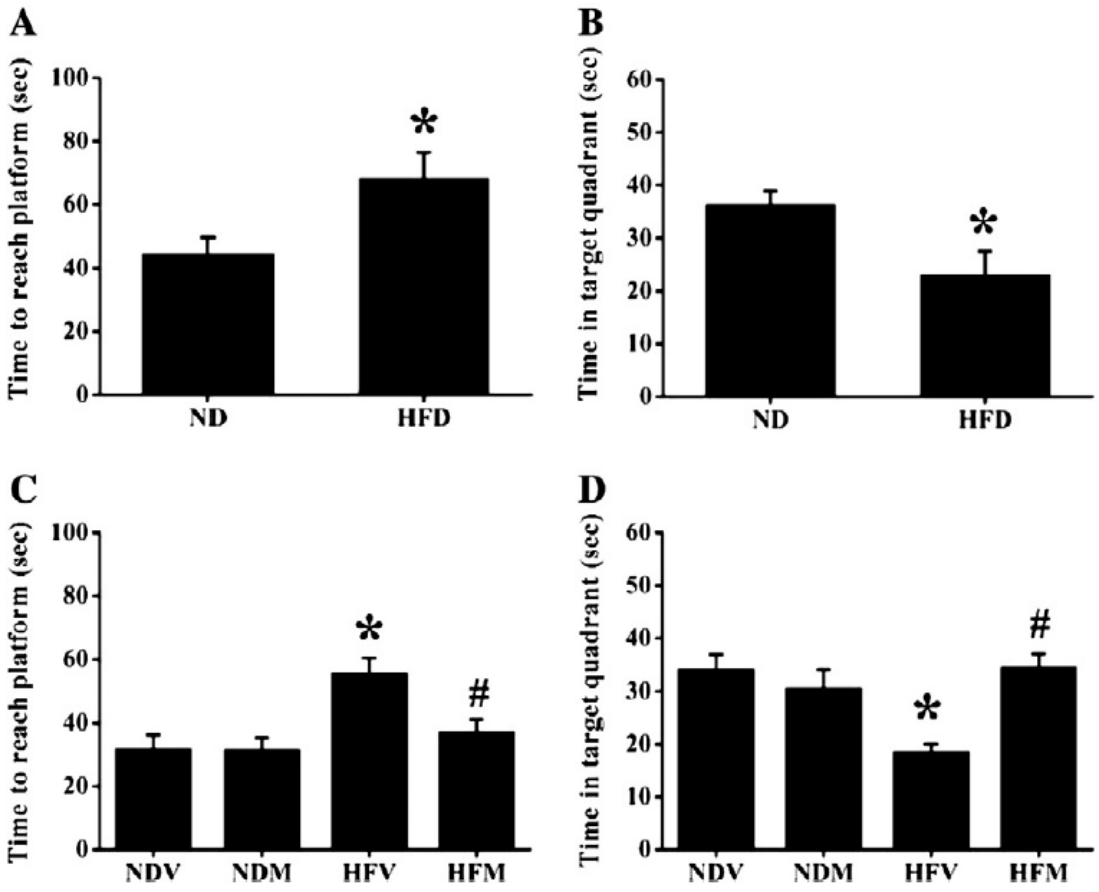

Figure 9. HFD consumption decreased learning and memory behaviors, indicated by increased time to reach the platform in acquisition test (A) and decreased time spent in target quadrant in the probe test (B). After 21 days of metformin administration, metformin treatment in HFD-fed rats significantly improved learning and memory deficit, indicated by the decreased time to reach the platform in acquisition test (C) and increased time spent in target quadrant in the probe test (D), $p < 0.05$; ND vs. HFD and NDV vs. HFV, #$p < 0.05$; HFV vs. HFM. ND: normal diet-fed rats, HFD: high fat diet-fed rats, NDV: normal diet-fed rats with vehicle treatment, NDM: normal diet-fed rats with metformin treatment, HFV: high fat diet-fed rats with vehicle treatment and HFM: high fat diet-fed rats with metformin treatment.

