Effect of Transcutaneous Electrical Stimulation of the Lower Abdominal Muscles on Degree of Hepatic Fatty Infiltration

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Abstract: Fatty liver is one of the most common liver diseases associated with easy fatigability. Dietary regime or exercise is one of the modalities helping in its treatment. Due to the exhausting physical activity and crash dieting together with the emergence of fast and easy methods for electrical muscles stimulation, the present study aimed to probe the response of fatty liver to this type of exercise. Male albino rats were divided into three groups. Group I-control rats, group II-fructose-fed rats for induction of fatty liver for five weeks (FR) and group III-fructose fed with electrical muscle stimulation (FR+EMS) of the lower abdominal muscles in the last three weeks of feeding. Electrical muscle stimulation was 45min/session, 5days/week. At the end of the study, final B.W, serum ALT& AST, plasma total protein and albumin were determined. Also, fasting glucose, fasting insulin, insulin resistance score (HMAS), serum TG and TC were determined. Both retroperitoneal fat bad & liver weights and their ratio to body weight as well as hepatic tissue content of TG, MDA and GSH all were determined. In addition microscopic examination of the hepatic tissue was done. Results: the fructose enriched-diet, group II, showed significant increase in fasting glucose, fasting insulin and elevated insulin resistant score. In addition serum AST, ALT, TG and hepatic tissue TG were significantly increased while total proteins and GSH were significantly reduced. The liver weight/body weight ratio was increased, while body weight and weight of retroperitoneal fat bud, plasma albumin and tissue MDA, all were non-significantly differing from that of control. Histopathological examination revealed periportal steatosis (zone 1&2), portal congestion and inflammatory cells infiltration. In group (FR+EMS), fasting blood glucose, fasting insulin and insulin resistance score all were decreased compared to (FR)group, but their values still higher compared to normal control. The weight of retroperitoneal fat bud and its ratio to body weight were decreased significantly. However, liver weight, its ratio to body weight hepatic TG content and liver enzymes AST, ALT all were increased non-significantly compared to (FR) rats. Hepatic tissue MDA was significantly increased while GSH was decreased non-significantly. Microscopic examination revealed more marked steatosis to include all zones of hepatic lobule. Conclusion: EMS stimulation improved partially the fructose-fed associated insulin resistance but it increased hepatic steatosis. This modality of exercise is suggested to increase rate of lipolysis in particular in abdominal area causing increased fatty acids influx to the liver and/ or the rate of fatty acid oxidation did not match the rate of lipolysis compared to other types of exercise.

Key words: fatty liver - transcutaneous electrical muscle stimulation- fructose enriched diet.

INTRODUCTION

Electrical muscle stimulation is a widely accepted therapeutic tool for muscle rehabilitation and recovery. In the sedentary society with, the emergence of mass produced portable battery operated systems and the simple operating instruction designed for individual home use, a variety of systems with various applications for toning, strengthening, body shaping and general fitness become available. In addition, because of the exhausting physical activity and crash dieting, the benefits of electrical stimulation became apparent, as it provides a safe, fast and effective method for exercising (Vrbova, et al., 2008).

Transcutaneous electrical stimulation in normal innervated muscle is achieved by excitation of the nerve rather than by excitation of the muscle directly. This is because nerve fibers and neurofibrils, which are true communicators of the stimulation current to the muscle cells, having a lower threshold to stimulation compared to muscle (Nelson, et al., 1999; Kahn, et al., 2000).
Despite in local electrical muscle stimulation, compared to general exercise regime, the contracting muscle mass is small and its contraction is not implemented in joint flexibility and muscle lengthening (Pette and Vrbová, 1999) but, it causes early contraction of the large motor units in the muscle and so stronger contraction (30% more powerful) with tendency for vascular modification and conversion of muscle fiber from fast to slow and fatigue-resistant one (Windisch, et al., 1998; Rana, et al., 2009). Thus, this stronger contraction may consume more energy.

