CANOLA OIL DIET SUPPLEMENTATION DECREASED LIPID ACCUMULATION PRODUCT, FATTY LIVER INDEX, AND LIVER STEATOSIS IN YOUNG OBESE FEMALES

I Wayan Weta[1], I.B. Putra Manuaba[2], W.P. Sutirtayasa[2], Safarina G. Malik[3], W. Citrawulan Sucipta[4], Firman P. Sitanggang[5].

Correspondence: wayan_weta06@yahoo.co.id

1. Department of Clinical Nutrition, Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia.
2. Post Graduated Program of Udayana University, Denpasar, Bali, Indonesia.
3. Ejkman Institute for Molecular Biology, Jakarta, Indonesia.
4. Department of Public Health, Faculty of Medicine , Udayana University, Denpasar, Bali, Indonesia.
5. Department of Radiology, Sanglah Hospital, Denpasar, Bali, Indonesia.

Abstract

Background: Morbidity of obesity increases in population of most countries. Lipid accumulation in obesity relates to low grade chronic inflammatory state and associates with nonalcoholic fatty liver disease (NAFLD). This condition might also due to high ratio of n-6:n-3 poly unsaturated fatty acid (PUFA) in daily intake of most population.

Methods: We conducted a randomized, triple blind (subjects, assessors, and investigators) clinical trial, consisting of 66 young obese females in Denpasar, Bali, Indonesia. Subjects were divided into two groups (33 with (+CO) and 33 without (-CO) canola oil intervention), for 12 weeks. Data were collected before the study (pre), at 6 weeks (mid) and 12 weeks (post). The +CO group was supplemented with 30 ml emulsion consisted of 10 g canola oil (2000 mg linoleic acid (LA), and 1000 mg and α-linolenic acid (ALA), n-6:n-3 PUFA ratio 2:1), and the -CO group with 30 ml emulsion of placebo. All subjects were recommended for restricted daily energy intake below 1500 kcal. Independent variables of body mass index (BMI), waist circumference (WC), triglyceride (TG), and γ-glutamyltransferase (GGT) were measured. Liver steatosis was assessed using ultrasonography (USG). Lipid accumulation product (LAP) and Fatty liver index (FLI) were calculated.

Results: There were significant decreased of LAP (pre vs mid and mid vs post, both P=0.033, and pre vs post, P=0.000), and FLI (pre vs mid, P=0.021, and pre vs post, P=0.036) in the +CO group, respectively. Prevalent of liver steatosis were decreased (P=0.000) in both the +CO and -CO groups. There were strong correlations among LAP, FLI and liver steatosis (P<0.001). Strong correlation was also observed between pre and mid LAP and FLI to post liver steatosis. FLI showed a consistently specific correlation to all stages of liver steatosis, and the correlationt was even stronger in higher stage of liver steatosis.

Conclusion: Supplementation of 10 g canola oil daily for 12 weeks decreased LAP, FLI, and liver steatosis in young obese females in Denpasar, Bali, Indonesia. LAP and FLI were good predictors of liver steatosis. However, FLI was a better and more specific predictor for progression of higher level of liver steatosis when compared to LAP.

Key words: young obese females, canola oil, LAP, FLI, liver steatosis.

Introduction

Prevalence of obesity is rapidly growing throughout the world. The morbidity and mortality related to its complications is also rising. Obesity is considered a gateway disease. Individuals with severe obesity have a high risk of comorbidities including nonalcoholic fatty liver disease (NAFLD), cardiovascular disease, and diabetes. Obesity is associated with an increased risk of NAFLD. Steatosis, the hallmark feature of NAFLD, occurs when the rate of hepatic fatty acid uptake from plasma and de novo fatty acid synthesis is greater than the rate of fatty acid oxidation and export (as triglyceride within very low-density lipoprotein). Therefore, an excessive amount of intrahepatic triglyceride (TG) represents an imbalance between complex interactions of metabolic events [1]. NAFLD now represents the most common of liver disorders and the most frequent cause of chronic liver disease [2]. It is now apparent that adipocytes are not simply a storage reservoir of fat but are active endocrine organs that play multiple roles in the body. Their metabolic role changes as they enlarge with increasing obesity [3]. Obesity is a chronic low grade inflammatory disease that marked by expression of pro-inflammatory cytokines such as TNF-α and IL-6 [4, 5].

