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Effect of Dietary Fats on Glucose Tolerance, Insulin Sensitivity and Membrane Free Fatty Acids in Rats

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Abstract: The present work was designed to assess the possible effects of n-3 polyunsaturated fatty acid (n-3 PUFA) as fish oil, monounsaturated fatty acid (MUFA) as olive oil (OO), saturated fatty acid (SFA) as butter oil (BO) and their combinations on glucose tolerance, insulin sensitivity and membrane free fatty acid levels. Relatively high fat (20% w/w, 40% energy) content diets were prepared and supplemented to adult male Wistar rats for 5-weeks. Body growth, intravenous glucose tolerance, insulin sensitivity and membrane free fatty acid levels in hepatic cells and erythrocytes were measured. Mean body weights and total body fats were significantly increased in both SFA and MUFA diets fed rats as compared to control and n-3 PUFA dietary groups respectively. Significant impaired glucose tolerance and insulin insensitivity was observed in rats supplemented with SFA diet as compared to all other dietary groups. However, MUFA diet has not shown any significant effect on glucose tolerance and insulin sensitivity, although it significantly induced obesity in rats. The presence of (10%) fish oil in the diet corrected the adiposity affect of SFA and significantly reduced the impairment in glucose tolerance and insulin insensitivity. In conclusion, the results provide evidence that replacing SFA with MUFA is most beneficial even though MUFA promotes the obesity. Fish oil proven the protective effect against the impairment of glucose tolerance, insulin insensitivity and obesity which induced by butter oil.

Key words: Corn oil, olive oil, butter oil, fish oil, glucose tolerance, insulin sensitivity, free fatty acids

Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) is a disease that has a high prevalence in Western nations and some indigenous populations (Jarrett, 1989; Granner and O'Brien, 1992). The first abnormality that can be detected in NIDDM is insulin resistance, although both insulin resistance and impaired β-cell function appear to be essential for full expression of the disease (DeFronzo *et al*., 1992). Evidence suggests that insulin resistance develops in genetically predisposed individuals in response to environmental triggers (Barnett *et al*., 1981). Factors responsible for the onset of the disease are not well characterized but may include the type of fat in the diet (Storlien *et al*., 1993), or the degree of obesity of the individual, as judged by body mass index (Scarfors *et al*., 1991). The most widely used animal model of insulin resistance is the high fat-fed rat (Storlien *et al*., 1986; Ramirez *et al*., 1990; Han *et al*., 1997).

Several lines of evidence indicate that fish oil affects glucose metabolism. Islin *et al*. (1991) noticed that eicosapentaenoic acid (EPA) suppressed hyperglycaemia in mice and improved glucose tolerance induced by high-fat diet. Bunce *et al*. (1992) reported a delay in the development of glucose intolerance with long-term feeding of fish oil in diabetic-prone BHE/cdb rats. Lardinois and Starich (1991) reported that, high n-3 fatty acids content diet increased the fasting plasma insulin level. Hepatocytes isolated from rats fed fish oil had a significantly greater affinity for insulin than those hepatocytes from rats fed coconut oil (Pan and Berdanier, 1991). It is also suggested that diets with PUFA augment insulin secretion in human (Lardinois *et al*., 1987). These effects of fish oil are thought to be caused by the increased EPA in the plasma membrane of β-cells and peripheral tissues, which would result in functional changes of these cells to improve glucose metabolism (Fischer *et al*., 1986). However, the beneficial effects of fish oil on diabetes remain to be examined. Glauber *et al*. (1988) and Hendra *et al*. (1990) reported the n-3 fatty acid supplementation resulted in an increase in fasting glucose as well as glucose area during a mixed meal profile in type II diabetic patients. Monounsaturated fatty acid diets are being increasingly advocated for use in the treatment of patients with type II diabetes (Wright, 1998; Garg, 1994; Rocca *et al*., 2001). Recently, Vessby *et al*. (2001) suggested that, decreasing SFA and increasing MUFA in diet, improves insulin sensitivity. Lower fasting plasma glucose concentrations have been observed after consuming diets enriched with olive oil (Sirtori *et al*., 1986; Garg *et al*., 1988).

This study was designed to evaluate the potential benefits of n-3 PUFA and MUFA on impaired glucose tolerance and insulin resistance induced by SFA in rats. And these effects were correlated with the concentrations of membrane free fatty acids in hepatic cells and erythrocytes.