Also electrical muscle stimulation can attain much higher levels of activity over time than any exercise regime because the central nervous, cardiovascular, and other systems will not interfere with and limit the amount of activity, as is the case in exercise (Petter and Vrbova 1999; Banerjee et al., 2005).

Exercise is a major challenge for other organs, particularly for the liver due to its central role in glucose and lipid homeostasis and its function as energy supplier for the working muscle. At the same time, fatty liver disease is the most common cause of chronic liver diseases (Duvnjak, et al., 2009). It is a component of metabolic syndrome where obesity, type 2 diabetes mellitus, and hyperlipidemia are coexisting conditions (Chitturi et al., 2004; Botezelli et al., 2010). There are multiple reports providing evidence for the role of active exercise in the regulation of hepatic metabolic enzymes such as lipoprotein lipase to deliver free fatty acids from its TG stores to supply the working muscle with energy (van der Heijden et al., 2010; Schoiswohl et al., 2010; Hoene, et al., 2010). Common protocol of exercise such as swimming showed to counteract fatty liver disease in rats and to improve insulin signaling (Botezelli, et al., 2010; Ropelle, et al., 2009). Also, aerobic exercise program showed to reduce hepatic fat accumulation and insulin resistance in obese adolescent’s girls (van der Heijden, et al., 2010). However, it is questionable whether all models of physical exercise have the same beneficial effects on adiposity, fatty liver, lipid profile, and body weight.

In addition, as easy fatigability is a common finding with fatty liver, this study tried to evaluate the ability of transcutaneous electrical stimulation, of the lower abdominal muscle, (where it is common to find fat deposition) to improve the fatty liver and its associated metabolic consequences.

MATERIAL AND METHODS

Animals:

Male albino rats (140-160 gm) were used in this study. They were purchased from local farm in Helwan and maintained in the hold facilities in Physiology Department, College of Medicine, Ain Shams University, Egypt, under standard condition of boarding with free access to their prepared food and tap water.

Experimental Groups and Food Design:

At the beginning of the study, rats were weighed, their height was determined from the nose to the anus (the height and weight were used for calculation of body mass index, the weight in gram was divided by the square height in cm²) (Novelli, et al., 2007). The animals were randomly divided into 3 groups.

Experimental Groups:

1. Group I- control rats.
2. Group II- fructose- fed rats for induction of fatty liver for five weeks (FR).
3. Group III- fructose fed with electrical muscle stimulation (FR+EMS) of the lower abdominal muscles in the last three weeks of feeding.

Diet Formulation:

The composition of food presented to control was Corn starch enriched diet (CSED) which was similar to the fructose enriched diet (FRED) that was presented to the other two groups except for fructose which was replaced by corn starch. The Fructose enriched diet (FRED) consisted of fructose powder 610 g (Uni Fructose, from Uni Pharma, Egypt), skim milk powder (casein,200g), wheat bran (96 g), peanut oil (50g), salt(35 g), L-Methionin (7g), vitamin fortification mixture 2g / kg of food (Multi-Sanostol, Chemipharm, each 10ml contains: vitamins A 2400 IU/g, D3 200 IU/g, B1 2mg, B2 2.0mg, B6 1.0 mg, B12 5μg/g, C 100mg, E 2mg/g, nicotinamide 10mg and other minerals). In addition, cellulose in form of vegetables was introduced to all groups and their food consumption was observed daily (Patel et al 2009).
**Transcutaneous Electrical Muscle Stimulation:**

