行政院國家科學委員會專題研究計畫　成果報告

魚油及炸油對大白鼠脂肪組織中 PPARalpha 與 PPARgamma 下游基因表現及脂肪細胞型態之影響

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摘要

細胞核轉錄因子 Peroxisome Proliferator Activated Receptor (PPAR)是固醇類荷爾蒙受器家族成員之一，目前已知有 PPAR α、δ(β)、γ三種 isoforms，其中 PPAR α 主要表現於肝臟，負責調控脂質代謝基因之轉錄表現；PPAR γ_2 主要表現於脂肪組織，調控脂肪細胞分化及脂質合成基因之表現。已知魚油與炸油均可活化 PPAR α，二者均可降低大鼠肝臟脂質，促進肝臟脂質代謝。本研究目的在探討魚油及炸油對大鼠脂肪組織細胞型態、脂質代謝影響。我們假設炸油與魚油均會抑制脂肪組織 PPAR γ 下游基因表現，因而抑制高脂飲食誘發之脂肪細胞增大作用(Hypertrophy)。此作用可能是經由活化肝臟 PPAR α 下游基因表現，促進肝臟脂質代謝，降低血液中富含 TG 之脂蛋白含量，進而影響周邊組織(脂肪組織)對 TG 之利用。當脂肪細胞合成的 LPL 減少，便會減少脂肪細胞內 TG 合成與堆積，導致脂肪細胞與組織量減小，這可能影響脂肪細胞激素分泌，進而影響血糖控制。

實驗設計將大白鼠分為低油組(LF)，餵予 5%新鮮黃豆油，三組高油組分別餵予 20%新鮮黃豆油(HF)、油炸黃豆油(HO)及魚油(HFO)，六週後犧牲，以 Northern 法偵測腹膜後及副睪脂 PPAR γ 下游基因(LPL、aP2) mRNA。脂肪細胞以鋨酸染色固定觀察脂肪細胞大小及數目，另以化學分析測定脂肪組織內 DNA、蛋白質、脂質含量。定量肝脂質、血漿脂質、leptin、血糖及胰島素。結果顯示餵食魚油(HF)與炸油(HO)均會表現 PPARα活化特徵，包括肝腫大，過氧化體增生，肝脂質降低及 PPARα下游基因 ACO 酵素活性增加。二者也會會抑制脂肪細胞增大作用(hypertrophy)，炸油並不影響脂肪組織中細胞總數，但脂肪細胞內脂質含量顯著降低 ($P<0.05$)，炸油抑制脂肪細胞增大作用更勝魚油。但餵食魚油或炸油對脂肪組織 LPL、aP2 mRNA 表現並無顯著影響。不同於預期的是，炸油與魚油在血糖調控上似乎走向不同途徑，炸油導致大鼠禁食血糖及胰島素顯著增加 ($P<0.05$)，似乎有造成胰島素抗性之趨勢。

關鍵詞：魚油、炸油、脂肪組織、PPAR α、PPAR γ、胰島素抗性

Abstract

PPAR (Peroxisome proliferator activated receptor) are transcription factor belonging to the nuclear receptor family. Three major isoforms, PPAR α、 δ (β) and γ had been identified in mammalian tissues. PPAR α is mainly expressed in liver and transcriptionally regulate the expression of genes for lipid metabolism. PPAR γ_2 is expressed exclusively in adipose tissue and regulate the differentiation of adipocytes as well as genes for lipid storage. This study was aimed at the effect of oxidized frying oil (OFO) and fish oil on the morphology and lipid metabolism of adipocytes. We hypothesized the expression of PPARγ down stream genes in adipocytes will be down-regulated by feeding dietary OFO or fish oil. As a result, the hypertrophy of adipocytes induced by high-fat diet will be inhibited. These effects could be inferred to the activated PPAR α , dietary OFO and fish oil enhance the lipid metabolism in liver and decrease the TG-rich lipoprotein in plasma, which may reduce the ability of utilization of TG in extra-hepatic tissue. When the expression of LPL is decreased in adipose, the synthesis and accumulation of TG in adipocyte will be reduced then result in smaller cellular size and decreased fat mass. The hormones secreted by adipocytes may be modified and affect the regulation of glucose homeostasis in rats.

To test this hypothesis, SD rats were separated into one low-fat group (LF) which fed with 5 % fresh soybean oil, and 3 experimental high fat diet groups which fed with 20% fresh soybean oil (HF), OFO (HO) or fish oil (HFO) respectively. After 6 weeks, rats were killed and the expression PPAR γ down-stream genes (LPL and aP2) were measured in epididymal and retroperitoneal fat pad. The size and numbers of adipocytes from the two fat tissues were detected and chemical composition of adipocytes, liver lipids, plasma lipids, leptin, insulin and glucose were also analyzed. The results show, feeding with dietary OFO or fish oil, liver enlargement, peroxisome proliferation, reduction of liver lipid and induction of PPAR α target gene—ACO activity, all of the PPAR α activation effects were induced. Both OFO and fish oil inhibit the hypertrophy of adipocytes. Feeding OFO had no effect on total adipocyte numbers, but the lipid content in adipocytes were significantly reduced ($P<0.05$). The anti-hypertrophy effect of OFO was more potent than fish oil. However, the expression of aP2 and LPL genes in epididymal and retroperitoneal fat tissue were not significantly influenced by feeding OFO or fish oil. Unexpected, the fasting serum glucose and insulin were significantly increased which imply an insulin resistance may be under developed.
Keywords : fish oil, oxidized frying oil, adipose tissue, PPAR α , PPAR γ , insulin resistance