Table 1: Composition of diets (g/100g)

Ingredients	Corn oil Diet (CO)	Olive oil Diet (OO)	Butter oil Diet (BO)	Fish oil + Olive oil (FOO)	Fish oil + Butter oil (FBO)
Casein	18	18	18	18	18
Corn starch	23.5	23.5	23.5	23.5	23.5
Sucrose	23.5	23.5	23.5	23.5	23.5
Cellulose	10	10	10	10	10
Corn oil	20	-	-	-	-
Olive oil	-	20	-	10	-
Butter oil	-	-	20	-	10
Fish oil	-	-	-	10	10
Standard mineral and vitamin mix	5	5	5	5	5

Table 2: Mean changes in body weight and organ weights of rats supplemented with RC, CO, OO, BO, FOO and FBO diets for the period of 5-weeks. (Mean ± S.E.)

Groups	Body weight increased (g) in 5-weeks	Organ weights (g/100g body weight)				
		Liver	Heart	Kidney	Spleen	Fat pads
RC	120.97±8.20^{c}	4.01±0.21	0.51±0.10	0.87±0.06	0.42±0.08	1.62±0.15^{b,c,d}
CO	119.07±3.70^{c,d}	3.78±0.21	0.40±0.02	0.81±0.03	0.50±0.03	2.16±0.07^{a,d}
OO	140.35±2.73^{a,b,e,f}	3.64±0.11	0.41±0.03	0.75±0.03	0.50±0.03	2.46±0.15^{a,e}
BO	138.33±2.25^{b,e,f}	3.60±0.13	0.43±0.02	0.86±0.05	0.45±0.08	2.69±0.26^{a,b,e,f}
FOO	105.95±2.45^{c,d}	3.73±0.07	0.42±0.01	0.77±0.02	0.56±0.05	1.78±0.12^{c,d}
FBO	119.85±6.47^{c,d}	3.71±0.10	0.42±0.02	0.78±0.01	0.44±0.05	2.06±0.12^{d}

Statistically significance was determined by one-way ANOVA and Duncan's test. Letters refers to significance 'a' RC, 'b' CO, 'c' OO, 'd' BO, 'e' FOO and 'f' FBO. at P <0.05 level. Six rats were used in each group.

Materials and Methods

Diets: Casein, corn starch, sucrose, cellulose, olive oil, fish oil, dietary standard mineral and vitamin mixture were received from WINLAB, UK. Pure corn oil and butter oil were purchased from local market. The composition of diets is shown in Table 1. The diets were prepared every week, packed in small packs of 500 g and stored in freezer (-20 ^{o}C).

Animals: A total of 36 male Wistar rats of similar age group, weighing 150-160g were received from Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh. They were maintained under the standard conditions of temperature (23 ± 1^{o}C), humidity (50-55%) and light (12h light and 12h dark cycle). The rats were housed in separate cages (one in each cage) and divided in 6 groups. After an adjustment period (a week), the animals were fed with rat chow (RC) and fats content diets (Table 1) for the period of 5-weeks. Control group of rats were kept on free access to food throughout the period. The prepared diets were fed 7.5g/100g body weight per day to each rat for first three weeks and the last two weeks continued with 7.0g/100g/day. This fix amount of dietary fats was given after measuring the food intakes of rats in pilot study. Body weights were recorded daily and mean weight increased after 5-weeks was calculated.

Intravenous glucose tolerance test was performed with little modification as described by Davidson and Garvey (1993). After an overnight fast, the rats were anaesthetized with urethane (20% w/v, 0.5 ml/100g i.p. body weight) and placed on a warming operating table. Through a ventral midline neck incision, the left carotid artery was catheterized. After a baseline sample (400 µl) was taken, first heparin (1,000 IU/kg) and glucose (50 mg/100g body weight) was rapidly loaded through the same catheter. Further blood samples were collected at 3,6,9,12 and 15 min in micro-centrifuge tubes and centrifuged at 5,000 rpm for 10 minutes. The plasma samples were stored at –20^{o}C till analysis for glucose and insulin levels. After the timed blood samples were drawn, blood was collected as much as possible through cardiac puncture and refrigerated for estimation of membrane fatty acids in erythrocytes. All the animals were sacrificed after the blood samples were drawn and dissected. Liver, heart, kidney, spleen and total body fat were excised and weighed. Two pooled liver samples from each group was kept in refrigerator to estimate the membrane phospholipids.

