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RESEARCH ARTICLE

Does n-3 polyunsaturated fatty acids diet affect the lipolytic activity of adipose tissue in insulin resistant rats?

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Abstract

The association between abdominal fat accumulation and risk of multiple chronic diseases has been established. Insulin resistance is the hallmark mechanism unifying obesity, dyslipidemia, hyperglycemia, diabetes, hypertension and cardiovascular diseases. This study investigates the effect of n-3 polyunsaturated fatty acids diet on the outcomes of insulin resistant rats as well as the lipolytic activity of adipose tissue. n-3 polyunsaturated fatty acids enriched diet for 3 months showed a significant reduction in body weight (BW) , systolic blood pressure (SBP) , triglyceride, cholesterol, LDL, fasting blood glucose (FBG) and insulin levels in insulin resistant rat with a significant rise in HDL level and lipolytic activity of both SC and visceral adipose tissue.

Introduction

The importance of the nutrient composition of the diet is recognized to account for metabolic disorders; as obesity and diabetes, which account to the incidence of cardiovascular diseases. Obesity is a serious disease affecting a large population worldwide (Bassesen, 2008 and Bary, 2004). Insulin resistance is the hallmark mechanism unifying obesity, dyslipidemia, hyperglycemia, diabetes, hypertension and cardiovascular diseases (Reavenet al.2004 and Nordmann, 2006).

The attempt to prevent; treat obesity and insulin resistant in the population by consuming low carbohydrate low fat diet shows great effect in reducing body weight and improving the metabolic processes, but the chronic consumption of low carbohydrate diet is associated with a potential problem ; as it contains a high percentage of fat - most in the form of saturated fat- to compensate for carbohydrate-calorie reduction (Nordmann et al., 2006, Volek et al.,2008 and Lara-Castro et al.,2004).High-saturated fat diet in humans and rats is associated with obesity , high risk of diabetes and cardiovascular disease (Surwit et al., 1988, Rossmeisl et al., 2003 , Stroline et al.,1991, Neschen et al., 2002 and Vessby et al., 2001) . What about polyunsaturated fatty acids diet? Does it help diabetic patients?

Several human and animal dietary studies confirmed the correlation between free fatty acids (FFAs) and insulin resistance, but the detected effect of different types of fatty acids diet - saturated and polyunsaturated - on insulin resistance has been controversial (Storlien et al., 1991, Yki-Jarvinen et al., 2002, Raffaele et al., 2007 and Jong Sam et al.2005).

Since dietary fat and its relation to obesity, lipid profile, blood pressure, and glycemic state has been a controversial issue for years, and with the fact that adipose tissue acts as endocrine gland secreting adipokine that affect metabolism, this study was designed to induce insulin resistance in rats , investigate the effect of polyunsaturated fatty acids (PUFAs) diet on body weight, blood pressure, lipid profile, glycemic state and adipose tissue function and compare the findings with the saturated fatty acids diet's effect.