前言

已知 PPAR(Peroxisome Proliferator Activated Receptor)是負責調控脂質代謝與脂肪細胞分化之轉錄因子，目前發現在哺乳類有三種 isoforms：PPARα、δ(β)、γ，PPARδ(β)雖分布最廣，但其功能並不清楚；PPARα主要表現於可代謝脂質組織，如肝、心、腎、棕色脂肪、骨骼肌，而 PPARγ_2 主要表現於脂肪組織。利用基因剔除小鼠(gene knockout mice)已清楚證實 PPARα角色在維持及調控脂質代謝；PPARγ則是脂肪細胞分化所必須。

除了降血脂藥 Peroxisome proliferator (PP)已知魚油可活化 PPARα 及γ，近來我們證實氧化炸油亦可活化 PPARα (Chao et al, 2001)。大鼠餵食 20% (w/w)炸油可促進肝臟 PPARα下游基因 ACO 及 CYP4A1 表現，Transactivation assay 亦證實炸油含有 PPARα活化物。炸油中的 PPARα活化物推測可能包含 CLA、Hydroxy fatty acids(如 Ricinoleic acid、9-HODE 或 13-HODE)或其他未知物。事實上，這些脂肪酸或其氧化物利用 transactivation assay 均已證實可同時活化 PPARα及γ，因此魚油或炸油的生理效應部分應可歸因於 PPAR 對肝臟及周邊組織作用，進而影響體內脂肪及碳水化合物代謝。

由於過去未曾探討炸油對肝外周邊組織之影響，再加上大鼠餵食炸油部分效應與魚油非常類似，例如體重降低、肝臟及血漿 TG 降低、肝臟解毒酵素 CYP450 量增加，脂肪組織量減小，因此本研究計劃比較魚油與炸油對大鼠脂肪組織脂質代謝及脂肪細胞型態之影響。

我們假設炸油與魚油均會抑制脂肪組織 PPARγ下游基因表現，因而抑制高脂飲食誘發之脂肪細胞增大作用(Hypertrophy)。此作用可能是經由活化 PPARα，促進肝臟脂質代謝，降低血液中富含 TG 之脂蛋白含量，進而影響周邊組織(脂肪組織)對 TG 之利用。由於 LPL 基質(富含 TG 之脂蛋白)減少，脂肪細胞合成分泌的 LPL 可能減少，因此降低脂肪細胞內 TG 合成與堆積，導致脂肪細胞與組織量減小，這可能影響脂肪細胞激素分泌，進而影響血糖控制。

研究方法

1. 炸油製備
   延續先前方法，為黃豆沙拉油以 205±5℃油炸麵片 24 小時(6hr/d×4d)。
2. 動物飼養
   剛離乳 SD 品系公鼠 32 隻分為四組：LF (5%新鮮黃豆油)、HF(20%新鮮黃豆油)、HO(20%炸油)、HFO(20%魚油)餵食六週。
3. 組織分析及檢測
   1) 組織稱重：肝、腎、腹睪脂、骨骼肌、棕色脂肪、心、腦。
   2) 組織取樣：抽血分離血漿，取部分肝、腹睪脂、骨骼肌以液態氮冷凍保存供抽取 RNA，肝、腹睪脂作組織切片。
   3) mRNA 表現：以 GTC 抽取 RNA，以 Northern 法偵測副睪脂及腹膜後脂肪組織 LPL、aP2 mRNA，並以 18S rRNA 作校正。

4) 細胞組織化學分析及成分分析：肝切片以 DAB 染色，觀察過氧化體增生情形。脂肪組織以 collagenase 處理收集脂肪細胞後以鋨酸固定染色以細胞計數器測定細胞直徑分布，另脂肪組織分別萃取脂質及 DNA 分析組織內粗脂肪、TG 含量、DNA 及蛋白質。
5) 血液生化分析：肝臟與血漿脂質包括 TG、TC、PL、NEFA，以市售套組進行。血清 leptin、insulin 及 glucose 以市售套組進行。

統計分析：以 Student t test 比較 LF 與 HF 組差異高油組(HF、HO、HFO)間則以 one-way ANOVA 進行檢定若有顯著差異則以 Duncan's multiple range test 分析組間差異。所有數據均事先檢定是否常態分布否則轉為對數值

結果與討論

由於炸油顯著抑制大鼠攝食量，魚油亦有輕微食量降低現象，因此餵食方式以對飼育進行，半數 LF 與 HF 組供給與 HO 組攝取熱量相當飼料量，其餘半數則比照 HFO 組提供熱量相當飼料量。由於 HO 與其對飼育大鼠攝食量及體重顯著低於 HFO 及其對飼育組，因此生長速率分別比較結果如圖一所示，在控制熱量相當下攝取炸油及魚油並不影響大鼠體重增加，同樣也不影響飼料效率 (Feed efficiency)。

除體重增加及攝食量外，不論 LF 或 HF，對照 HO 或 HFO 飼育並不影響以下結果，因此下述結果將兩種對飼育合併，回歸四組 LF (pair fed with HO/HFO) 、HF (pair fed with HO/HFO)、HO 及 HFO 統計。在相對組織重方面，高油飼料顯著增加腹睪脂及腹膜後白色脂肪組織量(HF vs. LF, $P<0.05$, 表一)比較 HF、HO 及 HFO 組，發現炸油及魚油顯著增加肝及腎重量，降低腹睪脂及腹膜後脂肪量，此外炸油顯著增加肩胛棕色脂肪量，而魚油不會。其中炸油造成肝腫大及降低腹膜後脂肪堆積效應顯著大於魚油 ($P<0.05$)。

