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Alteration in lipid metabolism induced by a diet rich in soya-oil and amylopectin in a rat model

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This present study was designed to evaluate the impact of different dietary regimens on lipid metabolism in brain, liver and plasma of albino rat model. Twenty (20) male Wister albino rats (110 g) were assigned to two dietary groups and housed individually. One group received a control diet enriched in 20% soy- oil and another enriched with 20% amylopectin. The starved group was fed primarily with carboxyl methyl cellulose (CMC). Daily growth rate and average condition factor of each group was monitored for 14 days. Lipid profiles of brain, liver and plasma were analyzed by colorimetric, immunological, thin layer chromatography and gas chromatography-mass spectrometry. Elevated serum total cholesterol was indicated in rats of starved, oil fed and starch fed rats by factors of 0.40, 0.65 and 0.75 mmol/L respectively when compared with the control. Low density lipoprotein (LDL) cholesterol was 0.9, 1.1 and 0.95 mmol/L higher in starved, oil fed and starch fed rat groups, respectively when compared with the control group. Alteration in feeding pattern revealed a marked decrease in the levels of the steroidal sex hormones in the starved and other dietary groups compared to the control. Hepatic and liver lipid profile revealed the polar lipids tentatively identified altered expression of diphosphatidyl glycerol, phosphatidyl choline, phosphatidyl serine, and cholesterol in order of decreasing polarity. GC-MS results showed a shift in the ratio of saturated to unsaturated fatty acids in the test groups. Lauric acid was observed to be predominantly present in the starved group.

Key words: Lipid metabolism, soy-oil, steroidal hormones, liver lipid, serum lipoproteins.

INTRODUCTION

Dietary intake has a lot of influence on our nutrition and health. The incidence of cardiovascular disease (CVD) and diabetes type 2 is increasing worldwide representing the two most influential lifestyle-dependent diseases at present (Anna et al., 2012). In addition, lipid droplets are implicated in a number of other cellular functions, ranging from protein storage and degradation to viral replication. These processes are functionally linked to many physiological and pathological conditions, including obesity and related metabolic diseases (Tobias and Robert, 2012). Consequently, multiple components of these metabolic syndrome including obesity and diabetes have been linked with Alzhemeirs disease (Frisardi et al., 2010; Luchsinger et al., 2012; Whitmer et al., 2008) and considerable evidence also implicate them in cognitive decline (Biessels et al., 2008; Talbot et al., 2012). Different feeding pattern is usually accompanied by pathophysiological conditions in affected organism and

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consequent serious morbidity. A high dietary fiber intake has been proposed to reduce the risk of diabetes type 2 ((Weickert and Pfeiffer, 2008) through improvement of glycemic and insulinemic response and also reduced insulin resistance (Ulmius et al., 2009).

The hydrophobic core of neutral lipids stores metabolic energy. Fatty acids liberated from triglycerides (TGs) in lipids have one of several fates. They can be re-esterified to TG, used for β-oxidation to generate energy, used as building blocks for membrane lipid synthesis, or used as cofactors for cell signaling or exported (Tobias and Robert, 2012). Lipid metabolism is of particular interest due to its high concentration in the central nervous system (CNS). The importance of lipids in cell signaling and tissue physiology is demonstrated by many CNS disorders and injuries that involve deregulated meta-bolism (Rao and Hatcher, 2007).

These resultant alterations in lipid metabolism might lead to multiple deleterious changes in cellular function that are prominent features of many lipid-related diseases in the societies. To critically assess the role of alterations in lipid metabolism and the role of the accumulation of lipids in organ, it would be necessary to identify the types of lipid molecular species that accumulate and next correlate their presence and abundance to pathological changes in cellular function. This will facilitate early diagnosis of disease and potentially provide a valuable measure of treatment efficacy.

Although considerable efforts have been directed at evaluating alterations in various metabolic and physio-logical functions, lipid metabolism in response to different feeding pattern in the rat -by extension human- has not been fully evaluated.

Previous lipid analytical approaches have been limited and lack a detailed analysis of specific lipid classes and their associated fatty acid metabolites. It is expedient to know the changes taking place in the various lipid composition of an organism as this plays a major role in the adaptation of animal to stress and in their respective cognitive performance.

In this study, we began with the identification and quantitation of different lipid classes in individual mole-cular species to determine the biochemical flux through cellular metabolic networks. We also analyzed and compared the lipid changes induced by different feeding patterns and starvation using thin layer chroma-tography and gas chromatography-mass spectrometry. A connec-tion between the lipid profile changes with meaningful pathological conditions of adaptation to stress, and cognitive function was observed in albino rats.

MATERIALS AND METHODS

Chemicals

Diphosphatidyl glycerol, phosphatidyl choline, phosphatidyl serine, di-isobutyl ketone, ethanol, acetic acid, ethylacetate , cyclohexane, chloroform, methanol, and isopropanol were purchased from Sigma

Chemical Co. (St. Louis, MO), USA. Precoated silica gel plates type F254 from Merck A. G. (Darmstadt, Germany). Bovine serum albumin (BSA) was R.I.A grade (Fraction V), pure steroids standard (11- ketotestosterone, testosterone, and estradiol) and the secondary antibody (Mouse monoclonal anti rabbit IgG) was purchased from SpiBio (Saclay, France). Ammonia solution was obtained from Aldrich (Steinheim, Germany). Triton X-100 was obtained from Boehringer Mannheim Gmbh (Mannheim, Germany). All other organic solvents were of analytical grade.

Animals and diets

Male Wistar rats of average weight 110 g were used according to the standard guideline of the Committee on care and use of experimental animal resources. They were housed one per cage, in wire-bottomed cages in a temperature-controlled room (22°C) with a 12-h light: dark cycle and maintained on a standard diet with water *ad libitum* for six weeks

Feeding pattern

Rats were randomly divided into four groups of five rats per group and fed for two weeks. The composition of this diet is shown in Table 1. Diet formulation was done as earlier described (Zulet et al., 1999). Rats (n=5) in groups A, B, C and D were fed with control, soybean oil enriched, starch rich and carboxyl methyl cellulose (CMC) diets, respectively. The weight of the rats in each group was monitored daily for 14 days. Mathematical model of Chatelier et al. (2006) was used for the evaluation of daily growth and condition factor as shown below:

Daily growth = $\{((M_f - M_i)/M_i) \times 100\} / n$

Condition factor = $(M_f/BL^3) \times 100$

Where, M_f is final mass in kg, M_i is initial mass in kg, n is the number of feeding days and BL is body length in cm.

