Effects of conjugated linoleic acid supplementation and exercise on body fat mass and blood lipid profiles among overweight Iranians

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ABSTRACT

Introduction: Conjugated linoleic acid (CLA) has been studied for its fat mass reduction effects. This study aimed to determine the effects of CLA supplementation on body fat mass (BFM) and selected blood lipid profiles among overweight Iranian. **Methods:** A total of 180 adults with BMI = $26-29 \text{ kg/m}^2$ and BFM exceeding 21%and 28% for men and women, respectively were recruited through voluntary participation from weight management clinics in Tehran. They were assigned randomly to three groups as follows: Group (1) (control group) receives weight loss diet only; Group (2) receives weight loss diet +3 gr/day CLA supplement (mixture of cis-9, trans-11 and trans-10, cis-12) twice a day and Group (3) weight loss diet +3 gr/day CLA supplement as Group (2) twice a day + regular exercise (walking at 5.5-6 km/h for at least 160 minutes/week). The trial was conducted for 12 weeks. Anthropometric measurements and blood lipid profiles were determined at weeks 0, 6 and 12. **Results:** Both Group 2 and Group 3 showed a significant between-group difference in reduction of BFM (1.3% and 2.6% respectively) compared to Group 1. Group 2 supplementation showed increased free fatty acid (FFA) (0.44 mM to 0.55 mM) and decreased HDL-chol (47.5 mg/dL to 42.0 mg/dL) between weeks 0 and 12. These results were not observed for Group 3. Conclusion: Combination of CLA supplementation with exercise showed BFM reduction in overweight Iranian adults. Further research is suggested to verify the findings of this study.

Keywords: Overweight, conjugated linoleic acid, body fat mass, lipid profiles, Iranians

INTRODUCTION

Prevalence of obesity is on the rise globally. The National Health and Nutrition Examination Survey in the United States reported that more than one-third of adults were obese, and this phenomenon is distributed equally between genders (Ogden *et al.*, 2014). In Malaysia the prevalence of overweight and obesity among adults were reported as 30% and 17.7%, respectively (IPH, 2015).

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In Iran, the prevalence of obesity is increasing. A recent review reported that the prevalence of obesity of Iranians aged below 18 years was 5.5%, and 21.5% for the older population (Mirzazadeh *et al.*, 2009). Obesity prevalence was considerably higher among women, and it was attributed to the effect of sedentary lifestyle among Iranian women.

Obesity is a risk factor for almost all chronic diseases (Finucane *et al.*, 2011). Obesity is preventable and manageable through a balanced, moderate, and varied diet along with regular exercising. Nevertheless, it is not easy for people to manage their diets and lifestyles; therefore, several weight management plans including the use of supplements have been studied to address this issue.

Conjugated linoleic acid (CLA) is an isomer of the essential fatty acid, linoleic acid, found in dairy products and meat. Research on CLA has increased as CLA has been found to have anti-carcinogenic effects in animals (Koronowicz & Banks, 2018). Common CLA isomers studied are trans-10, cis-12 and cis-9, trans-11 isomers. Previous studies used fatty acid or triglyceride form of CLA as a supplement added to drinks such as milk or as capsule or soft gel (Dilzer & Park, 2012). CLA seemed to have a body fat mass reducing effect some animals (Benjamin et al., 2015), but findings from human studies varied due to differences in materials, methodology, and design of the studies (Chen et al., 2012; Dilzer & Park, 2012).

In terms of lipid profiles, CLA has been shown to lower blood high density lipoprotein (HDL-chol) (Racine *et al.*, 2010). A study reported that the HDLchol lowering effect of the CLA might be mostly related to *cis*-9, *trans*-11 isomer (Wanders *et al.*, 2010). Alterations of low density lipoprotein (LDL-chol), total cholesterol, triglyceride (TG), and free fatty acid (FFA) in blood by CLA remain unclear as study results are inconsistent (Dilzer & Park, 2012). It has been shown that increase in FFA levels is a risk factor among obese, diabetic, and those suffering from cardiovascular disease (Boden, 2011). While *trans*-10, *cis*-12 CLA is reported to influence the FFA levels and insulin resistance in a short term, *cis*-9, *trans*-11 isomer does not appear to have such an effect (Dilzer & Park, 2012).