表二為血脂質及肝脂質定量結果：HF 與 LF 組相較有顯著較低血清總脂質 (Total lipid)，但較高肝臟 Total lipid、TG、TC ($P<0.05$)。魚油顯著降低大鼠血清 Total lipid 、TC 及 NEFA，炸油只顯著降低血清 NEFA；但在肝脂質方面，炸油如同魚油顯著降低肝臟 Total lipid、TG、TC 及 NEFA，二組肝脂質堆積量僅為 HF 組之 1/3~1/2。

根據過去經驗知道炸油降低肝脂質堆積是經由活化 PPARα，促進 PPARα下游基因，包括 ACO 及 CYP4A1 表現，刺激肝臟脂質走向氧化代謝途徑。圖二顯示炸油與魚油顯著增加肝臟 ACO 活性，其順序為 HO>HFO>HF ($P<0.05$)，而高新鮮油本身並不增加 ACO 活性 (HF vs. LF, $P>0.05$)(圖二)。此外肝切片以 DAB 染色觀察過氧化體，證明魚油及炸油均會造成肝臟過氧化體增生，尤其炸油的 peroxisome prolifeeration 效應更甚於魚油(圖三)。肝臟過氧化體增生、肝腫大、誘導 ACO 活性均是 PPARα活化特徵，由此顯示炸油活化 PPARα能力應大於魚油。

根據本研究假說，炸油及魚油會改變脂肪細胞脂質代謝及型態，因此可能

影響血糖調控，因此追蹤四週期間禁食血糖變化。結果顯示 HF 相較於 LF，或 HF 相較於 HO 及 HFO，血糖並無顯著差異，除了在第四週 HO 組血糖顯著高於 HF 及 HFO($P<0.05$，圖四)。有趣的是 HO 組大鼠在犧牲時有最高的血清胰島素，高油本身(HF vs. LF)或魚油油種 (HFO vs. HF)並不會增加血清胰島素(圖五)，因此炸油似乎會降低胰島素敏感性。血清 Leptin 雖懷疑與胰島素抗性有關，但在本研究卻顯示 HO 組有顯著最低 serum leptin (HO vs. HF, $P<0.05$) (圖五)。正如文獻所示，Serum leptin 與體脂肪有正相關，本研究各組間 serum leptin 變化亦呈現與脂肪組織量一致。

圖六及圖七分別為腹膜後及腹睪脂脂肪細胞大小分布圖。不論腹膜後或腹睪脂脂肪，HF 與 LF 相較，脂肪細胞大小並無差異；而 HO 及 HFO 組細胞直徑分布圖則有向左移趨勢，尤其 HO 組向左偏斜程度最大，顯見餵食炸油及魚油均會導致脂肪細胞變小，其中炸油抑制脂肪細胞增大(Hypertrophy)作用更甚魚油。

除了直接定量脂肪細胞大小，DNA 含量間接代表細胞數目。如圖八所示，每單位重(克)DNA 量(μg/g tissue)可以解釋脂肪細胞大小變化，每塊組織 DNA 量(μg/tissue)則提供脂肪細胞 Clonal expension 或 Apoptosis 可能受油脂量或來源影響。結果顯示，高脂飼料並不影響大鼠腹膜後及腹睪脂脂肪細胞數目(HF vs. LF)，而炸油組不論在腹膜後及腹睪脂脂肪，就每單位重來看均有最高的 DNA 含量，換言之每單位重量有最多細胞數目，這與前述觀察到它有最小的脂肪細胞相符。但魚油組每單位重之 DNA 量與 HF 組相較並無顯著差異，倒是腹睪脂有顯著較低總細胞數($P<0.05$)，顯示魚油或許可能促進脂肪細胞凋亡或抑制細胞增生。炸油則不影響此二組織細胞總數。此結果亦顯示炸油或魚油對不同部位脂肪組織有不等程度影響。

脂肪組織的粗脂肪及 TG 定量顯示(表三)，高脂飲食並不影響腹膜後及腹睪脂每單位重或每塊組織的脂質量，除了腹睪脂 TG 總量顯著增加 (3.18 vs. 2.28 mmol/tissue, HF vs LF)。餵食炸油顯著降低腹膜後脂肪組織粗脂肪及 TG 總量($P<0.05$)，魚油則顯著降低腹睪脂每單位重及每塊組織粗脂肪及 TG 量($P<0.05$)。若是將脂質定量結果以每單位重(克)DNA 表示則可突顯炸油組的 Anti-hypertrophy of adipocytes 效應。炸油組不論在腹膜後及腹睪脂細胞中均有顯著最低的粗脂肪或 TG 量；魚油組與 HF 組相較，雖然每單位 DNA 也有較低的脂質量，但未達統計差異。這些結果也暗示了炸油似乎對腹膜後脂肪組織，而魚油似乎對腹睪脂有較大效應。