At the end of the experiment, the animals were sacrificed by decapitulation. Blood was quickly collected and organs (brain and liver) isolated. Serum was separated from blood samples and was used immediately. The brain and liver from all rats were removed, weighed and frozen at -10°C until use.

Serum lipid profile

The blood serum obtained from the animals was analyzed for total cholesterol, triglycerides, high density lipoprotein C (HDL-C), and low density lipoprotein C (LDL-C). Chemical assays were performed by utilizing commercially available kit (Randox UK). Instrument set-up, run procedures, and maintenance policies were strictly adhered according to the manufacturer's instructions. Total cholesterol was measured after release from its esters by an ester hydrolase using the cholesterol oxidase technique. Triglyceride level was measured after hydrolysis by lipoprotein lipase by assay of released glycerol. LDL-C was calculated according to the method of Friedewald (1972).

Steroidal sex hormones assay

Immunological measurement of sex steroids was carried out using 0.5 mL aliquots of plasma. Plasma was thawed and the free steroids were extracted twice with 2 ml of a mixture made of ethylacetate/cyclohexane (1:1). After freezing at - 4°C, the organic

Table 1. Feed formulation

Feed composition	Control (Group A)	Group B	Group C	Group D
Corn Starch (g/100 g	42.2	42.2	42.2	-
Skimmed milk (g/100 g)	43.8	43.8	43.8	-
Premix (g/100 g)	4	4	4	-
Pure Starch (g/100 g)	-	10	-	-
Soy oil (g/100 g)	-	-	10	-
CMC (g/100 g)	-	-	-	100

1 g of premix contains: 3200 i.u vitamin A, 600 i.u vitamin D3, 2.8 mg vitamin E, 0.6 mg vitamin K3, 0.8 mg vitamin B6, 0.004 mg vitamin B12, 0.2 mg folic acid, 0.1 mg biotin H2, 70 mg choline chloride, 0.08 mg Cobalt, 1.2 mg Copper, 0.4 mg iodine, 8.4 mg iron, 16 mg manganese, 0.08 mg selenium, 12.4 mg zinc, 0.5 mg antioxidant. Skimmed milk contains 28% protein.

phase was collected in 5 ml glass tubes and dry evaporated using a Speed-Vacuum apparatus. The dry residue was then dissolved in 0.5 ml of assay buffer (potassium phosphate buffer 0.1 M, pH 7.4, 0.9% (w:v) NaCI, 1 mM ethylenediaminetetra acetate (EDTA), 0.1% (w:v) bouvine serum albumin (BSA) and 0.01% NaN$_3$). Each sample was analyzed in triplicate (Maclouf et al., 1987). Standard curve was obtained using pure 11KT dissolved in assay buffer, and ranging from 7.8 to 1000 pg/ mL. The crossing activity of specific anti-11KT antibody, (rabbit anti- 11KT serum) was as described in the instruction manual (Maclouf et al., 1987). Enzyme linked immuno sorbent assay (ELISA) for estradiol were adapted from schedule previously described for 11-ketotestosterone (Nash et al., 2000).

Thin layer chromatography

Lipids were extracted as described elsewhere (Bligh and Dyer, 1959). The inclusion of isopropanol rather than methanol in the extraction avoids any lipid degradation by endogeneous lipolysis. Analytical thin layer chromatography (TLC) was carried out using silica gel plates (E. Merck, Darmstadt, Germany). One dimensional polar lipid separations were achieved using a solvent system of Diisobutylketone: chloroform: methanol: acetic acid: distilled water (3: 4.5: 1.5: 2: 0.4 by vol.) Lipids were visualized by brief exposure in an iodine tank, or using a pray of 8-anilino-1- naphthalene sulphonic acid (0.2% in methanol) followed by viewing under UV. Lipids were identified by comparison with authentic lipid standards and later using spraying reagents.

Gas chromatography - mass spectra analysis

The crude lipid extract was transmethylated using 2.0 M KOH in methanol and *n*-heptane according to the method as described (Ichihara et al., 1996) with minor modification. Extracted lipids (10 mg) were dissolved in 2 ml heptane followed by 4 ml of 2.0 M methanolic KOH. The tube was then vortexed for 2 min at room temperature.

After centrifugation at 4000 rpm for 10 min, the heptane layer was taken for GC-MS analyses. The lipid composition was analysed by GC Clarus 500 with autosampler (Perkin Elmer, USA) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m × 0.32 mm, ID × 0.25 μm, BP 20 0.25 UM, USA) coupled to MALDI-TOF (Applied Biosystems, 4800). Various fatty acids were identified by comparing the retention times of various peaks obtained from the mass spectra of the samples.

Statistical analysis

Significance of differences between starved and normal tissue total lipids and metabolite concentrations were assessed using a one-way ANOVA with a Tukey's post test, with $P < 0.05$ considered statistically significant. Results were reported as means ± SD or as percent change from normal control.

RESULTS

Daily growth and condition factor

Figure 1 shows the daily growth pattern of differentially fed rats. Control group exhibited a significantly higher daily growth rate in mass per day compared to the other three dietary groups. The control had a mean specific growth rate of 2.565 g/day; oil fed, 1.585 g/day; starch fed, 1.68 g/day and the starved, 6.7 g/day (Figure 1). The control group also exhibited a significantly higher $(P < 0.05)$ mean condition factor of (0.058) than estimated mean condition factor of 0.048, 0.047 and 0.042 for starch-fed, oil-fed and starved groups, respectively (Figure 2).