This study aimed to determine effects supplementation the of of a mixture of two main isomers of CLA supplementation and exercise intervention anthropometric on indicators and blood lipid profiles (HDLchol, LDL-chol, and FFA) in overweight Iranians. To the best of our knowledge, this is the first study which assesses the effects of CLA supplementation on overweight Iranians.

MATERIALS AND METHODS

Research design and study subjects

This study was a randomised controlled trial (RCT) in which volunteers were assigned randomly to three groups. The sample size of 180 participants determined by considering were previous studies and based on available guidelines (Machin & Campbell, 2005) with a possibility of 15% dropout during the study. The subjects (100 women and 80 men) were recruited through voluntary participation from three weight management clinics in Tehran. Duration of the study was 12 weeks.

The inclusion criteria were apparently healthy Iranian volunteers aged between 20-50 years old, and with a BMI between 26-29 kg/m². These subjects must have a body fat mass (BFM) of more than 28% for women and 21% for men, and were not taking any medication or supplement. Pregnancy, lactating, history of hospitalisation, or previous or current health condition were exclusion criteria.

Subjects (n=180) were randomly assigned into three different groups namely Group 1 (control), Group 2 (CLA), and Group 3 (CLA + Exercise). All subjects were on a balanced weight loss diet which means their diet had been adjusted to provide 50-55% of calories from carbohydrates (CHO), 15-20% from protein and not more than 30% calories from fat. Group 1 received only the weight loss diet; Group 2 received the weight loss diet plus conjugated linoleic acid supplement (a mixture of the two bioactive isomers in the form of a 1500 mg soft gel -50% cis-9, trans-11 and 50% trans-10, cis-12- containing 78-84% CLA twice a day); Group 3 received the weight loss diet plus the same CLA supplement as Group 2 plus performing moderate intensity exercise (walking at 5.5-6 km/h for at least 160 minutes per week).

Ethics, consent and permissions

The present study was performed following the ethical guidelines of the Declaration of Helsinki, and the Good Clinical Practice rules. The study was approved by The Human Research Ethics Committee of the University Putra Malaysia (JKEUPM) as FPSK Mei (13)01 and all subjects signed an informed written consent form. The trial was registered in UMIN-CTR as UMIN000020284.

Clinical assessments

first During the session. general information pertaining to characteristics, demographic background and medical history of the subjects was collected. anthropometric Data related to measurements including body weight, height, body mass index (BMI), waist to hip ratio (WHR), and body fat mass (BFM) percentage and dietary assessment (24-hour recall) were collected at each visit. Blood samples were drawn for the analysis of total triglycerides (TG), low density lipoprotein (LDL-chol), high density lipoprotein (HDL-chol), fasting blood sugar (FBS), and free fatty acid content of blood (FFA).

Adverse effects

Adverse effects (AEs) were self-recorded by the subjects and defined as any unexplainable unfavourable effect. Subjects were instructed to record symptoms, frequency, severity, and duration of each AE. During each visit, the investigator reviewed recorded AEs, and subjects could visit physicians for treatment with the study covering their treatment. Subjects would be excluded from the study if they were concerned about the AE or if the physician considered them ineligible for the study.

Diet

A 3-day, 24-hour dietary recall was used to analyse the caloric intake of the subjects. This was done at weeks 0, 6 and 12 of the study. Subjects were interviewed by a dietitian to recall food consumed during the previous 24 hours. Nutritionist Pro software (Axxya Systems, 2006) were used to analyse the dietary information.

Exercise

For the Group 3, the exercise was defined as walking at 5.5-6 km/h for at least 160 minutes per week. The subjects had the choice to conduct the 160 mins exercise three or four times a week. This was set to meet the criteria for a moderate intensity exercise defined by Ainsworth *et al.* (2000). Subjects kept track of walking speed and distance using treadmill or software installed on their cell phone. Their exercise activity was provided to the dietitian at each visit.

Anthropometric measurements

Body weight was reported in kg. Height was measured with a standing scale

in meters with accuracy of 0.005 meters and body mass index was computed using those measurements. Waist circumference was measured with a measuring tape recorded in cm Jackson-Pollock four-site formula (from abdomen, suprailiac, triceps, and thigh) was used to calculate the body fat mass percentage (BFM). For the calculation of the skin fold thickness a Harpenden caliper was used.