Material and Methods

The Experimental Research Committee of the Physiology Department and the Scientific and Ethics committees of College of Medicine Cairo University approved all procedures. Male rats were supplied by Animal Care facility of Cairo University. The study was conducted in accordance with World Helsinki Declaration. 20 Rats of average body weight 129.4 g were divided equally into two groups ; control group (C gp) and Experimental group (E gp), both groups were fed commercial rat chow; had free access to sweetened water with 25% fructose for 3 months to induce insulin resistance (Eldeeb et al.,2012). After induction of insulin resistance the control group (C gp) continued feeding on normal commercial rat chow while the experimental group (E gp) 10% of n-3 polyunsaturated fat - Sunflower oil-was added to its chow. Both groups were maintained in a room at 23oC with light on from 7:00 till 18:00. After 3 months the body weight (BW) , systolic blood pressure (SBP) , fasting blood glucose (FBG) and insulin, serum triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol levels were measured, Lipolytic activity of both visceral and subcutaneous adipose tissue were measured. Rats were killed by decapitation; subcutaneous adipose tissue and visceral (omental) adipose tissue from each group were immediately transported to the laboratory in Ringer's solution at room temperature and digested by Rodbell methods (eldeeb et al., 2012, Rodbell, 1964 and Martin et al., 1971). Lipolytic activity was determined by measuring free glycerol released from fat cells by (Colorimetric kits DIALAB Co.). All serum variables were performed on samples taken from overnight unfed rats. Blood glucose was measured by glucose oxidase method (kit510-A, Sigma), Insulin level was assessed by ELISA Kit purchased from (DRG Company). Serum triglycerides were measured by lipoprotein lipase technique (Colorimetric triglyceride kits DIALAB Co.). Total serum cholesterol (esterified and free forms) by (Colorimetric Cholesterol kit (352-20, Sigma). HDL-C was measured by Colorimetric method with (Stanbio kit No. 0599). LDL-C serum level was calculated by using the following equation:

$$LDL\text{-}C = TC - (HDL\text{-}C + \frac{TG}{5})$$

Drugs used: Fructose, Bovine serum albumin (BSA; A 6003, essentially fatty acid-free, Sigma); collagenase IV (Sigma Co.); adrenalin (Sigma); Insulin (EIL LILLY Co.). Results are expressed as mean ±SD. The t-test was used to compare the two groups. Statistical significance was set at $P<0.05$ level.

Result and Discussion

Results:

Adding 10% saturation of n-3 polyunsaturated fatty acids (sunflower oil) to the diet of insulin resistant rats for 3 months showed a significant reduction in body weight (BW), systolic blood pressure (SBP), triglycerides, cholesterol, LDL, fasting blood glucose (FBG) and insulin levels by 21.27%, 7.10%, 8.39%, 1.68%, 6.29%, 18.24%, 47.8 % respectively with a significant increase in HDL level by7.95%.

Lipolytic activity of subcutaneous adipose tissue showed a significant increase by 22.9% in presence of adrenalin while in presence of insulin it decreased significantly by 20.7% respectively. Lipolytic activity of visceral adipose tissue showed a significant increase in presence of adrenalin by 23.41% while in presence of insulin it decreased significantly by33.18%. Moreover the lipolytic activity of the visceral adipose tissue showed a significant high lipolytic activity compared to that of subcutaneous adipose tissue in both conditions; presence of adrenalin and insulin. (Table 1)

Table 1: Effect of n-3 polyunsaturated fatty acid diet on insulin resistant rats

Parameters	Control group		Experimental group		% change	p
	Mean	SD	Mean	SD		
Body weight (BW) (g)	247.7	47.11	195	14.14	-21.27	<0.05
SBP(mmHg)	137.9	9.573	128.1	9.55	-7.10	<0.05
TG	66.7	6.515	61.1	5.281	-8.39	<0.05
cholesterol	87.44	3.25	85.97	2.285	-1.68	<0.05
HDL	30.1	1.91	28.3	1.766	7.95	<0.05
LDL	43.65	2.802	43.2	2.379	-6.29	<0.05
Glucose(mg/dl)	165.5	12.57	135.3	9.117	-18.24	<0.05
Insulin(µg/l)	0.524	0.322	0.2735	0.089	-47.8	<0.05
Sc-adrenalin	274.27	40.095	337.082	30.754	22.9	<0.05
Visceral-adrenalin	375.19	20.1415	463.04	141.59	23.41	<0.05
Sc-insulin	23.139	8.068	18.329	2.239	-20.7	<0.05
Visceral-insulin	72.208	23.492	48.248	6.211	-33.18	<0.05

SBP- Systolic Blood pressure SC- Subcutaneous

Discussion:

In the present work, insulin resistant rats fed 10% sunflower oil for 3 months had a significant reduction in BW which was in accordance with Gang et al., (2007) who found that adding polyunsaturated fatty acids (PUFAs) to the diet of insulin resistant rats showed significant decrease in BW, as PUFAs increase: energy expenditure in mice; resting metabolic rate and the thermic effect of food and fat oxidation in human (Ziegler et al., 2001 and Yu et al. 2004). In 2003 Peyron-Caso et al., found that insulin resistant rat fed PUFAs lost weight due to PUFA's ability to limit adipose tissue hypertrophy - more in visceral fat; increase its lipid oxidation at expense of storage; increase energy expenditure and limit the development of visceral adipose tissue by suppressing the late phase of adipocyte differentiation through modifications of peroxisome proliferator-activated receptor (Halvorsen et al., 2001).

This study showed a significant reduction in SBP of insulin resistant rats after fed 10% sunflower oil for 3 months by10.7% ; similar to Valensi, in (2005) who found that PUFAs were efficient in vivo in delaying and decreasing the rise in blood pressure, suggesting a positive effect in the prevention of hypertension associated with hyperinsulinemia. PUFAs readily incorporate in cardiac membranes are able to prevent the increase in heart rate and QT interval length that is also associated with insulin resistance. PUFAs reduce SBP by many mechanisms which are related to the regulation of cardiac adrenergic function by membrane PUFAs (blocker-like activity) as it : improve membrane cell fluidity and prostanoic balance in favour of arterial dilation (eicosapentaenoic acid (EPA); stimulate the synthesis of prostacyclin; inhibit the synthesis of thromboxan-A2); improve endothelium function and reduce cardiac adrenergic activity (Delarue et al., 2004).

Our study showed that feeding insulin resistant rats 10% of saturation of Sunflower oil for 3moths had a significant reduction in TG, Cholesterol, LDL, Insulin and glucose levels with a significant increase in HDL level, in accordance with Thirunavukkarasu et al in (2004) who found that the levels of plasma and tissue lipids were lowered significantly when PUFAs enriched diet was administered along with fructose. HDL-C concentration was increased whereas those of LDL-C and VLDL-C were decreased. These effects of PUFAs on lipid metabolism may be related to their effect on glucose utilization. PUFAs increase glucose disposal, insulin sensitivity, and insulin action in fructose-treated rats (Berman et al., 2003).This could result in efficient regulation of the key enzymes of lipid metabolism and thus normalizing the circulatory lipid concentrations. Lowering of plasma TG concentrations may be attributed to the reduced availability of the precursor FFAs and to enhanced peripheral tissue clearance through increased LPL activity. Moreover stimulation of LPL leads to a rise in HDL production and reduction in VLDL constituents (Peyron-Caso et al., 2003).In (2007) Raffaele et al., suggested that the rise in plasma concentrations of HDL-C in PUF-treated fructose rats may be due to delayed clearance and increased synthesis of HDL constituents. Others suggested that maintenance of ambient levels of phospholipids in plasma and tissues of PUFAs -treated fructose fed rats could be due to their exerted antioxidant property. PUFAs quench reactive oxygen species that could act on membranes of the biological systems (Delarue et al., 2004). Surprisingly, data reported in human clinical studies reported a slight or no change in HDL cholesterol values in type 2 diabetes after treatment with PUFAs. In obese men with insulin resistance, a decrease in both the catabolism and the production of HDL apoA-I and HDL apoA-II has been observed after treatment with PUFAs (Ziegler et al., 2001). In 2004, Thirunavukkarasu et al., found that administration of 1,656 mg olive oil daily for 8 weeks in type 2 diabetic subjects did not significantly affect the concentrations of VLDL apoB. However, a significant decrease in its level was reported when receiving a higher concentration of 3 g/day during two consecutive 8-week periods. The mechanism by which dietary PUFAs prevent insulin resistance remains unclear. Adipose tissue releases FFAs and adipocytokines into the circulation, and its metabolic activity is the main contributor to the development of insulin resistance (Rousseau et al., 2002). (Bohov et al., 1997) suggested that a decrease in adipocyte lipolysis by PUFAs could play an important role in increasing insulin sensitivity by reducing the fatty acids (FA) availability and increasing glucose utilization .They proposed that the FA composition of membrane phospholipids of insulin target tissues is an important factor influencing insulin sensitivity through their effect on insulin receptor and the glucose transporter (GLUT4). In 2000 Clarke, showed that the PUFA content in adipose tissue and skeletal muscle phospholipids contribute to efficient insulin action. Ghafoorunissa et al., in (2005) demonstrated that dietary fish oil supplementation increased the expression of GLUT4 mRNA in skeletal muscle of diabetic rats. The observed increase of PUFAs in diaphragm phospholipids and therefore possibly in all skeletal muscle and adipocyte plasma membrane could have contributed to the reversal of insulin resistance in sucrose-fed rats. According to Elliott et al., 2002, PUFAs may improve insulin action through its specific effect at the level of insulin release from the islets or through higher receptor mediated hormone clearance. However, the precise mechanism is not known. It is also possible that PUFAs may improve insulin sensitivity through up-regulation of the intracellular insulin signaling pathway. Alternatively, PUFAs could prevent insulin resistance by a direct membrane independent effect on gene expression as PUFAs regulate the transcription of several genes involved in glucose and lipid metabolism (Baillie et al., 1999). Indeed, cell culture and animal studies reported that PUFAs up-regulate leptin mRNA expression and

