JOURNAL OF OLEO SCIENCE

J. Oleo Sci., Vol. **53**, No. 2, 57-62 (2004)

JOS

Dietary Effects of Natural Conjugated Triene Fatty Acid in Comparison with *Trans* Fatty Acids of Hydrogenated Fat on Plasma and Tissue Lipid Profile

Pubali DHAR[1], Santinath GHOSH[2] and Dipak Kumar BHATTACHARYYA[3]*

[1] Krishnanagar Govt. College
(Krishnagar, Nadia, West Bengal)
[2] Department of Chemical Technology Calcutta University
(92, A.P.C. Road Calcutta-700009 INDIA)
[3] Department of Chemical Technology, Calcutta University, University Colleges of Science & Technology
(92, A.P.C. Road, Calcutta-700009, INDIA)

Edited by K. Miyashita, Hokkaido Univ., and accepted September 26, 2003 (received for review August 4, 2003)

Abstract: An attempt has been made to compare the nutritional quality between naturally occurring conjugated octadecatrienoic fatty acid with trans configuration and the trans fatty acids being produced in hydrogenated fats. The karela seed oil (*Momordica charantia*) (29.2% trans isomer) was used as the source of conjugated trienoic trans fatty acid and hydrogenated fat for trans fatty acid (39.5%). The two fats were fed to male albino rats (Charles Foster strain) for four weeks. Feeding experiment showed comparable results in terms of growth rate, food efficiency ratio (FER) and serum lipid profile. In the third week the growth rate of rats fed hydrogenated fat with trans showed a significant increase than the karela seed oil with trans conjugated trienoic fatty acid fed group. Total cholesterol level of liver was significantly higher in rats fed karela seed oil containing conjugated trienoic fatty acid with trans configuration. The phospholipid content of heart and brain of rats fed karela seed oil was significantly higher than the hydrogenated fat with trans fed group.

Key words: conjugated octadecatrienoic fatty acid, *Momordica charantia*, trans fatty acid, hydrogenated fat

1 Introduction

Trans fatty acids (TFA) are unsaturated fatty acids invariably formed during industrial hydrogenation of triglyceride oils. Hydrogenated fats for use in shortening, cooking fat and margarine products are produced by partial and selective hydrogenation of liquid oils such as sunflower, rapeseed, cottonseed rice bran oil and palm olein and constitute 30-60% of TFA, depending on the nature of the liquid oils.

The seed oils of some species of cucurbitaceae family like *Tricosanthes anguina* commonly known as snake gourd and *T. diocia* commonly known as parwal and *Momordica charantia* commonly known as karela contain about 30-60% of conjugated octadecatrienoic fatty acid. The fatty acid in karela seed oil commonly called α-elaeostearic acid which is 9 cis 11 trans 13 trans. This natural trans fatty acid is found in tung oil (*Aleurites fordii*, montana) at 80% level. Karela seed oil is not a normal vegetable oil for human consumption but karela is a common vegetable in different parts of the world.

*Correspondence to: Professor D.K. BHATTACHARYYA, Emeritus Professor, Department of Chemical Technology, Calcutta University, University Colleges of Science & Technology, 92, A.P.C. Road, Calcutta -700009, INDIA
E-mail: dkb_oilteck@yahoo.com

The metabolic effects of trans isomers are today a matter of controversy generating diverse extreme positions in light of biochemical, nutritional and epidemiological studies.

Trans fatty acids of hydrogenated fat products have been reported to contribute to several health problems including thrombogenesis leading to coronary heart disease (1). In British and U.S. report from 1984-89 the trans fatty acids were more or less acquitted of unhealthy effects. During the last 5-6 years, investigations have been made on the connection between the consumption of trans fatty acids and the occurrence of coronary heart disease and the impact on the lipoprotein level in plasma.

Nestel *et al*.(2) showed that the LDL-cholesterol is raised in partially hydrogenated fats and oils and with specified trans fatty acids than with the corresponding unhydrogenated oils and the levels are marginally lower than with highly saturated fats such as butter or palm oil.

Less certain are the effects of trans fatty acids on HDL-cholesterol lowering and Lp (a) concentration (1,2). Most controversial is the alleged link between trans fatty acid consumption and coronary heart disease (CHD). Of some concern is that in the two most influential studies, by Ascherio *et al*. (3) and Willett *et al*. (4) who ranked CHD risk by quintile and not in the lowest quintile.

This would lead to the conclusion that eating around 3-4gm TFA daily is more protective against coronary heart disease than eating none at all.