Biochemical analyses

Participants were asked to fast 12 hours before each blood collection. At weeks 0, 6, and 12, 10 ml blood was collected from each subject. Lipid profiles (TG, LDL-chol, HDL-chol), FBS and FFA contents were determined. Detergent Solubilisation/ Enzymatic Analytical method was used to determine blood HDL-chol, and LDLchol, and the Quantitative Enzymatic method was used for determination of TG contents. FFA content was analysed using quantitative spectrophotometry, while FBS level was determined using a quantitative enzymatic method.

Statistical analyses

All of the analyses were done using SPSS 22 software (IBM Corp. Released 2013. Armonk, NY, USA). Chi-square test was used to test categorical variables for significant differences between groups. Shapiro-Wilk test was used for test of normality. For analysing within-group differences, repeated measures ANOVA and Friedman test for parametric and non-parametric data, respectively were applied. Between-group comparisons were performed with one-way ANOVA and Kruskal-Wallis test for parametric and non-parametric data, respectively, and post hoc analysis were performed with a Bonferroni adjustment. A significance level of 0.05 was indicated for all tests.

RESULTS

Study subjects

After 12 weeks of follow up, 171



Figure 1. Flowchart of the total number of subjects recruited and analysed

participants completed the study (95%) (Figure 1). Five participants were excluded because having to take medication (Group 1, n=2; Group 2, n=1; Group 3, n=1), one because of stomach upset as an adverse effect (Group 2, n=1), two did not show up for follow up sessions (Group 1, n=1; Group 3, n=1), and two participants did not follow the prescribed calorie diet (Group 1, n=1; Group 3, n=1).

Among all the participants who started the study only one (n=1)reported an AE which was related to an upset stomach. While the AE was not considered serious by the subject and physician, the subject decided to quit the study. The completed sample size of 171 was higher than the calculated minimum number needed for this study (153).

Table 1 summarised the baseline characteristic of the subjects in three study groups. Majority of subjects were female (55%, n=94). No significant difference existed between the groups for gender, age, marital status, income, and education level. All participants reported no alcohol use as alcohol consumption is prohibited in the country. Only ten subjects (all male) reported tobacco use. The daily caloric intake within different groups was not significantly different during the 12 weeks of study (Table

 Table 1. Baseline characteristic of the subjects

		Group				
Variables	Group 1 n=56	Group 2 n=58	Group 3 n=171	Total	р	x^2
Gender [‡]					0.94	0.135
Female	30 (31.9%)	32 (34.0%)	32 (34.0%)	94		
Male	26 (33.8%)	26 (33.8%)	25 (32.5%)	77		
Education level [‡]					0.51	5.231
Primary	7 (46.7%)	5 (33.3%)	3 (20.0%)	15		
Secondary	7 (46.7%)	5 (33.3%)	3 (20.0%)	15		
Diploma	14 (38.9%)	11 (30.6%)	11 (30.6%)	36		
University	28 (26.7%)	37 (35.2%)	40 (38.1%)	105		
Income [‡]					0.66	4.090
≤500 USD	10 (34.5%)	11 (37.9%)	8 (27.6%)	29		
500 <x<≤1000< td=""><td>15 (38.5%)</td><td>9 (23.1%)</td><td>15 (38.5%)</td><td>39</td><td></td><td></td></x<≤1000<>	15 (38.5%)	9 (23.1%)	15 (38.5%)	39		
1000 <x≤1500< td=""><td>14 (27.5%)</td><td>18 (35.3%)</td><td>19 (37.3%)</td><td>51</td><td></td><td></td></x≤1500<>	14 (27.5%)	18 (35.3%)	19 (37.3%)	51		
>1500	17 (32.7%)	20 (38.5%)	15 (28.8%)	52		
Marital status [‡]					0.55	4.958
Single	14 (31.8%)	15 (34.1%)	15 (34.1%)	44		
Married	34 (29.7%)	39 (35.1%)	39 (35.1%)	112		
Divorced	4 (50.0%)	2 (25.0%)	2 (25.0%)	8		
Widowed	5 (62.5%)	2 (25.0%)	1 (12.5%)	8		
Age [§]	35 (29)	36.5 (30)	35 (30)		0.66	
BFM§						
Female	29.0 (0.79)	29.0 (1.76)	29.3 (1.1)	-	0.80	
Male	23.4 (1.24)	22.9 (1.07)	22.9 (1.17)	_	0.25	