In group III (FR+EMS), the rats were subjected to bilateral transcutaneous electrical stimulation of the lower abdominal muscles. The rats were first allowed to adapt to the sensation associating the electric stimulation of the muscle and the abdominal muscle was prepared for the protocol of exercise. The adaptation was performed throughout 5 sessions /week in the first two weeks starting by one minute /session then the duration and intensity gradually increased. Mild anesthesia was used in first 2-3 sessions, then the rats showed adaptation to the sensation of muscle stimulation in the following sessions. The purpose of the adaptation was to reduce the stress of the animals without promoting the physiological changes that might arise from the physical training. Then 5 sessions per week, 45 minutes for each session throughout the last 3 weeks was applied. Alpha wave healthtronic device (model B.B-1006) was used to produce passive exercise by sending electrical impulses or signals to the selected muscle or muscle group to contract and relax. This exercise cycle is repeated automatically once every two seconds, where the frequency was adjusted at intermittent position. Each device has three pair of leads, each pair of leads was connected and covered with wet cotton and applied bilaterally to the lower abdominal muscle (Rectus abdominus and External oblique) of fixed rats under mild anesthesia using ether. The power knob turned slowly clockwise and the leads moved over the muscle searching for the motor endpoint, where better response occur and rhythmical muscle movement felt and seen. The leads fixed in its places by cotton coated plaster straps (EL-Kafoury and Abdel-Rahman, 2004).

**Collection of Blood Samples:**

At the end of the study and after 12 h of overnight fasting, the rats were anesthetized using pentobarbital sodium in a dose 40mg / kg B.W. intraperitoneally and final body weight and height were recorded for all rats. Tail blood sample was used for fasting blood glucose level determination in whole blood using the On-Call EZ blood glucose meters and its corresponding strips On-Call Plus (Acon Laboratories, Inc.) purchased from United Co. for Biological Industries.

Then, blood samples were taken from the abdominal aorta. For each rat, the first blood sample was collected in dry test tube and left for one hour at room temperature until complete clotting. Clotted blood samples were subjected to centrifugation for 15 min at 4000 rpm to separate serum. Serum samples was stored at -20 C° for further analysis (serum TG, TC, AST, ALT and insulin).

Another blood sample was taken in a heparinized tube and centrifuged for separation of plasma at 4000 rpm for 10 min. plasma stored at -20C° for total plasma protein and plasma albumin determination.

**Measurement of TC and TG:**

Serum TC and TG were determined by enzymatic colorimetric methods at 500nm, using kite provided by Stanbio, USA for; total cholesterol level (Richmond, et al., 1973) and triglyceride level (Siedel, et al., 1983).

**Determination of Liver Enzyme Activity:**

Aspartate amino transferase (AST and alanine amino transferase (ALT) were measured colorimetrically (Rietman and Frankel 1957, 1957). Commercial kits obtained from Bio labo SA, France were used.

**Plasma Albumin:**

was determined in plasma by colorimetric BCG method according to (Doumas, et al., 1971) using kits supplied by Biolabo SA, France. Absorbance was read at 630 nm using UNICO 7200 Series spectrophotometer (Shanghai Instrument CO., Ltd., China)

**Plasma Total Proteins:**

were measured in plasma by colorimetric Biuret method described by Gornall et al., (1949) using kits supplied by Biolabo SA, France. Absorbance was read at 550 nm using UNICO 7200 Series spectrophotometer (Shanghai Instrument CO., Ltd., China)

**Determination of Insulin and Insulin Resistance Score:**

-Serum Insulin was determined by Insulin (ELISA) kit which was supplied by United Biotech Inc., USA. (Clark and Hales et al., 1991) -Insulin resistance was calculated by the Homeostasis Model Assessment Score HMAS [fasting plasma insulin (µU/mL) x fasting plasma glucose (mmol/L)/22.5] according to Bonora et al., (2000).

After taking the blood samples the bilateral retroperitoneal fat bud was excised, washed by saline, dried using filter paper and weighed. The weight of retroperitoneal fat to the body weight was later on calculated. Lastly the liver tissue was excised.
Liver Tissue Handling:
The liver tissue dissected carefully, rinsed with ice cold saline, examined gently for macroscopic fatty appearance and then weighed after brought dry using filter paper. The coefficient of hepatic weight was calculated where liver weight (gm) was divided by body weight (100 g). Lastly, the hepatic tissue was divided rapidly in ice-cold saline into three samples. Two of the samples were wrapped in Para film layer and were stored at -80 C°. The first sample used for measurement of hepatic TG, and the second one for hepatic MDA and reduced glutathione measurement. The third sample placed in 10% formalin for histological examination. On preparation of tissue homogenate the liver pieces allowed to thaw.