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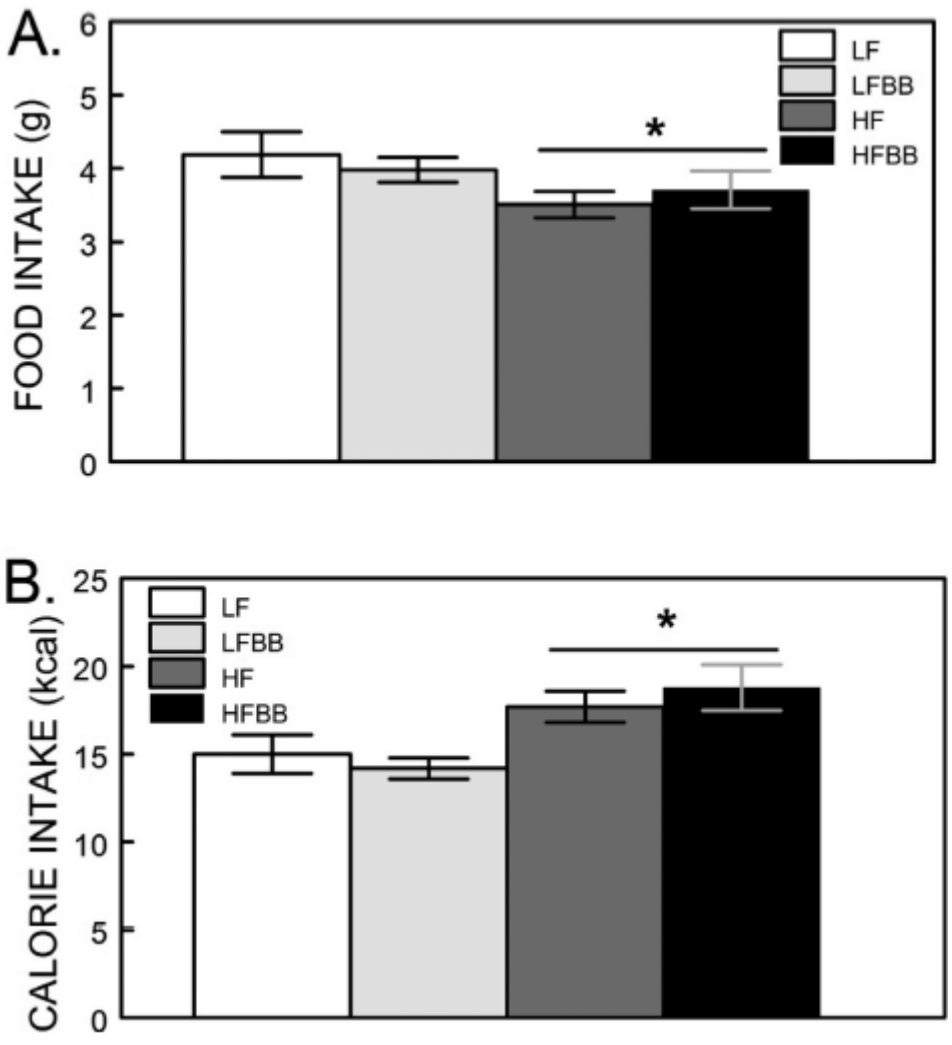

Figure 10. Food (A) and caloric (B) intake. An average of two 24 h food intake measurements indicated that overall the mice that ate HF diets consumed less than the mice that ate LF diets. However, mice on HF diets consumed significantly more calories than those on LF diets. (* $p < 0.05$, significant difference from LF diets, two-way ANOVA.)

