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ORIGINAL ARTICLES

Comparative Study To Evaluate The Role Of Flaxseed Oil To Alleviate Complications Due To Consumption Of High Fat And Carbohydrate Diet

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ABSTRACT

Regular consumption of high fat and or carbohydrate diet lead to health complications that results in obesity, type 2 diabetes and cardiovascular disease. Oils rich in omega 3 fatty acids are assumed to prevent from such disorders. This study aims to test the efficiency of flaxseed oil to protect from these hazards and to compare its effect with that of a standard dietary supplement containing EPA/DHA and wheat germ oil rich in vitamin E. Four groups of rats (Sprague Dawley) were used. Group1 was a control, fed on a standard diet**;** groups 2, 3 &4 were fed on a high fat and carbohydrate diet (HFHC). Flaxseed oil was added to diet 3 and the formula eicosapentaenoic acid /Decosahexaenoic acid (EPA/DHA) to diet 4.Feeding period continued for 8 weeks. At the end of the experiment fasting blood samples were obtained from animals in all groups and analyzed for several biochemical parameters. The results obtained showed that animals in groups 2, 3 &4 fed on the HFHC diet consumed less food than control but attained more body weight. The fasting blood glucose, serum malondialdehyde, serum total lipids, total cholesterol, LDL cholesterol, and triglycerides were all increased in rats fed on the HFHC .The total antioxidants and HDL were decreased. Addition of either flaxseed or (EPA/DHA) to the diet caused a marked improvement of all these parameters. These parameters returned back to near normal values. The effect of flaxseed oil supplementation was more or less comparable to that of the food supplement containing the (EPA/DHA) and wheat germ oil. The conclusion is that flaxseed oil supplementation can protect against health hazards exerted due to consumption of high fat or carbohydrate by correcting body fat accumulation, blood glucose, oxidation stress and lipid profile. The effect is comparable to that of omega-3 fatty acids and vitamin E.

Key words: Flaxseed oil, high fat diet, high carbohydrate diet, obesity, type2 diabetes, oxidation stress, blood lipids, omega fatty acids.

Introduction

There is a link between dietary fat or carbohydrate and each of obesity, insulin resistance, and non-insulin dependent diabetes mellitus. Energy intake that exceeds energy expenditure induces an increase in body weight, which leads to obesity (Moller & Kaufman , 2005). It is evidenced that type 2 diabetes increases with the degree and duration of obesity (Nkondjock & Receveur 2003).

Obesity is associated with an adipose tissue storage depot for energy in excess of requirements. This adipose tissue act as an active endocrine organ associated with low-grade systemic inflammation through the expression of proinflammatory mediators such as tumor necrosis factor-α, interleukin-6 (Weisberg *et al*., 2003), C-reactive protein (Blake *et al.,* 2005) and leptin (Lau *et al.,* 2005). Adipose tissue also secretes the anti-inflammatory mediator, adiponectin, which has direct positive effects on vascular function (Nesto, 2005). The increased body fat can be a critical indicator of disease risk which includes insulin resistance, type 2 diabetes mellitus, hypertension and dyslipidemia (Fruhbeck 2007). This is in addition to impaired fasting plasma glucose (Nesto 2005), raised levels of triglycerides and of low-density lipoprotein cholesterol and reduced levels of high-density lipoprotein cholesterol.

Oxidative stress was also reported to be increased with High fat high sugar diet (Feillet-Coudray *et al*., 2009). During the last few years, several investigations were traced that point to oxidative stress that favor impairment in glucose homeostasis, being a main cause and initiating event in the pathogenesis of insulin resistance associating type 2 diabetes, (Clemence Blouet *et al*., 2007)

There are conflicting data regarding the benefits of omega-3 fatty acids, in patients with type 2 diabetes. Normal individuals who receive regular doses of essential (n-3) fatty acids or their sources usually show improved endothelial and myocardial functions with triglyceride-lowering, anti-inflammatory, antithrombotic, and antiarrhythmogenic effects (Filion *et al*., 2010). High dietary intake of oily fish, or some plant sources such

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as flaxseed oil that are usually rich in *n*-3 fatty acids, is also associated with improved cardiovascular disease outcomes (Chris *et al*., 2013).