Linoleic acid (LA; n-6 poly unsaturated fatty acid (n-6 PUFA)) and α-linolenic acid (ALA; n-3 PUFA), have the same metabolic pathway. In human and animal, LA and ALA were desaturated and elongated competing against each other by three enzymes, δ-6 desaturase, elongase and δ-5 desaturase, to produce long chain PUFA (LC-PUFA), arachidonic acid (AA) and ecosapentanoic acid (EPA), docosapentanoic acid (DPA) and docosahexanoic acid (DHA). AA and EPA produce antagonistic physiologic eicosanoids. AA produce pro-inflammatory eicosanoid, like prostaglandin 2 (PG2), leukotriene 4 (LT4), but EPA formed PG3 and LT5 series as anti-inflammatory eicosanoid [6,7]. Only a small amount of ALA from diet is converted into n-3 LC-PUFA. The conversion of ALA into EPA, DPA and DHA, were found to be higher in females compared to males. In males, only 15% of ALA was converted into EPA and only five percent into DHA. While in females, 35% of ALA was converted into n-3 LC-PUFA (21% EPA, 6% DPA and 9% DHA). This condition may be due to higher β-oxidation process of fatty acid in males compared to females [8, 9]. EPA also potentially inhibits the activity of δ-5 desaturase. AA also produces PG2 series that inhibits δ-6 and δ-5 desaturase. These enzymes converted n-6 PUFA derivative, dihomo γ-linolenic acid (DGLA) into AA. DGLA also produce anti-inflammatory eicosanoid PG1 series. This condition enables the control mechanism of the AA composition in the cell membrane. This is an auto-regulation mechanism to balance the pro-inflammatory mediators produced by AA with anti-inflammatory

mediators produced by EPA and DGLA [10, 11]. Other benefit effect of n-3 PUFAs derived reduce lipid accumulation in obese, through two mechanisms N-3 PUFAs, especially EPA and DHA, could control pathways that involved in hepatic metabolism, by regulating gene transcription factors (PPARα, PPARγ, SREBP-1, ChREBP) [12]. The first mechanism of n-3 PUFA decrease lipid synthesis through inhibition of sterol regulatory element-binding protein-1c (SREBP-1c) transcription. The second mecanism increase β-oxidation of fatty acid through expression activatation of peroxisome proliferator-activated receptor- α (PPAR-α) to stimulate oxidation of fatty acid, inhibit pro-inflammatory mediator (TNFα, IL-6). N-3 PUFAs also activate PPARγ, to increase fatty acid oxidation and improve insulin sensitivity [12, 13, 14, 15].

In obese individuals, total n-3 PUFA(ALA) and LC-PUFA (EPA,DHA) are 30 % and 35% lower compared those with normal weight. This means that n-6 PUFA (LA) and LC-PUFA (AA) are relatively higher in obese than non-obese individuals [16]. Increasing n-6:n-3 ratio willinfluence pro- versus anti-inflammatory eicosanoid production within the liver that contribute to the development of non-alcoholic fatty liver disease (NAFLD) [8].

Evidence from autopsy and imaging studies demonstrate that NAFLD was found in 20-35% of populations worldwide, with 10% of these cases were being NASH. The prevalence of NAFLD is much higher among obese patients and patients with type 2 diabetes. In those conditions, NAFLD is found in 70-80% of patients. Among those patients, 25-70% were reported to develop into advance diseasessuch as NASH and fibrosis [17, 18, 19]. The incidence of paediatric NAFLD has risen sharply in the last three decades, corresponding with worldwide increase in childhood obesity. The estimates of NAFLD prevalence in obese children using ultrasonography is ranging from 45-60%. The International Obesity Task Force has concluded that the lowest estimated prevalence of hepatic steatosis is 28% among obese children in EU [19].

NAFLD is the pathological accumulation of fat, mainly triglycerides in hepatocytes that exceeds 5% of the liver weight in the absent of alcohol intake [17]. Disease can progress from macrovesicular lipid accumulation in the hapatocytes (steatosis) to non-alcoholic steatohepatitis (NASH) to outright fibrosis, cirrhosis and even hepatocellular carcinoma. A combination of environmental and genetic factors determines individual risk of NAFLD development and progression, with nutrition as modifiable environmental risk factor. Obesity, insulin resistance, type 2 diabetes, hypertriglyceridemia, low HDL cholesterol and hypertension are primary causes of

NAFLD, and the secondary causes are nutritional problem, drug, metabolic disarrangement, toxin and infection [17, 20].

The pathogenesis of NAFLD proposed to be a 'two hit process', with fat accumulation in hepatocytes viewed as the primary insult, and increased oxidative stress leading to inflammation being the second 'hit' in progression to NASH and fibrosis. Only a minority of patients with hepatic steatosis progress to necro-inflammatory NASH and develop fibrosis, it was originally conceptualized that a second 'hit' is required to induce cellular event (e-g. oxidative stress) leading to inflammation, cell death and fibrosis [19, 20].

The diagnosis of NAFLD needs confirmation on imaging studies or liver biopsy, together with exclusion of individual who regularly consume more than 20 g ethanol per day. In clinical setting, there is still no consensus about weather or not liver biopsy is required to confirm diagnosis of NAFLD. Those are invasive and expensive. Presently, the available noninvasive marker for NAFLD including a set of clinical sign and symptoms, laboratory tests, imaging tests, and combinations of clinical and biochemical tests result. Although several of these markers in general useful for diagnostic evaluation of suspected NAFLD, they lack sensitivity and specificity [21].

Bedogni et al (2006) have developed a fatty liver index (FLI) to predict NAFLD. FLI was constructed from BMI, WC, TG and GGT [22]. Lipid accumulation product (LAP) is an index to predict the risk of more general impacts of body lipid excess. LAP was constructed by value of waist circumference (WC) and triglycerides (TG) [23,24,25].