(N. Carey *et al*., 2014)

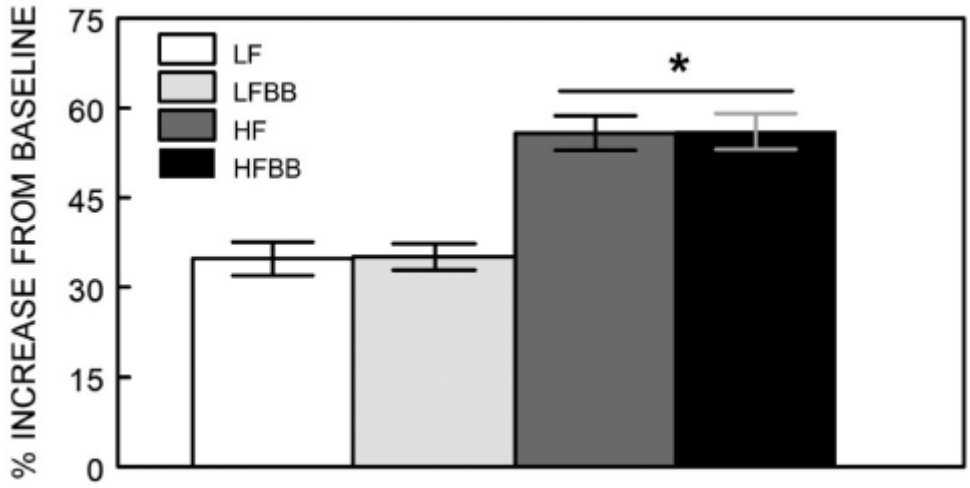

Figure 11. Percent increase in weight after 5 months on diets. Mice on HF diets gained significantly more weight than mice on LF diets. Blueberry had no effect on weight. (* $p < 0.05$, significant difference from LF diets, two-way ANOVA.

(N. Carey *et al*., 2014)