Plasma samples were analyzed for glucose concentrations by using a diagnostic kit (Human Diagnostics, Germany). Glucose disappearance rate

Table 3: Mean changes in fasting plasma glucose, $K_{glucose}$ value, fasting plasma insulin, insulin area under the curve (AUC), insulin sensitivity and insulinogenic index of rats supplemented with RC, CO, OO, BO, FOO, FBO diets for the period of 5-weeks. (Mean ± S.E.)

Groups	Fasting plasma glucose (mMol/l)	$K_{glucose}$ value (%/min)	Fasting plasma insulin (µU/ml)	Insulin area under the curve (AUC)	Insulin sensitivity ($K_{glucose}$-value/ AUCx10^3)	Insulinogenic index (isulin/glucose) at 15 min
RC	9.20±0.45	3.75±0.27[d]	31.79±2.50	902.58±72.32	4.25±0.37[d]	5.82±0.74
CO	9.65±0.86	3.96±0.25[d]	31.94±1.32[e]	837.10±41.55	4.77±0.36[d]	4. 94±0.44[d]
OO	8.53±0.71	3.77±0.26[d]	28.25±1.72[d]	769.74±24.72[d]	4.90±0.30[d]	4.46±0.29
BO	10.23±0.60	1.99±0.13[a,b,c,e,f]	37.30±2.22[c,e]	974.20±53.09[c,e]	2.07±0.15[a,b,c,e,f]	6.14±0.23 [b,c,e,f]
FOO	9.32±0.37	3.79±0.19[d]	26.40±1.32[b,d]	775.42±35.27[d]	4.91±0.19[d]	4.21±0.29[d]
FBO	9.98±0.18	3.65±0.17 d	30.34±2.75	838.41±33.05	4.36±0.16[d]	4.89±0.29[d]

Statistically significance was determined by one-way ANOVA and Duncan's test. Letters refers to significance 'a' RC, 'b' CO, 'c' OO, 'd' BO, 'e' FOO and 'f' FBO. at P <0.05 level. Six rats were used in each group.

Table 4: Free fatty acids (percentage) composition in hepatic cell membrane of rats supplemented with RC, CO, OO, BO, FOO and FBO diets for the period of 5-weeks

Fatty acid	C-Number	RC	CO	OO	BO	FOO	FBO
Lauric acid	C12:0	2.56	0.81	-	1.24	-	-
Myristic acid	C14:0	0.41	0.87	-	1.05	-	0.32
Palmitic acid	C16:0	20.73	21.09	18.76	24.09	17.19	20.16
Stearic acid	C18:0	19.84	20.45	21.96	26.11	19.84	19.73
Oleic acid	C18:1n-9	19.17	15.23	27.54	23.32	21.01	20.14
Linoleic acid	C18:2n-6	12.15	23.70	9.81	6.89	9.11	8.20
Eicosanoic acid	C20:1n-3	0.54	2.31	0.52	0.87	1.01	0.36
Arachidonic acid	C20:4n-6	23.19	15.54	21.41	15.40	9.05	10.47
Eicosapentae-noic acid	C20:5n-3	-	-	-	-	8.99	6.18
Docosadienoic acid	C22:2n-6	0.58	-	-	-	1.99	0.40
Docosatetrae-noic acid	C22:4n-6	-	-	-	-	1.45	-
Docosahexae-noic acid	C22:6n-3	0.83	-	-	1.03	10.36	14.04

($K_{glucose}$ value) was calculated as described by Davidson and Garvey (1993). Plasma insulin levels were measured by a radioimmunoassay kit (Coat-A-Count INSULIN) supplied by DPC, Los Angeles, USA. The insulin area under the curve (AUC) at 15 minutes, insulinogenic index (insulin/glucose) and insulin sensitivity ($K_{glucose}$ value/AUC) were calculated by using computer software "Mini Stat".

Statistical Analysis: Statistical significance of the results were assessed by one-way ANOVA and Duncan's test applied to individual groups. 'P' value less than 0.05 were considered as significant.

Results

The results showed that, rats fed with OO and BO diets for five weeks significantly increased the body weights as compared to control. The fish oil (10%) showed significant (P<0.001) effect on body weight as controlling the obesity induced by olive and butter oil diets. Mean weight of total fat pads were significantly increased in CO, OO, BO diets fed animals as compared to controls. However, fish oil presence in the diets of olive oil and butter oil, significantly reduced the adiposity (Table 2).