A study from England shows that there was again no association between the trans isomers of oleic and linoleic acids and sudden cardiac death (5). Wauben IP *et al*. (6) illustrated that TFA combined with a marginal EFA status do not affect growth or brain long chain PUFA during pre and post-natal periods in B6D2F mice. Vermunt *et al*. (7) showed that high trans α-linolenic acid diet significantly increased plasma LDL : HDL cholesterol and total cholesterol : HDL cholesterol ratios. Loi *et al*. (8) showed that dietary trans isomers of linolenic acid alter the fatty acid profile of rat liver, platelet and heart tissues.

De Roos *et al*. (9) tested whether trans fatty acids and saturated fatty acids had different effects on flow mediated vasodilatation (FMV), a risk marker of coronary heart disease (CHD). Replacement of dietary saturated fatty acids by trans fatty acids impaired FMD of the brachial artery, which suggested increased risk of CHD. In a recent study by Noguchi *et al*. (10) the dietary fat from bitter gourd oil decreased the formation of 18:2 n-6 and increased the concentration of 22:6 n-3.

In our previous observation (11) the nutritive value of conjugated octadecatrienoic acid (C18:3 Δ9, 11,13 α-elaeostearic acid) as occurred in karela seed oil has been compared with linseed oil, containing non-conjugated octadecatrienoic fatty acid (C18:3 Δ9, 12,15 α-linolenic acid). It has been observed that the concentration of total cholesterol, triglyceride, VLDL-cholesterol, LDL-cholesterol and LDL/HDL-cholesterol in serum were significantly higher in karela seed oil than in linseed oil. In another *in vivo* experiment on rats (12) conjugated octadecatrienoic acid (C18:3 Δ9, 11,13 α-elaeostearic acid) of karela seed oil has reduced plasma and erythrocyte membrane lipid peroxidation at 0.5% level in comparison with sunflower oil group.

By considering the controversial effect of trans fatty acids interest has been generated to know whether the natural trans fatty acid containing oil like karela seed oil will behave in a similar fashion or in a different way in comparison with hydrogenated fat containing artificial trans fatty acids. In the present study rats were fed with two dietary fats (karela seed oil and vanaspati) for six weeks and their growth rate and lipid profiles of serum, heart, brain and liver had been compared.

2 Experimental

2·1 Meterials

Dietary fat sources : Oil was extracted from authentic karela (***Momordica charantia***) seed, obtained from the local market at Calcutta, India, in a Soxhlet apparatus with food grade n-hexane. The oil after carefully alkali refining and bleaching was stored under vacuum. Conventional vanaspati, a proprietary brand (Dalda, Hindustan lever Ltd., Kolkata, India), was used as control fat.

2·2 Analysis of Fat Products

The slip-melting point of vanaspati and karela seed oil was determined by standard method (13). Gas-liquid chromatography was employed for determining the fatty acid composition of the two dietary oils after their conversion into methyl esters (14). Trans isomer content in karela seed oil was determined by infra-red spectrophotometry according to Allen (15).

2·3 Feeding Experiment

Animal experiments were designed and carried out as per the reports published from the laboratory earlier (11). Male albino rats of Charles foster strain (selected for the authenticity of the strain) were housed in individual cages and were fed the dietary oils and fresh water *ad libitum*. Daily food consumption and weekly body weight gain were recorded. The feeding experiment was conducted to evaluate the dietary attributes of the two dietary fats one containing conjugated linolenic acid called α-elaeostearic acid which is a trans triene and other containing trans monoene.

24 rats (70-80g body weight) were divided into two groups each consisting of 12 rats having equal average body weight. They were fed experimental diets composed of fat free casein, 18%; fat, 20%; starch, 55%; salt-mixture 4% (composition of salt mixture No.12 (in g) : NaCl 292.5, KH_2PO_4 816.6; $MgSO_4$ 120.3; $CaCO_3$ 800.8; $FeSO_4$, $7H_2O$ 56.6; KI 1.66; $MnSO_4.2H_2O$, 9.35; $ZnCl_2$ 0.5452; $CuSO_4.5H_2O$, 0.9988, $CoCl_2$ $6H_2O$ 0.0476) (16), cellulose 3%; one multivitamin capsule (vitamin A I.P. 10,000 units, Thiamine mononitrate I.P. 5mg, Vit B I.P. 5mg, Calcium pantothenate USP 5mg, Niacinamide I.P. 50mg, Ascorbic acid I.P. 400 units, Cholecalciferol USP 15 units, Menadione I.P. 9.1mg, Folic acid I.P. 1mg, Vitamin E USP 0.1 mg) per kg of diet. The diets were adequate in all nutrients.

