

Research Article

Effect of Soybean Oil Supplemented Diet on Fatty Acid Level and Lipid Profile of Albino Rats

¹Friday O. Uhegbu, ¹Amadike E. Ugbogu, ¹Kingsley C. Nwoku and ²Victor C. Ude

¹Department of Biochemistry, Abia State University, PMB 2000, Uturu-Nigeria

²Department of Medical Biochemistry, College of Medicine, Enugu State University of Science and Technology, PMB 01660, Enugu, Nigeria

Abstract: This aim of this study is to evaluate the effect of soybean oil supplemented diet on the fatty acid level and lipid profile of male albino rats. Forty healthy male albino rats weighing between 96-110 g and aged 6 weeks were used. The rats were placed randomly into four groups of ten animals each. Group 1 served as control and had no soybean oil in their feed. Group 2-4 was placed on soybean oil supplemented diet 10, 20 and 30% respectively. The experiment lasted for 14 days. The results showed that the animals had significant ($p<0.05$) increase in body weight compared with the control. Triacylglycerol, phospholipid and total cholesterol level were also significantly ($p<0.05$) decreased, 80.46 ± 3.76 to 78.46 ± 3.22 ; 15.36 ± 0.34 to 10.12 ± 0.75 ; and 116.65 ± 6.38 to 110.95 ± 6.36 mg/dL respectively. Very Low Density Lipoprotein (VLDL) and Low Density Lipoprotein (LDL) were also significantly decreased, 15.56 ± 2.56 to 13.45 ± 2.76 ; 68.88 ± 2.54 to 56.59 ± 2.14 mg/dL respectively. However High Density Lipoprotein (HDL) showed significant ($p<0.05$) increase 60.37 ± 1.36 to 65.75 ± 1.89 mg/dL. Cholesterol, triacylglycerol and fatty acids are significant and independent risk factors of adverse cardiovascular events. The clinical and nutritional implication of these results is discussed.

Keywords: Arthrosclerosis, cardiovascular disease, cholesterol, lipogenesis, osteoarthritis

INTRODUCTION

Fatty acids are carboxylic acids with hydrocarbon chains ranging from 4 to 36 carbons. The fatty acids that occur in natural fats are usually straight chain derivatives containing an even number of carbon atoms (Robert and Harwood, 1988). The main pathway for de-novo synthesis of fatty acids (lipogenesis) occurs in the cytosol. This system is present in many tissues, including the kidney, liver, brain, lung, mammary gland and adipose tissue (Kim, 1997). The nutritional state of the organism regulates lipogenesis. Thus the rate is high in the well-fed animal whose diet contains a high proportion of carbohydrate. It is depressed under conditions of restricted caloric intake, especially on a fat diet or when there is a deficiency of insulin as in diabetes mellitus (Wardlaw and Kessel, 2002).

Cholesterol is the major steroid found in animal tissues in the form of lipoproteins either in free forms inside the cells or combined form as esters in the blood circulation. Free cholesterol is usually converted to bile acids, while the cholesterol esters are the major storage and transport forms of cholesterol (Murray *et al.*, 1990).

Unsaturated fatty acids in phospholipids of the cell membrane are important in maintaining membrane fluidity. The transport of cholesterol to and from the liver is mediated by Low Density and High Density Lipoproteins (LDH and HDL). HDL is called the 'good cholesterol since it can exchange it with other lipid carriers and transports it to the liver for disposal in bile (Witztum, 1994; Vance and Vance, 1996).

The majority of clinical problems related to fatty acid metabolism are associated with processes of oxidation. Triacylglycerides are the most abundant family of the lipids and the major component of depot or storage lipids in plants and animal cell. It is a combination of glycerol and fatty acids (Chow, 1992; Vessby, 1994).