Method

Research design, subjects and intervention

This was a randomized control group pre and post test design, with triple blind (subject, assessor, investigator) study [26], involving young obese females (18 to 25 years) in Denpasar, recruited from May to September 2013.

Samples size was calculated using below formulation [26], the minimal sample size requirement of each group was 33 subjects.

$$n = \frac{2\sigma^2 f\,(\alpha.\ \beta)}{(\mu 1 - \mu 2)^2}$$

Seventy four motivated subjects were enrolled in the study, allocated randomized into intervention and control group (37 subjects of each group). Informed consent was obtained for enrollment into study. The protocol of study was approved by Research Ethic Committee of Udayana University/Hospital of Sanglah (No. 787/UN.14.2/ Litbang/2012, 17 September 2012).

The intervention group were supplemented with 30 ml of emulsion contain 10 g canola oil (2000 mg LA, 1000 mg ALA; n-6:n-3 ratio 2:1) (+CO) and control group with 30 ml of placebo (-CO) daily. Supplementation was conducted for 12 weeks (June until September 2013). Subjects were also recommended to restrict their daily energy intake below 1500 kcal. To maintain subjects' compliance, we designed weekly meetings (every Sunday). In this moment, subjects could participate in one hour exercise trained by a gym instructor. They also were given 250 ml emulsion for a week supplementation, and any complained or adverse effects of supplementation were also monitored. We also make sure, that the supplement for the last past week taken as instructed, by checking left over found in the used bottle container.

During the period of intervention, 8 subjects (4 subjects of each group) were lost to follow up. A total of 66 subjects (33 each group) completed and followed the study protocol and were included in analysis.

Data collection

Research variables were assessed 3 times: pre on the first day of week 1, mid on the last day of week 6, and post on the last day of week 12, except for energy intake and fatty liver assessment that were conducted twice (pre and post) only. Energy intake was measured using semi quantitative food frequency questionnaire (SQ-FFQ) method. Body weight (BW) was assessed using digital scale (Omron HBF-362 model) with critical value of 0.1 kg. Body height (BH) was assessed using stature meter (General Care No.26SM) with critical value of 0.1 cm. Waist circumference (WC) was measured using flexible non elastic tape, in middle level of abdomen, with critical value of 0.1 cm. Body mass index (BMI) was calculated as BW (kg)/(BH(m))^2. Triglyceride (TG) was measured from serum using colorimetric method with critical value of 1 mg/dl. γ-glutamyltransferase (GGT) was measured from serum using colorimetric method with critical value of 1 µg/l. Fatty liver was assessed using ultrasonography (US) and interpreted independently by three radiologist. Fatty liver criteria were [28]: (1) Normal liver, absent of steatosis and other liver disorder, (2). Mild steatosis, marked by appearance of liver parenchymal or hepatorenal echo contrast a little bit bright without disorder of intrahepatic vascular, (3). Moderate

steatosis, marked by liver parenchymal or hepatorenal appearance brighter in more area without intrahepatic vascular disorder, and (4). Severe steatosis, marked by diffuse and brighter liver appearance with blunting intrahepatic vascular. Lipid accumulation (LAP) [23,24,25] and fatty liver index (FLI) [22] were calculated using these formulae:

LAP (females) = (WC(cm)-58)*(TG(mmol))

$FLI = (e^{0.953* \log e(TG) +0.139*BMI +0.718*\log e(GGT) +0.053*WC -15.745})/ (1 + e^{0.953* \log e(TG) +0.139*BMI +0.718*\log e(GGT) +0.053*WC -15.745}) * 100$

Statistical analyses

Statistical analysis was performed using Stata 12.1 (Stata Corp, College Station, TX, USA). Distribution of normally continuous data were presented in mean ± standard error of mean (SE). Differences between groups were analyzed by independent t-test. Differences within group with two variables (pre and post) were analyzed by pair t-test, and with three variables (pre, mid, and post) were tested by Repeated Measured Analysis Variance (Anova). Ordinal generalized linear model (OGLM); a repeated ordinal logistic regression were use for assess OR of more severe vs less severe ordinal data of liver steatosis. Repeated measures linear regression to observe the trend repeated assessed of continous data. Multiple linear regression method backward were used for analyzed the strength and direction of correlation between continuous data. Significant level was defined at P value <0.05 (CI 95%) [29].

Result

Subjects' average age were 20.7±1.6 years old, with age range of 18 to 25 years (+CO 20.9±1.8 vs -CO 20.6±1.5). Most subjects were students of Udayana University (90.0%), and the others were staff (7.0%), and unemployment (3.0%). Based on ethnicity, most of them were Balinese (90.0%), and the others were Indian (4.5%), Javanese (3.0%) and Chinese (1.5%).

Energy intake of both the +CO and the -CO groups were observed to be decreased (P= 0.000 and P= 0.001) compared to base line (pre) as shown in table 2, meet the study recommendation. There was no difference in intake between group, which means that energy intake of both group are comparable.