Glucose disappearance pattern in between 3 min to 15 min was shown in Fig. 1. After the glucose load (50 mg/100 mg body weight), the plasma glucose levels reached their highest peak at 3 min and followed falling rapidly in all groups. Glucose decay was seen slower in BO group as compared to control and other dietary groups.

In all dietary groups the insulin levels were start increasing till min-15 with little decay at min 6. Butter oil diet increased highest insulin level from 37.20 ± 2.22 to 99.96 ± 3.53 which followed FBO from 30.34 ± 2.75 to 69.52 ± 3.06, CO from 31.94 ± 1.32 to 67.61 ± 4.61, OO from 28.25 ± 1.72 to 60.61 ± 3.88 and FOO from 26.40 ± 1.32 to 59.16 ± 3.95 (Fig. 2).

In Table 3, the mean fasting glucose levels were not significantly altered in dietary groups and as compared to control. Glucose disappearance rate ($K_{glucose}$ value) was significantly decreased (P<0.001) in BO supplemented rats as compared to controls and other experimental fats fed rats. Highest rise in fasting insulin level was seen in BO group and it is significant to OO and FOO group respectively. Insulin area under the curve (AUC) at minute 15 was significantly elevated

Table 5: Free fatty acids (percentage) composition in erythrocytes membrane of rats supplemented with RC, CO, OO, BO, FOO and FBO diets for the period of 5-weeks

Fatty acid	C-Number	RC	CO	OO	BO	FOO	FBO
Lauric acid	C12:0	2.79	2.53	-	1.76	-	-
Myristic acid	C14:0	0.38	0.78	0.32	0.97	0.44	0.32
Palmitic acid	C16:0	20.83	22.55	19.80	30.12	18.48	20.16
Stearic acid	C18:0	19.91	19.64	20.85	24.00	21.92	22.94
Oleic acid	C18:1n-9	16.09	19.49	27.69	21.80	19.56	20.14
Linoleic acid	C18:2n-6	13.51	22.23	9.15	7.28	7.84	5.11
Eicosanoic acid	C20:1n-3	0.69	1.20	0.70	1.21	0.94	0.36
Arachidonic acid	C20:4n-6	22.89	11.58	21.49	10.15	8.22	10.47
Eicosapentaenoic acid	C20:5n-3	-	-	-	-	6.87	6.18
Docosadienoic acid	C22:2n-6	1.00	-	-	-	0.67	0.40
Docosatetraenoic acid	C22:4n-6	0.35	-	-	-	2.39	-
Docosahexaenoic acid	C22:6n-3	1.56	-	-	2.71	12.67	14.04

in BO group as compared to OO and FOO diet groups respectively. Insulin sensitivity ($K_{glucose}$ value/AUC) was significantly lowest ($P<0.001$) in BO diet supplemented group as compared to other dietary groups. FOO diet fed rats found most insulin sensitive than other fat diets fed rats. The ratio of plasma insulin to glucose concentrations has been used to compute insulinogenic index or an index of β-cell response to changing glucose stimulus. A significantly higher value at 15 min was observed for BO as compared to other dietary groups (Table 3).

Percentage phospholipids of hepatic cell and erythrocytes membrane were shown in Table 4 and 5. In both cell membranes, Eicosapentaenoic (C20:5), docosadienoic and docosehexaenoic (C22:6) and docosatetraenoic (C22:2) acids were detected mostly in fish oil (FOO and FBO) supplemented dietary groups.

In the hepatic cells, total saturated fatty acids were highest (52.49%) in BO and lowest (37.03%) in FOO diets fed groups. Sum of PUFA was highest in FOO (41.96%) and lowest in BO diet group. The lowest ratio of PUFA/SFA was seen in BO fed group where it was highest in FOO. The highest total of n-3 PUFA was estimated in fish oil supplemented groups. The total of n-6 PUFA (39.24%) found as a highest in CO diet fed group. However, the highest ratio of n-6 and n-3 PUFA (n-6/n-3 PUFA) was calculated (60.0%) in OO group and it followed the control group. (Fig. 3a). Similar results were seen in erythrocytes membrane phospholipids, highest total saturated fatty acids (56.85%) in BO diet fed rats. Total PUFA percent was estimated highest 39.6% in FOO group. The ratio in between n-6 PUFA and n-3 PUFA was highest 43.77% in OO diet group which followed 28.17% in CO diet supplemented group (Fig. 3b).