Rats were maintained on the above diets *ad libitum* for 6 weeks. The amount of daily diet consumed by each rat and weekly body weight gain were noted. Rats were sacrificed under anesthesia, blood was collected, and liver, heart, brain were immediately excised blotted, and stored at deep freeze temperature (-20℃) for analysis.

2·4 Lipid Analysis

The total lipids were extracted from liver, brain, heart with chloroform / methanol mixture and estimated gravimetrically (17). According to the standard methods, the lipid components such as total Cholesterol (18), HDL-Cholesterol (19) Triglyceride (20), LDL (21) and VLDL (22) of plasma were analyzed using enzymatic kits supplied by Ranbaxy. By the same methodologies total cholesterol (18), triglyceride (20), phospholipid (23) and total lipid of brain, heart and liver tissues were estimated. For statistical analysis of result, student's test (24) was performed.

3 Results

3·1 Fatty Acid Composition

The fatty acid composition, the trans fatty acid content and the slip melting point of vanaspati (hydrogenated fat) and of karela seed oil are included in **Table 1**. The slip melting point of vanaspati is 39.1℃ but that of karela oil is 31.5℃. Karela seed oil has high melting point due to its stearic acid (31.3%) and conjugated octadecatrienoic acid (50.4%) having 58% of it in trans configuration. Vanaspati has also high trans oleic acid (39.5%).

3·2 Growth Rate

The growth rate of rats raised on the hydrogenated fat (vanaspati) and natural trans fatty acid containing oil (karela seed oil), as shown in **Fig. 1** was nearly identical.

The hydrogenated fat control group showed a satisfactory growth promoting effect which agreed well with the observation of Mattson (25), who suggested that trans fatty acid dietary fat had little effect on growth.

3·3 Lipid Profile

The serum lipid profiles of rats (**Table 2**) did not reveal any significant difference in the content of the total cholesterol, HDL-cholesterol, VLDL-cholesterol, LDL-cholesterol, triglycerides and LDL/HDL between two dietary fats.

A significantly higher liver cholesterol was found in

Table 1 Fatty Acid Composition of Vanaspati and Karela Seed Oil.

Test fat	Fatty acid composition, (w/w%)						%Trans	Slip Point ℃
	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$		
Karela oil	—	2.4	31.3	8.0	7.9	*50.4	29.2	31.5
Vanaspati	1.0	29.6	11.0	49.7	8.7	—	39.5	39.1

*58% of conjugated triene

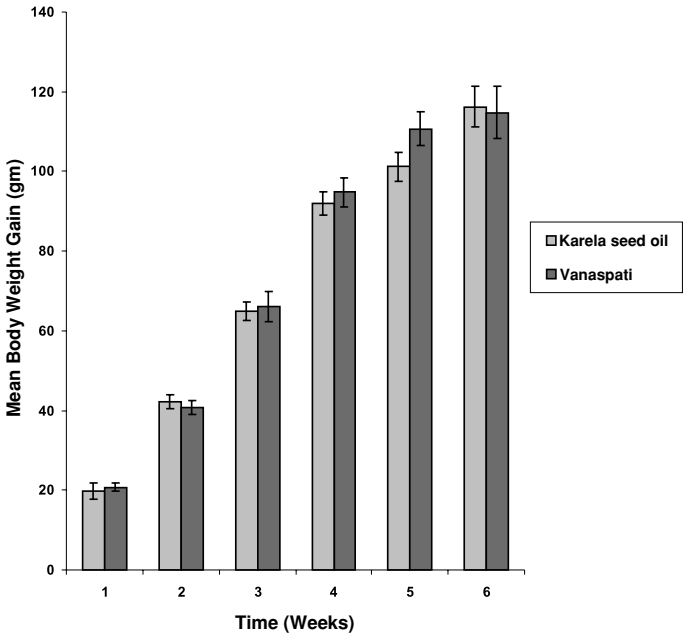

Fig. 1 Mean Body Weight Gain of Rats Fed Vanaspati and Karela Seed Oil at 20 % Level.

rats fed karela seed oil. Phospholipid content of brain and heart was also significantly higher in case of the karela seed oil fed group (**Table 3**).

4 Discussion

The primary purpose of the present study was to obtain a comparative information on the nutritional quality of natural trans in the form of conjugated triene and the trans fatty acid formed by hydrogenation.