In the +CO group compared to base line (pre), all anthropometric variables were decreased. BMI decreased after six weeks (mid) (P=0.007) and 12 weeks (post) (P=

0.005), and WC decreased significantly at six weeks (mid) (P= 0.000) and 12 weeks (post) (P= 0.000) intervention. However, in the -CO group, only WC was significantly decreased (P= 0.007) after 12 weeks (post). In the +CO group, all biochemical variables (TG and GGT) were decreased. TG decreased significantly (P= 0.021) at 12 weeks (post test) and GGT decreased (P= 0.002) at 6 weeks (mid test) compared to base line (pre). No significant changed TG and GGT were observed in −CO group. Figue 1 plots the trend changes of BMI, WC, TG and GGT over the time of intervention. BMI and WC were lower in +CO than –CO group (P 0.045 and P 0.049) after 12 weeks intervention. GGT lower in +CO than –CO group (P 0.034) after six weeks intervention, but not after 12 weeks intervention (P>0,05). There was no significant difference decreased (P>0.05) between +CO and –CO group after intervention.

Compared to base line in the +CO group, LAP decreased significantly after six weeks (mid) (P= 0.033) and 12 weeks (post) intervention (P= 0.000). FLI also decreased at mid (P= 0.021) and post (P= 0.036) intervention. But no significant decreased of LAP and FLI were observed in the −CO group.

Table 2 observed Odd ratio (OR 0.0856, p 0,001) of more severe vs less severe liver steatosis in + CO vs –CO group was very small during 12 weeks intervention of canola oil. Figure 2 plots the probability of liver steatosis as estimate from OGLM. This is a cumulative probability of four degrees of liver steatosis (none, light, moderate and severe) sums to 1 at any time point. The figure shows 12 weeks +CO intervention decreased morbidity and severity of liver statosis in compared to –CO group.

Independently from supplementation intervention, there a positive strong correlation (P<0.001) was found between each of LAP, FLI, and liver steatosis. There was also positive strong correlation between pre and six week (mid) intervention of LAP and FLI to 12 weeks (post) intervention of liver steatosis. This mean that LAP and FLI were good predictor of liver steatosis.

Table 1. Changes of research variables value of subjects based on groups and moment of assessment at base line, six and 12 weeks of intervention (pre, mid, and post).

Parameter Group	Pre (mean±SE)	Middle (mean±SE)	Post (mean±SE)	*P**
Energy Intake (kcal)				§
+CO (n=33)	1802±107		1351±110	0.000
−CO (n=33)	1811±118		1368± 97	0.001
*P***	0.956		0.909	
BMI (kg/m^2)				
+CO (n= 33)	30.343±0.967Ⱨǂ	29.946±0.938Ⱨ	29.840±0.964ǂ	Ⱨ 0.007 ǂ 0.005
−CO (n= 33)	30.340±0.769	30.554±0.729	30.578±0.741	NS
*P***	0.998	0.610	0.540	
Waist Circumference (cm)				
+CO (n= 33)	92.273±2,150Ⱨ ǂ	89.064±2,078Ɑⱦ	86.867±2,078 ⱦǂ	Ⱨ 0.000 ⱦ 0.002 ǂ 0.000
−CO (n= 33)	92.661±2,150 ǂ	91.076±2,078	89.924±2,078 ǂ	ǂ 0.007
*P***	0.899	0.496	0.302	
Triglyceride (mg/dl)				
+CO (n= 33)	115.152±9.986ǂ	108.879±9.181	102.818±7.046 ǂ	ǂ 0.021
−CO (n= 33)	102.879±9.986	94.636±9.181	97.455±7.046	NS
P**	0.388	0.277	0.592	
GGT (µg/l)				
+CO (n= 33)	25.788±2.911Ⱨ	21.152±2.758 Ⱨ	21.818±3.001	Ⱨ 0.002
−CO (n= 33)	25.152±2.911	24.970±2.758	24.697±3.001	NS
*P***	0.878	0.331	0.500	
Lipid Accumulation Product (LAP)				
+CO (n= 33)	47.904±7.010 Ⱨǂ	41.576±6.019 Ɑⱦ	36.303±4.974 ⱦǂ	Ⱨ 0.033 ⱦ 0.033 ǂ 0.000
-CO (= 33)	44.208±7.010	38.551±6.019	37.555±4.974	NS
*P***	0.711	0.723	0.859	
Fatty Liver Index (FLI)				
+CO (n= 33)	6.302±2.884 Ⱨ ǂ	5.350±2.764 Ⱨ	5.181±2.782 ǂ	Ⱨ 0.021 ǂ 0.036
−CO (n= 33)	5.763±1.962	5.023±1.693	4.744±1.559	NS
*P***	0.878	0.920	0.891	

*P within-group tested by Repeated Anova, ** P between-group tested by independent t test, § tested by Paired Sample t test.