Soybean is a frost sensitive annual herb belonging to the family fabacea with diploid number of chromosomes (Hymowitz and Newell, 1981). It is grown commercially in over thirty-five countries of the world, with about 90% of the current world production coming from USA, Brazil, China and Argentina (Brown *et al.*, 1985). As a garden plant, soybean can be eaten green shelled (though they are not easy to

Corresponding Author: Friday O. Uhegbu, Department of Biochemistry, Abia State University, PMB 2000, Uturu-Nigeria, Tel.: +234 (0) 8035426500

This work is licensed under a Creative Commons Attribution 4.0 International License (URL: <http://creativecommons.org/licenses/by/4.0/>).

separate from the pods), salted, roasted or dried. More commonly, soybean are used in human food as flour, incorporated with wheat flour to round out the protein content in bread, cakes, cookies and crackers. It is also used in soups and as an extender in potted meats, confectionaries and butter (Cowman, 1973). Soybean as nutritional supplement is very essential; it is the only plant source that offers all eight essential amino acids in the amount needed. It has more non-essential amino acids than animal proteins (Cowman, 1973). The soybean seed is composed of $5.0 \pm 0.94\%$ water, 44.6 to 50.3% protein, 13.5 to 24.2% fat, 14 to 23.9% carbohydrate, 2.8 to 6.3% fiber and 3.3 to 6.4% ash. The soybean accounts for approximately 50% of the production of oil seed crops in the world.

Soybean oil is produced from the seeds of the soybean plant. Its seed provides approximately 60% of the world supply of vegetable protein and 30% of the oil (Brown *et al.*, 1985). On crushing the mature beans, around 18% oil could be obtained, the rest being the oil cake or meal, which forms the prime source of protein in animal feeds (Ostland Jr, 2002).

Soybean oil is 61% polyunsaturated fat and 24% monounsaturated fat which is comparable to the total unsaturated fat content of other vegetable oils (~85%). Like other vegetable oils, soybean oil contains no cholesterol. Soybean oil is a highly concentrated source of feed energy. Its caloric value is the major reason for its increased use. The special processing method of soybean oil provides a fat source that is resistant to rancidity (Walter and George, 1922; Murray, 1993).

Liquid soybean oil is low in saturated fat, contains no trans fat and is high in poly- and monounsaturated fats. It is also the principal source of omega-3 fatty acids in the United State diet and the primary commercial source of vitamin E as well. Soybean oil is also used as a nutritional supplement in intravenous feedings (Zhang *et al.*, 1997; Ostland Jr, 2002).

The present study investigated the effects of soybean oil supplemented diet on fatty acid levels and lipid profile of Albino rats.

MATERIALS AND METHODS

Animals: Forty healthy male albino rats weighing between 96-110 g and aged 6 weeks were used for this study. The rats were placed randomly into 4 groups of 10 animals each.

Group 1 served as control and had no soybean oil in the feed.

Groups 2 to 4 was placed on the soybean oil supplemented diet 10, 20 and 30%, respectively for 14 days (Table 1). They were allowed feed and water *ad libitum*. All processes involved in the handling and the experiment was carried out according to standard protocols approved by the animal ethics committee of the faculty of Biological and Physical Sciences, Abia State University, Uturu.

Table 1: Dietary composition of soybean oil supplemented feed

Diet composition	Control diet (control) g	Soybean oil supplemented diet (g)		
		10%	20%	30%
Corn	69.0	59.0	49.0	39.0
Fish meal	5.0	5.0	5.0	5.0
Groundnut	10.0	10.0	10.0	10.0
Bone meal	5.0	5.0	5.0	5.0
Palm oil	5.0	5.0	5.0	5.0
Soybean oil	-	10.0	20.0	30.0
Vitamin premix	1.0	1.0	1.0	1.0
Corn starch	5.0	5.0	5.0	5.0
Total	100.0	100.0	100.0	100.0

Collection of blood samples and serum preparation:

At the 15th day the animals were sacrificed after overnight starving. Incisions were made into their thoracic cavity. Blood samples were collected by heart aorta puncture using a 10 mL hypodermic syringe and allowed to clot in sample vials. The samples were centrifuged at 3000 rpm for 5 min. using the Bran Scientific and Instrument Company England centrifuge. The supernatant was harvested by simple aspiration with Pasteur pipette and stored in clean tube at -4°C until analysis.

Serum lipid profile: Serum was separated by centrifuging the blood. Diagnostic kits for the lipid profile were purchased from BioSystems (S.A Costa Brava of Barcelona, Spain). Very Low Density Lipoprotein-Cholesterol, (VLDL-C); triacylglycerol, cholesterol and phospholipids in serum were measured using enzyme assay kits from Wako Pure Chemicals (Tokyo, Japan). Lipids were extracted and purified according to the method of Folch *et al.* (1957). The concentrations of triacylglycerol, cholesterol and phospholipids were measured according to the methods of Fletcher (1968), Richmond (1973) and Bartlett (1959). The assays were performed according to the manufacturer's instruction. VLDL-C concentrations were estimated using the method of Burnstein and Sammaile (1960).