Karela seed oil contains in addition to trans structure some amount of 18:2 (cis-cis) essential fatty acid and shows a normal growth pattern which corroborates the report of Alfin-Slater and Aftergood (26). The karela seed oil though rich in conjugated fatty acid, showed no significant difference in total cholesterol, LDL cholesterol, VLDL and TG of serum. This suggests that conjugated trienoic fatty acid, α-elaeostearic acid containing polyenoic trans and hydrogenated fat having isolated trans are more or less equivalent from the nutritional point of view.

Our result showed similar effects of dietary conjugated trienoic fatty acid as indicated by lipid profiles of serum presumably due to the adequate supply of essential fatty acid to both the groups.

5 Conclusion

Trans isomer fatty acids whether natural or polyenoic in character or whether in the form of isolated trans as occurring in modified fats like hydrogenated fats exhibit similar pattern of dietary effects.

Table 2 Lipid Composition of Serum of Rats fed 20% Vanaspati and Karela Oil.

Test fat	TC mg/dl	HDL mg/dl	TG mg/dl	VLDL mg/dl	LDL mg/dl	LDL/HDL
Karela oil	101.82±9.51	31.68±3.78	82.65±7.47	15.18±1.45	53.62± 6.08	1.83±0.28
Vanaspati	119.93±9.51	39.97±3.76	76.95±7.25	15.39±1.45	64.57±11.91	1.89±0.52

Values are mean±SEM, n = 12

Table 3 Tissue Lipid Profile of Rats Fed 20% Karela Oil and Vanaspati.

Test fat	Organs	Weight of Tissue % body weight	Lipids(mg/g) in tissues			
			Total lipid	Total Cholesterol	Phospholipid	Triglyceride
Vanaspati	Liver	3.57±0.04	49.99±3.17	1.85±0.17*	17.32±0.33	13.05±2.43
	Heart	0.34±0.01	54.06±3.8	2.61±0.22	15.62±0.48**	8.24±1.71
Karela Seed Oil	Brain	0.67±0.03	78.67±4.15	12.97±1.2	35.32±2.69***	0.00
	Liver	3.43±0.01	44.71±1.15	2.29±0.19*	18.26±1.73	8.24±1.71
	Heart	0.34±0.02	55.23±3.89	3.42±0.34	26.42±2.09**	10.32±1.27
	Brain	0.68±0.03	91.52±5.57	12.81±0.16	44.43±3.16***	0.00

Values are mean±SEM, n = 12

* Values are significantly different at $p < 0.1$.

** Values are significantly different at $p<0.05$.

***Values are significantly different at $p<0.001$

Acknowledgement

The study was financially supported by University Grants Commission (Govt. of India).