secretion (Peyron-Caso et al., 2003). Further, PUFAs were shown to ameliorate conjugated linoleic acid–induced insulin resistance by up-regulating the expression of leptin and adiponectin (Clarke, 2001). Leptin and adiponectin were shown to prevent insulin resistance (Shapiro et al., 2008).

This study showed that feeding insulin resistant rats n-3 polyunsaturated fatty acids enriched diet for 3 months increased significantly the lipolytic activity of subcutaneous (SC) and visceral adipose tissue in the presence of adrenaline which was in accordance with Baillie et al. (1999), who found that; fish oil consumption prevents sucrose-induced insulin resistance in adipose tissue and thus might indirectly mediate the regulation of LPL activity. Modifications at the enzyme level, however, cannot be excluded as certain dietary PUFAs have been reported to inhibit the expression of lipogenic enzyme genes. This is also consistent with Rustan et al. (1997), Ryan and Elahi, (1996), who found that dietary PUFAs increased total lipolysis in mesenteric and subcutaneous fat cells compared with adipocytes derived from lard-fed animals. Increased lipolysis in fish oil–fed rats is in agreement with the fact that the enrichment of adipose tissue in specific long-chain highly unsaturated fatty acids increases their selective mobilization in vitro.

In this study, visceral adipose tissue lipolytic activity was higher than SC lipolytic activity in insulin resistant rats fed PUFAs. Increased lipolytic activity can be justified by Bernardo in (2000) who found that diabetic subjects fed PUFAs lost weight and became less hyperinsulinemic and adipose LPL activity increased further. They showed increase in visceral adipose tissue lipolysis more than SC adipose tissue. This probably indicates that adipose tissue LPL activity may represent an adipocyte "set point" that is intended to limit adipocyte shrinkage induced by a hypocaloric diet, this increase in LPL activity is, as indicated, due to posttranslational changes in the LPL enzyme. Measuring the insulin-antilipolytic activity of SC and visceral adipose tissue in this study showed that PUFAs supplementation in insulin-resistant rats significantly decreased the amount of released glycerol (as an index of lipolytic activity). It has been shown that number and binding properties of insulin receptors are influenced by the physico-chemical properties of plasma membranes (Ginsberg et al. 1991). This relationship may be expressed simply by the fact that higher membrane fluidity such as that induced by dietary PUFAs is associated with higher numbers of insulin receptors and/or greater sensitivity to insulin. Field et al. (1999) observed that a high ratio of PUFAs to saturated FA in phospholipids induced by dietary manipulation increased not only insulin binding but also coupling between insulin receptors and glucose transport, glucose oxidation and lipid synthesis in adipocytes. In (1998) Fickova et al., found that dietary fatty acid manipulation modified the effects of insulin on glucose metabolism and transport; did not observe major impact on the antilipolytic properties of insulin; because different post-receptor mechanisms have been proposed for the antilipolytic action of insulin and its effects on glucose metabolism, they assume that intracellular pathway was not affected in the rats of their study. However, they cannot exclude the possibility that a higher lipolytic response, together with a weaker antilipolytic activity of insulin, could account partially for the lower lipid content and hence smaller size of adipocytes in the PUFAs diet group.