[†]Group 1, diet only; Group 2, diet + conjugated linoleic acid (CLA) supplement; Group 3, diet + CLA supplement + moderate-intense exercise; Values are represented as number of subject (percentage) or median (IQR)

[‡]Chi-square test

[§]Non-parametric test, Kruskal-Wallis, α =0.05

			Grou	μp^{\dagger}			
Variable (kcal)	1		2	2		3	
	Median	IQR	Median	IQR	Median	IQR	
Calories, week 0	1575	350	1650	950	1650	1000	
Calories, week 6	1600	350	1625	1000	1650	900	
Calories, week 12	1575	413	1650	850	1650	950	
p-value [‡]	0.95	50	0.9	37	0.1	22	

Table 2. Dietary intake of the subjects

[†]Group 1, diet only; Group 2, diet + conjugated linoleic acid (CLA) supplement; Group 3, diet + CLA supplement + moderate-intense exercise

Unit: Calories (kcal)

^{$+}Non-parametric test, Kruskal-Wallis, \alpha=0.05$ </sup>

Table 3	3	Anthro	pometric	measureme	ents	of	the	subi	ects
								- /	<pre>/</pre>

Variables				
vunubles -	1	2	3	– p
BW‡				
Week 0	80.30±10.5	82.95±10.16	83.11±9.78	0.27
Week 6	78.80±10.4	81.13±10.03	80.96±9.63	0.38
Week 12	76.90±10.3	79.26±9.91	78.80±9.65	0.40
BMI§				
Week 0	27.70 (2.98)	27.6 (2.90)	27.6 (2.74)	0.73
Week 6	27.10 (3.01)	27.0 (3.12)	26.8 (2.76)	0.14
Week 12	26.50 (2.97)	26.4 (2.76)	26.1 (3.15)	0.12
WHR [‡]				
Week 0	0.89±0.11	0.89±0.09	0.88±0.09	0.85
Week 6	0.88±0.11	0.88±0.09	0.87±0.08	0.67
Week 12	0.87±0.10	0.87±0.08	0.85±0.08	0.37
BFM§				
Week 0	28.00 (9.80)	27.70 (9.80)	27.30 (8.6)	0.99
Week 6	28.10 (10.60)	26.60 (9.70)	25.20 (9.5)	0.004
Week 12	28.10 (10.80)	26.40 (10.40)	24.70 (9.8)	< 0.0005
∆Baseline [¶]	-	2.09	2.54	_
ΔCLA¶	-	-	0.51	_

[†]Group 1, diet only; Group 2, diet + conjugated linoleic acid (CLA) supplement; Group 3, diet + CLA supplement + moderate-intense exercise

*Parametric test is one-way ANOVA (α =0.05)

^sNon-parametric test is Kruskal-Wallis (α=0.05)

[¶]Difference in differences (DID) as a measure of intervention effect for variables with significant between group differences. Δ Baseline: intervention effect compared to baseline. Δ CLA: intervention effect compared to CLA group

*Between-group Differences (comparing three groups)

Notes:

- 1. BW, body weight; BMI, body mass index; WHR, waist-to-hip ratio; BFM, body fat mass; Values are represented as mean±SD or median (IQR).
- 2. BW (kg); BMI (kg/ m^2); WHR is a ratio; BFM (%).
- 3. All within-group Differences (comparing three weeks of 0, 6 and 12) are significant across AM variables with *p*<0.0005.
- 4. Bonferroni corrected α =0.0167 (for tests based on each variable), α =0.0028 (when considering all AM variables).

