Double Blind Comparison of Plasma Lipids in Healthy Subjects Eating Potato crisps Fried in Palmolein or Canola Oil

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Abstract

The effect of palmolein and of canola oil plasma lipids was examined in double blind experiments in healthy human adults. Subjects are 53g (men) of 35g (women) of the oil on potato crisps in randomized crossover experiments. The remainder of their diets was low in fat. The mean 3 per cent rise of total cholesterol on palmolein compared with usual (Australian) diet was predominantly due to a 10 percent rise of HDL-cholesterol. This was seen in both series of experiments (n = 21 in 1990 and n = 30 in 1991). Results with canola were less consistent. Plasma total and LDL cholesterols were lower than on usual diet in both series of experiments (less so in 1991); plasma HDLs did not rise above levels on usual diet in 1990, but they rose in the 1991 experiments. Part of the explanation for inconsistency may be that the canola oil used in 1991 had been inadvertently mixed with some palmolein in the factory that made the crisps.

Key words: Palmolein, Canola oil, Plasma lipids, Australian diet.

Introduction

Potato crisps are usually fried in palmolein, which has excellent technical properties. Polyunsaturated oils are thought to be unsuitable. Recently canola oil crisps have appeared in the market with the implication that their lower proportion to saturated fatty acids would have beneficial effects on plasma cholesterol. We decided to examine this experimentally because snack foods can be an important part of the diet in modern city dwellers. The Research and Development department of a major manufacturer agreed to fry batches of potato crisps in canola oil or the conventional palmolein for a human trial. This provided an opportunity for us to run a double blind crossover trial.

We have carried out two series of experiments, in 1990 and 1991, on apparently healthy, free-living men and women, mostly members of the University community, who volunteered in response to advertisement. The men were asked to eat in an otherwise low fat diet, 3 x 50g (3 bags) of experimental potato crisps each day, providing (at about 35 percent of fat by weight) 53 g of fat from the oil on the crisps. The women were asked to ear 2 x 50g (2 bags) of the crisps, providing 35g of fat daily. These amounts of fat from the fried crisps are about half the reported averages for Australian men and women respectively (105 and 70g/d)(1).

Methods

Subjects were asked to eat about a third of the day's crisps at the beginning of each meal and then, in the rest of their diets, to eat foods that were low to moderate on fat from a list of such foods. They were instructed to avoid cheese (except low fat variants), chocolates, fat/ oil on meat, fatty meats, meat products, cakes, biscuits (except crispbread), cream, full cream milk, butter, ice cream, etc. The emphasis of what they should eat was on breakfast cereals, bread, toast, thin smears of polyunsaturated margarine, jam, marmalade or 'vegemite'; fruit, boiled potatoes, pasta or rice, boiled or microwaved vegetables, fish grilled or lightly fried in oil, and low fat soups. Alcohol was allowed in moderation if subjects were accustomed to it but they abstained for at least 12 hours before blood sampling. The subjects recorded all items of food and drink consumed each day on daily record forms throughout the experiment. The crisps were supplied in plain bags, unmarked except for two code characters: "T4" or "B9" in 1990 and "D5" or "G8" in 1991. Until the end of the experiment, the type of oil was known only by the food scientist who prepared the crisps.

In 1990, 21 subjects (12 men, 9 women) completed the 5 week experiment. They were randomly allocated to take palmolein ("B9") or canola ("T4") crisps for the first 3 weeks, then (without a washout period) changed over to the other type, canola or palmolein for another 2 weeks. Thus half the subjects ate (new) canola crisps for 3 weeks and the (usual) palmolein crisps for the next 2 weeks. The other half of the subjects ate palmolein crisps for 3 weeks and canola crisps for the next 2 weeks. The first week of the 3 weeks, served as an adjustment period. Fasting venous bloods were taken once at the start (usual diet), then for the last 3 mornings of both the first and second experimental periods. Venous blood was taken from an ante-cubital vein with the subject in the supine position after on overnight fast (except water) of at least 12 hours. Bloods were taken in our Metabolic House and subjects were then given breakfast before going on to their day's work. Blood was drawn into 10ml plastic test tubes containing potassium EDTA, and plasma was obtained by centrifugation in a bench top refrigerated centrifuge at 3,000 rpm for 10 minutes. Several small aliquots were stored in Eppendorf tubes at -80°C; the rest of the sample was kept at 4° C in the cold room. At the end of each period, the plasmas were analysed for total cholesterol and triglyceride. HDLs were analyzed by LDL precipitation in a batch for each subject at the end of the 5 weeks trial.