圖九顯次 HF 不論與 LF 相較或與 HO 或 HFO 相較，脂肪組織 aP2 及 LPL mRNA 表現均無顯著差異。

以上結果證明，炸油與魚油均會抑制大鼠脂肪細胞增大作用。雖然魚油及炸油均具有 PPARα活化能力，二者抑制肝脂質堆積程度也相當，但炸油抑制脂肪細胞脂質堆積能力大於魚油。炸油對脂肪組織 PPARγ下游基因影響需再探討。且與魚油不同的是，炸油造成大鼠禁食血糖及胰島素增加，似乎有造成胰島素抗性之趨勢。這或許是炸油與魚油活化 PPARα能力不同，或是魚油與炸油影響不

同部位脂肪組織，而在血糖調控走向不同後果。此外，炸油造成胰島素抗性之途徑，以及此不利成分究竟為何，是否與先前發現之 PPARα活化物及降低血液或肝臟脂質堆積之成分相同，均是未來值得研究方向。

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Table 1 Relative tissue weight of rats fed experimental diets for 5 wk[1]

	Relative liver wt.	Relative epididymal fat wt.	Relative retroperitoneal fat wt.	Relative brown adipose wt.	Relative kidney wt.
			g/100 g body		
LF	3.21 ± 0.32	1.05 ± 0.27*	1.24 ± 0.20*	0.15 ± 0.03	0.88 ± 0.06
HF	3.37 ± 0.27^{c}	1.35 ± 0.16^{a}	1.53 ± 0.37^{a}	0.17 ± 0.03^{b}	0.83 ± 0.06^{b}
HO	5.69 ± 0.37^{a}	1.14 ± 0.22^{b}	0.62 ± 0.31^{c}	0.23 ± 0.04^{a}	0.99± 0.06^{a}
HFO	4.18 ± 0.36^{b}	1.02 ± 0.26^{b}	1.14 ± 0.33^{b}	0.20 ± 0.05^{ab}	1.01 ± 0.07^{a}

[1] Values are means ± SD, n = 8. * denote a significant difference (*P*<0.05) between LF and HF group which was analyzed by student t-test. The significance of differences among HF, HO and HFO groups were analyzed by one-way ANOVA and Duncan's Multiple Range Test. Values not sharing a superscript letter are significantly different (*P*<0.05).

Table 2 Serum and liver lipids of rats fed experimental diets for 5 wk[1]

	Serum lipids				Liver lipids			
	Total lipid	TG	TC	NEFA	Total lipid	TG	TC	NEFA
	g/L		*mmol/L*		*mg/g*		*μmol/g*	
LF	6.29 ± 0.87*	1.09 ± 0.40	2.54 ± 0.63	0.48 ± 0.12	34.6 ± 5.3*	25.0 ± 6.5*	34.6 ± 5.3*	22.1 ± 4.6
HF	5.22 ± 1.14^{a}	0.71 ± 0.19^{a}	2.71 ± 0.79^{a}	0.44 ± 0.07^{a}	53.5 ± 14.3^{a}	40.5 ± 9.9^{a}	53.5 ± 14.3^{a}	24.2 ± 4.7^{a}
HO	5.57 ± 1.21^{a}	0.82 ± 0.30^{a}	2.55 ± 0.40^{a}	0.33 ± 0.06^{b}	19.1 ± 9.0^{b}	17.4 ± 10.1^{b}	19.1 ± 9.0^{b}	10.5 ± 3.3^{b}
HFO	3.17 ± 0.81^{b}	0.68 ± 0.18^{a}	1.63 ± 0.38^{b}	0.35 ± 0.02^{b}	20.2 ± 4.5^{b}	12.1 ± 5.4^{b}	20.2 ± 4.5^{b}	10.7 ± 1.7^{b}

[1] Values are means ± SD, n = 8. * denote a significant difference (*P*<0.05) between LF and HF group which was analyzed by student t-test. The significance of differences among HF, HO and HFO groups were analyzed by one-way ANOVA and Duncan's Multiple Range Test. Values not sharing a superscript letter are significantly different (*P*<0.05).

Table 3 Lipid content in retroperitoneal or epididimal fat pad of rats fed experimental diets for 5 wk[1]

	Crude fat			TG		
	(g/g tissue)	(g/tissue)	(g/g DNA)	(mmol/gtissue)	(mmol/tissue)	(mmol/gDNA)
Retroperitoneal fat pad						
LF	0.48 ± 0.05	1.59 ± 0.22	1132 ± 384*	0.49 ± 0.07*	1.60 ± 0.27	1148 ± 421
HF	0.48 ± 0.03[a]	2.07 ± 0.71[a]	2000± 1015[a]	0.41 ± 0.04[a]	1.76 ± 0.59[a]	1664 ± 720[a]
HO	0.46 ± 0.05[a]	0.61 ± 0.48[b]	585 ± 221[b]	0.45 ± 0.10[a]	0.61 ± 0.49[b]	595 ± 267[b]
HFO	0.44 ± 0.07[a]	1.28 ± 0.67[b]	1275± 615[ab]	0.46 ± 0.07[a]	1.38 ± 0.78[a]	1365 ± 661[a]
Epididymal fat pad						
LF	0.67 ± 0.05	1.97 ± 0.57	1322 ± 387	0.78 ± 0.11	2.28 ± 0.71*	1518 ± 419
HF	0.73 ± 0.32[a]	2.76 ± 1.47[a]	1470 ± 711[a]	0.86 ± 0.09[a]	3.18 ± 0.69[a]	1730 ± 645[a]
HO	0.67± 0.03[ab]	1.77± 0.49[ab]	854 ± 300[b]	0.91 ± 0.07[a]	2.36± 0.55[ab]	1141 ± 375[a]
HFO	0.43 ± 0.22[b]	1.52 ± 1.12[b]	1053± 496[ab]	0.58 ± 0.30[b]	2.06 ± 1.46[b]	1429 ± 664[a]

[1] Values are means ± SD, n = 8. * denote a significant difference (P<0.05) between LF and HF group which was analyzed by student t-test. The significance of differences among HF, HO and HFO groups were analyzed by one-way ANOVA and Duncan's Multiple Range Test. Values not sharing a superscript letter are significantly different (P<0.05).