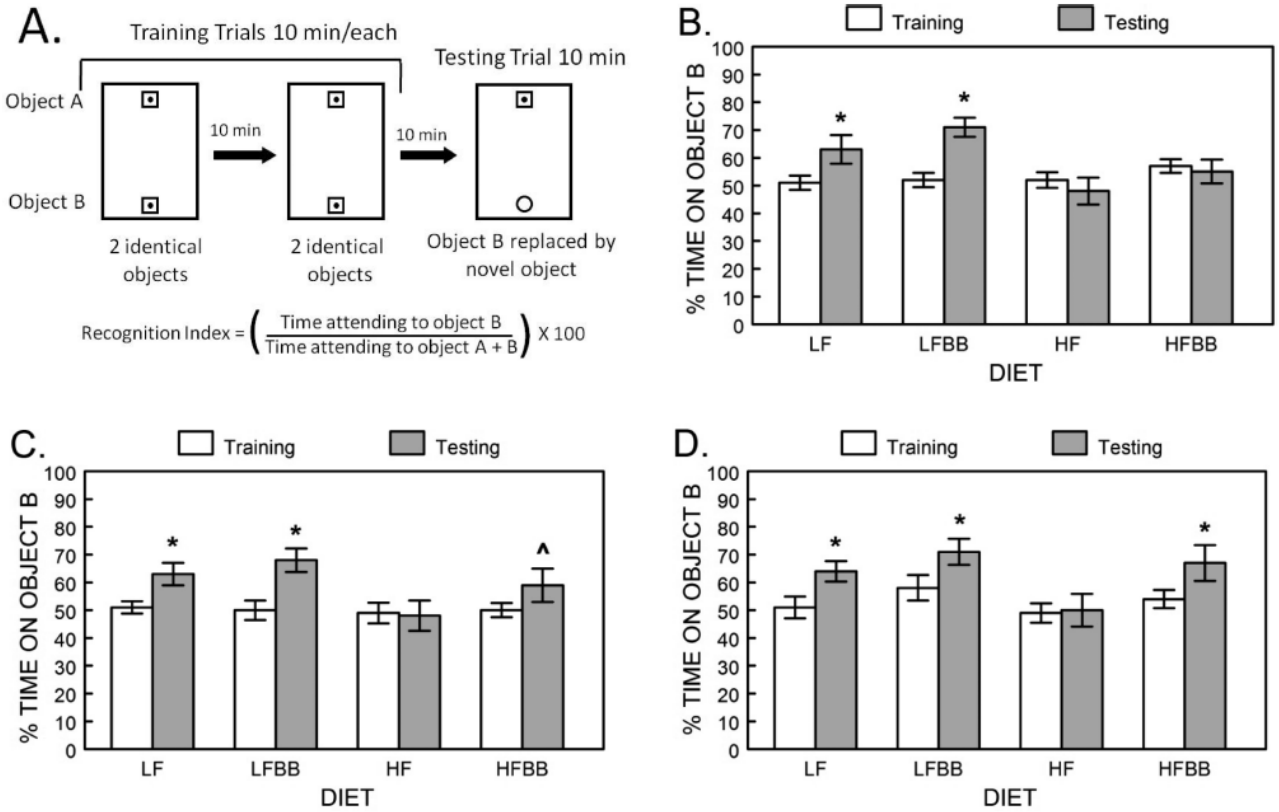

Figure 12. Novel object recognition performance after 2 (B), 3 (C), and 4 (D) months on the diets. (A) The schematic represents testing cages as they were arranged in each trial of testing. (B) After 2 months on the diets, mice on LF and LFBB diets showed a significant increase in RI in the testing trial compared to the training trial, suggesting intact object recognition learning/memory. Mice on HF and HFBB diets did not demonstrate an increase in RI in the testing trial, suggesting impairment. (C) After 3 months on the diets, mice consuming HFBB diet showed a trend toward an increase in RI in the testing trial ($p = 0.056$). (D) After 4 months on the diets, mice on LF, LFBB, and HFBB diets showed a significant increase in RI in the testing trial compared to the training trial, whereas the mice on the HF diet did not. (* $p < 0.05$, testing trial is different from training trial, Wilcoxon signed ranks test.)

(N. Carey *et al*., 2014)