Several large randomized studies have shown significant improvements in mortality and a reduction in lipid parameters leading to major CVD events after treatment with *n*-3 fatty acids (Yokoyama *et al*., 2003, GISSI-Prevenzione, 1999 and Tavazzi *et al*., 2008). Most of these symptoms are usually the outcome of diabetic conditions hence it is expected that diabetic individuals most probably will benefit from n-3 fatty acids.

In spite of this, dietary n-3 PUFA supplementation during a weight loss program does not appear to assist weight loss. Poor dietary compliance may be a contributing factor in accurate assessment of the role of these fatty acids in weight loss, (Irene A. Munro and Manohar L. Garg, 2013)

Recently, Origin (2012) evaluated the impact of *n*-3 fatty acids versus placebo on the risk of progression to all-cause mortality and major cardiovascular events in individuals with dysglycemia, reported no difference in risk between the drug and placebo in >12,000 subjects, with an average 6 years of follow up. Again, Meropi *et al*., (2013) found that daily consumption of flaxseed oil did not confer any benefit in inflammatory or biochemical markers in normal weight young adults who traditionally use olive oil as the main edible oil. On the other hand, Nivedita M. Jangale, *et al*., (2013) reported that dietary flaxseed oil reduced glycation in streptozotocin-nicotinamide diabetic rats. Both diets reduced oxidative stress and improved antioxidant status in liver.

Because of this controversy, the present study was designed to evaluate the role of excessive consumption of fat and carbohydrate on body and organs weight, blood sugar, production of reactive oxygen species, and lipid parameters. Moreover, to evaluate the protective effect of flaxseed oil compared to that of omega -3 fatty acids against hazards of high fat or carbohydrate consumption.

Material and Methods

The ingredients used for the preparation of the diet given to the animals were purchased from the local market. These items were Corn starch, Sucrose, Corn oil. Flaxseed oil was purchased fresh from specialized stores.

Casein was obtained from Sisco Research Laboratories PVT. LTD, India. The (EPA/DHA) supplement was obtained from Sedico, Egypt.

Salts and vitamins used for the preparation of the salt and vitamin mixtures were obtained from Merck, Germany, and prepared according to (AIN 93) Reeves *et al*. (1993).

The diets given to the animals (HCHF) and the standard normal diet were formulated and composed as shown in table 1.

Animals used in this experiment were Sprague –Dawley rats, obtained from the animal house of the National Research Centre, their body weight ranged between 90-110 g, and comprised both sexes.

Kits used for the estimation of the analyzed parameters were obtained from Biodiagnostic, Egypt.

Design of Animal Experiment:

The animal experiment comprised 4 groups each of 8 rats.

The 1st group was fed on the standard normal diet.

The 2nd group was fed on the high fat and carbohydrate diet (HFHC).

The 3rd group was fed on the (HFHC) and flaxseed oil (25 g/kg Diet)

The 4th group was fed on the (HFHC) and the formula EPA/DHA (25 g/ kg Diet) given from a dietary supplement in the form of gelatin capsules, each contain 1000 mg fish oil (18% EPA and 12% DHA) together with 100 mg wheat germ oil.

Animals were kept individually in stainless steel cages, water was allowed adlibitum, and the room temperature was adjusted at 25º C. The feeding period continued for 8 weeks. During the experimental period the food consumption and body weight of the animals were followed. The experimental procedure was carried out according to the institutional Animal Ethics Community of the NRC, Egypt. At the end of the experimental period, animals were fasted overnight and in the morning blood samples were taken from each rat by open heart puncture under slight ether anesthesia. Blood samples were left to clot at room temperature then centrifuged at 3500 rpm and serum was separated. The abdomen was opened and the liver was excised then washed with saline plotted between sheets of filter paper and weighed, samples were kept in the deep freeze at -20 ºC till analysis.

Blood sugar was determined according to the method of Trinder, P (1969), serum malondialdehyde according to the procedure of Satoh, K (1979), total antioxidants according to Koracevic *et al*., (2001), total lipids as described by Knight *et al*., (1972), , serum triglycerides according to Fossat I & Prencipe (1982) . Total cholesterol by the technique described by Allain *et al*., (1974), LDL according to Levy A.L. (1981) and HDL according to Burstein (1970).

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Table 1: Composition of Standard normal diet and the High Fat and Carbohydrate Diet (g /100 g)

Ingredients	*Standard normal diet	High fat and carbohydrate diet
Casein	12.5	14
Fat (corn oil)	8	-----
Sucrose	10	40
Corn starch	61	17.5
Milk fat	-----	24
Vitamin mixture	1	1
Salt mixture	3.5	3.5
Cellulose	4	-----

*The Standard normal diet was formulated according to AIN (1993).