Variables		$Group^{\dagger}$				
variables -	1	2	3	— p		
FBS [‡]						
Week 0	81.5 (35)	82.0 (32)	81.0 (31)	0.99		
Week 6	82.0 (37)	82.0 (24)	79.0 (28)	0.16		
Week 12	83.0 (30)	79.0 (28)	78.0 (24)	0.003		
p^{**}	0.98	0.94	0.002			
T G §						
Week 0	90.9±20.51	87.7±19.02	86.9±16.69	0.48		
Week 6	89.2±17.19	86.5±17.34	84.8±12.07	0.33		
Week 12	88.8±19.08	84.5±16.99	81.9±10.62	0.07		
p^{**}	0.21	0.02	0.002			
HDL-chol [‡]						
Week 0	48.0 (36)	47.5 (40)	46.0 (38)	0.46		
Week 6	47.0 (33)	46.0 (27)	50.0 (31)	0.003		
Week 12	49.0 (35)	42.0 (33)	52.0 (28)	< 0.0005		
p^{**}	0.43	0.006	< 0.0005			
∆Baseline [¶]	-	2.09	2.54	_		
ΔCLA^{\P}	_	-	0.51	_		
LDL-chol [§]						
Week 0	85.6±15.20	85.0±17.97	85.1±17.19	0.98		
Week 6	84.8±14.16	84.2±16.63	81.3±11.79	0.39		
Week 12	84.5±13.25	82.8±14.62	76.5±11.30	0.004		
p^{**}	0.67	0.16	< 0.0005			
FFA [‡]						
Week 0	0.42 (0.73)	0.44 (0.81)	0.42 (0.86)	0.94		
Week 6	0.40 (0.71)	0.51 (0.95)	0.32 (0.75)	< 0.0005		
Week 12	0.35 (0.66)	0.55 (1.03)	0.31 (0.83)	< 0.0005		
p^{**}	< 0.0005	< 0.0005	0.003			
∆Baseline [¶]	_	2.09	2.54	_		
ΔCLA^{\P}	_	-	0.51			

Table 4. Blood profiles of the subjects

[†]Group 1, diet only; Group 2, diet + conjugated linoleic acid (CLA) supplement; Group 3, diet + CLA supplement + moderate-intense exercise

^{*}Non-parametric test is Kruskal-Wallis (α =0.05)

[§]Parametric test is one-way ANOVA (α =0.05)

¹Difference in differences (DID) as a measure of intervention effect for variables with significant between group differences. Δ Baseline: intervention effect compared to baseline. Δ CLA: intervention effect compared to CLA group

*Between-group Differences (comparing three groups)

**Within-group Differences (comparing three weeks of 0, 6 and 12)

Notes:

1. FBS, fasting blood sugary; TG, triglyceride; HLD, High Density Lipoprotein; LDL-chol, Low Density Lipoprotein; FFA, Free Fatty Acid; Values are represented as mean±SD or median (IQR).

2. FBS, TG, LDL-chol, and HDL-chol are reported in mg/dl; FFA is reported in mmol/l.

3. Bonferroni corrected α =0.0167 (for tests based on each variable), α =0.0033 (considering all blood profile variables).

2). Furthermore, no between-group differences were found at baseline for anthropometric status (Table 3) and blood profiles (Table 4).

Effects of CLA on anthropometric measurements

Summary of changes in anthropometric status is presented Table in 3. Compared to body weight at baseline, all groups experienced significant weight loss (Group 1, $\Delta = -3.5 \pm 0.7;$ Group 2, Δ =-3.7±0.8; Group 3, Δ =- 4.3 ± 0.9) with weight reduction among Group 3 (CLA+exercise) being clinically significant (Pi-Sunyer, 1996) as subjects lost 5.4% of their weight, on average. However, no significant between-group differences were found. Similarly, all groups experienced significant BMI reduction (Group 1, p<0.001; Group 2, p<0.001; Group 3, p<0.001–BMI at week 12 compared to baseline BMI) but with no statistically significant differences between the groups. A similar result was shown for WHR compared to the baseline WHR.

Noticeably, there was a significant between-group difference for BFM between Group 2 and Group 3, compared to Group 1 (Group 2 compared to Group 1, p<0.005; Group 3 compared to Group 1, p<0.005), while the difference between Group 2 and Group 3 is not significant (p=1.0). This finding is in addition to significant within-group difference in



Figure 2. Changes of BFM and select blood profiles across treatment groups during the study (a. BFM changes, b. HDL-chol changes, c. LDL-chol changes, d. FFA changes during the study) ${}^{*}p < 0.05$ ${}^{**}p < 0.01$

BFM reduction among all groups. Figure 2a illustrates the differences in BFM among the three study groups.