Measurement of TG in Rat Liver Tissue:
The hepatic lipid was extracted from the liver tissue using a chloroform/ methanol mixed solution (2/1, v/v) in a ratio 1 gm liver tissue to 5 ml solution. The homogenization was done for 3 minutes using Ultrasonic homogenizer 4710 series, Chicago. The homogenate was filtered using filter paper, and as the extract now contain lipid in lower phase and non lipid on upper phase, the extract was mixed thoroughly with 0.2 its volume of water(water washing ) to remove non lipid substance. The extract plus water was subjected for centrifugation at 2400rpm for 20 minutes. The supernatant was removed by pipette and the lipid content was measured in the lower phase (Folch, et al., 1957) according to colorimetric methods as that used for serum TG assay.

Measurement of MDA and Reduced Glutathione in Liver Tissue:
For MDA determination liver tissue homogenized in 5 ml cold buffer (50Mm potassium phosphate, PH7.5) per gram liver tissue using tissue homogenizer (IK-A-WERK, Ultra-Turrax, West Germany) at 4000 rpm for 15 min (10%, w/v).For reduced glutathione (GSH) the buffer was (50 mM potassium phosphate, PH7.5 plus1Mm EDTA).
The obtained supernatants were used for colorimetric assay. Reduced glutathione (GSH) was measured at absorbance wave length 405nm (Beutler, et al., 1963) and Malondialdhyde (MDA) at absorbance 543nm (Ohkawa, et al., 1979). The kits provided by Bio-diagnostic-Egypt.

Histopathology:
For the histo-pathological study, rat liver specimens were fixed in 10% buffered formalin and processed for embedding in paraffin blocks. Sections (4-6 um thick) were cut and stained with Hematoxyline and Eosin (HE) for histo-pathological examination under light microscope (Bancroft, et al., 2008).
The degree of steatosis is generally determined by evaluating the proportion of hepatocytes containing fat droplets counted in 40X fields. The acinar architecture was followed and the liver parenchyma was divided in thirds and the percentage involvement by steatotic hepatocytes was assessed. (grade 0, < 5%), (grade 1,mild, 5%-33%), (grade 3, moderate, 33%-66%) or (grade 4, sever, > 66%).
Also, scoring for inflammation according to the presence of inflammatory cells was done (0: non, 1: <2, 2: 2-4, 3: > 4). Moreover, hepatocyte ballooning score was estimated (0: None, 1: Few ballooned cells 2: Many ballooned cells) (Kleiner, et al., 29). The histological evaluation of the liver sections was performed blindly.

Statistical Analysis:
Results were expressed as mean ± SEM. The statistical significance of differences between means was determined by Student’s ‘t’ test for unpaired data at a level of significance P <0.05.All Statistical data, statistical significance and correlation study were analyzed by ANOVA, using SPSS program (Statistical Progression for Social Science) statistical package (SPSS Inc.) version 8.0.1

Results:
Changes in the Weights Of, Body, Liver and Retroperitoneal Fat Bud:
As shown in table (1), the fructose fed rats (FR) failed to gain weight compared to normal control (% of change in their weight was 8.49 ± 1.5% versus 32.49± 1.5% in control rats). Also, the electric muscle stimulated fructose -fed rats (FR+EMS) failed to gain weight although they exhibited increased food consumption (% of change in their weight was 16.33 ±4.6%).
In addition, the absolute liver weight and liver weight /body weight ratio were significantly increased in fructose fed rats (FR ) and electric muscle stimulated fructose -fed rats (FR+EMS) compared to control group. (p<0.001for all). in FR+EMS group, the weight of retroperitoneal fat bud was significantly reduced compared
Changes in Blood Glucose, Insulin and Insulin Resistance Score:
As shown in table (2) and figure (1, A, B & C) in the fructose fed rats (FR) the fasting blood glucose, fasting insulin and insulin resistance score (HOMA) all, were significantly (p<0.001 for all ) increased compared to control group I. On the other hand, the rats in the electric muscle stimulation group III (FR+EMS) exhibited significant and partial decrease in all of these parameters compared to FR group (p< 0.05, p<0.001 for glucose, insulin and HOMA score respectively). But, they still have significant higher values (p<0.01, p<0.05 & p<0.01 respectively) compared to control group I.