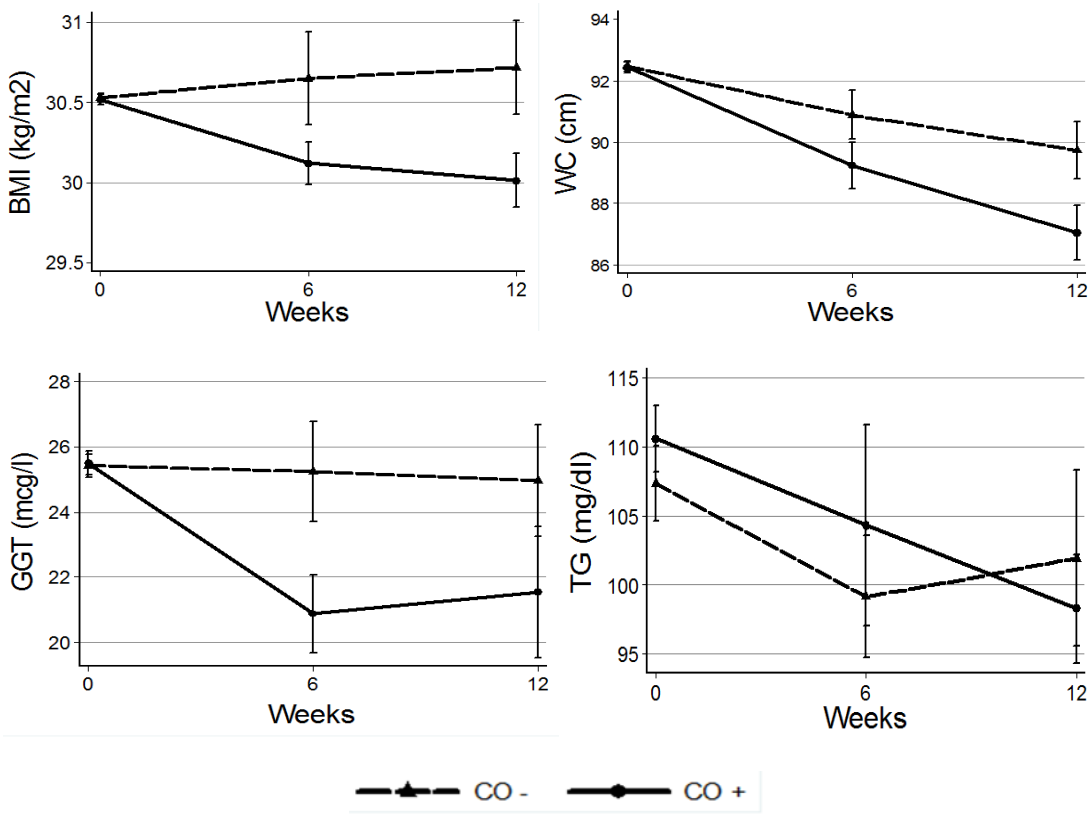

Figure 1. Changes in body mass index (BMI), waist circumference (WC), gamma-glutamyl transferase (GGT) and triglycerides (TG), in mean and standard error for repeated linear regression.

Table 2. Odd ratio (OR) of more severe versus less severe liver steatosis (LS) of +CO compared to –CO group, analyzed for ordinal logistic regression

LS	OR	SE	Z	P	95% CI	
SL based line		144.0855	6.33	0.000	35.59402	874.4502
	176.4233					
1.Canola	.9035432	.1959138	-0.47	0.640	.5907236	1.382018
12. Canola	.0856324	.0610272	-3.45	0.001	.0211844	.3461464
Cut1	8.342597	1.252533			5.887677	10.79752
Cut2	13.72544	2.127812			9.555008	17.89588
Cut3	17.59521	3.023079			11.67008	23.52034