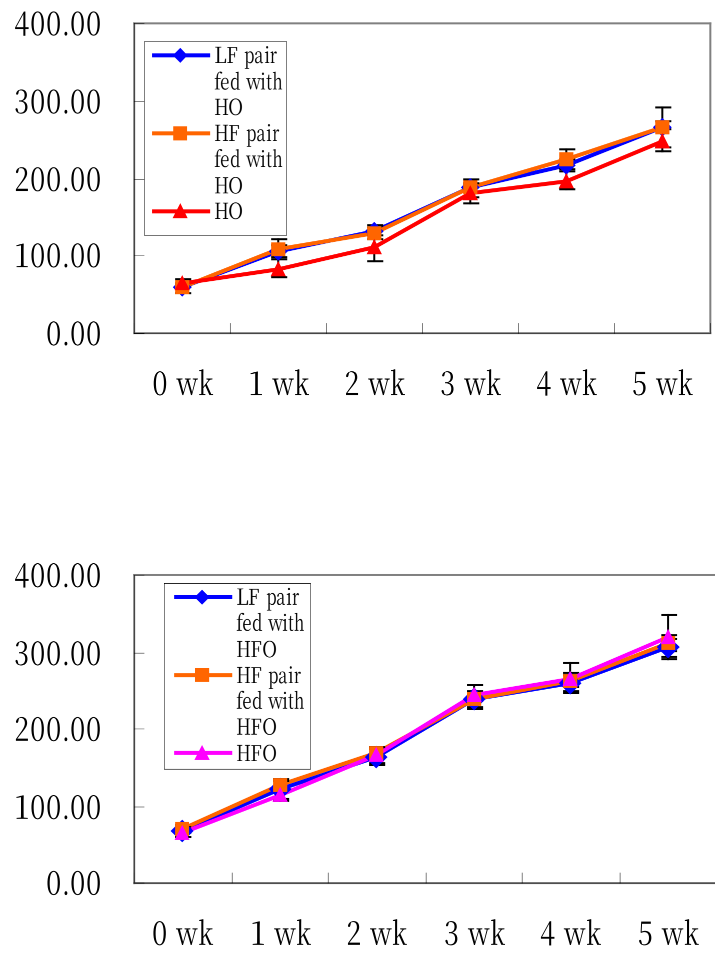

Figure 1 The growth curve of rats fed experimental diets for 5 weeks. There was no significant difference of body weight gain of rats fed with HO or HFO diet when compared with their pair fed controls.

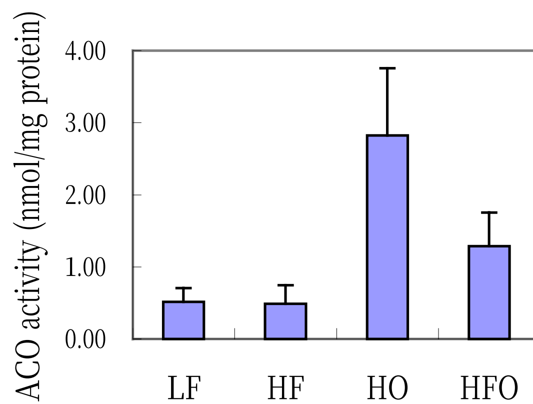

Figure 2 The acyl-CoA oxidase (ACO) activity in liver of rats fed experimental diets for 5 weeks. The significance of differences among HF, HO and HFO groups were analyzed by one-way ANOVA and Duncan's Multiple Range Test. Values not sharing a superscript letter are significantly different ($P<0.05$).

