

科技部補助專題研究計畫報告

生酮飲食或合併運動介入對產後母鼠體重滯留、體組成、血液
生化值變化與耐力運動表現之影響 (L03)

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本研究具有政策應用參考價值：否 是，建議提供機關
(勾選「是」者，請列舉建議可提供施政參考之業務主管機關)
本研究具影響公共利益之重大發現：否 是

中華民國 109 年 10 月 19 日

中文摘要：肥胖已成為全球流行的疾病，預估到了西元2050年時，女性過重及肥胖的盛行率超過50%。對女性而言，懷孕期間與產後一年內是體重增加的關鍵期，易導致婦女產後體重過多的滯留。產後體重滯留過多是婦女將來肥胖的危險因子。生酮飲食已被證實對於不運動的人在短期內的減重效果最好；此外，對於耐力運動員可能也有助於提升運動表現。然而生酮飲食合併運動訓練是否有助於產後婦女減少產後的體重滯留甚至增加耐力運動表現仍未有相關研究探討。因此本研究目的為探討生酮飲食或合併運動介入對產後體重滯留、體組成、血液生化值變化與耐力運動表現之影響。本研究方法將應用產後母鼠的動物模式進行6週生酮飲食或合併運動訓練介入。10週齡大之產後ICR雌性小鼠，隨機分成四組(n=8/組)：(1)標準飲食組(CN)、(2)生酮飲食組(LCKD)、(3)標準飲食合併運動組(CN-EX)、(4)生酮飲食合併運動組(LCKD-EX)。以前肢抓力測試與跑步力竭時間及運動後疲勞相關生化指標評估運動表現。實驗結束後，收集血液進行一般血液生化值分析；收集肝臟與肌肉及其他器官、子宮脂肪等進行脂肪與組織病理分析。結果顯示介入結束後，低醣生酮飲食組組織體重顯著較其他各組高；而低醣生酮飲食合併運動訓練組組織體重與標準飲食或標準飲食合併運動組比較無顯著差異。低醣生酮飲食組之運動表現相較於其他組差，而低醣生酮飲食組合併運動組則相反。由本研究結果推論，低醣生酮飲食減少產後母鼠體重的滯留與增加體能表現之效果有限，但合併運動組雖有較佳的效果對於產後體重滯留之減少，但仍與有或無合併運動的標準飲食之效果相當。因此，低醣生酮飲食是否能應用於產後體重管理以及提升體能表現，仍須更多的研究評估。

中文關鍵詞：產後體重滯留、低醣生酮飲食、運動表現、肥胖

英文摘要：Obesity has been officially recognized by the World Health Organization (WHO) as a global epidemic. It is projected that more than 50% of women will be overweight or obese by 2050. For women, in addition to pregnancy, the first year postpartum is another key period to gain excess weight, resulting in long-term weight retention. Excessive postpartum weight retention can be a significant contributor to long-term maternal obesity and related diseases. The ketogenic diet (KD), also known as low-carbohydrate ketogenic diet (LCKD), has been proven to be very effective for short-term weight loss in sedentary people. However, whether LCKD coupled with exercise training will be an effective strategy for postpartum women to reduce their retained weight and enhance their endurance exercise performance remains unclear. Hence, the objective of the present study was to examine the effect of the LCKD with or without exercise training intervention on maternal postpartum weight retention, body composition, biochemical profiles and endurance exercise performance in female mice. In this study, 10-week-old postpartum female ICR mice were assigned to 4 groups (n=8/group): (1) postpartum mice with

a normal diet and no exercise training (CN); (2) postpartum mice with a normal diet and exercise training (CN+EX) 3); postpartum mice with a low-carbohydrate-ketogenic-diet and no exercise training (LCKD); (4) postpartum mice with a LCKD and exercise training (LCKD+EX). The intervention was conducted for 6 weeks. Exercise performance was evaluated by forelimb grip strength and exhaustive running time. For the biochemical analysis, the serum levels of glucose, ketone body and tissue damage markers including lactate, ammonia, glucose, blood urine nitrogen (BUN) and creatinine kinase (CK) were measured. The weight of the uterine fat pads (UFP) and brown adipose tissue (BAT) were recorded for calculating body fat levels. Other organs were examined by histopathology. Our results showed that a 6-week LCKD intervention alone had the least effect on postpartum weight loss in all postpartum mice, whereas a standard diet and exercise training combined with a standard diet or LCKD interventions had similar effects. A 6-week LCKD intervention alone seemed to have a negative effect on physical fitness. More studies are warranted to examine these issues for the management of postpartum weight retention and physical fitness by the use of a LCKD intervention.