Discussion

The high-fat-fed rat model of insulin resistance has been used extensively to elucidate physiological mechanisms of non-pharmacological (Kraegen *et al.*, 1989; Storlien *et al.*, 1991) and pharmacological strategies (Storlien *et al.*, 1987; 1993; Oakes *et al.*, 1994) for reversing or preventing insulin resistance. Results in the present study showed that the rats supplemented with MUFA and SFA for five weeks significantly increased the body weight and visceral fats compared to control. Similar dietary effects has been observed by Parrish *et al.* (1991) and Pan *et al.* (1994) in mice and concluded that both MUFA and SFA diets favor adiposity by increasing lipogenesis. The lowering effects of fish oil consumption on adiposity is well established (Parrish *et al.*, 1991; Kim *et al.*, 2000) as significantly lower fat size in fish oil fed rats compared to rats consumed saturated animal fat (Hill *et al.*, 1993; Luo *et al.*, 1996). Similar dietary effects has been observed by Mulrooney and Grimble (1993) and Macho *et al.* (1993) that fish oil supplemented rats reached lower final body weight compared to other dietary fats and carbohydrates. The concept that central/visceral obesity causes insulin resistance is largely based on the finding in numerous studies, that these two phenomena are closely correlated (Kissebah and Peiris, 1989; Bjorntorp, 1990; 1992; Kissebah, 1991). However, the existence of a strong correlation does not prove a cause-effect relationship and it is possible that visceral obesity develop in parallel with and serves as a marker for, some other phenomenon that is the actual cause of the insulin resistance. Thus, in the present study this would not explain the impairment induced by the SFA since the level of adiposity as judged by visceral fat pads weight and body weight did not differ between the rats fed the MUFA or SFA diets. In a human study which has studied the influence of SFA and MUFA on glucose homeostasis are consistent with the present findings as butter (100g) was shown to suppress the blood glucose response area in subjects with NIDDM while neither 40g or 80g MUFA had any influence on the glucose tolerance (Rasmussen *et al.*, 1996). Nydahl *et al.* (1995) concluded that, lipid-lowering diets containing either

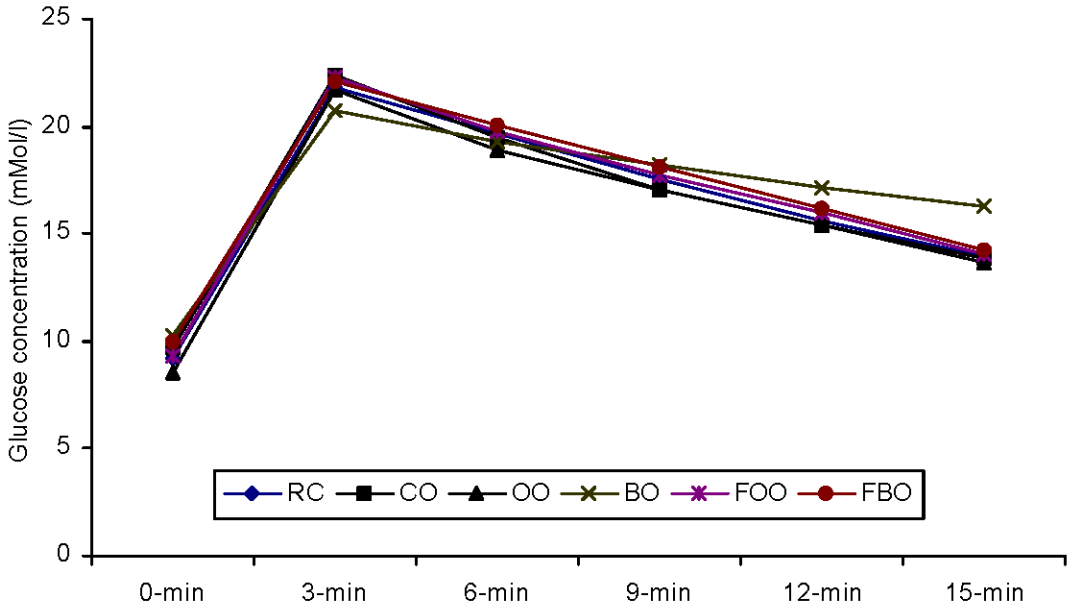

Fig. 1: Glycogenic response to a glucose load (50 mg/100 g body weight, iv) in rats supplemented with RC, CO, OO, BO, FOO and FBO diets for the period of 5 weeks