Lipid composition

Estimated mean serum lipid triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol concentrations at the end of each diet period are shown in Figure 3. Serum total cholesterol was greater by 0.40 mmol/L in the starved rats (4.30 mmol/L), 0.65mmol/L in the oil fed rats (4.55 mmol/L), 0.75 mmol/L in the starch fed rats (4.65 mmol/L) when compared with the control (3.90 mmol/L), respectively. LDL-cholesterol was greater by 0.9 mmol/L (2.10 mm/L), 1.1 mmol/L (2.3 mmol/L) and 0.95 mmol/L (2.15 mmol/L) in subjects starved, fed with oil, and fed with starch, respectively, when compared with the control (1.2 mmol/L). However, triglyceride was lowered by 1.3, 1.4 and 0.65 mmol/L in starved, oil fed and starch fed rats, respectively, when compared with the control (2.7
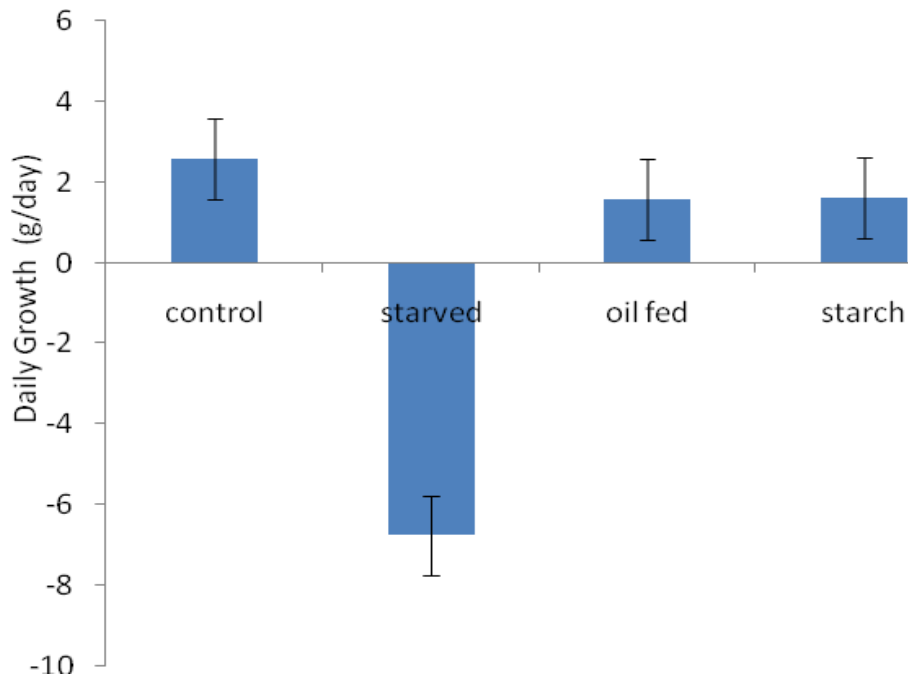
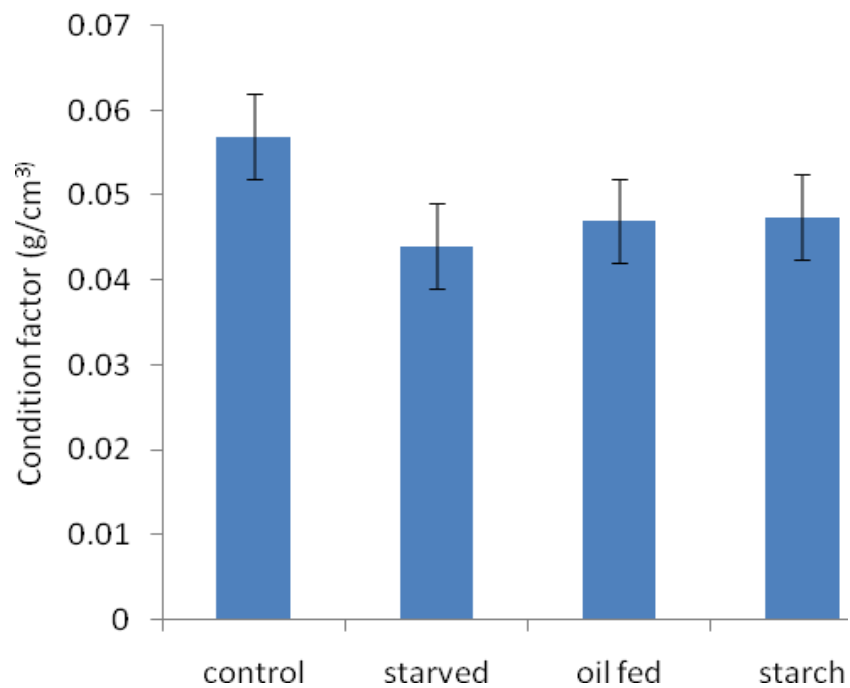**Figure 1.** Daily growth pattern of the differentially fed rats.

Figure 2. Average condition factor of the differentially fed rats.

mmol/L) while HDL-cholesterol was also lowered by 0.1 and 0.15 mmol/L in starved and oil fed groups, respectively, when compared with the control (1.7 mmol/L), but there was an increase by factor of 0.75 mmol/L in the HDL-cholesterol of starch fed group when compared with the control. All differences were significant. The polar lipids that were tentatively identified were designated diphosphatidyl glycerol (DPG), phospha-
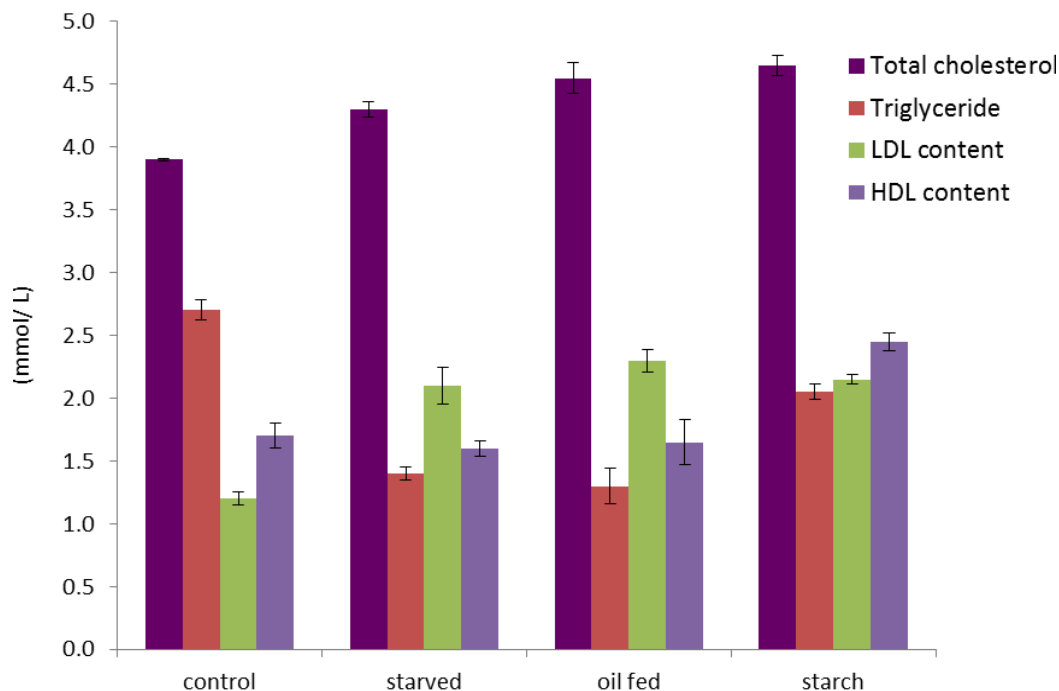
Figure 3. Serum Lipid profile of the differentially fed rats. Values are means ± SD are given in mmol/l.