Statistical analysis: All values are expressed as means±S.D. Data were analyzed by one-way ANOVA and all differences were inspected by Duncan's new multiple-range test. Differences were considered to be significant at $p < 0.05$.

RESULTS

The effect of soybean oil supplemented diet on the body weight of the experimental animals (Table 2)

Table 2: Effect of soybean oil supplemented diet on animal weight (g)

Control	Soybean supplemented diet		
	10%	20%	30%
97.86±0.56	101.56±1.53	101.64±1.74	102.65±1.06

Values are Mean±S.D. of 10 (n = 10)

Table 3: Effect of soybean oil supplemented diet on fatty acid levels (mg/dL)

	Control	Soybean supplemented diet		
		10%	20%	30%
Triacylglycerol	79.46±3.78	78.95±4.32	78.54±2.65 ^a	78.46±3.22 ^a
Phospholipid	15.36±0.34	11.54±0.87	10.86±0.42	10.12±0.75
Total cholesterol	116.65±6.38	112.38±7.12	110.75±7.46 ^b	110.95±6.36 ^b
VLDL	15.56±2.54	14.58±1.24	13.12±1.79	13.45±2.76
LDL	68.88±2.54	64.23±2.16	60.56±2.85	56.59±2.14
HDL	60.37±1.36	62.76±1.78	65.45±2.37 ^c	65.75±1.89 ^c

Values are mean±S.D. of 10 (n = 10); Means in the row with the same letter are not significantly different at p<0.05

shows that there was a significant (p<0.05) increase in body weight of the animals compared to control. The increase seems to be percentage soybean oil dependent.

Fatty acid levels decreased significantly (p<0.05) in test animals compared to control, while HDL increased significantly compared to control (Table 3). The effect seems to be percentage soybean oil dependent.

DISCUSSION

The experimental animals gained weight which was significant (p<0.05) compared to the control (Table 2). The significant increase in weight of the animals may have been caused by the soybean oil. Soybean oil is rich in polyunsaturated fatty acids, including the two essential fatty acids, linoleic and linolenic, that are not produced in the body. Linoleic and linolenic acids aid the body's absorption of vital nutrients and are required for human health. These two essential acids are also precursors to hormones that regulate smooth muscle contraction, blood pressure and the growth of healthy cells (Ostland Jr, 2002). The soybean oil is a vegetable oil whose fats are more digestible than animal fats. Specially processed soybean oil is used to treat osteoarthritis (Murray, 1993).

Total cholesterol level was significantly decreased (p<0.05) in the experimental animals (Table 3). Soybean oil is low in saturated fat and high in polyunsaturated fatty acids. Pure soybean oil is about 50% linoleic acid and 8% linolenic acid. Soybean oil works to lower cholesterol levels by decreasing cholesterol absorption in the gut. A high ratio of polyunsaturated fatty acids to saturated fatty acids in the diet is a major factor in lowering plasma cholesterol concentrations and is considered to be beneficial in preventing coronary heart disease (Brown and Goldstein, 1984; Anderson *et al.*, 1995). Specific processed parts of soybean oil called unsaponifiables may have a beneficial effect on joints (Murray, 1993). In both clinical trials and population studies, polyunsaturated fats in the diet have been shown to actively lower serum cholesterol levels (Austin and Hokanson, 1994; Glew, 2006; Ayoola *et al.*, 2008).

Nutrition experts recommend limiting total fat consumption to 30% or less of the total daily calories

and limiting saturated fats to 10% or less. Saturated fatty acids raise blood cholesterol which can thicken arterial walls and increase the risk of heart disease (Kwiterovich, 2000; Steinberg, 2006).