英文關鍵詞： Postpartum weight retention, low carbohydrate ketogenic diet, exercise performance, obesity

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中文摘要

肥胖已成為全球流行的疾病，預估到了西元 2050 年時，女性過重及肥胖的盛行率超過 50%。對女性而言，懷孕期間與產後一年內是體重增加的關鍵期，易導致婦女產後體重過多的滯留。產後體重滯留過多是婦女將來肥胖的危險因子。生酮飲食已被證實對於不運動的人在短期內的減重效果最好；此外，對於耐力運動員可能也有助於提升運動表現。然而生酮飲食合併運動訓練是否有助於產後婦女減少產後的體重滯留甚至增加耐力運動表現仍未有相關研究探討。因此本研究目的為探討生酮飲食或合併運動介入對產後體重滯留、體組成、血液生化值變化與耐力運動表現之影響。本研究方法將應用產後母鼠的動物模式進行 6 週生酮飲食或合併運動訓練介入。10 週齡大之產後 ICR 雌性小鼠，隨機分成四組(n=8/組)：(1)標準飲食組 (CN)、(2)生酮飲食組(LCKD)、(3)標準飲食合併運動組(CN-EX)、(4)生酮飲食合併運動組(LCKD-EX)。以前肢抓力測試與跑步力竭時間及運動後疲勞相關生化指標評估運動表現。實驗結束後，收集血液進行一般血液生化值分析；收集肝臟與肌肉及其他器官、子宮脂肪等進行脂肪與組織病理分析。結果顯示介入結束後，低醣生酮飲食組織體重顯著較其他各組高；而低醣生酮飲食合併運動訓練組織體重與標準飲食或標準飲食合併運動組比較無顯著差異。低醣生酮飲食組之運動表現相較於其他組差，而低醣生酮飲食組合併運動組則相反。由本研究結果推論，低醣生酮飲食減少產後母鼠體重的滯留與增加體能表現之效果有限，但合併運動組雖有較佳的效果對於產後體重滯留之減少，但仍與有或無合併運動的標準飲食之效果相當。因此，低醣生酮飲食是否能應用於產後體重管理以及提升體能表現，仍須更多的研究評估。

中文關鍵詞：產後體重滯留、低醣生酮飲食、運動表現、肥胖

Abstract

Obesity has been officially recognized by the World Health Organization (WHO) as a global epidemic. It is projected that more than 50% of women will be overweight or obese by 2050. For women, in addition to pregnancy, the first year postpartum is another key period to gain excess weight, resulting in long-term weight retention. Excessive postpartum weight retention can be a significant contributor to long-term maternal obesity and related diseases. The ketogenic diet (KD), also known as low-carbohydrate ketogenic diet (LCKD), has been proven to be very effective for short-term weight loss in sedentary people. However, whether LCKD coupled with exercise training will be an effective strategy for postpartum women to reduce their retained weight and enhance their endurance exercise performance remains unclear. Hence, the objective of the present study was to examine the effect of the LCKD with or without exercise training intervention on maternal postpartum weight retention, body composition, biochemical profiles and endurance exercise performance in female mice. In this study, 10-week-old postpartum female ICR mice were assigned to 4 groups (n=8/group): (1) postpartum mice with a normal diet and no exercise training (CN); (2) postpartum mice with a normal diet and exercise training (CN+EX) (3); postpartum mice with a low-carbohydrate-ketogenic-diet and no exercise training (LCKD); (4) postpartum mice with a LCKD and exercise training (LCKD+EX). The intervention was conducted for 6 weeks. Exercise performance was evaluated by forelimb grip strength and exhaustive running time. For the biochemical analysis, the serum levels of glucose, ketone body and tissue damage markers including lactate, ammonia, glucose, blood urine nitrogen (BUN) and creatinine kinase (CK) were measured. The weight of the uterine fat pads (UFP) and brown adipose tissue (BAT) were recorded for calculating body fat levels. Other organs were examined by histopathology. Our results showed that a 6-week LCKD intervention alone had the least effect on postpartum weight loss in all postpartum mice, whereas a standard diet and exercise training combined with a standard diet or LCKD interventions had similar effects. A 6-week LCKD intervention alone seemed to have a negative effect on physical fitness. More studies are warranted to examine these issues for the management of postpartum weight retention and physical fitness by the use of a LCKD intervention.