(1) LS none, (2) LS light, (3) LS moderate, (4) LS severe

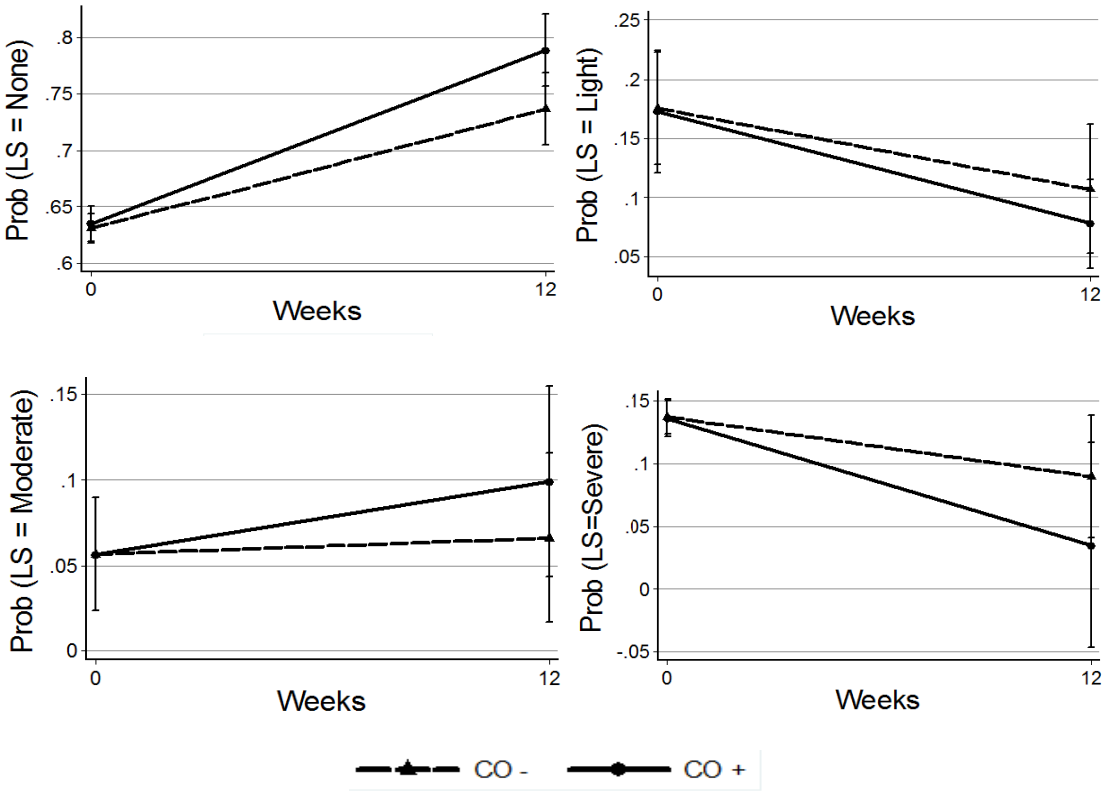

Figure 2. Probability of four degrees of liver steatosis (LS) weeks 12 based on LS week 0. The depicted mean probabilities were obtained from OR in table 2.

Table 3.Correlation matrix of Lipid Accumulation Product, Fatty Liver Index, and level of Liver Steatosis. (n=66)

	LAP1	LAP2	LAP3	FLI1	FLI2	FLI3	LS1	LS3
LAP1	1							
LAP2	.911**	1						
	.000							
LAP3	.858**	.921**	1					
	.000	.000						
FLI1	.747**	.807**	.860**	1				
	.000	.000	.000					
FLI2	.696**	.795**	.850**	.989**	1			
	.000	.000	.000	.000				
FLI3	.637**	.759**	.840**	.969**	.989**	1		
	.000	.000	.000	.000	.000			
LS1	.564**	.516**	.579**	.553**	.506**	.494**	1	
	.000	.000	.000	.000	.000	.000		
LS3	.521**	.529**	.624**	.623**	.590**	.589**	.871**	1
	.000	.000	.000	.000	.000	.000	.000	

Presented in Correlation coefficient and two-tail significant (P).** P< 0.001
LAP, Lipid Accumulation Product, 1, pre, 2, mid, 3, post.
FLI, Fatty Liver Index, 1, pre, 2, mid, 3, post.
LS, Liver Steatosis, 1, pre, 3, post.