Phospholipid and Low Density Lipoprotein (LDL) were significantly decreased in the animals (Table 3). The soybean oil supplemented diet may have LDL lowering activity in the animals, hence the significant decrease in phospholipid and LDL. Plant sterols, chemicals derived from soybean oil, are used to lower total cholesterol and "bad" Low-Density Lipoprotein (LDL) cholesterol (Potter, 1995; Ostland Jr, 2002; Holzbeierlein *et al.*, 2005). There was significant (p<0.05) increase in High Density Lipoprotein (HDL). The increase may be due to the presence of omega-3 fatty acid. Canola oil; obtained from rapeseeds, flax seed oil and soybean oil contain high levels of omega-3 fatty acid which lowers the risk and predisposition to cardiovascular diseases (Arterburn *et al.*, 2008). But it has been generally agreed among nutritionists that edible oils such as coconut and peanut oils should be avoided due to high content of saturated fatty acids (Enig, 1996). Excessive intake of any fat is undesirable. Other research collected over many years from around the world has shown that populations with diets low in saturated fats have the lowest death rates. As a result, the replacement of saturated fats with reasonable amounts of polyunsaturated fats, such as those found in soybean oil, is recommended. Cholesterol, triacylglycerol and fatty acids are significant and independent risk factors of adverse cardiovascular events (Wierzbicki and Mikhailidis, 2002; Cromwell and Otvos, 2004).

The results of the present study showed that consumption of soybean oil can help in reducing the risk of hyperlipidemia and atherosclerosis which is in agreement with the report of Luc and Fruchart (1991) and Poveda *et al.* (2005).

Considering the reported reduction in complications of cardiovascular diseases with lowering of plasma lipids concentrations, the use of this oil especially in resource-poor economies by an impoverished populace should be strongly promoted. There may also be need to encourage food processing industries to incorporate this important seed oil in their products.

CONCLUSION

The present study observed that soybean oil supplemented diet caused a significant ($p < 0.05$) decrease in TC, LDL, VLDL, TG and phospholipid and a significant ($p < 0.05$) increase in the level of HDL compared with the control. The results show that the effect of the soybean supplemented diet on the lipid profile of the animals is dose dependent. In conclusion, soybean oil supplemented diets have blood cholesterol and triacylglycerol lowering effect, which may be of clinical benefit to individuals at risk of cardiovascular disease.

ACKNOWLEDGMENT

The authors wish to thank Prof. Chibuzo Ogbuagu, Vice Chancellor, Abia State University, Uturu, Nigeria for his financial support. We also appreciate the input of Mr. C. Asumugha for carrying out some of the analysis.