Total cholesterol was measured enzymatically with the CHOD-PAP Monotest kit (Boehringer Mannheim, Germany) and triglyceride with UNI-KIT-II TRYGLYCERIDE PAP (Rosche Diagnostica, Basel, Switzerland) in our laboratory using a Cobas-Fara centrifugal auto-analyzer. High density lipoprotein cholesterol was measured after precipitation of other lipoproteins by heparin and manganous chloride (2). LDLs were calculated by the Friedwald equation (3).

By capillary gas chromatography of ethyl esters of the hexane phase the palmolein

contained 14:0 - 1%, 16:0 - 39%, 18:0 - 5%, 18:1 - 45% and 18:2 - 11%; the canola oil contained 16:0 - 5%, 18:0 - 3%, 18:1 - 63%, 18:2 - 20% and 18:3 - 8%. Gas chromatographic results in our laboratory agreed closely with those supplied by the manufacturer.

Result

First series experiments (1990)

The subjects kept healthy. Changes in body weight were small and not significantly different between palmolein and canola periods. Plasma total cholesterols for the two (order) groups of subjects are in **table 1**. Since there were no obvious effects of order (4) (though the total cholesterols were lower after 3 weeks on canola than after 2 weeks) the 2 groups were combined **(table 2)**. By t-test than the mean differences between canola and palmolein were significant ($P < 0.001$). Total cholesterol rose an average of 0.14 mmol/L from usual diet on palmolein and fell 0.39 mmol/L on canola but the usual diet values were based on a single blood sample. Although the women ate two-thirds the weight of crisps that the men ate, differences in cholesterols between canola and palmolein were about the same: -0.55 mmol/L in the women and -0.50 mmol/L in the men. The lower cholesterols on canola oil crisps had been expected, because the percentage of saturated (mostly palmitic) fatty acids is much lower in canola oil than in palmolein.

High density lipoprotein (HDL) cholesterols were, however, higher on palmolein crisps, whether these were taken before of after the canola crisps **(Table 3)**. Because this result was unexpected, all the plasma HDL - cholesterols were repeated on extra samples which had been kept unopened at -80° C. The results in **Table 3** are for the first run and the second run combined. For each subject, the HDLs for both periods were measured in the same batch as before. As there was again no obvious interaction across the change over, results of the two groups were combined in **Table 4**. HDL-cholesterols were significantly higher on palmolein than on canola. They appeared to rise from usual diet values on palmolein and stay the same on canola. Naturally the female subjects had higher HDL-cholesterols but the ratio of these on palmolein/canola were identical between men and women.

Table 1. Plasma Total Cholesterols (mmol/L) First Series.

Plamolein (3 wks) 5.05;	Canola (2 wks) 4.67
(difference -0.38 lower in canola; lower in 8/9 subjects)	
Canola (3 wks) 4.40;	Palmolein (2 wks) 5.03
(difference -0.63 lower in canola; lower in 10/12 subjects)	

Table 2. Plasma Total Cholesterol (mmol/L) First Series.

	two ways combined (n = 2)	difference
Plamolein (mean of 3 bloods)	5.04	0.14
Usual diet (1 blood)	4.90	0.39
Canola (3 wks)	4.51	

Plasma (fasting) triglycerides were higher in the men, but they were not significantly different between bloods at the end of the canola and palmolein periods.

Table 3. Plasma HDL Cholesterols (mmol/L) First Series.

Plamolein (3 wks) 1.501;	Canola (2 wks) 1.41
(different + 0.09 on palmolein; higher in 5/9)	
Canola (3 wks) 1.28;	Palmolein (2 wks) 1.44
(difference +0.16 on palmolein; higher in 11/12)	

Mean of last 3 days of periods - RELATED

Table 4. Plasma HDL Cholesterols (mmol/L) First Series.

		Difference
Plamolein (mean of 3 bloods)	1.46	
		+0.12
Usual diet (Single blood)	1.32	
		+0.02
Canola (mean of 3 bloods)	1.34	

Two ways combined (n =21). Repeated analyses.

Table 5. Plasma Total Cholesterol (TC), Cholesterol (HDL-c) and ratio (TC/HDL)

	mmol/L		
Plamolein			
	TC = 5.04	HDL = 1.46	TC/HDL 3.45
Canola			
	TC = 4.51	HDL = 1.34	TC/HDL 3.37

Means both ways combined (n =21). First Series.