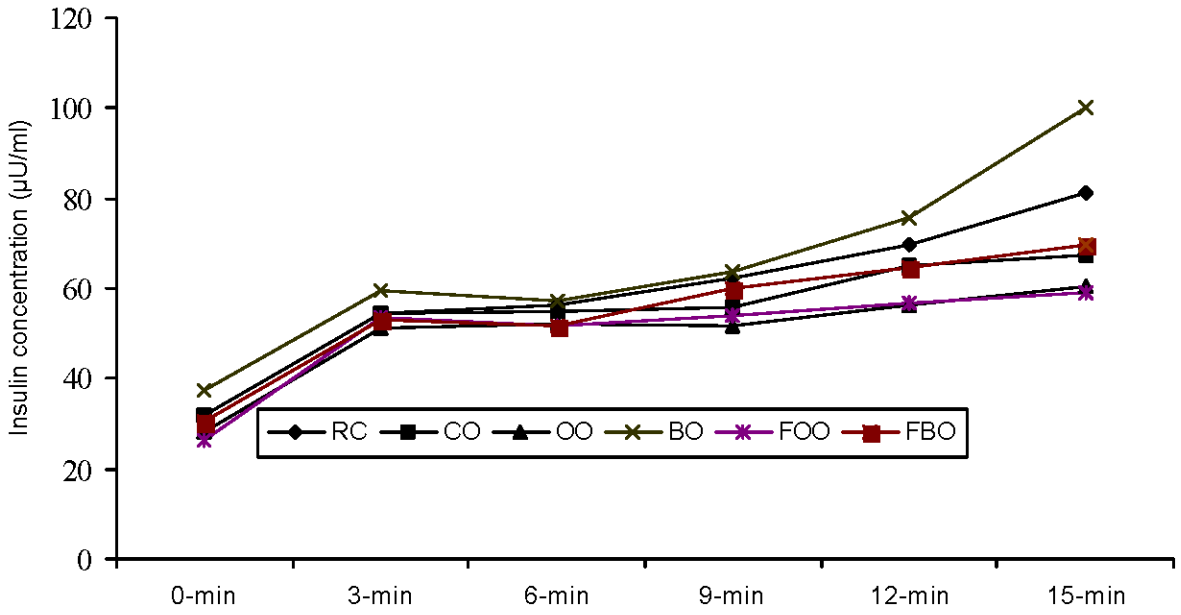

Fig. 2: Insulinemic response to a glucose load (50 mg/100 g body weight, iv) in rats supplemented with RC, CO, OO, BO, FOO and FBO diets for the period of 5 weeks

rapeseed oil or olive oil, improved the intravenous glucose tolerance. Furthermore, Rasmussen *et al.* (1995) confirmed that diets rich in MUFA improved glucose tolerance as compared to high carbohydrate diet.

The effects of dietary fish oil on glucose tolerance have been studied by several groups in a high fat insulin resistance rat models (Storlien *et al.*, 1987; Hainault *et al.*, 1993) with different results. In rats fed a 59% fat diet, Storlien *et al.* (1987) reported that replacement of 6% n-6 PUFA with n-3 PUFA, increased insulin-stimulated glucose metabolism in liver and skeletal muscle but not