REFERENCES

- Anderson, J.W., B.M. Johnstone and M.E. Cook-Newell, 1995. Meta-analysis of the effects of soy protein intake on serum lipids. *New Engl. J. Med.*, 333: 276-282.
- Arterburn, L.M., H.A. Oken, H.E. Bailey, J. Hamersley, C.N. Kuratko and J.P. Hoffman, 2008. Algal-oil capsules and cooked salmon: Nutritionally equivalent sources of docosahexaenoic acid. *J. Am. Diet. Assoc.*, 108: 1204-1209.
- Austin, M.A. and J.E. Hokanson, 1994. Epidemiology of triglyceride, small dense low density lipoprotein and lipoprotein as risk factors for coronary heart disease. *Med. Clin. North Am.*, 78: 99-115.
- Ayoola, G.A., S.S. Ipav, M.O. Sofidiya, A. Aderonke, A. Bello, H.A.B. Coker and T.O. Odugbemi, 2008. Flaxseed and cardiovascular risk factors: Results from a double blind, randomized, controlled clinical trial. *J. Amer. Coll. Nutr.*, 27: 65-74.
- Bartlett, G.R., 1959. Colorimetric assay methods for free and phosphorylated glyceric acids. *J. Biol. Chem.*, 234: 469-471.
- Brown, M.S. and S.L. Goldstein, 1984. How LDL receptor influences cholesterol and arteriosclerosis. *Sci. Am.*, 251: 58-66.
- Brown, A.H.D., J.E. Grant, J.J. Burdon, J.P. Grace and R. Pullen, 1985. Collection and utilization of annual perennial glycine. In: Shibles, R. (Ed.), *Proc. World Soybean Research Conference*. Wetern Press, Boulder, pp: 345-352.
- Burnstein, M.A. and J. Sammaille, 1960. A rapid determination of cholesterol bound to A and B-lipoprotein. *Clin. Chemical Acta*, 5: 601-609.
- Chow, C.K., 1992. Fatty acids in foods and their health implications. Marcel Dekker Inc., New York, pp: 237-262.
- Cowman, J.C., 1973. Processing and Products. In: Caldwell, B.E. (Ed.), *Soybean: Improvement, Production and Uses*. Am. Soc. Argron. Madison Wis., pp: 619-664.
- Cromwell, W.C. and J.D. Otvos, 2004. Low density lipoprotein particles number and risk for cardiovascular disease. *Curr. Atheroscler. Rep.*, 6: 381-387.
- Enig, M.G., 1996. Health and nutritional benefits from coconut oil: An important food for the 21st century. Proceeding of the Paper Presented in the Seminar AVOC Lauric Oils Symposium in Ho Chi Min City, Vietnam.
- Fletcher, M.J., 1968. A colorimetric method for estimation of serum triglycerides. *Clin. Chim. Acta*, 22: 393-397.
- Folch, J., M. Lee and G.H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226: 497-509.
- Glew, R.H., 2006. Lipid Metabolism II: Pathways of Metabolism of Special Lipids. In: Delvin, T.M. (Ed.), *Textbook of Biochemistry with Clinical Correlations*. 6th Edn., Wiley Liss, New Jersey, pp: 695-741.
- Holzbeierlein, J.M., J. Mcintosh and J.B. Thrasher, 2005. The role of soy phytoestrogens in prostate cancer. *Curr. Opin. Urol.*, 15(1): 17-22.
- Hymowitz, T. and C.A. Newell, 1981. Taxonomy of the genus, glycine, domestication and uses of soybeans. *Econ. Bot.*, 35: 272-288.
- Kim, K.K., 1997. Regulation of mammalian acetyl coenzyme A carboxylase. *Ann. Rev. Nutr.*, 17: 77-99.
- Kwiterovich, P.O., 2000. The metabolic pathways of high-density lipoprotein, low density lipoprotein and triglycerides: A current review. *Am. J. Cardiol.*, 86(12): 5-10.
- Luc, G. and J.C. Fruchart, 1991. Oxidation of lipoproteins and atherosclerosis. *Am. J. Clin. Nutr.*, 53: 2065-2095.
- Murray, M.T., 1993. *The Healing Power of Foods*. Prima Publishing, Rocklin, pp: 211-212.
- Murray, I.K., P.A. Mayer, O.K. Granner and V. Rodwel, 1990. Cholesterol Biosynthesis, Degradation and Transport. In: *Harper's Biochemistry*. 2nd Edn., Prentice Hall International Inc., pp: 240-254.
- Ostland Jr, R.E., 2002. Pyhtosterols in human nutrition. *Annu. Rev. Nutr.*, 22: 533-549.
- Potter, S.M., 1995. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. *J. Nutr.*, 125: 606S-611S.

- Poveda, E., P. Ayala, R. Milena, E. Ordonez, C. Baracaldo, W. Delgado and M. Guerra, 2005. Effects of vegetable oil supplementation on the lipid profile of Wistar rats. *Biomedica*, 25: 101-109.
- Richmond, N., 1973. Determination of cholesterol in serum. *Clin. Chem.*, 19: 1350-1356.
- Robert, K. and J.L. Harwood, 1988. Fatty acids metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 39: 101-138.
- Steinberg, D., 2006. Thematic review series: The pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy, part V: The discovery of the statins and the end of the controversy. *J. Lipid Res.*, 47: 1339-1351.
- Vance, D.E. and J.E. Vance, 1996. *Biochemistry of Lipids, Lipoproteins and Membranes*. 3th Edn., Elsevier, Amsterdam, pp: 553, ISBN: 0444823646.
- Vessby, B., 1994. Implications of long-chain fatty acid studies. *Inform*, 5: 182-185.
- Walter, F.B. and S.J. George, 1922. The chemical composition of soybean oil. *J. Am. Chem. Soc.*, 44(12): 2947-2952.
- Wardlaw, G.M. and M. Kessel, 2002. *Perspectives in Nutrition*. 5th Edn., McGraw Hill, New York, pp: 418-461.
- Wierzbicki, A.S. and D.P. Mikhailidis, 2002. Beyond LDL-C: The importance of raising HDL-C. *Curr. Med. Res. Opin.*, 18(1): 36-44.
- Witztum, J.L., 1994. The oxidation hypothesis of atherosclerosis. *Lancet*, 344: 793-795.
- Zhang, R., Y. Li and W. Wang, 1997. Enhancement of immune function in mice fed high doses of soy daidzein. *Nutr. Cancer*, 29(1): 24-28.