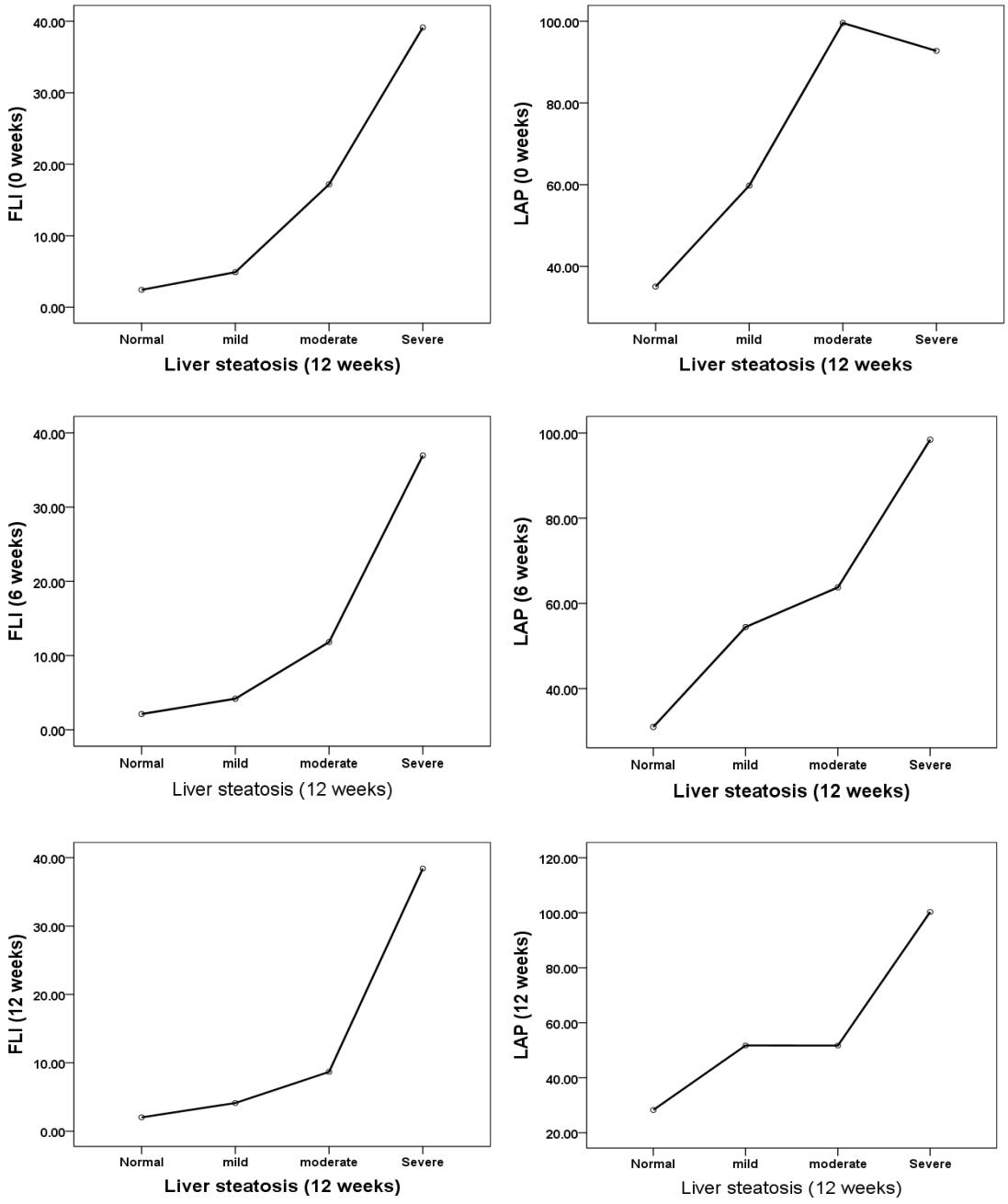

Figure 3. Relationship of Fatty Liver Index (FLI) (left) and Lipid Accumulation Product (LAP) (right) to Liver Steatosis.

Above, the index was measured 12 weeks before Liver Steatosis was diagnosed
Middle, the index was measured 6 weeks before Liver Steatosis was diagnosed
Below, the index was measured at the same time of Liver Steatosis was diagnosed

Although there were positive strong correlation (P <0.001) among BMI, WC, TG and TG to liver steatosis, however, using multiple linear regression analysis, GGT was the most accurate marker to predict liver steatosis, while BMI and WC were more accurate than TG. All of four regression equation (Table 4) showed that liver steatosis could always be determined by GGT. GGT is an enzyme that is released by hepatocyte progressively in response to oxidative stress. This fact might explaine the reason of more specific correlation of FLI to liver steatosis than of LAP to liver steatosis, as shown in Figure 3.

Table 4. Multiple linear regression between Liver Steatosis to BMI, WC, TG, and GGT.

Dependent Variables	Predictor Variables	Unstandardized Coefficient		R^2
		B	CI 95%	
Liver Steatosis (base line/pre)	(Constant)	-2.371	-4.005_-0.737	0.436
	WC	0.038	0.019_ 0.0.57	
	GGT (base line/pre)	0.022	0.008 _ 0.036	
Variation of Liver Steatosis value determined by WC and GGT (base line) 43.6%.				
Liver Steatosis (post)	(Constant)	-2.335	-3.675_-.995	0.426
	WC	0.038	0.022_0.053	
	GGT (base line/pre)	0.011	-0.001_0.023	
Variation of Liver Steatosis value (post) determined by WC and GGT (base line) 42.6%.				
Liver Steatosis (post)	(Constant)	-1.492	-2.664_-0.319	0.384
	BMI	0.088	0.045_ 0.130	
	GGT (mid)	0.012	-0.001_0.025	
Variation of Liver Steatosis value (post) determined by BMI and GGT (mid) 38.4%.				
Liver Steatosis (post)	(Constant)	-1.570	-2.675_-0.464	0.398
	BMI	0.092	0.052_0.131	
	GGT (post)	0.010	-0.001_0.022	
Variation of Liver Steatosis value determined by BMI and GGT (post) 39.8%.				

Analysed by multiple linear regression method *backward*, PIN 0.05, POUT 0.10, Independent variables; BMI, body mass index, WC, waist circumference, TG, triglycerides, GGT, γ- glutamyltransferase. R^2 (Coefficient Correlation).CI 95%, Dependent variable: Liver Steatosis; 1. Normal liver, 2. Mild, 3. Moderate, 4. Severe.

Discussion

Canola oil high in n-3 PUFA (ALA), desaturated and elongated in human body to forms LC-PUFA (EPA, DPA and DHA). N-3 LC-PUFAs have potential effects to reduce lipogenesis and induce lipid oxidation. EPA also produces anti-inflammatory cytokine (PG3, LT5). The mechanism could protect and prevent the process of "the two hit theory". They can reduce the first hit-liver fat accumulation, and second hit - stress oxidative. Reducing lipogesis, will be marked by reducing anthropometric parameter such as; body weight, BMI, WC, and lipid blood parameter such as TG. And decrease level of inflammatory and oxidative stress will be shown by decrease liver inflammatory enzymes, such as GGT, ALT, and AST.