Changes in the Liver Related Parameters:
As shown in table (2) and figure (2, A, B&C), the fructose fed rats (FR) showed significant increase in both AST&ALT serum levels (p<0.001 & p<0.01 respectively) and significant (p<0.05) decrease in total plasma proteins compared to control group. The plasma albumin levels did not differ significantly from those of control. On the other hand, the FR+EMS group showed also significant higher AST&ALT compared to control (p<0.001 & p<0.05 respectively) and higher though non significant compared to FR group. The total plasma protein and plasma albumin all, were non- significantly differ from control or FR group.

Changes in Lipid Profile:
As shown in table (2) the serum TG showed significant increase (p<0.05) and serum TC increased non-significantly in fructose fed rats (FR) compared to control group. In FR+EMS, the serum TG was significantly increased compared to control (p<0.05) and increased non-significantly compared to FR group, while the TC level were non- significantly differ from both control and FR group.
Fig. 1: Changes in blood glucose, insulin and insulin resistance score (HOMA) in the different studied groups.

Fig. 2: Changes in liver related parameters in the different studied groups.

Fig. 3: Changes in hepatic tissue content of triglyceride (TG), MDA & GSH content.

**Correlation Analysis:**

As shown in table (3-a), in fructose fed rats the TG content of the hepatic tissue showed significant positive correlation with insulin level ($r=0.996$, $p<0.001$, $n=6$) and significant positive correlation with weight of retroperitoneal fat bud ($r=0.815$, $p<0.04$, $n=6$). Liver TG content in FR group showed also positive non significant correlation with liver weight, liver weight /body weight ratio, serum ALT and HOMA score. In addition, retroperitoneal fat showed positive significant correlation with insulin level ($r=0.825$, $p<0.043$, $n=6$ non -tabulated).
As shown in table (3-b), in FR+EMS the TG content of hepatic tissue showed positive non significant correlation with insulin and MDA content of the liver. On the other hand hepatic TG content showed negative non- significant correlation with GSH content of hepatic tissue.

Table 3-a: correlation analysis in fructose-fed group (FR).

<table>
<thead>
<tr>
<th>Liver Weight (gm)</th>
<th>Liver weight/body weight ratio</th>
<th>Weight of retroperitoneal Fat bud (gm/100gm)</th>
<th>Insulin (u mol/L)</th>
<th>HOMA score</th>
<th>ALT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver TG r=</td>
<td>+0.735</td>
<td>+0.527</td>
<td>+0.815*</td>
<td>+0.996*</td>
<td>+0.751</td>
</tr>
<tr>
<td>p=</td>
<td>0.084</td>
<td>0.283</td>
<td>0.04</td>
<td>&lt;0.001</td>
<td>0.085</td>
</tr>
<tr>
<td>N=</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 3-b: correlation analysis in FR+EMS.

<table>
<thead>
<tr>
<th>Insulin (u mol/L)</th>
<th>MDA (nmol/g)</th>
<th>GSH (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver TG r=</td>
<td>-0.778</td>
<td>-0.627</td>
</tr>
<tr>
<td>p=</td>
<td>0.069</td>
<td>0.183</td>
</tr>
<tr>
<td>N=</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Histo-pathological Results:

Microscopic examination of the hematoxyline and eosin stained sections from liver tissues in control group (Figure-4) showed a normal morphology with the classic hepatic lobule traversed by central vein “C” and peripherally - situated portal triad “P” containing bile duct and branches of hepatic artery and portal vein (figure 1-A, B). Between the lamina (sheets) of hepatic cells are venous sinusoids (S) radiating from the central vein towards the periphery. The liver cells posses distinct cell outline, prominent darkly staining nuclei and eosinophilic cytoplasm (Figure 4-C, D).