Conclusion:

n-3 polyunsaturated fatty acids enriched diet for 3 months in insulin resistant rats showed a significant reduction in body weight (BW) ,systolic blood pressure (SBP) , triglyceride, cholesterol, LDL, fasting blood glucose (FBG) and insulin levels with a significant rise in HDL level and lipolytic activity of both SC and visceral adipose tissue.

Conflict of Interest: None

References:

1. Bessesen, D.H. Update on obesity (2008).*J. Clin. Endocr. Metab.*,*93*, 2027-2034.

2. Bray, G.A. Medical consequences of obesity (2004).*J. Clin. Endocr. Metab.*,*89*, 2583-2589.

3. Reaven, G.; Abbasi, F.; McLaughlin, T. (2004). Obesity, insulin resistance, and cardiovascular disease. *Recent Prog. Horm. Res.*, *59*, 207-223.

4. Nordmann, A.J.; Nordmann, A.; Briel, M.; Keller, U.; Yancy, W.S., Jr.; Brehm, B.J.; Bucher, H.C. (2006) Effects of low-carbohydrate *vs.* low-fat diets on weight loss and cardiovascular risk factors: Ameta-analysis of randomized controlled trials. *Arch. Intern. Med.*, *166*, 285-293.

5. Volek, J.S.; Fernandez, M.L.; Feinman, R.D.; Phinney, S.D. (2008). Dietary carbohydrate restriction induces a unique metabolic state positively affecting atherogenic dyslipidemia, fatty acid partitioning, and metabolic syndrome. *Prog. Lipid Res.*, *47*, 307-318.

6. Lara-Castro, C.; Garvey, W.T. (2004). Diet, insulin resistance, and obesity: Zoning in on data for Atkins dieters living in South Beach. *J. Clin. Endocr. Metab.,89*, 4197-4205.

7. Surwit, R.S.; Kuhn, C.M.; Cochrane, C.; McCubbin, J.A.; Feinglos, M.N. (1988). Diet-induced type II diabetes in C57BL/6J mice. *Diabetes*, *37*, 1163-1167.

8. Rossmeisl, M.; Rim, J.S.; Koza, R.A.; Kozak, L.P. (2003). Variation in type 2 diabetes—Related traits in mouse strains susceptible to diet-induced obesity. *Diabetes*, *52*, 1958-1966.

9. Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW. (1991). Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. Diabetes; 40: 280-289.

10. Neschen S, Moore I, Regittnig W, Yu CL, Wang Y, Pypaert M, et al. (2002) Contrasting effects of fish oil and safflower oil on hepatic peroxisomal and tissue lipid content. Am J PhysiolEndocrinolMetab; 282: E395-401.

11. Vessby B, Unsitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, et al. (2001). Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. Diabetologia; 44:312-319.

12. Yki-Järvinen H, Kauppila M, Kujansuu E, et al., (2002). "Comparison of insulin regimens in patients with non-insulin-dependent diabetes mellitus". N. Engl. J. Med.; 327 (20): 1426–33.