Statistical analysis:

Statistical analysis was carried out according to Fisher (1970). LSD (Least significant difference) test was used to compare the significant differences between means of treatment (Waller and Duncan, 1969). The statistical package for social science S.P.S.S. (1999) program version was used for all analysis.

Results:

The food consumption, body and liver weights of animals used in the experiment are shown in table 2&3. The food consumption of the control group rats amounted to a mean value of 212.5 ± 10.63 g, the food consumption of the other groups was less. Animals in group(2) fed on the HFHC diet consumed a mean value of 147.5 ± 4.61 g., those of group (3) who were given flaxseed oil with the diet consumed a mean value of 150.0 ± 3.87 ±g. and those in group (4) who were given EPA/DHA consumed a mean value of 157.5 ± 5.12 g. At the end of the experiment, control rats attained gain in body weight amounted to 72.67 ± 9.27 g., animals in group (2) attained higher gain in body weight (88.5 ± 10.42 g.), those in groups (3 and 4) attained relatively higher body weight gain than control but still lower than rats in group (2) (76.0 ±5.85 g. and 86.67 ± 13.4 g.). The liver weights of rats in the experimental groups were relatively higher than that of controls. The values obtained were 8.63 ± 1.23g for group (2), 5.22 ± 0.38g, for group (3), 5.46± 0.42 g. for group (4), relative to a value of 4.88 ± 0.57g for controls.

As shown in table 4, the fasting blood glucose of normal control rats has a mean value of 64.4 ± 3.33 mg/dl. When rats were put on the HFHC diet for 8 weeks, the blood sugar level was increased to a value of 124.76 ± 5.66 mg/dl. However, when flaxseed oil was added to the diet, an appreciable relative decrease occurred in the blood sugar concentration (89.12 ± 2.80 mg/dl) and when EPA/DHA were added to the diet a similar drop occurred in blood sugar concentration 82.42 ± 3.93 mg/dl.

Serum malondialdehyde value for rats fed on the HFHC diet was significantly higher than that of controls (4.66 ± 0.51 mM/ml) compared to a normal value of 1.82 ± 0.31 nm/ml. Addition of either flaxseed oil or the EPA/DHA caused a reduction of the values of serum malondialdehyde relative to the value obtained for rats fed on the HFHC diet. The values obtained were 2.05 ± 0.19 for group 3 and 1.79 ± 0.17 mM/ml for group (4).

The total antioxidants found in serum of control rats amounted to 1.42 ± 0.08 mM/L. After rats were fed on the HFHC diet for8 weeks, this value was decreased to 0.68 ± 0.12 mM/L. Again, when each of flaxseed oil or the mixture of EPA/DHA were added to the HFHC diet, the antioxidant value raised again to values of 1.34 ± 0.07 for group (3) and to 1.53 ± 0.06 for group (4).

The values reported for serum total lipids, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides are shown in table 5. It can be noticed that all lipid parameters except HDL cholesterol were elevated in rats that were fed on the HFHC diet. Addition of flaxseed or EPA/DHA mixture caused significant limitation to this rise; meanwhile, serum HDL cholesterol was relatively increased. The change in lipid parameters was markedly less when flaxseed oil or the mixture of EPA/DHA was added to the diet. The values reported for serum total lipids were 318.68 ± 14.87 mg/dl for control rats, 529.5 ± 40.64 mg/dl for rats fed on the HFHC diet, 350.8 ± 36.36 mg/dl for rats in group (3) and 327.31 ± 17.99 mg/dl for rats in group (4). Serum total cholesterol was 59.54 ± 4.38 mg/dl in control rats, increased to 109.37 ± 10.45 mg/dl for rats in group (2), 88.05 ± 6.95 and 57.22 ± 4.63 mg/dl for groups (3 &4) respectively. The values reported for serum triglycerides were 161.0 ± 14.30, 247.36 ± 14.91, 200.27 ± 13.78 &198.34 ± 13.37mg/dl for the control, groups 2, 3&4 respectively.

Table 2: Food intake, body weight gain and feed efficiency ratio (FFR) of rats fed on the different diets.