tidylcholine (PC) phosphatidyl serine (PS) and Cholesterol (CL) in order of decreasing polarity. Neutral lipids were also present in all the four groups and was designated neutral lipids (NL) at the solvent front on the TLC plate (Figure 4). The polar lipids from starch fed rats seemed to be almost identical with that of the lipid enriched diet rat group with significant differences only in the proportion of the components. The polar lipids from the starved rats seemed to be almost identical with that of the control group also with significant differences in the proportion of the components. DPG was much more pronounced in starch fed rats and lipid fed rats when compared with the control group.

Gas chromatography - mass spectra (GC/MS) analysis

A typical GC/MS profile of crude lipid extracts of the brain and the liver was analyzed from negative electrospray ionization in the full-scan mode. This approach allowed us to obtain the spectra (Figure not shown). Analysis and interpretation of MS spectra of each ion in the MS spectra allowed for the identification of fatty acyl chain composition of brain and liver.

Brain profile

The chromatogram from the GC-mass spectra analyses shows that palmitic acid, oleic, lignoceric, stearic, arachi-

donic acid, docosatetraenoic and docosahexaenoic acid, were present in the control group in different percentages with no traces of lauric acid (Table 2). The starch fed rats also had a similar profile with that of the control except for the complete absence of linoleic acid which made up only 0.61% of the control's fatty acid profile. However, the dominant fatty acid in the starved rat was lauric acid which made up 53.77% of the fatty acid profile of this rat with just traces of oleic acid, docosatetraenoic acid and docosahexaenoic acid. The oil fed group has all the fatty acids present in the control although in varying percentages but also with an additional presence of 8.94% lauric and 1.74% lignoceric acid (Figure 6).

Liver profile

The chromatogram revealed the presence of palmitic acid, oleic, stearic, linoleic, arachidonic, behenic , lignoceric, docosatetraenoic and docosahexaenoic acid in varying percentages in the liver of the control (Table 3). The starved group lacked arachidonic and behenic acid but had 7.81% palmitoleic acid when compared with the control. No traces of linoleic, behenic and lignoceric were found in the liver of the oil fed group.

About half of the total fatty acids in the liver of the starch fed group was oleic acid, this group had the highest amount of oleic acid when compared with the control and other groups, it was also the only group that gave the presence of lauric acid, although, it lacked stearic, linoleic, and arachidonic acid when compared
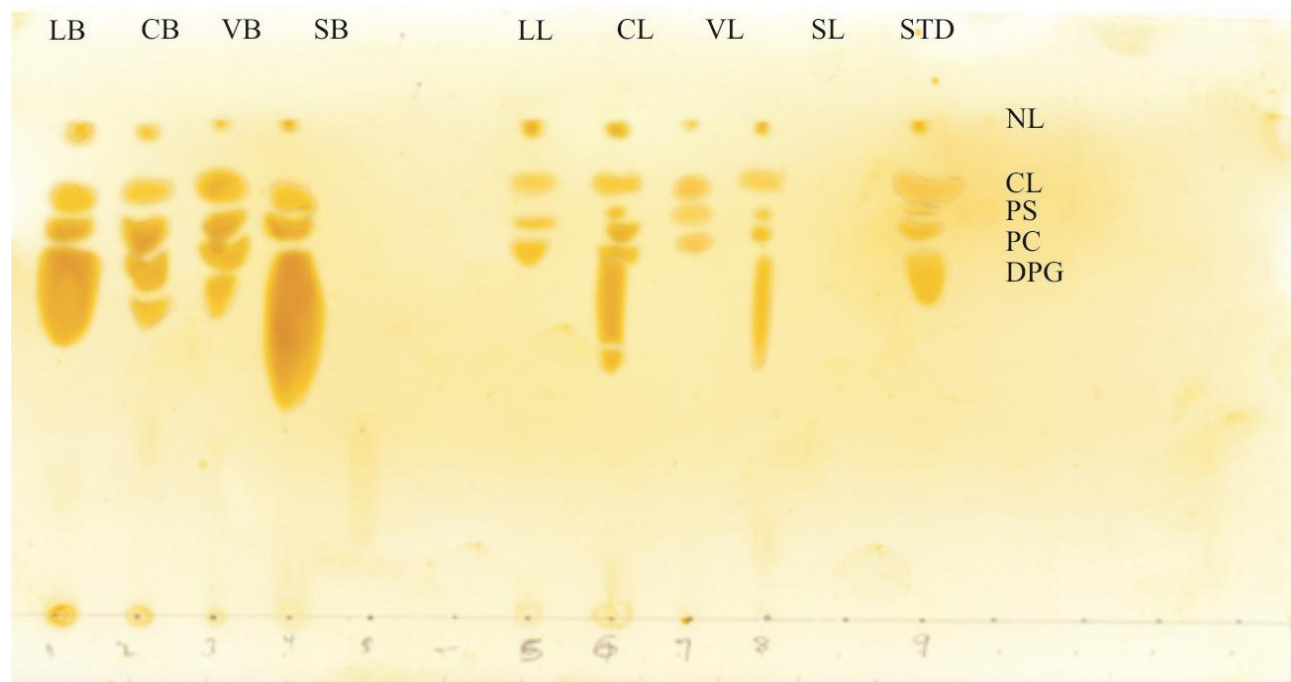
Figure 4. The separation of total lipids and lipid fractions from the brain and liver of lipid fed rats, starch fed rats, starved rats, and control group. The solvent was diisobutyl ketone - chloroform - methanol acetic acid - H2O (30: 45: 15: 20: 4 v/v). The samples were: 1, Total brain lipids from lipid fed rats; 2, total brain lipids from control group; 3, total brain lipids from starved group; 4, total brain lipids from starch fed rats; 5, total liver lipids from lipid fed rats; 6, total liver lipids from control group; 7, total liver lipids from starved group; 8, total liver lipids from starch fed rats; 9, standard mix standards: DPG, Diphosphatidyl glycerol; PC, phosphatidylcholine; PS, phosphatidyl serine; CL, cholesterol; NL, neutral lipids developed with iodine chrystal.