Key words: Postpartum weight retention, low carbohydrate ketogenic diet, exercise performance, obesity

1. Introduction

Obesity is considered as a worldwide epidemic affecting both genders across all ages (Chooi, Ding, & Magkos, 2019). It is worth noting, however, that the rate of obesity is greater in women than in men when looking at trends in adult obesity (Farpour-Lambert, Ells, Martinez de Tejada, & Scott, 2018). Therefore, more focus is needed to address the impact of obesity on women's health issues, and particularly on their reproductive health. Maternal obesity has raised public health concerns as it can adversely affect both mothers' and children's health outcomes. For instance, excessive gestational weight gain strongly predicts the risk of postpartum weight retention (PPWR) (Rong et al., 2015), which may induce additional weight gain and obesity in midlife and the postmenopausal period (Gunderson, 2009). Lifestyle modification encompassing diet, exercise, and behavior therapy is the cornerstone of successful weight control (Nicodemus, 2018; Wadden, Webb, Moran, & Bailer, 2012). The ketogenic diet, a low carbohydrate, adequate-protein and high fat diet, may be a promising opportunity to target PPWR, as a body of research is emerging which shows that the ketogenic diet can produce significant weight loss for overweight or obese adults in a short time period (2-6 months)(Kirkpatrick et al., 2019). The objective of the present study was to investigate the effects of a 6-week low-carbohydrate-ketogenic diet (LCKD) alone, exercise-training alone or combined LCKD and exercise training on postpartum weight retention, physical performance, body composition, and blood biochemistry in postpartum mice.

2. Materials and Methods

2.1. Diet, animal experimental design and the treadmill training protocol

Female Institute of Cancer research (ICR) mice aged 6 weeks were purchased from BioLASCO (Yilan, Taiwan) and were mated with ICR males. Females were given ad libitum access to standard chow diets and water during pregnancy (No. 5001, PMI Nutrition International, Brentwood, MO). After giving birth, 32 female mice, aged 10 weeks, were randomly assigned to four groups (n= 8 mice per group): control (CN), control plus exercise (CN+EX), low carbohydrate ketogenic diet (LCKD), and low carbohydrate ketogenic diet plus exercise (LCKD+EX). Postpartum mice in the CN and CN+EX groups were fed a standard chow diet with or without aerobic exercise-training for 6-8 weeks, respectively; whereas the postpartum mice in the LCKD and LCKD+EX groups were fed a low carbohydrate ketogenic diet with or without aerobic exercise-

training for 6-8 weeks, respectively. All animals were housed at the National Taiwan Sport University's animal facility with controlled temperature (22 ± 2 °C) and humidity ($60 \pm 10\%$), and a 12-h light-dark cycle. All animal protocols (Approval Number: IACUC-10817) were approved by the Institutional Animal Care and Use Committee (IACUC) of National Taiwan Sport University and were compliant with the guide for Laboratory Animal Facilities and Care, which is promulgated by the Council of Agriculture, Executive Yuan, Taiwan.

Postpartum mice in the CN+EX and LCKD+EX groups were subjected to a 6-week aerobic exercise training program, which consisted of running on a motorized treadmill (MK680C, Muromachi Kikai Co. Ltd. Tokoyo, Japan) for 30 minutes, five times weekly for 6 weeks. After a 6-week diet and treadmill exercise intervention, treadmill exhaustion and 15-min swimming tests, as well as limb grip strength assays, were conducted with all animals to examine the effects of LCKD, treadmill training or a combination of both on muscle performance, physical performance and metabolic parameters. The animals were then fasted overnight and euthanized with CO₂ inhalation at the end of the study (the beginning of week 8). After sacrifice, blood was collected via cardiac puncture, and the liver, kidneys, gastrocnemius muscle, caecum, and fat pads were manually dissected, rinsed in saline solution, blotted dry and weighed. Food and water intakes and the body weights of all postpartum mice were measured weekly throughout the total study duration.