Parameters Groups	Food intake (g)	Body wt. gain (g)	Feed efficiency ratio (FER)
1)Control normal (-ve control)	212.50±10.63[a]	72.67±9.27[a]	0.346±0.051[b]
2) Fed on the HFHC diet (+ve control)	147.50±4.61[b]	88.50±10.42[a]	0.601±0.100[a]
3) Fed on HFHC diet+ flaxseed oil	150.0 ± 3.87[b]	76.0±5.85[a]	0.507±0.047[a]
4) Fed on the HFHC diet + EPA/DHA	157.50±5.12[b]	86.67±13.40[a]	0.566±0.192[a]

- All data are presented as mean ± standard error.
- Values with different superscript letters are significantly different at $p \leq 0.05$.

Table 3: Body and liver weight of animals (g.)

No. of groups	Body weight	Liver weight	L/B %
1)Normal control (-ve control)	179.6±10.19[a]	4.88_0.57[b]	2.78±0.41[b]
2)Fed on the HFHC dietl (+ve control)	191.33±11.70[a]	8.63±1.23[a]	4.44±0.44[a]
3) Fed on HFHC + flaxseed oil	177.2±6.62[a]	5.22±0.38[b]	2.94±0.19[b]
4)Fed on the HFHCdiet + EPA/DHA	190.1±14.86[a]	5.46±0.42[b]	2.92±0.25[b]

L/B: Liver to body weight ratio All data are presented as means ± S.E.
Values that share the same letter are not significant.
Values that share differences letters are significant at $p \leq 0.05$.

Table 4: Blood glucose, serum malondialdehyde and total antioxidant of rats after the feeding period.

Parameters Groups	Glucose mg/dl	Malondiadelhdye (mmol/ml)	Total Antioxidant(mM/l)
1)Normal control (-ve control)	64.38±3.33[c]	1.82±0.31[b]	1.42±0.08[a]
2) Fed on the HFHC diet (+ve control)	124.76±5.66[a]	4.66±0.51[a]	0.68±0.12[b]
3) Fed on the HFHC diet + flaxseed oil	89.12. ±2.80[b]	2.05±0.19[b]	1.34±0.07[a]
4) Fed on the HFHC diet + EPA/DHA	82.42±3.93[b]	1.79±0.17[b]	1.53±0.06[a]

- All data are presented as mean ± SE.
- Values that share the same letters are not significant.
- Values that share different letters are significant at $p \leq 0.05$.

Table 5: Lipid profile of rats at the end of the experiment.

Parameterss Groups	Total lipids mg/dl	Total cholesterol mg/dl	LDL-cholesterol mg/dl	HDL-cholesterol mg/dL	Triglycerides mg/dl
1)Normal control (-ve control)	318.68±14.87[b]	59.54±4.38[c]	48.31±2.87[b]	56.03±5.60[a]	161±14.30[b]
2) Fed on the HFHC dict (+ve control)	529.50±40.64[a]	109.31±10.45[a]	77.07±2.42[a]	33.42±4.82[b]	247.36±14.91[a]
3)Fed on the HFHC diet + flaxseed oil	350.81±36.36[b]	88.05±6.95[b]	53.922.71[a]	57.79±4.97[a]	200.27±13.78[b]
4) Fed on the HFHC diet + EPA/DHA	327.31±17.99[b]	57.22±4.63[c]	52.98 ±2.14[b]	58.97±2.43[a]	198.34±13.37[b]

- All data are presented as means ± S.E.
- Values that share the same letter are not significant.
- Values that share different letters are significant at $p \leq 0.05$.

Discussion:

As expected rats that were fed on the HFHC diet gained more body weight than control animals, in spite of the relatively lower amount of food consumed. Previous studies have proved that this dietary regimen leads to accumulation of fat in the body and onset of hyperglycemia in addition to impairment of glucose tolerance (Uma Bhandari *et al*., 2013). The HFHC diet given to the animals provides 502 K cal /100 g diet relative to a value of 394.6 K cal for the standard diet. In spite of the drop in food consumed by the HFHC fed rats (753 K cal relative to 805 K cal for controls) yet there was an appreciable increase in body weight. This shows that the amount of calories consumed may not be the sole factor contributing to the accumulation of fat in the body, still the fat and sugar content and in turn the imbalance of the diet is contributing. The intake of a high fat diet leads to excessive accumulation of lipids in the body and development of obesity which results from extensive adipose

tissue remodeling by adipocyte hypertrophy, adipocyte hyperplasia and angiogenesis (Avram *et al*., 2007). The increase in body weight was associated with an increase in liver weight either absolute or relative to body weight. Such observation was also reported by Yu-Hsin Hsieh & Sheng-Yang Wang, (2012). It has been stated that chronic inflammation which usually associate obesity induced by high fat diet evidenced by accumulation and activation of macrophages/dendritic cells and T cells occurs in adipose tissue and the liver (Weisberg *et al*., 2003).