Effects of CLA on blood profiles

Table 4 summarises the results of analyses on subjects' blood profiles among the study groups. After 12 weeks, there was no significant between-group differences pertaining to FBS, TG, and LDL-chol compared to the baseline values. For FBS, the only significant difference was the within-group reduction among Group 3 (p=0.002). We observed difference pertaining to reduction in TG among Group 2 (Δ =-3.2±8.9) and Group 3 $(\Delta = -5.0 \pm 11.4)$. LDL-chol reduction was only significant among Group 3 (p<0.0005). Furthermore, participants in Group 3 experienced significant withingroup increase of HDL-chol (p<0.0005) while HLD blood levels of those in Group decreased significantly (p=0.006), 2 HDL3 (HDL-chol at week 12) compared to HDL1 (HDL-chol at week 0).

There was a significant decrease in HDL-chol level among Group 2 when comparing HDL-chol at week 12 compared to baseline level. There was also a significant between-group difference when comparing HDL-chol at week 12 between Group 2 and Group 1. Figures 2b and 2c show the alterations in HDL-chol & LDL-chol levels among the three groups, respectively.

There was a significant betweengroup difference when comparing FFA levels between Group 2 at the 6-week and 12-week point compared to the other groups. Another finding was that FFA level increased in Group 2 (p<0.0005) while Group 1 and Group 3 showed decreases in their FFA levels (p<0.0005 and p=0.003, respectively) (Figure 2d) shows differences in FFA levels during the study among all groups.

DISCUSSION

This study showed that consuming CLA as a soft gel supplement containing two isomers for 12 weeks significantly decreased BFM. The present study found significant changes in BW, BMI, WHR, and BFM within all groups. This could be due to on the weight reduction diet taken by all groups. However, both the intervention groups 2 and 3 experienced almost five times more reduction in BFM compared with the control group. This finding suggests an improvement in body weight reduction obtained through CLA consumption, an effect that was not improved any further by adding aerobic exercising for 160 minutes/week. This might be due to an increase in lean body mass (LBM) that was reported in other studies (Steck et al., 2007; Dilzer & Park, 2012). Unfortunately, this study did not consider LBM for evaluation.

This study revealed statistically significant reduction of FBS in Group 3 after 12 weeks compared to the other groups. This is in accordance to findings of other studies that have shown exercising or physical activity decreases fasting blood sugar levels among both healthy and diabetic population (Ossanloo, Najar & Zafari, 2012). Nevertheless, other studies reported a non-significant difference of FBS with CLA supplementation used among diabetic subjects (Racine et al., 2010; Sluijs et al., 2010).

No significant between-group difference was recorded for blood triglyceride levels. This finding is in accordance with of other investigations (Racine *et al.*, 2010; Sluijs *et al.*, 2010). There is inconsistency in previous studies for the effects of CLA supplementation on LDL-chol concentrations (Dilzer & Park, 2012). We found that exercising appears to exert a significant effect on LDL-chol, with the participants in Group 3 experiencing significant LDLchol decrease compared to other groups. Other studies have reported exercising decreases LDL-chol levels among healthy, diabetic, and atherosclerosis patients irrespective of their age (Lira et al., 2010; Kelley & Kelley, 2007). This study confirmed the reduction of HDLchol level in Group 2 compared to the other groups. This negative effect of CLA has been reported by many studies (Gaullier et al., 2005; Racine et al., 2010; Sluijs et al., 2010).

Perhaps the most important finding of this study was that CLA supplementation elevated FFA concentrations significantly comparing between groups 1 and 2. This unfavourable increase was controlled by aerobic exercise (comparing between groups 2 and 3).

Overall, this study found that CLA supplementation for 12 weeks has a positive impact on body fat mass reduction of overweight individuals with marginal BFM percentages along with the negative effects of decreasing HDLchol and increasing FFA levels. The combination of CLA with exercise will be beneficial on body composition and would not add adverse effects to health. This could be one of the way to reduce increased adiposity and potentially lower the risk of other diseases associated with obesity.

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Authors' contributions

Hanieh F and Loh SP designed the study. Hanieh F and Abas M helped in data collection. Hanieh F analyzed the data. All authors discussed the results and commented on the manuscript.

Conflict of interest

The authors declared no conflict of interests.

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