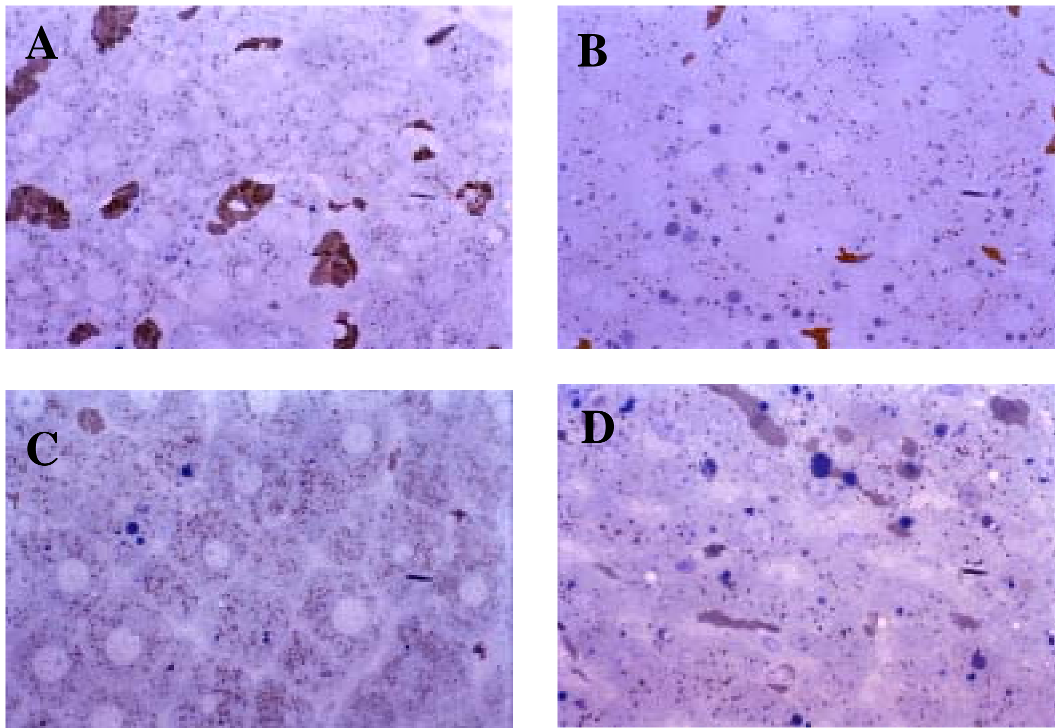

Figure 3 The proliferation of peroxisome in liver of rats fed experimental diets for 5 weeks. A: LF group, B: HF group, C: HO group, D: HFO group. The liver sections were stained by DAB and observed under light microscope.

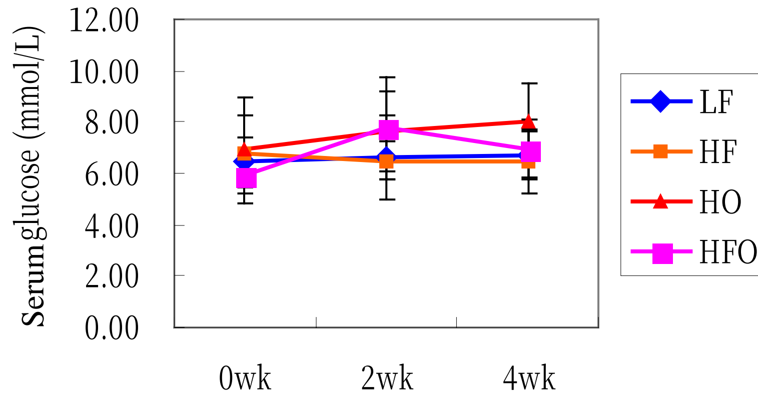

Figure 4 The profile of serum glucose change of rats fed experimental diets for 5 weeks. * indicate a significant difference between HO and HF groups (P<0.05).

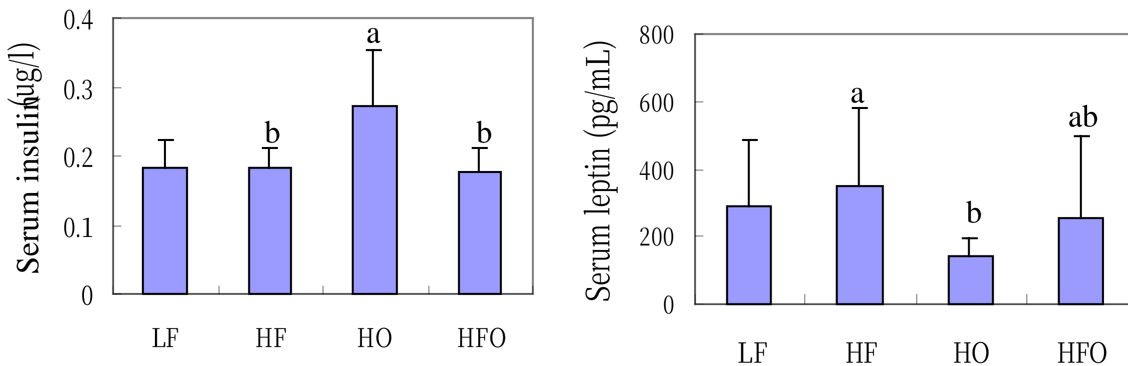

Figure 5 The concentration of serum insulin and leptin of rats fed experimental diets for 5 weeks. The significance of differences among HF, HO and HFO groups were analyzed by one-way ANOVA and Duncan’s Multiple Range Test. Values not sharing a superscript letter are significantly different (P<0.05).