The increase in blood sugar concentration reported in rats fed on the HFHC diet was previously reported by several investigators and is attributed to the high sugar content of the diet and the expected insulin resistance occurring in rats due to this dietary regimen (Uma Bhandari *et al*., 2013). The relative drop in blood glucose concentration that occurred in rats given flaxseed oil with the HFHC diet can be attributed to multiple factors among which the relative decrease in body weight gain and the consequent drop in fat accumulation in the body that is expected to lead to improvement in insulin resistance that associate overweight or obesity. This also occurred in rats given EPA/DHA with the HFHC diet; however the decrease in body weight of the rats was less than that reported for rats given flaxseed oil with the HFHC diet. This shows that flaxseed oil was more effective to realize a reduction in body weight than the mixture of EPA/DHA. This is an advantage to flaxseed oil. In the present study reduction in body weight gain of rats given either the flaxseed oil or the EPA/DHA is expected to be accompanied by a depletion of body fat stores. Such observation was reported before by Mancini *et al*., (2001).

On the other hand, the accumulation of triglycerides in adipocytes leads to deterioration of the responsiveness of glucose metabolism in other tissues such as the liver which lead to insulin resistance (Frayn , 2001). The results obtained from this study showed that rats given the omega 3 fatty acids or flaxseed oil had a relatively lower triglycerides concentration. This goes in parallel with the finding of Chris D Pole (2013), who reported that essential omega 3 fatty acids supplementation is associated with improved endothelial and myocardial function with triglycerides lowering effect. The lowering effect of both omega 3 fatty acids and flaxseed oil on triglycerides or blood sugar level is more or less equal which means that flaxseed alone can perform the role exerted by omega 3 fatty acids. The FER of rats given the EPA/DHA was more than that of rats given the flaxseed oil, however this does not mean a net growth of muscle mass or better food assimilation since fat accumulation can be contributing.

The high fat and carbohydrate diet given to the rats caused an imbalance between reactive oxygen species and antioxidants. It has been reported that obesity and hyperlipidemia synergistically promote systemic oxidative stress. (Staiger & Haring, 2005). The results obtained in this study proved significant increase in serum malondialdehyde which confirm the imbalance in oxidation state in rats given the HFHC diet. Malondialdehyde is a by-product of lipid peroxidation and reflect the degree of oxidation in the body (Friedewald, *et al*., 1972). Oxidative stress in obesity may be caused by hyperglycemia and elevated lipid levels, the conditions that are prevailing in obese animals used in the present study and evidenced by the obtained results. Addition of either omega fatty acids or flaxseed oil to the HFHC diet caused a marked reduction in malondialdehyde concentration and an improvement of the total antioxidants. The reduction and the improvement were more or less similar in both cases, again confirming the effectiveness of flaxseed oil approximately to the same extent like omega 3 fatty acids. It has been reported that omega3 fatty acids can abrogate chemically induced diabetes in experimental animals and attenuate the oxidant stress that occurred in diabetes mellitus (Surech *et al*., 2003). Also, our finding support that reported by Nevidita *et al*., (2013) that dietary flaxseed oil and fish oil reduced oxidative stress and improved antioxidant status in liver.

The disturbed lipid pattern that occurred due to consumption of HFHC diet was more or less corrected when either EPA/DHA or flaxseed oil were given with the diet. The TL, TG, TC &LDL were relatively normalized and HDL was raised. The effect was more or less similar in both cases of n-3 fatty acids or flaxseed oil. Once again confirming that the effect of flaxseed oil is approximately similar to that of the n-3 fatty acids. Flaxseed oil is low in saturated fat, moderate in mono-unsaturated fatty acids and rich in poly-unsaturated fatty acids, especially n-3 α-linolenic fatty acid (ALA). (Singh,*et al*., 2011) . These characters are in favor to anti-inflammatory effect of the oil (Simopoulos A.P., 2008). The anti-inflammatory effect of ALA present in flaxseed oil may point to efficient conversion to the more anti-inflammatory active EPA.