2.2. Forelimb grip strength test

The forelimb grip strength of all postpartum mice was measured at the end of the exercise intervention for maximal force (grams) to indicate the muscle strength of the animals after the interventions using a low-force testing system (Model-RX-5; Aikoh Engineering, Nagoya, Japan). The measurement was described in detail in our previous study (Hsu et al., 2016; Huang et al., 2014; Lin et al., 2015).

2.3. Treadmill exhaustion test

After the 6-week diet and exercise interventions, the postpartum mice in all groups performed a treadmill run to exhaustion test, which was a progressive exercise test, for the determination of endurance capacity. The running time to exhaustion was determined, where exhaustion was defined as the inability of the mouse to run on the treadmill and stay in the front for 5 seconds, despite receiving mild electric stimuli (1.22 mA, 2 Hz) generated at the back of the treadmill (Dougherty, Springer, & Gershengorn, 2016).

2.4. Analysis of blood biochemistry

Serum was obtained from blood immediately collected at sacrifice and centrifuged at 1500 ×g, 4 °C for 10 min. It was used for assessments of AST, ALT, albumin, total protein (TP), blood urea nitrogen (BUN), creatinine, CPK, UA, TC, TG and glucose levels using an autoanalyzer (Hitachi7060, Hitachi, Tokyo, Japan). Furthermore, the levels of ketone bodies of all animals were measured using a portable meter and a test strip (Optium FreeStyle, Abbott, North Chicago, USA).

2.5. Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical differences among groups were analyzed using one-way ANOVA followed by Duncan's post hoc test with SAS 9.0 (SAS Inst., Cary, NC, USA). P-value < 0.05 was considered as statistically significant.

3. Results and discussions

The postpartum weight of the mice in the LCKD group was significantly heavier than that of the CN, CN+EX and LCKD+EX groups from the 4th week of the intervention ($p < 0.05$). However, it should be noted that the exercise-trained mice on either the LCKD or control diet produced similar results of postpartum weight loss as compared with untrained mice on the control diet alone in the present study. Presumably, a well-balanced diet with limited caloric intake in the postpartum period is an essential component of fighting postpartum weight retention (Bertz et al., 2012).

The forelimb grip strength marginally decreased in the LCKD group in comparison with the CN group ($p > 0.05$). Exercise-trained mice on the control diet or LCKD showed no differences in grip strength in comparison to un-trained mice on the control diet ($p > 0.05$). However, there is a statistically significant difference in the forelimb grip strength of the exercise-trained and un-trained mice on the LCKD ($p < 0.05$). The running time to exhaustion of the postpartum mice in the LCKD group was significantly shorter than that of the other groups ($p < 0.05$). However, no significant difference was seen between the CN and CN+EX groups. LCKD in combination with aerobic exercise increased the running time to exhaustion in the present study, indicating the improved endurance exercise performance that could be attributed to the beneficial effect of aerobic exercise training for 6 weeks. It has been shown that aerobic exercise training can protect muscle against damage events (Powers, Bomkamp, Ozdemir, & Hyatt, 2020).

To examine the effects of LCKD and/or aerobic exercise training on non-lipid metabolic parameters, blood was collected at the end of the study. The final blood test