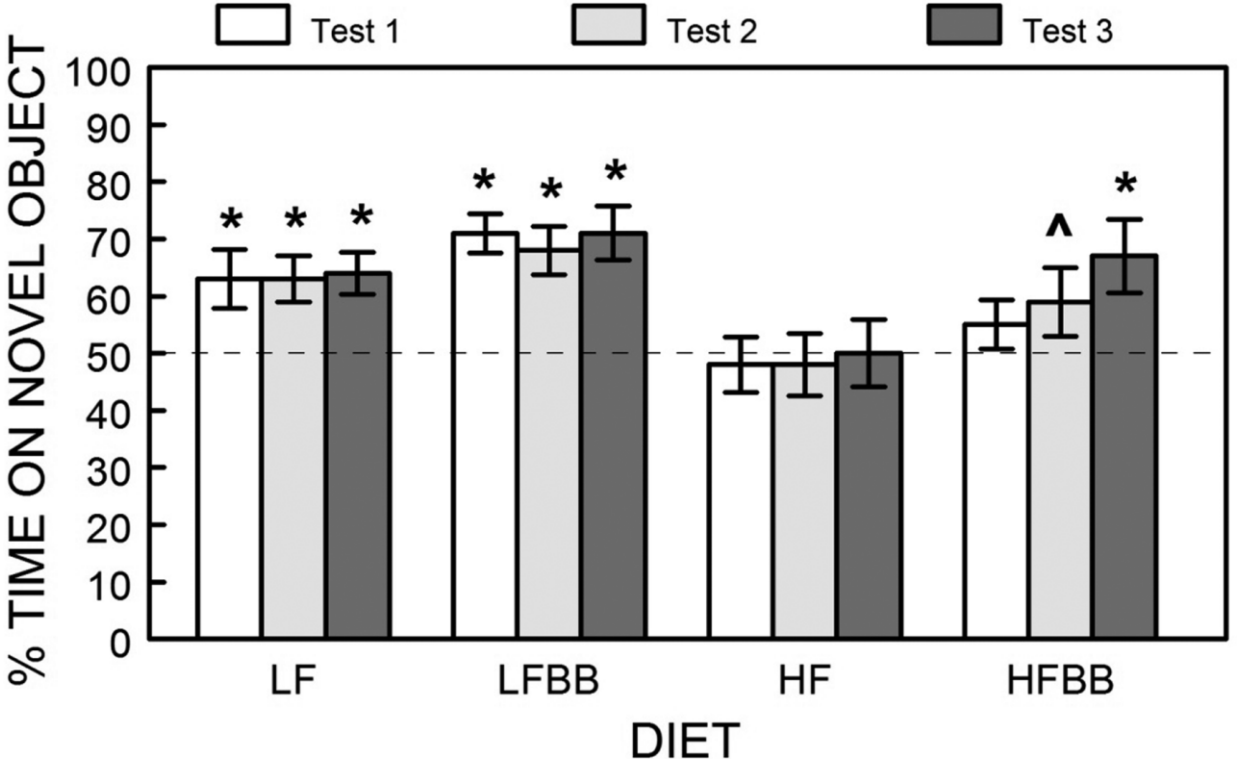

Figure 13. Testing trial performance in the novel object recognition task over three test sessions. The LF- and LFBB-fed mice, but not the mice fed the HF diet, performed at levels greater than chance when tested after 2, 3, and 4 months on the diets (chance is 50% time on the novel object and is indicated by the dotted line). The HFBB-fed mice demonstrated a trend ($p = 0.15$) after 3 months on the diet, and after 4 months on the diet, the mice demonstrated performance that was significantly greater than chance. (* $p < 0.05$, one-sample t test compared to a mean of 50%.)

(N. Carey *et al*., 2014)

Table 1. Effects of 12-week high fat diet (HFD) consumption on body weight, cholesterol, glucose, insulin and HOMA index.

Parameters	12-week ND	12-week HFD
Body weight (g)	450 ± 7.1	550 ± 9.42*
Plasma total cholesterol (mg/dl)	83.34 ± 10	129.43 ± 8.61*
Plasma glucose (mg/dl)	140.38 ± 6.24	148.8 ± 7.67
Plasma insulin (ng/ml)	2.26 ± 0.3	3.2 ± 0.48*
HOMA index	20.13 ± 2.23	35.45 ± 4.44*

ND: normal diet group, HFD: high-fat diet group.

$P < 0.05$ vs. ND.

(Pintana *et al*., 2012)

Table 2.Effects of metformin administration on peripheral insulin sensitivity parameters and MDA levels in rats fed with normal or high-fat diet.

Parameters	NDV	NDM	HFV	HFM
Body weight (g)	443.33 ± 9.55	445 ± 14.55	561.67 ± 19.05*	558.33 ± 20.56*
Visceral fat (g)	29.07 ± 3.74	23.13 ± 3.40	47.30 ± 4.30*	44.75 ± 4.31*
Plasma total cholesterol (mg/dl)	90.80 ± 4.33	89.30 ± 11.02	136.02 ± 7.36*	108.57 ± 5.84#
Plasma glucose (mg/dl)	143.89 ± 4.94	131.69 ± 8.79	150.11 ± 7.52	139.08 ± 4.49
Plasma insulin (ng/ml)	2.19 ± 0.33	2.20 ± 0.43	3.46 ± 0.43*	2.11 ± 0.48#
HOMA index	21.94 ± 3.74	16.24 ± 2.05	43.41 ± 6.61*	21.96 ± 4.02#
Plasma MDA (mmol/ml)	2.52 ± 0.15	2.63 ± 0.19	7.13 ± 0.13*	6.43 ± 0.20*,#
Brain MDA (mmol/ml)	1.46 ± 0.23	1.36 ± 0.28	2.30 ± 0.32*	1.55 ± 0.17#

* $p < 0.05$ vs. NDV (NDV: normal diet rats with vehicle treatment, NDM: normal diet rats with metformin treatment, HFV: high fat diet rats with vehicle treatment and HFM: high-fat diet rats with metformin treatment).

# $p < 0.05$ vs. HFV (NDV: normal diet rats with vehicle treatment, NDM: normal diet rats with metformin treatment, HFV: high fat diet rats with vehicle treatment and HFM: high-fat diet rats with metformin treatment).

(Pintana *et al*., 2012)

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