As shown in table (3), calculating the score for both steatosis and hepatocytes ballooning it was grade zero for both in 6/6 of the rats while the inflammation score was grade zero in 5/6 of the rats and grade 1 in 1/6 of the control rats.

Grossly, in the fructose fed rats, and in fructose fed plus electrical muscle stimulation, the liver appeared enlarged with lightening in color.

In addition, microscopic examination as shown in figure (5), revealed that in fructose fed rats zone 3 surrounding central vein showed less fatty degeneration and preserved eosinophilic cytoplasm (5-A,B,C,D,E&F). On the other hand steatosis was very diffuse in zones 1,2 near to the portal vein in almost all rats (5-E &G) with little fatty infiltration (microvesicular) in zone 3 in 2 rats from six. (5-H). In the periportal zones microvesicular and macrovesicular steatosis with distortion of the nucleus were seen with observation of vaculated cells where the lipid content has been cleared during histological fixation. In addition, the livers of fructose fed rats showed mixed inflammatory cells infiltration around the central vein, in the portal areas and in the blood sinusoids with minimal signs of peri-sinusoidal fibrosis.

From table (4), calculating the score for steatosis, it was grade 3 in 4/6 of the rats (moderate=33%-66%) and grade 4 in 2/6 of the rats (sever = > 66%). The inflammatory score was 3 in all of the rats (> 4 inflammatory cells) and the hepatocyte ballooning score was 2 (many ballooned cell).

Regarding fatty liver changes in fructose fed rats plus transcutaneous electric muscle stimulation (FR+EMS), figure (6) showed the white appearance of the hepatocytes lobules in the small magnification overcoming the eosinophilic appearance (fig 6-A&B). In addition hepatocytes ballooning & inflammatory cells infiltration were observed markedly (6-C). Portal congestion (6-D), macrovesicular and microvesicular steatosis all over the three hepatic zones that reach to zone 3 around central vein were also recorded (6-E &F).

Table 4: Grading of histopathological changes of the liver, examined by hematoxylin and eosin, in the different studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Steatosis score</th>
<th>Inflammatory Score</th>
<th>Hepatic ballooning score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0   1  2  3  4</td>
<td>0   1  2  3</td>
<td>0   1  2</td>
</tr>
<tr>
<td>Normal control  (6)</td>
<td>6 - - - -</td>
<td>5  - - -</td>
<td>6 - - -</td>
</tr>
<tr>
<td>FFR (6)</td>
<td>- - - 4 2</td>
<td>- - - 6</td>
<td>- - -</td>
</tr>
<tr>
<td>FR+EMS(6)</td>
<td>- - - 6</td>
<td>- - - 6</td>
<td>- - -</td>
</tr>
</tbody>
</table>

In between bracket: number of studied rats in each group.

-Steatotic score : (grade 0, < 5%), (grade 1, mild, 5%-33%), (grade 3, moderate,33%- 66%) and (grade 4, sever,> 66% ).
-Scoring for inflammation according to the presence of inflammatory cells: (0: non, 1: <2, 2: 2-4, 3: > 4).
-Hepatocyte ballooning score (0: None, 1: Few ballooned cells 2: Many ballooned cells). (Kleiner et al., 2005)
Discussion:

In human and rats compared to other species there is no conversion of fructose to glucose in the intestine during absorption due to lack of converting enzymes. That is why fructose (in fruits and honey) was initially thought to be advisable for patients with diabetes due to its low glycemic index (it is about 23% compared to 100% in glucose) (Mayes PA, 1993; Ackerman et al., 2005). Fructose is highly extracted by the liver from the portal circulation via the glucose transporters-5 (GLUT5) in an insulin-independent mechanism. Fructose is immediately converted into pyruvate and lactate that enter the mitochondria, increasing acetyl COA and lipogenesis. It caused shift in the balance from oxidation to estrification of non estrified fatty acids resulting in increase of lipogenesis (Mayes PA, 1993; Tappy, and Lê KA, et al., 2010; Botezelli, et al., 2010).