References

1. R.P. MENSINK and M.B. KATAN, Effect of Dietary Trans Fatty Acids on High Density Lipoprotein Cholesterol Levels in Healthy Subjects, *N. Engl J. Med*., Vol. **323**, 439-445 (1990).
2. P. NESTEL, M. NOAKES, B. BELLING, R. MCARTHUR, P. CLIFTON, E. JANUS and M. ABBEY, Plasma Lipoprotein Lipid and Lp(a) Changes with Substitution of Elaidic Acid for Oleic Acid in the Diet, *J. Lipid Res*., Vol. **33**, 1029-1036 (1992).
3. A. ASCHERO, C.H. HENNEKENS, J.E. BURING, C. MASTER, M.J. STAMLER, W.C. WILLETT, Trans Fatty Acid Intake and Risk of Myocardial Infarction, *Circulation*, Vol. **89**, 94-101 (1994).
4. W.C. WILLETT, J.M. STAMPFER and J.E. MANSON, Intakes of Trans Fatty Acids and Risk of Coronary Heart Disease Among Women, *Lancet*, Vol. **341**, 581-586 (1993).
5. T.L. ROBERTS, D.A. WOOD, R.A. RIEMERSMA, P.L. GALLAGHER and F.C. LAMPE, *Lancet*, Vol. **345**, 278-282 (1995).
6. I.P. WAUBEN, H.C. XING, D. MCCUTCHEON and P.E. WAINWRIGHT, Dietary Trans Fatty Acids Combined with a Marginal Essential Fatty Acid Status During the Pre- and Postnatal Periods do not Affect Growth or Brain Fatty Acids but may Alter Behavioral Development in B6D2F (2) Mice, *J. Nutr*., Vol. **131**, 1568-1573 (2001).
7. S.H. VERMUNT, B. BEAUFRERE, R.A. RIEMERSMA, J.L. SEBEDIO, J.M. CHARDIGNY and R.P. MENSINK, Dietary Trans Alpha-linolenic Acid from Deodorized Rapeseed oil and Plasma Lipids and lipoproteins in Healthy Men: the Trans Line Study, *Br. J. Nutr*., Vol. **85**, 249-250 (2001).
8. C. LOI, J.M. CHARDIGNY, S. ALMANZA, L. LECLERE, C. GINIES and J.L. SEBEDIO, Incorporation and Metabolism of Dietary Trans Isomers of Linolenic Acid Alter the Fatty Acid Profile of Rat Tissues, *J. Nutr*., Vol. **130**, 2550-2555 (2000).
9. N.M. DE ROOS, M.L. BOTS and M.B. KATAN, Replacement of Dietary Saturated Fatty Acids by Trans Fatty Acids Lowers Serum HDL Cholesterol and Impairs Endothelial Function in Healthy Men and Women, *Arterioscler Thromb. Vasc. Biol*., Vol. **21**, 1233-1237 (2001).
10. R. NOGUCHI, Y. YASUI, R. SUZUKI, M. HOSOKAWA, K. FUKUNAGA and K. MIYASHITA, Dietary Effects of Bitter Gourd Oil on Blood and Liver Lipids of Rats, *Arch. Biochem. Biophys*.,Vol. **396**, 207-212 (2001).
11. P. DHAR and D.K. BHATTACHARYYA, Nutritional Characteristics of Conjugated Octadecatrienoic Fatty acid Containing Oil, *Ann. Nutr. and Metb*., Vol. **42**, 290-296 (1998).
12. P. DHAR, S. GHOSH and D.K. BHATTACHARYYA, Dietary Effects of Conjugated Octadecatrienoic Fatty Acid (9cis,11trans, 13trans) Levels on Blood Lipids and Nonenzymatic *In Vitro* Lipid Peroxidation in Rats, *Lipids*., Vol. **34**, 109-114 (1999).
13. Indian Standard I.S. Methods of Sampling and Tests for oils and Fats (Revised), Fourth reprint, May 1975, Indian Standard Institution (ISI), *IS*, 548 (part I), p. 33 (1964).
14. L.D. METCALFE and A.A. SCHMITZ, The Rapid Preparation of Fatty Acid Esters for Gas Chromatographic Analysis, *Anal. Chem*., Vol. **33**, 363-364 (1961).
15. R.R. ALLEN, A Rapid Method for Determination of Trans Saturation in Fats and Derivatives, *J. Am. Oil Chem. Soc*., Vol. **46**, 552-553 (1969).
16. J.H. JOANES and C. FOSTER, A Salt Mixture for Use with Basal Diet Either Low or High in Phosphorus, *J. Nutr*., Vol. **24**, 245-256 (1942).
17. M. KATES, *Techniques of Lipidology*, New York, American Elsevier Publishing Co., pp. 349-351 (1972).
18. C.C. ALLAIN, L.S. POON, C.S.G. CHAN, W. RICHMOND and P.C. FU, Enzymatic Determination of Total Serum Cholesterol, *Clin. Chem*., Vol. **20**, 470-475 (1974).
19. G.R. WARNICK, T. NGUYEN and A. ALBERS, Comparison

of Improved Methods for Quantification of High-density Lipoprotein, *Clin. Chem*., Vol. **31**, 217-222 (1985).
20. G. BUCOLO and M. DAVID, Quantitative Determination of Serum Triglyceride by the Use of Enzymes, *Clin. Chem*., Vol. **19**, 476-482 (1973).
21. W.T. FRIEDWALD, R.I. LEVY and D.S. FREDRICKSON, Estimation of the Concentration of Low Density Lipoprotein Cholesterol in Plasma Without use of Preparative Ultracentrifuge, *Clin. Chem*., Vol. **18**, 499-502 (1972).
22. F.T. HATCH and R.S. LEES, Practical Methods for Plasma Lipoprotein Analysis, *Adv Lipid Res*., Vol. **6**, 1-68 (1968).
23. P.S. CHEN, T.Y. TORIBARA and H. WARNER, Micro Determination of Phosphorous, *Anal. Chem*., Vol. **28**, 1756-1758 (1956).
24. S. PEARSON and H.O. HARTLEY (eds), *Biometrika Tables for Statisticians Vol. 1*, Cambridge, (1966).
25. F.H. MATTSON, An Investigation of the Essential Fatty Acid Activity of Some of the Geometrical Isomers of Unsaturated Fatty Acids, *J. Nutr*., Vol. **71**, 366-370 (1960).
26. R.B. ALFIN-SLATER and L. AFTERGOOD, Geometrical and Positional Fatty Acid Isomers, (E.A. EMKEN and M.J. DUTTON, ed.), Champaign, American Oil Chemist's Society, pp. 53-74 (1979).