In conclusion, it can be safely stated that consumption of flaxseed oil can help to relief many of the complications that occur due to consumption of high fat and carbohydrate diet such as increase in body weight due to accumulation of fat, high blood sugar, oxidation stress and deranged lipid profile. Flaxseed oil was able to replace a blend of EPA/DHA and wheat germ oil rich in the antioxidant vitamin E and exert more or less the same effect. It is recommended to use flaxseed oil as daily use particularly as dressing or mixed with other food preparations when ever this is suitable.

References

Allain, C.C., L.S. Poon, C.S. Chan, W. Richmoud and P.C. Fu, 1974. Enzymatic Determination of total serum Cholesterol. Clin. Chem, 20: 470-475.

Avram, M.M., A.S. Avram and W.D. James, 2007. Subcutaneous fat in normal and diseased states 3. Adipogenesis: from stem cell to fat cell. Journal of the American Academy of Dermatology, 56: 472-492.

Blake, G.J, and P.M. Ridker, 2005. High sensitivity C-reactive protein for predicting cardiovascular disease: an inflammatory hypothesis. Eur Heart J, 22: 349-352.

Burstein, M.A., 1970. Fully Enzymatic Colorimetric Determination of HDL Cholesterol in the Serum. Lipid Res., 11: 583-595.

Chris, D.P., J.P. Halcox, S. Jenkins-jones, S.M. Emma, G.M. Schifflers, K.K. Ray and C.J. Cumie, 2013. Omega -3 fatty acids and mortality outcome in patients with and without type 2 Diabetes After Myocardial –Infarction: A retrospective , Matched-Cohort study. Clinical Therapeutics, 35: 40-51.

Clemence, B., F. Mariotti, D.A. Marniche, V. Mathe, T. Mikogarni, D. Tome and J.F. Hueau, 2007, Dietary cysteine alleviates sucrose- induced oxidative stress and insulin resistance. Free radical Biology & Medicine, 42: 1089-1097.

Feillet-Coudray, C., T. Sutra, G. Fouret, J. Ramos, C. Wrutniak-Cabello, G. Cabello, J.P. Cristol and C. Coudray, 2009. Oxidative stress in rats fed a high-fat high-sucrose diet and preventive effect of polyphenols: Involvement of mitochondrial and NAD(P)H oxidase systems. Free Radical Biology and Medicine, 46: 624-632.

Filion, K.B., F. El Khoury and M. Bielinski, 2010. Omega-3 fatty acids in high risk cardiovascular patients: meta-analysis of randomized controlled trials BMC. Cardiovasc. Disord, 10: 24-3.

Fisher, R.A., 1970. Statistical method for research workers Edinburgh ed. 14, Oliver and Boyed, pp: 140.

Fossati, P. and I. Prencipe, 1982. Serum triglyceride determination calorimetrically using an enzyme that produce hydrogen peroxide. Clin. Chem., 28: 2077-2080.

Frayn, K.N., 2001. Adipose tissue and the insulin resistance syndrome. Proceedings of the Nutrition Society, 60: 375-380.

Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry, 18: 499-502.

Fruhbeck, G., 2007. Vasoactive factors and inflammatory mediators produced in adipose tissue .G. Fantuzzi, T. Mazzone (Eds.), Adipose tissue and adipokines in health and disease. Human. Press Inc., Totowa, 63-77.

GISSI- Prevenzione Investigators (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico), 1999. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI- Prevenzione trialLancet, 354: 447-455.

Irene A.M. and M.L. Garg, 2013. Dietary supplementation with long chain omega-3 polyunsaturated fatty acids and weight loss in obese adults. Obesity Research & Clinical Practice, 7(3): e173-181.

Koracevic, D., G. Koracevic, V. Djordjevic, S. Andrejevic and V. Cosic. 2001. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol, 54: 356-361.

Knight, J.A., S. Anderson, and J.M. Rawle, 1972. Chemical basis of the sulfo-phosph-vanillin reaction for estimating total serum lipid. Clin. Chem., 18: 199-202.

Lau, D.C., B. Dhillon, H. Yan, P.E. Szmitko and S. Verma, 2005. Adipokines: molecular links between obesity and atheroslcerosis. Am J Physiol Heart Circ Physiol, 288: 2031-2041.