Canola oil diet supplementation observed has potential effects to prevent and treat liver steatosis, decreased LAP (WC and TG) and FLI (BMI, WC, TG, and GGT) in young obese female subjects of this study. The proportion of liver steatosis also decreased. This finding showed that if subjects were given supplementation for a certain duration, the risk of developing diseases such as NAFLD and NASH could be reduced even prevented. Other study, Nobilli et al (2012) also reported that DHA supplementation improves liver steatosis in children with NAFLD, doses of 250 mg/day and 500 mg/day appear equally effective [30].

FLI was calculated based on BMI, TG, WC, and GGT [20], and LAP was a simple index formulated from WC and TG, both were reported as good markers to detect liver steatosis in adult from Northern Italy [24]. LAP was reported to be better than BMI for identifying cardiovascular risk of adult in the US [23], and as a simple and accurate predictor of metabolic syndrome in Taiwanese people aged 50 years and over [25].

The findings in this study were similar to other reported studies. Olivera et al (2009) concluded that alanine aminotransferase (ALT) and TG should be considered as screening for suspected fatty liver disease in overweight/obese youth [31]. Omagiri et al (2009) investigated that BMI, body fat, and body lipid parameter such as total cholesterol and TG were strongly associated with fatty liver in Japanese adult [32]. Park et al (2011) reported that NAFLD has significant odds ratio with ALT (2.22), GGT (2.15), TG (1.92) and BMI>25 kg/m2 (7.65), in Korean [27]. Sartrio et al (2007) reported, NAFLD in Italian obese children, most of the prediction explained by ALT and Z score of BMI [33].

Derivatives of n-3 PUFA have potential effect to inhibit lipid synthesis, and reduced LAP and FLI. Supplementation with n-3 PUFA derivate in this study correlated with

decreased body lipid accumulation, expressed by the decreased of BMI, WC, and TG. Oxidative stress was also reduced, marked by the decreased of GGT. Sander et al (2006) reported that decreasing n-6:n-3 PUFA ratio intake (3:1) lowered plasma TG in older subjects (45-75 years) [34]. Parra et al (2007) also reported, fish intake (cod, salmon, and fish oil) with moderate energy restriction for 8 weeks in obese person could decreased body weight, WC, fat mass, total cholesterol, and TG, respectively. Only cod supplementation decreased oxidative stress (blood MDA/AOP) significantly [35]. Other study concluded, LC n-3 PUFA consumption during energy reduction exerts positive effects on insulin resistance in young overweight persons, independently from changes in body weight, TG, erythrocyte membrane or adiponectin [36]. Rossmeis et al (2009) reported, α-ethyl DHA ethyl ester exhibit a similar range of beneficial effects on obesity and associated metabolic traits as naturally occurring n-3 LC-PUFA, but with a higher efficacy. Therefore, this compound could qualify as a novel drug for the treatment of obesity, dyslipidemia, and insulin resistance [37]. In relation to the 'two hit theory' in NAFLD progression [18, 19], the reduction of LAP and FLI indicated the inhibition of the first 'hit', and the observed GGT decreased as a marker for reduced inflammation indicated the inhibition of the second 'hit'. Dixon et al (2006) concluded, weight loss, reduced GGT and AST could predict the improvement of lobular inflammation and fibrosis, positive signs of prognostic features of NAFLD [38].

Patients with NAFLD are mostly asymptomatic and the disease was usually suspected by hyperechoic liver appearance on abdominal ultrasonography (USG) or increases of liver enzymes. Although USG is less sensitive than magnetic resonance imaging (MRI) in detecting a minor liver steatosis, nevertheless it is relatively good enough and practical to be applied for the diganosis of liver steatosis. Hamaguchi et al (2007) reported, in general Japan population the sensitivity of USG to detect NAFLD was 91.7% (95% CI 87.0-95.1, P<0.001) and the specificity was 100% (95% CI 95.4-100.0, P<0.001), within-observer reliability was 0.95 (95% CI 0.93-0.97, P<0.001) and between-observer reliability was 0.95 (95% CI 0.93-0.97, P<0.001). The AUC to diagnose NAFLD was 0.980 [28]. But, the sensitivity of USG to assess steatosis decreases relatively, with increased degree of fat infiltration and obese patients [21]. Barsic et al reported that, USG has been demonstrated to be potential to improve diagnosis of NAFLD and NASH. Other wise the liver enzymes commonly elevated in liver steatosis are ALT and GGT [39].

Preventing progressivity of liver steatosis to advance steatosis such as NASH, fibrotic, cirrhotic, and hepatoma is an important issue to be conducted, especially in obese

individuals. Advance steatosis is irreversible, thus no point of return. Diet high in n-3 PUFA could decrease body lipid accumulation, which in turn would prevent and reduce the risk to develop NAFLD, and blocked progressivity to advance steatosis through inhibition of the "two hit process" in liver. Increasing n-3 PUFA intake could be recommended as an answer of these objectives, especially in obese individuals.

In conclusion, daily supplementation of 10 mg Canola oil for 12 weeks has decreased LAP and FLI, and improved liver steatosis in young obese females. There were positive strong correlation of FLI, LAP, and liver steatosis, independently from supplementation intervention. FLI was more specific to predict liver steatosis than LAP.

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