13. Raffaele De Caterina, Rosalinda Madonna, Alessandra Bertolotto and Erik Berg Schmidt. (2007). n-3 Fatty Acids in the Treatment of Diabetic Patients. Biological rationale and clinical data Diabetes Care. vol. 30 no. 4 1012-1026.

14. Jong Sam Lee, Srijan K. Pinnamaneni, Su JuEo, In Ho Cho, Jae Hwan Pyo,ChangKeun Kim, Andrew J. Sinclair, Mark A. Febbraio, and Matthew J. Watt. (2005) Saturated, but not n-6 polyunsaturated, fatty acids induce insulin resistance: role of intramuscular accumulation of lipid metabolites. J Appl Physiol.; 100(5) 1467–1474.

15. Eldeeb R, Gamal-Eldin MH, Khowailed EA, Fathy MM, Shantakumari N, Saleh ME. (2012). Effect of Insulin Resistance on Lipolytic activity of Adipose Tissue in Male Rats. Nepal Journal of Medical sciences; 1(2): 68-73.

16. Rodbell M.(1964) Metabolism of isolated fat cells. The journal of chemistry; 230(2):375-380.

17. Martin Rl, Lutz B, Stephen LP, et al.,(1971). The Glucagon-sensitive AdenylCyclase System in Plasma Membranes of Rat Liver The journal of biological chemistry; 246(6):1877-1882.\

18. Gang Y., Juanjuan D., Tao W., Chunxia Z., Xizheng Z., Peihua W., James W.,Matthew L., Xiao X.(2007). Tissue Kallikrein Reverses Insulin Resistance and Attenuates Nephropathy in Diabetic Rats by Activation of Phosphatidylinositol 3-Kinase/Protein Kinase B and Adenosine 5'-Monophosphate-Activated Protein Kinase Signaling Pathways Endocrinology Vol. 148, No. 5 2016-2026.

19. Ziegler O, Quilliot D, Guerci B, Drouin P.(2001). Macronutrients, fat mass, fatty acid flux and insulin sensitivity. *Diabetes Metab27* : 261-70.

20. Yu Y, Suo L, Yu H, Wang C, Tang H (2004). Insulin resistance and endothelial dysfunction in type 2 diabetes patients with or without microalbuminuria. Diabetes Res ClinPract 65(2):95–104

21. Peyron-Caso E, Quignard-Boulangé A, Laromiguière M, Feing-Kwong-Chan S, Véronèse A, Ardouin B, Slama G, Rizkalla SW. (2003).Dietary fish oil increases lipid mobilization but does not decrease lipid

storage-related enzyme activities in adipose tissue of insulin resistant, sucrose-fed rats. J Nutr. Jul; 133(7):2239-43.

22. Halvorsen, B., Rustan, A. C., Madsen, L., Reseland, J., Berge, R. K., Sletnes, P. & Christiansen, E. N. (2001) Effects of long-chain monounsaturated and n-3 fatty acids on fatty acid oxidation and lipid composition in rats. Ann. Nutr. Metab. 45:30-37

23. Delarue J, LeFoll C, Corporeau C & Lucas D (2004). N-3 long chain polyunsaturated fatty acids: a nutritional tool to prevent insulin resistance associated to type 2 diabetes and obesity? *Reproduction, Nutrition,Development* 44(3) 289–299

24. Valensi P. (2005).Hypertension, single sugars and fatty acids; Journal of Human Hypertension.19, S5–S9. doi:10.1038/sj.jhh.1001954.

25. Thirunavukkarasu V., AnithaNandhini A. T., and Anuradha C. V. (2004). Effect of α-Lipoic Acid on Lipid Profile in Rats Fed a High-Fructose Diet. ExpDiabesity Res. 2004 Jul-Sep; 5(3):195-200.5(3):195-200.

26. Berman PAM, (2003). Effect of oral fructose on ethanol elimination from the blood stream. South Afr J Sci 2003; 99: 47-50.