Because these results have important implications in applied nutrition, we have waited to repeat the crossover trial before publishing a definitive report. In the second set of experiments made in 1991, four improvements were made to the design. More subjects, 30 (17 women, 13 men), were studied; apo-lipoprotein A_1 and B were also measured by timed immunonephelometry and the periods were longer. The first type of crisps were taken for 5 weeks (the first 2 weeks for adjustment) and the other type of crisps for the 3 weeks immediately following the changeover. Three bloods were now taken on consecutive mornings immediately before the start of the crisps (usual diet), as well as at the end of 5 and 8 weeks. As before, the subjects were allocated to start with palmolein or canola crisps by randomization. Plasma total cholesterol, HDL-cholesterol and triglyceride methods were as before.

Second series of experiments (1991)

These are presented in Tables 6-10 first showing the two order groups (canola -

palmolein and palmolein - canola) separately. Because 3 bloods were taken at the start while subjects were eating their usual diets, there is more confidence in the baseline plasma lipids in the second series.

Total cholesterols were lower at the end of the canola period than at the end of the palmolein period in both order groups. Differences were smaller in the palmolein-canola group. While plasma total cholesterol were lower on canola than at baseline, apo B-lipoproteins were higher on both canola and palmolein.

HDL- cholesterols and apo A_1 lipoprotein were higher than baseline on canola and on palmolein. When the two order groups are combined (**Table 10**), it can be seen that the mean HDL-cholesterol for 30 subjects was the same on canola as on palmolein. Mean apo A_1 lipoproteins were 2% higher on palmolein than on canola, but this difference was not significant.

In the second series, subjects tended to gain weight so that by the end of the 8 week experiment, group A had gained an average of 1.01 kg and group B 0.95kg. There was a little more weight gain in the canola periods.

The crisps in the second series were supplied in two batches. The first batch (1991 A) was a smaller quantity and most of the crisps eaten by the subjects were from the second batch (1991 B). **Table 11** compares our gas chromatographic analysis of the fat from the three batches of crisps: 1990 (all one batch) and the two used in 1991. The composition of the palmolein was very consistent in all 3 batches, but the third lot of canola oil used for most of the 1991 experiments was found to contain substantially more palmitic acid and less polyunsaturated fatty acids.

First series and Second series considered together **(Tables 11 and 12)**

It would probably be incorrect to combine the result for the 21 subjects in the first series with the 30 in the second series because the diet periods were of different duration.

In the first series total cholesterols were 0.14 mmol/L (3 percent) higher on palmolein than on usual diet, but HDL cholesterols rose 0.12 mmol/L (11 percent). There was therefore, no significant rise of LDL but a small rise of HDL cholesterol. The values for usual diet were however based on single blood samples.

In the second series of experiments there is more confidence in the baseline (usual diet) values as they too were based on 3 consecutive-day blood samples. Total cholesterols were again 0.14 mmol/L (3

Table 6. Plasma Total Cholesterols (mmol/L) Second Series

Baseline (usual diet)	Canola (5 wks)	Palmolein (3 wks)
4.83	5.52	5.02
(Difference -0.30	+0.2)	
Baseline (usual diet)	palmolein	Canola
5.17	5.25	5.11
(Difference +0.08	-0.06)	

(Differences are from the baseline). (n = 15 in each group).

Table 7. Apo B Lipoproteins (g/l) Second Series

Baseline (usual diet)	Canola	Palmolein
0.74	0.90	1.10
(Difference +0.16	+0.20)	
Baseline (usual diet)	palmolein	Canola
0.82	0.99	0.01
(Difference +0.17	+0.02)	

(Differences are from the baseline). (n = 15 in each group).

Table 8. HDL-Cholesterols (mmol/L) Second Series

Baseline (usual diet)	Canola	Palmolein
1.124	1.156	1.167
(Difference +0.032	+0.043)	
Baseline (usual diet)	palmolein	Canola
1.216	1.379	1.389
(Difference +0.173)		

(Differences are from the baseline for each type of oil). (n = 15 in each group).

Table 9. Apo A Lipoprotein (g/L) Second Series

Baseline (usual diet)	Canola	Palmolein
1.136	1.363	1.421
(Difference +0.227	+0.285)	
Baseline (usual diet)	palmolein	Canola
1.290	1.466	1.470
(Difference +0.173)		

(n = 15 in each group).