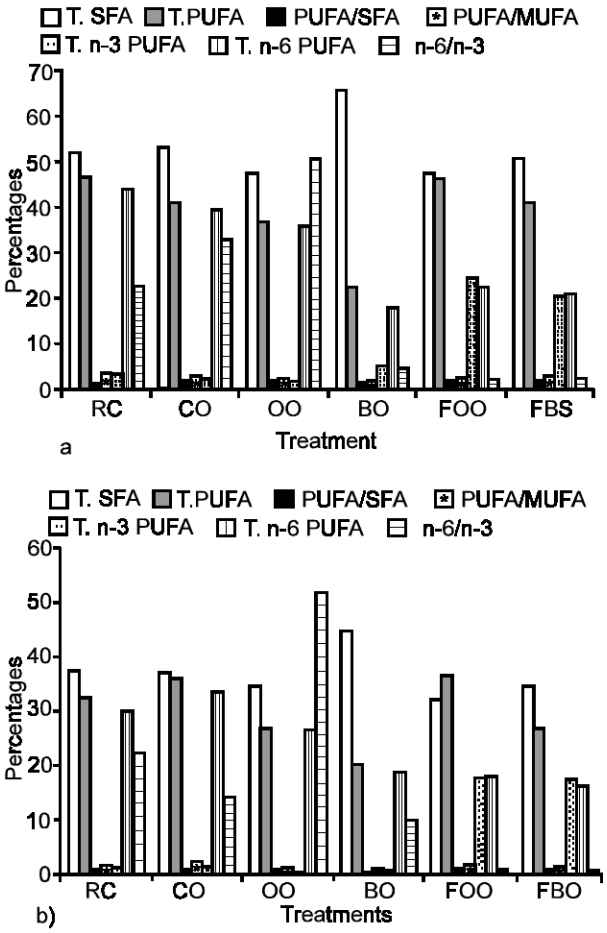

Fig. 3: Free fatty acid profile (total SFA, total PUFA, total n-3 PUFA, total n-6 PUFA, PUFA/SFA, PUFA/MUFA and n-6 PUFA/n-3 PUFA) in (a) hepatic cells membranes (b) erythrocytes membranes of rats supplemented with RC, CO, OO, BO, FOO and FBO diets for the period of 5 weeks

in white adipose tissue. In contrast, Hannault *et al.* (1993) reported that a 15% fish-oil, 50% total fat diet did increase insulin-stimulated glucose transport in adipocytes *in vitro*. Recently Ajiro *et al.* (2000) concluded that fish oil improves glucose tolerance by enhancing insulin secretion from pancreatic β-cells. However, the present results showed, FBO diet improved glucose tolerance compared with BO alone ($P < 0.001$), it can be concluded that fish oil has a beneficial effect on glucose tolerance.

In the present study, SFA diet fed rats showed hyperinsulinogenia effect as compared to other dietary groups. This data is consistent with the insulinogenic effect of the consumption of saturated fat reported in the previous studies of Collier *et al.* (1988) and Rasmussen *et al.* (1996). The mechanism of the increase is not known, however, it has been suggested that SFA appear to potentiate insulin release from isolated mouse islets through an increase in intracellular calcium (Warnotte *et al.*, 1994). Because fat is the most potent dietary stimulus of gastric inhibitory peptide (GIP) (Collier and O'Dea, 1983) an indirect effect via GIP-mediated glucose-dependent insulin secretion might also operate (Sarson *et al.*, 1984). Supplementation with PUFA's are reported to exert an augmentation of insulin secretion in few studies (Ajiro *et al.*, 2000; Lardinois *et al.*, 1987) and a reduction in others (Panzram, 1987; Glauber *et al.*, 1988; Lardinois and Starich, 1991). Chicco *et al.* (1996) concluded that, fish oil administration to normal Wistar rats, significantly reduced the plasma insulin levels without changing in glucose tolerance, which may be the result of increased peripheral insulin sensitivity. Similar results have been reported in pig studies by Behme, (1996) i.e. n-3 PUFA significantly increases the insulin sensitivity compared to n-6 PUFA without changing the glucose disappearance rate. Through the present results it is clear that with an increased insulin level and a reduced glucose disposal rate insulin sensitivity of the SFA fed animals was markedly impaired.

The nature of the fatty acids within the phospholipid bilayer determines the physicochemical properties of membrane which in turn would be expected to influence cellular functions, including transport phenomena and hormone responsiveness (Field *et al.*, 1988). The way that fatty acids can influence membrane and cellular function can involve several different mechanisms (Tappia and Grimble, 1994), an important one is through the balance of precursors for eicosanoid production and through altering membrane fluidity. The membrane fluidity on the basis of PUFA/SFA ratio in both liver and erythrocyte membrane lipids, was least in butter oil group and the highest in fish oil group. However fluidity is a complex function of membrane composition since it can be influenced by chain length, the degree of unsaturation and the n-6:n-3 ratio (Tappia and Grimble, 1994). As would be expected eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels were elevated only in fish oil diet fed group. Similar results have been reported in earlier studies (Stinson *et al.*, 1991; Sirtori *et al.*, 1992; Jones *et al.*, 1995) and in the adipocytes membrane phospholipids of fish oil fed rats (Mulrooney and Grimble, 1993). In conclusion, the results of the present study provide evidence that replacing SFA with MUFA or PUFA are most beneficial. Even MUFA increases body fats and weight, it does not impair glucose intolerance or insulin sensitivity. n-3 PUFA has shown protective effect against body fat accumulation and beneficial affects on impaired glucose tolerance and insulin resistance.

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