Table 2. Distribution of fatty acids in the brain of the selectively fed rats as identified by GC-MS.

Systemic name	Fatty acid	No. of carbon	Control (m%)	Starved (m%)	Oil fed (m%)	Starch fed (m%)
Dodecanoic acid	Lauric acid	12	0±0.0002	53.77±1.34	8.94±0.02	0±0.0001
Hexadecanoic acid	Palmitic acid	16	26.26±1.4	19.89±0.004	21.14±0.1	31.93±0.02
9-Octadecenoic acid	Oleic acid	18	34.33±0.07	5.83±0.003	21.77±0.3	42.05±0.05
9,12-Octadecadienoic acid	Linoleic acid	18	0.61±0.002	0±0.0001	0.53±0.04	0±0.0002
Octadecanoic acid	Stearic acid	18	16.26±0.005	0±0.0002	14.41±0.01	23.14±0.07
5,8,11,14-Eicosatetranoic acid	Arachidonic acid	20	8.44±0.002	0±0.0002	20.48±0.08	2.88±0.001
Tertacosanoic acid	Lignoceric acid	24	0±0.0001	0±0.0001	1.74±0.02	0±0.0001
Docosatetraenoic acid	Adrenic acid	22	11.21±1.2	8.21±0.2	11.24±0.12	18.56±1.55
Docosahexaenoic acid	Cervonic acid	22	10.41±1.01	3.21±0.05	10.05±0.7	9.23±0.96

Data represents mean ± SD of 3 independent experiments P ≤ 0.05 vs. control; Tukey's post-test.

with the control (Figure 5).

Sex steroidal hormones

The result of the determination of steroid sex hormones is shown in Figure 7. Estimated mean value of estradiol in the starved, starch fed and oil fed were 539.5, 683.5 and 679.5 pg/ml, respectively, while the control had 710.5 pg/ml. Estimated values of testosterone in the starved, starch fed and oil fed groups were 710, 823 and 1027, pg/ml respectively, while the control group had estimated testosterone value of 1132.5 pg/ml. Estimated 11-ketotestosterone mean values of the starved, starch fed and oil fed rats were 584, 722.5 and 928.5 pg/ml, respectively, while that of the control was 1120 pg/ml.