Table 10. Combined Results of Second Series (n = 30)

	Usual diet	Canola	Palmolein
Plasma Total Cholesterol (mmol/L)	5.0	4.82	5.14
Plasma LDL cholesterol (mmol/L)	3.80	3.57	3.83
Plasma Apo B lipoprotein (g/L)	0.78	0.95	1.05
Plasma HDL-cholesterol (mmol/L)	1.170	1.273	1.273
Plasma Apo A1 lipoprotein (g/L)	1.213	1.417	1.444
Plasma triglycerides (mmol/L)	0.664	0.669	0.728
Body weight (kg)	61.26	62.05	61.91

percent) higher on palmolein than on usual diet, but most of this was accounted for by a statistically significant rise of 0.10 mmol/L (8.5 percent) of HDL -cholesterol. LDL-cholesterols were almost unchanged, a difference of only 0.03 mmol/L on palmolein compared with usual diet. Thus results on palmolein were very much the same in the second series as in the first.

The results were somewhat more conflicting with the canola oil crisps. In the first series, total cholesterols were 0.39 mmol/L (8 percent) lower on canola than on usual diet and 0.53 mmol/L lower than on palmolein. HDL-cholesterols however, did not appear to rise on canola in this series, and were significantly lower than on palmolein. LDL-cholesterols (3) were lower on canola than on usual diet or palmolein.

In the second series, the fall of plasma total cholesterol on canola oil was less than in the first series. Differences were -0.18 mmol/L (4 percent) compared with usual diet and -0.32 mmol/L compared with palmolein. This is reflected in the LDL-cholesterol values. Another difference is that HDL cholesterols rose on canola in the second series by as much as they rose on palmolein.

Table 11. Plasma Lipids on Palmolein Crisps Compared with Usual Australian Diet

	1990	1991
Total Cholesterol (mmol/L)	+0.14 (3%)	+0.14 (3%)
HDL-cholesterol (mmol/L)	+0.14 (11%)	+0.10 (8.5%)
LDL cholesterol (mmol/L)	No change	No change

Table 12. Plasma Lipids on Canola Crisps Compared with Usual Australian Diet

	1990	1991
Total Cholesterol (mmol/L)	-0.39 (8%)	-0.18 (4%)
HDL-cholesterol (mmol/L)	No change	+0.10 (8.5%)
LDL cholesterol (mmol/L)	-0.17 (5%)	-0.23 (6%)

Table 13. Fatty Acid Percentages in Oils On Crisps

		1990	1991	1991B
Palmolein				
	14:0	1	1	-
	16:0	39	42	43
	18:0	5	4	4
	18:1	45	43	43
	18:2	11	9	9
	18:3	-	-	-
	20:0	-	-	-
	20:1	-	-	-
	P/S ratio	0.24	0.19	0.19
Canola				
	14:0	-	-	-
	16:0	5	6	16
	18:0	3	3	3
	18:1	63	61	60
	18:2	20	19	16
	18:3	8	8	5
	20:0	-	1	-
	20:1	-	2	-
	P/S ratio	3.5	3.0	1.1

Discussion

The diminished effect on total and LDL cholesterol of canola in the second series can probably be explained by the higher proportion of saturated fatty acids in the bulk of the 1991 canola oil. When lipid extracts of the crisps were analyzed the same capillary gas chromatography in our laboratory at the end of the second series of experiments (**Table 13**), we found a much higher percentage of 16.0 (16 percent of previously 6 percent), with correspondingly lower percentages of unsaturated acids, 18:1, 18:2, and 18:3. Further inquiry disclosed that the main batch of canola oil had become inadvertently mixed in the food factory with some palmolein.

It appears that on palmolein there was a rise of HDL-cholesterol averaging about 10 percent. This was seen as statistically significant in both series of experiments. A rise of HDL-cholesterol was not seen on canola oil in the first experiment, when gas chromatography showed that the oil had the fatty acid pattern of pure canola oil. It is difficult to interpret the HDL-cholesterol changes on canola in the second experiment when the canola oil had been admixed with some palmolein.

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References

1. Department of Community Services and Health (1987) National Dietary Survey of Adults; 1983. No 2 Nutrient Intakes, Australian Government Publishing Services, Canberra.

2. **Warnick GR**, Nugyen T, Allers AA. Comparison of improved precipitation method for quantification of high-density lipoprotein cholesterol. *Cin Chem* 1958; 31: 217- 222.

3. **Freidwald WT**, Levy RI, Fredrickson DS. Estimation of concentration of low-density lipoprotein without the use of the preparative ultracentrifuge. *Cin Chem* 1972; 18: 499-502.

4. **Hills B, Armitage** P. The two-perios cross-over clinical trial. *Brit J. Pharmacol 1974*; 8:7-20.