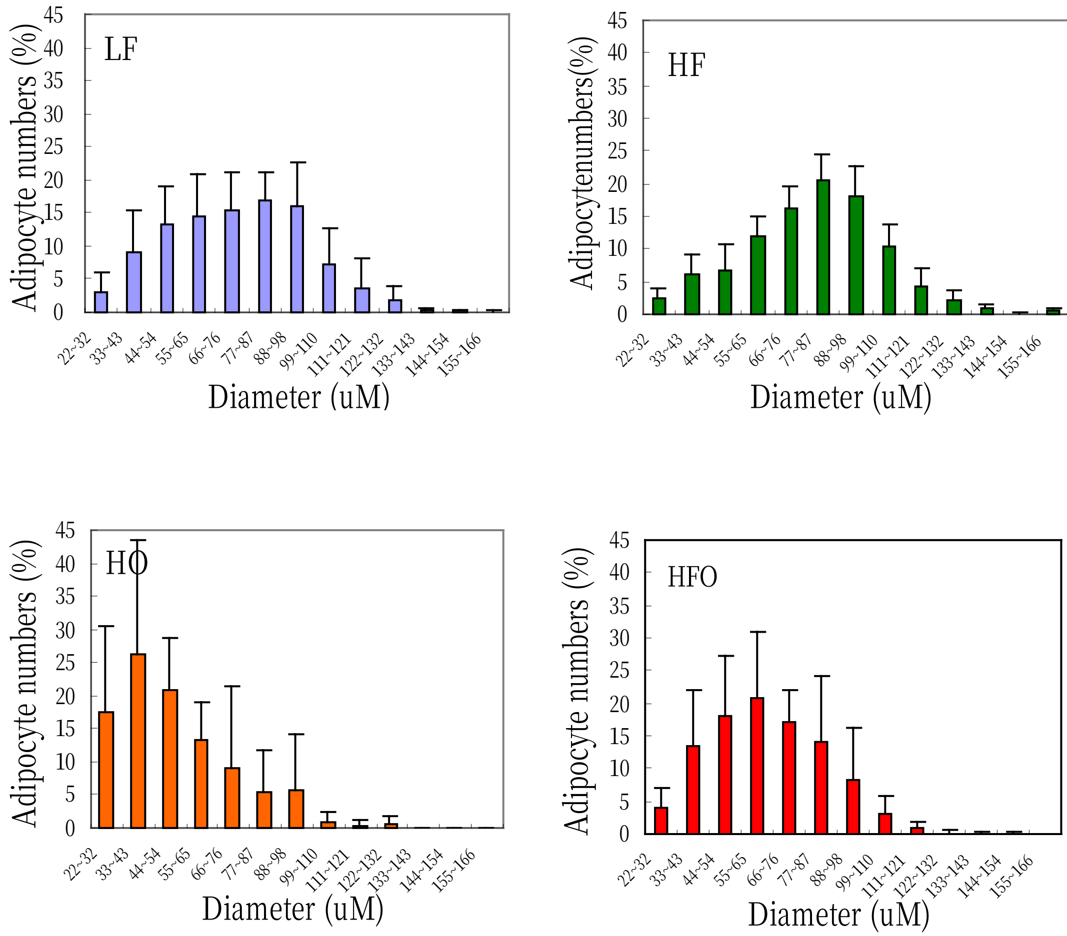

Figure 6 Distribution of sizse of adipocytes from retroperitoneal fat pad of rats fed experimental diets for 5 weeks.

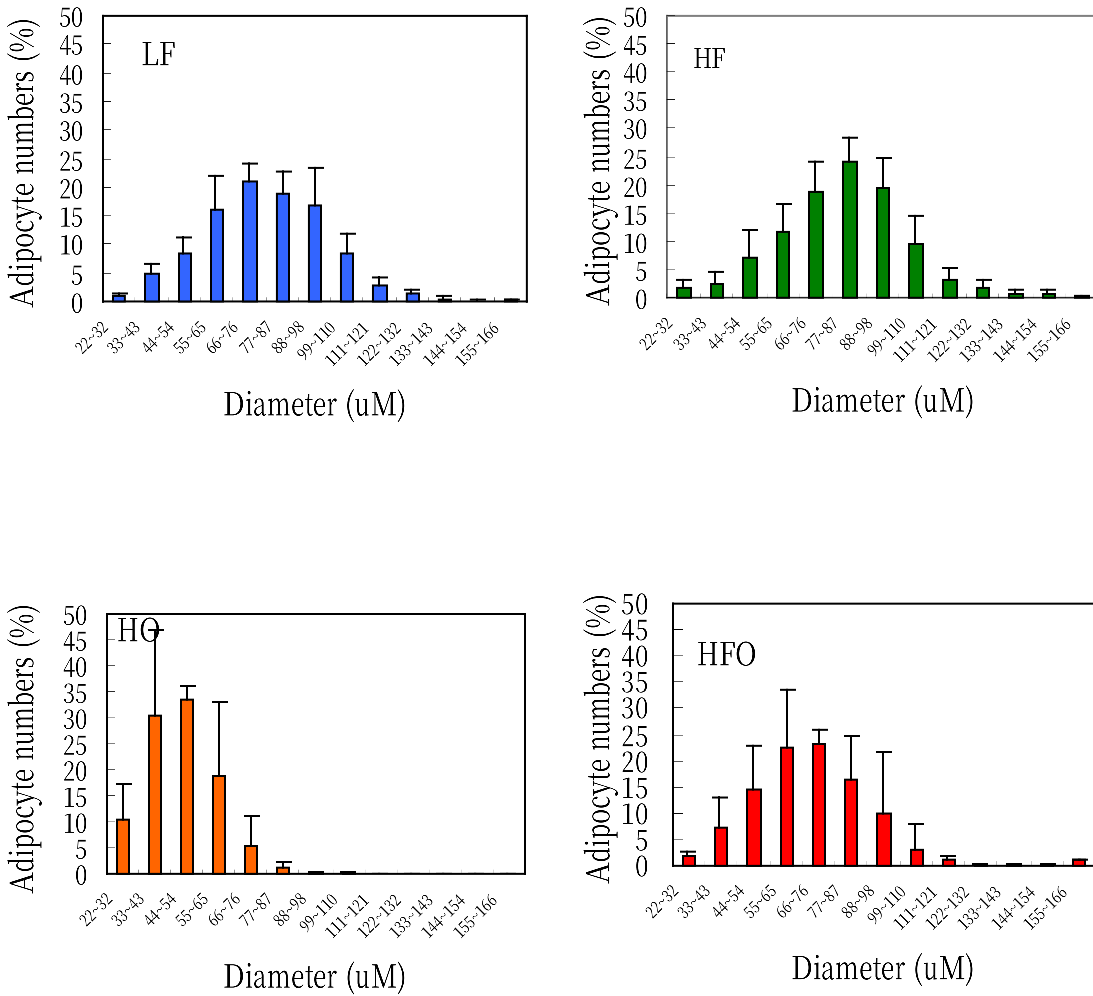

Figure 7 Distribution of sizes of adipocytes from epididymal fat pad of rats fed experimental diets for 5 weeks.