DISCUSSION

The results demonstrate that diet-related changes in the fatty acid composition of the tissues could have signifi-
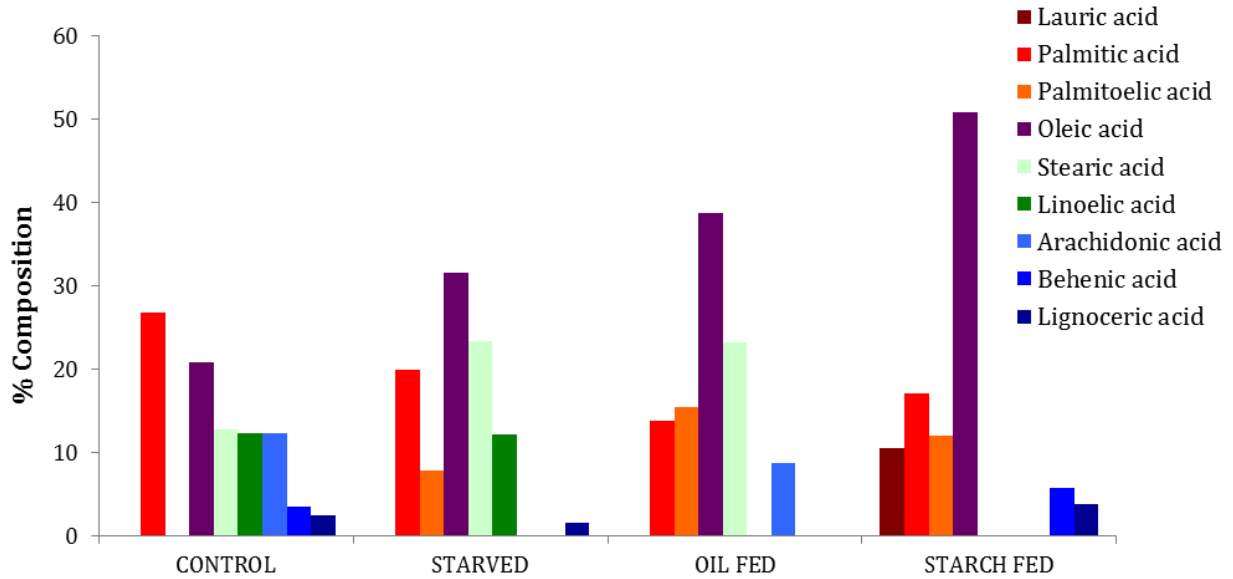
Figure 5. Histogram of the various fatty acids identified by GC-MS in the liver of the rats. Data represents mean ± SD of 3 independent experiments P≤ 0.05 vs. control; t test.
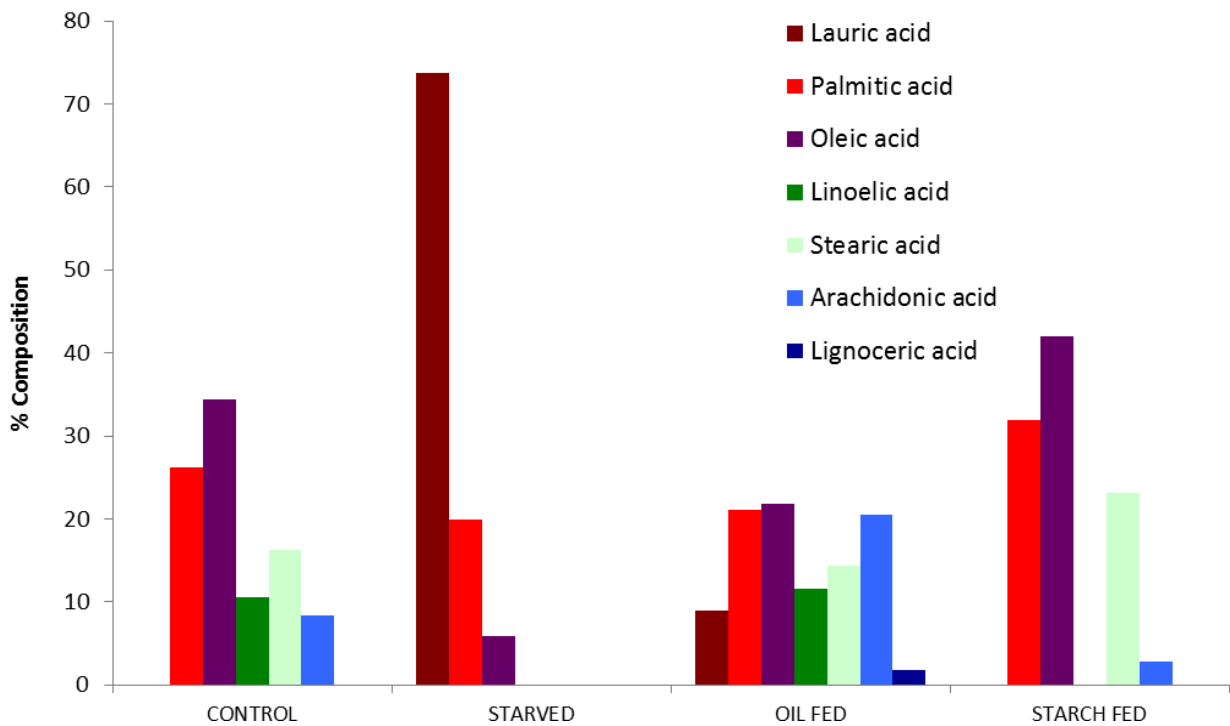

Figure 6. Histogram of the various fatty acids identified by MS in the brain of the rats. Data represents mean ± SD of 3 independent experiments. P≤ 0.05 vs. control; t, test.

cant effects on major physiological traits of performance and metabolism in rats. The starch fed groups and lipid fed groups revealed a number of associations between the percentage content of specific fatty acid in the percentage and/or types of fatty acids pools of both brain and liver and particular traits of growth, metabolism and steroidal hormones. It has been well established that nutrition plays a vital role in the etiology of hyperlipide-

Table 3. Distribution of fatty acids in the liver of the selectively fed rats as identified by GC-MS.

Systemic name	Fatty acid	No. of carbons	Control (m%)	Starved (m%)	Oil fed (m%)	Starch fed (m%)
Dodecanoic acid	Lauric acid	12	0±0.0002	0±0.0003	0±0.0001	10.46±0.02
Hexadecanoic acid	Palmitic acid	16	26.87±0.02	19.96±0.08	13.75±0.05	17.08±1.01
9-Hexadecenoic acid	Palmitoleic acid	16	0±0.0001	7.81±0.01	15.47±0.02	12.08±0.004
9-Octadecenoic acid	Oleic acid	18	20.8±0.04	31.52±1.02	28.78±0.2	40.12±1.58
Octadecanoic acid	Stearic acid	18	12.81±0.1	23.33±0.1	0.23±0.003	0±0.0004
9,12-Octadecadienoic acid	Linoleic acid	18	12.37±0.003	12.18±0.02	0±0.0001	0±0.0001
5,8,11,14-Eicosatetranoic acid	Arachidonic acid	20	12.36±0.01	0±0.0001	8.76±0.02	0±0.0002
Docosanoic acid	Behenic acid	22	3.54±0.02	0±0.0002	0±0.0001	5.78±0.12
Tetracosanoic acid	Lignoceric acid	24	2.4±0.02	1.59±0.04	0±0.0002	3.78±1.02
Docosatetraenoic acid	Adrenic acid	22	9.21±1.2	2.21±0.2	1.14±0.12	4.52±1.55
Docosahexaenoic acid	Cervonic acid	22	1.42±1.01	1.11±0.05	10.01±0.7	5.03±0.96