27. Raffaele De Caterina, Rosalinda Madonna,AlessandraBertolottoand Erik Berg Schmidt,(2007).n-3 Fatty Acids in the Treatment of Diabetic Patients. Biological rationale and clinical data Diabetes Care. vol. 30 no. 4 1012-1026

28. Ziegler O, Quilliot D, Guerci B, Drouin P. (2001). Macronutrients, fat mass, fatty acid flux and insulin sensitivity. *Diabetes Metab 27:* 261-70.

29. Rousseau D, Héliès-Toussaint C, Moreau D, Raederstorff D, Grynberg A. (2003)Dietary n-3 PUFAs affects the blood pressure rise and cardiac impairments in a hyperinsulinemia rat model in vivo.Am J Physiol Heart Circ Physiol. 285(3):H1294-302.

30. Bohov P, Balaz V, Sebokova E, and Klimes I. (1997). The effect of hyperlipidemia on serum fatty acid composition in Type 2 diabetics. Ann NY AcadSci 20;827:561-7.

31. Clarke SD. (2000)Polyunsaturated fatty acid regulation of gene transcription a mechanism to improve energy balance and insulin resistance. British Journal of Nutrition (4), 83, Suppl. 1, S59–S66

32. Ghafoorunissa A., Ahamed I., Laxmi R. and Vani A.(2005)*Dietary (n3) Long Chain Polyunsaturated Fatty Acids Prevent Sucrose Induced Insulin Resistance in Rats. J. Nutr. Nov.135:26342638.*

33. Elliott Sharon S , Nancy L Keim, Judith S Stern, Karen Teff, and Peter J Havel (2002)Fructose, weight gain, and the insulin resistance syndrome. American Journal of Clinical Nutrition, Vol. 76, No. 5, 911-922.

34. Baillie RA, Takada R, Nakamura M & Clarke SD (1999). Coordinate induction of peroxisomal acyl-CoA oxidase and UCP-3 by dietary fish oil: a mechanism for decreased body fat deposition. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 60(5-6), 351–356.

35. Clarke SD. (2001). Polyunsaturated fatty acid regulation of gene transcription: A molecular mechanism to improve the metabolic syndrome. J. Nutr. 131:1129–1132.

36. Shapiro A, Mu W, Roncal C, Cheng KY, Johnson RJ, Scarpace PJ (2008) Fructose-induced leptin resistance exacerbates weight gain in response to subsequent high-fat feeding. Am J PhysiolRegulIntegr Comp Physiol. Nov; 295(5):R1370-5. doi: 10.1152/ajpregu.00195.

37. Rustan AC, Nenseter MS, Drevon CA. Ann N Y Acad Sci. (1997) Omega-3 and omega-6 fatty acids in the insulin resistance syndrome. Lipid and lipoprotein metabolism and atherosclerosis. 20; 827:310-26.

38. Ryan AS, Elahi D., (1996). The effects of acute hyperglycemia and hyperinsulinemia on plasma leptin levels: its relationships with body fat, visceral adiposity, and age in women. J ClinEndocrinolMetab. 81:4433–4438.

39. Bernardo LéoWajchenberg (2000) Subcutaneous and Visceral Adipose Tissue: Their Relation to the Metabolic Syndrome. Endocrine Reviews 21 (6): 697-738

40. Ginsberg B. H., Brown T. J., Simon I., Spector A. A. (1991). Effect of the membrane lipid environment on the properties of insulin receptors. Diabetes 1981; 30:773-780.

41. Field C. J., Goruk S. D., Wierzbicki A. A., Clandinin M. T. (1999). The effect of dietary fat content and composition on adipocyte lipids in normal and diabetic states. Int. J. Obesity 1989; 13:747-756

42. Fickova M., Hubert P., Wierzbicki A., Clandinin M., (1998). Dietary (n-3) and (n-6) polyunsaturated fatty acids rapidly modify fatty acid composition and insulin effects in rat adipocytes. J. Nutr. 128: 512-519.