Levy, A.L., 1981. A fully Enzymatic Colorimetric Determination of LDL Cholesterol in the Serum. Clin. Chem., 27: 653-662.

Mancini, F.P., A. Lanni, L. Sabatino, M. Moreno, A. Giannino and F. Contaldo, 2001. Fenofibrate prevents and reduces bodyweight gain and adiposity in diet-induced obese rats. FEBS Letters, 491: 154-158.

Meropi, D.K., A. Vlassopoulos, A. Gatzieva, A. Farmaki, S. Katsiougiannis, D.B. Panagiotakos, N. Kalogeropoulos and F.N. Skopouli .Flaxseed oil does not affect inflammatory markers and lipid profile compared to olive oil, in young, healthy, normal weight adults. Metabolism, 62: 686-693.

Moller D.E. and K.D. Kaufman, 2005. Metabolic syndrome: a clinical and molecular perspective. Annu. Rev Med., 56: 45-62.

Nesto, R.W., 2005. Obesity: a major component of the metabolic syndrome. Tex Heart Inst J, 32: 387-389.

Nivedita, M.J., P.P. Devarchi, A.A. Dubal, A.E. Ghule, S.J. Koppikar, S.L. Budhamker, A.D. Chougat, M.J.Kulkami and A.M. Harsulkar, 2013. Dietary Flaxseed oil and Fish Oil Modulation Expression of Antioxidants and Inflamatory Jenes with Alleviation of Protein Glycation Status and Inflamation in Liver of Streptozotecen Nicotinamide Induced Diabetic Rats. Food Chemistry available on line 13 March 2013.

Nkondjock, A. and O. Receveur, 2012. Fish-seafood consumption, obesity, and risk of type 2 diabetes: an ecological study. Diabetes & Metabolism, 29: 635-642.

ORIGIN. 2012. n-3 fatty acids and cardiovascular outcomes in patients with dysglycemia . N Engl J Med, 367: 309-318.

Reeves, PG., F.H. Nielsen and G.C. Fahey, 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr., 123: 1939-51.

Satoh, K., 1979. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Cinical Chemical Acta, 20: 37-43.

Simopoulos, A.P., 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med (Maywood), 233: 674-688.

Singh, K.K., D. Mridula, J. Rehal and P. Barnwal, 2011. Flaxseed: a potential source of food, feed and fiber. Crit Rev Food Sci Nutr, 51: 210-222.

Staiger, H. and H.U. Haring. Adipocytokines: fat-derived humoral mediators of metabolic homeostasis. Experimental and Clinical Endocrinology and Diabetes, 113: 67-79.

Surech, Y. and U.N Das, FAMS, 2003. Long chain Polyunsaturated atty Acids and Chemically Induced Diabetes Mellitus. Nutrition., 10: 223-228.

Tavazzi, A.P., A.P. Maggioni and Gissi-H.F. Marchioli, 2008. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. Lancet, 372: 1223-1230.

Trinder, P., 1969. Determination of blood glucose using 4-amino phenzone oxygen acceptor. J Clin. Path., 22: 246-52.

Uma, B., H.S. Chaudhari, A.N. Bisnoi, V. Kumar, G. Khanna and K. Javed, 2013. Anti-obesity effect of standardized ethanol extract of Embelia ribes in murine model of high fat diet-induced obesity. Pharma Nutrition, 1: 50-57.

Waller, R.A. and D.B. Duncan, 1969. A bayes rule for the symmetric multiple comparisons problem. J. Am. Statistical Assoc., 64(328): 1484-1503.

Weisberg, S.P., D. McCann, M. Desai, M. Rosenbaum, R.L. Leibel and A.W. Ferrante Jr., 2003. Obesity is associated with macrophage accumulation in adipose tissue , J Clin Invest, 112: 1796-1808.

Yokoyama, M. and H. Origasa, 2003. Effects of eicosapentaenoic acid on cardiovascular events in Japanese patients with hypercholesterolemia: rationale, design, and baseline characteristics of the Japan EPA Lipid Intervention Study (JELIS). Am Heart J, 146: 613-620.

Yu-Hsin, H. and S.Y. Wang, 2012. Lucidone from Lindera erythrocarpa Makino fruits suppresses adipogenesis in 3T3-L1 cells and attenuates obesity and consequent metabolic disorders in high-fat diet C57BL/6 mice. Phytomedicine, Available 0n online 21 December.