Data represents mean ± SD of 3 independent experiments P≤ 0.05 vs. control; Tukey's post-test.
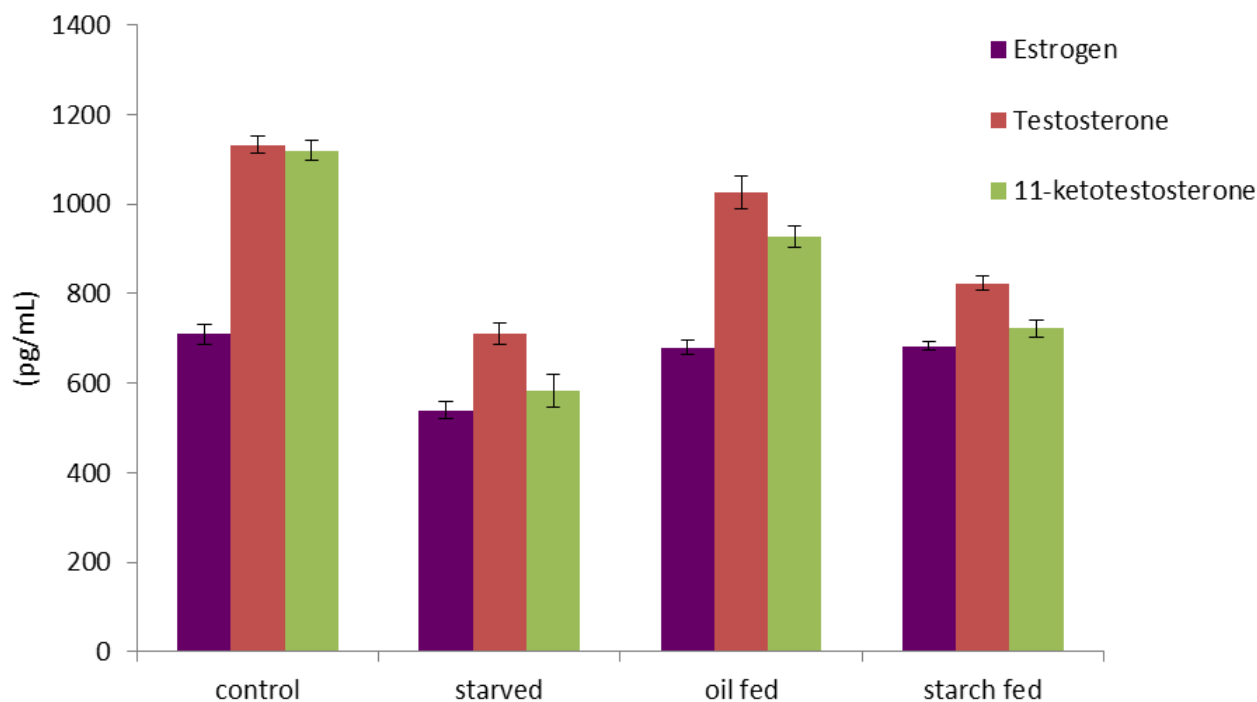

Figure 7. Steroid sex hormones of the selectively fed rats. Estradiol, testosterone, and 11-ketotestosterone are decreased in the starved, oil fed and starch fed groups when compared with the control.

mias, impaired cognitive function and alteration in sex steroidal hormones. Several animal and human studies have confirmed the hypercholes-terolemic properties of saturated fatty acids and cholesterol which include increasing total cholesterol and altering lipoprotein pattern and whose mechanism remain under study (Enos et al., 2013). High fat diets are strongly linked with the accumulation of excess body fat, chronic inflammation, and metabolic perturbations, ultimately leading to poorer

health outcomes (Bhargava and Lee, 2012) most of the available supporting literature is limited by the lack of control for various nutrients (for example, protein:carbohydrate:fat among others). We examined the effect of different dietary regimen on lipid profile changes in serum, brain and liver, metabolism. From the results of the daily growth and average condition factor carried out in this study, the control group exhibited a non-significantly higher daily growth rate in mass per day

compared to the other two dietary (starch enriched and soy oil enriched) groups, but there was a significant decline in weight in the group fed with carboxyl methyl cellulose, (starved) when compared with the control. The significant decline in the growth rate of the CMC fed rat might be attributed to unavailability of food for synthesis of building blocks for healthy living. The energy needs of the brain are supplied by metabolism of glucose and oxygen for the phosphorylation of adenosine di phosphate (ADP) to adenosine tri phosphate (ATP). Most of the ATP generated is used in the brain in maintaining intracellular homeostasis and trans- membrane ion gradients of sodium, potassium, and calcium. Energy failure results in rapid loss of ATP and uncontrolled leakage of ions across the cell membrane that results in membrane depolarization (Adibhatla and Hatcher, 2005).

Furthermore, increased total serum cholesterol and LDL-cholesterol in the starved rat is most likely due to the degradation of the energy stores (in form of glucose, amino acids and fats) since the breakdown of these store of energy produces acetyl CoA while releasing energy in form of ATP. This acetyl CoA is a precursor to cholesterol synthesis and it can also be used in the synthesis of new fatty acids but the reduction in the amount of triglyceride noticed in these starved rat and because free unesterified fatty acids are released from triglycerides during fasting to provide a source of energy and of structural components for cells, the possibility of fatty acid synthesis is ruled out in the starved rat, thus, the acetyl CoA produce may have been shuttled to cholesterol synthesis.

Dietary saturated fat intake has been associated with an increased risk of atherosclerotic cardiovascular disease and metabolic diseases, such as obesity and type 2 diabetes. This effect is thought to be mediated by an increase in plasma cholesterol, mainly low-density lipoprotein cholesterol l (Joan et al., 2011). This may further butress why triglycerides levels are also reduced in the starch fed and lipid fed rats.

However, acetyl CoA is also formed during degradation of carbohydrates, it is understandable how an excess of carbohydrates can result in the formation of fats, which can be kept in the adipose tissue (Tobias and Robert, 2012). This could account for the significant increase in the HDL-cholesterol of the starch fed group. Strong evidence supports a HDL-C-raising effect of diets enriched in saturated fat and cholesterol in humans (Kotseva et al., 2008).

In a well fed animal, the glycerol, fatty acids, mono- and di-glycerides resulting from digestion pass across the intestinal membrane and are resynthesized to triglyceride in the epithelial cells, therefore poor dieting may also account for the reduced levels of triglycerides in the starved, starch fed and lipid fed groups. Also the rate of absorption of the glycerides may differ according to diet composition of ingested lipids. Positive associations between cholesteryl ester transfer protein activity, LDL-cholesterol concentrations were found in animals fed a diet high in saturated fat. The changes in LDL cholesterol was explained by diet-induced down-regulation of LDL receptor activity through cleavage inhibition of the precursor protein (sterol regulatory element binding protein (Lars et al., 2007). This chain of events would allow for less hepatic clearance of LDL cholesterol. Overall, this relation is of interest, but its mechanism requires further elucidation, particularly during weight loss.