results show that no significant changes in the parameters including TP, CPK, CREA, ALB, TP and glucose were seen among any of the four groups ($p > 0.05$, data not shown for brevity). Similarly, no significant differences in plasma GOT and GPT were observed between the LCKD or LCKD+EX and the CN groups ($p > 0.05$). The postpartum LCKD feeding led to a significant increase in plasma LDH levels on both the untrained and trained mice, compared with their respective CN-fed mice ($p < 0.05$). Although plasma LDH levels were slightly higher in the LCKD+EX group than in the LCKD group, this difference did not reach statistical significance ($p > 0.05$). To further determine which tissues were involved in the LCKD-induced abnormalities, plasma GOT, GTP and CPK levels were measured. The results of this study show that postpartum mice in the LCKD and LCKD+EX groups had unchanged levels of plasma GOT, GPT and CPK, together with significantly elevated levels of blood LDH, which suggests that the elevated blood LDH levels were not likely the consequence of liver or muscle abnormalities. Postpartum mice in the LCKD, CN and CN+EX groups exhibited similar blood ketone levels ($p > 0.05$). Compared to the CN, CN+EX and LCKD groups, significantly elevated levels of blood ketones were noted in the LCKD+EX group ($p < 0.05$). In the LCKD-fed postpartum mice with aerobic exercise training, blood ketone levels could be enhanced by the effect of aerobic exercise in the context of the carbohydrate-restricted diet that was provided (Koeslag, Noakes, & Sloan, 1980).

Average daily water and food intake of the LCKD-treated mice with or without aerobic exercise was significantly lower than that of control with or without aerobic exercise ($p < 0.05$, **Table 1**). It should be noted that all animals were fed isocalorically during the study period, which led to the LCKD-treated mice eating less food; this is also evidenced by the fact that average daily energy intake was similar in all four groups (**Table 1**), suggesting that the postpartum mice tolerated the LCKD and the LCKD+EX well.

Table 1. Average daily water, food and energy intake in the postpartum mice fed CN and LCKD diets during the intervention

Parameters	Groups			
	CN	CN+EX	LCKD	LCKD+EX
Water intake (ml/day)	20.15 ± 14.31 ^b	20.71 ± 13.69 ^b	11.04 ± 8.17 ^a	10.03 ± 8.25 ^a
Food intake (g/day)	5.96 ± 1.08 ^b	6.07 ± 1.14 ^b	3.41 ± 0.70 ^a	3.42 ± 0.67 ^a
Energy intake (Kcal/day)	19.97 ± 3.62 ^a	20.32 ± 3.81 ^a	19.42 ± 4.01 ^a	19.49 ± 3.81 ^a

4. Conclusions

In this study, the analysis of other data, such as body composition, hepatic lipid profiles and physical performance is still the work in progress. To sum up, we showed that the postpartum mice in all groups progressively decreased their body weights during 6 weeks postpartum. However, the 6-week LCKD alone was associated with the least reduction in postpartum weight retention. Compared with the LCKD approach, the combination of LCKD and aerobic exercise intervention had a greater effect on promoting postpartum weight loss. However, the amount of weight loss in the LCKD+EX group did not differ from that in the control group. Furthermore, the LCKD feeding alone had negative effects on outcomes related to physical fitness, but the combined LCKD and EX approach showed the opposite effects. There is a controversy over the efficacy of LCKD on weight loss in the present study, and thus further studies are warranted to examine this issue for the management of postpartum weight retention.

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108年度專題研究計畫成果彙整表

計畫主持人：林崢怡		計畫編號：108-2629-B-424-001-			
計畫名稱：生酮飲食或合併運動介入對產後母鼠體重滯留、體組成、血液生化值變化與耐力運動表現之影響 (L03)					
成果項目		量化	單位	質化 (說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等)	
國內	學術性論文	期刊論文	1	篇	目前實驗數據仍分析、彙整撰寫中，預計將研究成果撰寫成一篇期刊論文並投稿至SCI期刊。
		研討會論文	0		
		專書	0	本	
		專書論文	0	章	
		技術報告	0	篇	
		其他	0	篇	
國外	學術性論文	期刊論文	0	篇	
		研討會論文	0		
		專書	0	本	
		專書論文	0	章	
		技術報告	0	篇	
		其他	0	篇	
參與計畫人力	本國籍	大專生	0	人次	參與本研究計畫之動物實驗執行，包含飲食或有氧運動介入，體組成、運動表現測驗、血液、器官組織等之收集，目前相關實驗部份皆已執行完畢。
		碩士生	2		
		博士生	0		
		博士級研究人員	0		
		專任人員	0		
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士級研究人員	0		
		專任人員	0		
其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)		此計畫目前所有的研究數據仍在分析中，預計年底將研究結果彙整並撰寫成一篇期刊論文並投稿至相關領域的SCI期刊。			