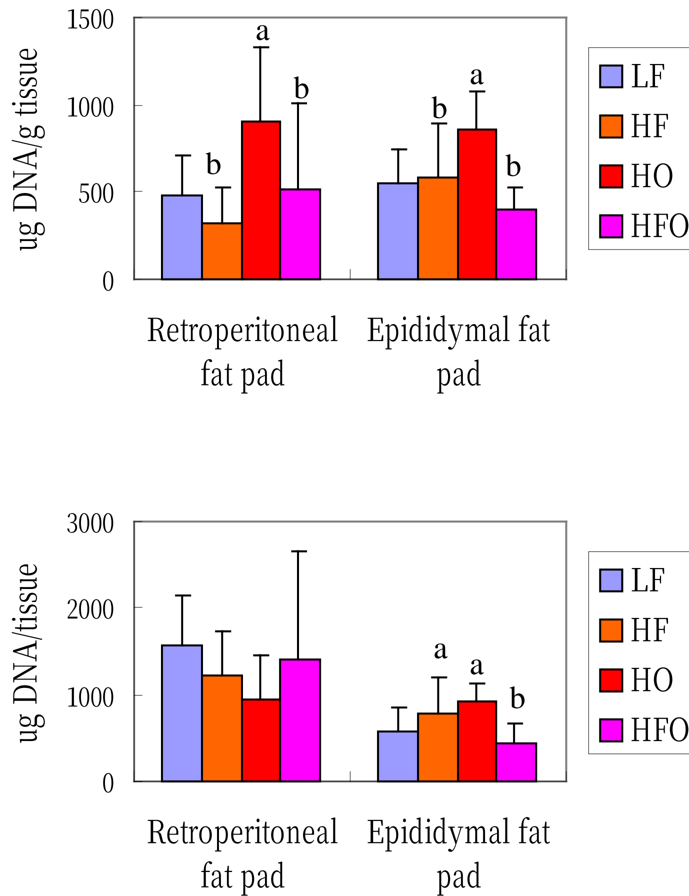

Figure 8 DNA content in retroperitoneal or epididymal fat pad of rats fed experimental diets for 5 weeks. The significance of differences among HF, HO and HFO groups were analyzed by one-way ANOVA and Duncan's Multiple Range Test. Values not sharing a superscript letter are significantly different ($P<0.05$).

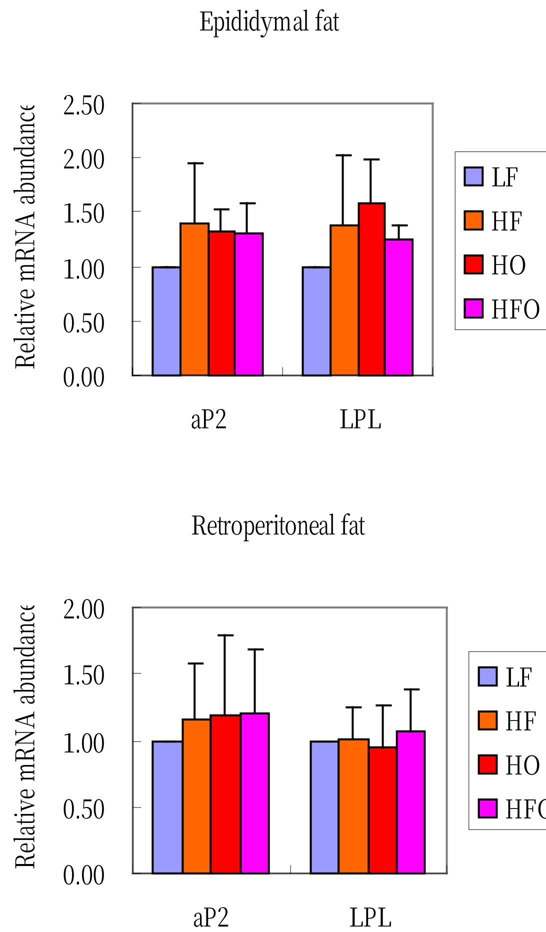

Figure 9 aP2 and LPL mRNA content in retroperitoneal or epididymal fat pad of rats fed experimental diets for 5 weeks. Each value was normalized by 18S RNA.

計劃成果自評

結果部分與假設不符，但意外發現炸油似乎有導致胰島素抗性之趨勢，除了血脂，炸油對體脂肪生成、胰島素敏感性、血糖控制將是未來值得探討方向，由於炸油成分之複雜及其呈現生理效應之多貌性，不同效應或許歸因於不同成分。研究成果除可發表學術期刊，未來將有助於食品科技應用開發針對不同保健需求最有利油炸用油或油炸條件。