From the results TLC analyses, the polar lipids that were tentatively identified were designated DPG, PC, PS, CL in order of decreasing polarity. Neutral lipids were also present in all the four groups and was designated NL at the solvent front on the TLC plate. The polar lipids from starch fed rats seemed to be almost identical with that of the Lipid group with significant differences only in the proportion of the components. The polar lipids from the starved rats seemed to be almost identical with that of the control group also with significant differences in the proportion of the components. DPG was much more pronounced in starch fed rats and lipid fed rats when compared with the control group. The similarities in the type of lipid identified in all the four groups supported previous reports on the general similarities of membrane and tissue lipids of different cells (Edward et al., 2010).

GC-MS results that show alterations in the feeding pattern of the albino rats has led to a marked imbalance in the ratio of saturated to unsaturated fatty acids in the carbohydrate fed rats, lipid fed rats and CMC fed rats when compared with the control. The major fatty acids produced by the CMC fed rats were the saturated fatty acids; there was only a trace of unsaturated fatty acid in the brain of the rats in this group. It can be inferred that diet may be a major determinant in the balance of saturated to unsaturated fatty acids in animals. Convincing data exist that show the ability of dietary saturated fatty acids to increase LDL cholesterol in animals and humans, also showing how mono-unsaturated fatty acids, carbohydrate and saturated fatty acids influence lipid profiles (Lars et al., 2007).

The marked reduction in unsaturated and poly unsaturated fatty acids in the starved rats could mean that organisms under stress may not be able to produce the essential fatty acids needed for healthy living and reproduction. The marked reduction of the unsaturated and polyunsaturated fatty acids could also be termed as one of the ways by which organisms adapt to stress and starvation. There was also an increase in the amount of polyunsaturated fatty acid in the oil fed when compared with the control. The starch fed group had approximately the same percentage of saturated fatty acid with the control group but the animals had most of the remaining fatty acids in the saturated form and only about 20% of polyunsaturated fatty acids. The differences in the amount of these varying fatty acids could be as a result of adjusting membrane saturation in the brain of these animals. Cognitive impairment is a major component of

dementing syndromes and influences the individual's ability to function independently (Crichton et al., 2010). Alzheimer's disease (AD), the most common form of dementia, is a progressive brain disorder affecting regions that control memory and cognitive functions, gradually destroying a person's memory and ability to learn, reason, communicate and carry out daily activities (Rao and Hatcher, 2007). Altered lipid metabolism is also believed to be a key event which contributes to CNS injury (Adibhatla et al., 2006). Adjusting membrane saturation induced by alteration in feeding pattern may be a contributory factor in the reduction of the cognitive performance of the CMC fed rats when compared to the control. This can also be correlated to results of growth rate and condition factor. High LDL and total cholesterol levels are also associated with cognitive impairment (Tristano et al., 2013).

Based on the fact that nutrition has a significant impact on the listed factors for cognitive dysfunction (for example, cardiac function) (Lemon et al., 2010), poor dietary habits may also contribute to poor cognitive performance. In turn, poor diet (for example, lack of fruits and vegetables, high fat and sodium intake) has been linked with reduced cognitive function among persons with medical illnesses that are frequently comorbid with heart failure. For instance, lack of fruits and vegetables, reduced adherence to Mediterranean diets, and diets high in fat intake have been shown to reduced cognitive functioning in vascular dementia, type 2 diabetes, Alzheimer's disease, and mild cognitive impairment (Crichton et al., 2010; Devore et al., 2009; Barberger et al., 2007).

The liver was observed to have appreciable more fatty acids than the brain, this may be due to the central role the liver plays in fatty acid metabolism. Next to adipose tissue, liver has the greatest capacity to store lipids in lipid droplets (Tobias and Robert, 2012).The adaptation of the liver to the periods following meals and the periods of fasting may be easy to upset by nutrition. Lauric acid, which is a 12-carbon straight chain fatty acid, was observed to be the predominant fatty acid in the brain of the starved rat as opposed to the complete absence in the control. This may be as a result of a breakdown by β-oxidation of the longer chain and unsaturated fatty acids that may have been stored in the starved rats. The predominance of lauric acid may also be as a result of the body scavenging for the available unsaturated and longer chain fatty acids from the animal in order to break it down and generate energy since fats are high-energy stores (Tobias and Robert, 2012). Linoleic acid which is a precursor to the synthesis of arachidonic acid has been found to be completely absent also in the brain of the starved rat, linoleic and arachidonic acid have been implicated during early brain development as well as during the development of late life cognitive decline and dementia (Innis, 2008; Oken et al., 2008; Boudrault et al., 2009; Das, 2008) since animals are expected to get linoleic acid from the diet (Muldoon et al., 2010); it is not

surprising that the starved animal lacked this essential fatty acid. The starved animals were observed to be lean and wrinkled; this skin trouble may have been as a result of a deficiency in the essential fatty acids. Estrogen, testosterone, and 11- ketotestosterone were decreased in the starved, oil fed and starch fed groups when compared with the control, but the marked decrease was more pronounced in the starved animal. The reduction of unsaturated fatty acids may be linked to this reduction in sex steroidal hormones since vitamin E deficiency has been observed in certain people suffering from congenital problems of fat absorption and since the requirement in man is a function of diet (Tobias and Robort, 2012; Vaisman et al., 2008).

Sex steroid hormones such as estradiol-17β and testosterone are particularly important in regulating the development and function of reproductive activity in mammals (Gallagher et al., 2001). In addition, 11-ketotestosterone is considered to be a dominant androgen in males and responsible for the development of the testis and sexual behavior (Cuisset et al., 2011). Due to the importance in reproduction and the fact their level can be quantitated in the plasma of the vertebrates, sex steroid profile can be used as biomarkers of altered-feeding induced reproductive stress. Although measure of plasma concentrations of sex steroids indicates the overall effects on circulating steroid concentration, they do not provide information as to the mechanism underlying how different feeding pattern exerts their effects. Hormones alteration can be caused by a number of factors, including changes in hormone synthesis, secretion, metabolism, hepatic catabolism or binding to plasma proteins. In general, increased in plasma steroid in others over the starved group can be the result of increased steroid biosynthesis or decreased hepatic catabolism by biotransformation enzymes. In sum, feeding rats on a diet rich in soy oil and carbohydrate for a 14-day period produced marked alterations in lipid profile in serum and tissues, as well as modifications in steroidal hormones.

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