In contrast to glucose, fructose in the liver cant be stored as glycogen and only stored as lipid. This characteristic makes fructose a highly lipogenic nutrient (Basciano, et al., 2005). Thus, rats fed this type of food have been used as an experimental model of human metabolic syndrome in which Non-alcoholic fatty liver disease is emerging as an acknowledged component (Botezelli, et al., 2010; Basciano, et al., 2005).
Fig. 5: Photomicrographs in fructose-fed rats. the zone 3 surrounding central vein showed less fatty degeneration and preserved eosinophilic cytoplasm (5-A,B,C,D,E&F). Steatosis was marked and restricted to zones 1, 2 near to the portal vein in almost all rats with inflammatory cells infiltration (I) (5-E &G). Little fatty infiltration in zone 3 appeared in 2 rats from six.(5-H).

In the present study, in fructose fed (group II) a lesser gain in body weight was noticed even when these rats and the control animals displayed comparable body weight at the onset of the period. These findings are in line with other findings in relatively short studies (3-6 weeks) (Cancelas, et al., 2008; Axelsen, et al., 2010). Our results did not corroborate with other studies that used fructose-rich diets to induce obesity and overweight in rats which seem to be dependent on rodent strain and on the administration period. The presence of fatty liver without overweight has been reported in human (St George, et al., 2009) and in the presence of insulin resistance overweight is not a must finding (Kotronen, et al., 2008).

The non significant change in adipose tissue mass (retroperitoneal) could be explained by shift of Lipogenesis to the liver rather than to adipose tissue particularly with shorter duration in rats. Fructose feeding activates lipogenic enzymes such as fatty acid synthase and malic enzyme in the liver but not in the adipose tissue and depresses conversion of glucose to lipids in adipose tissue, nevertheless, a recent study demonstrated that very long periods(6 months) on high fructose enriched diet might increase adipose tissue fat in rats (Abdel-Sayed et al., 2008).
The increased blood glucose level and the hyperinsulinaemia in fructose enriched group II suggests impaired insulin action which could be supported by the associated higher insulin resistance score (HOMA score). Resistance to insulin has been implicated in accumulation of hepatic TG (Bugianesi, et al., 2005) and hence steatosis in both rodents (Samuel, et al., 2004) and humans (Seppala-Lindoos, et al., 2002). The increased glucose, insulin and HOMA score were previously observed with fructose enriched diet and drinking water in rats (Cancelas, et al., 2008; Kannappan, et al., 2006; Kawasaki, et al., 2009).

Taking in consideration that fructose unlike glucose as when it reaches the blood stream it does not stimulate insulin secretion by pancreas beta cells and it is converted mainly to TG and could not be converted into glycogen (Botezelli, et al., 2010) these data refer to the presence of another mechanism by which fructose diet leads to hyperglycemia and increased insulin level.

It was reported before that excessive intrahepatic TG release fatty acids into the cytoplasm which can cause hepatic insulin resistance and inflammation. The localized intrahepatic inflammation can contribute to peripheral insulin resistance as a fatty liver secretes cytokines like TNF α into the systemic circulation that can cause peripheral insulin resistance. In addition, the high circulating TG and fatty acids thought to affect the insulin receptors with alterations in the insulin signaling cascade (Griffin, et al., 1999; Collison et al, 2009; Korenblat, et al., 2008). In the present study, the positive significant correlation between hepatic TG content and serum insulin level and positive correlation with HOMA score support the role of accumulated hepatic TG in inducing insulin resistance.
Consequently, the assumed hepatic insulin resistance is associated with increased glucose production by the liver via glycogenolysis and gluconeogenesis. Also, skeletal muscle insulin resistance causes decreased glucose uptake and adipose tissue insulin resistance leads to increased rate of lipolysis (Deivanayagam, et al., 2008).

The increased serum and hepatic TG with fructose enriched diet in group II is attributed to the ability of fructose to increase fatty acids esterification in the liver and to decrease its oxidation (Tappy, et al., 2010). This finding is in line with finding of Ackerman et al., (2005).

In addition the increased AST with fructose diet in this study was previously observed in fructose -fed rats (Botezelli, et al., 2010). The increased liver enzyme AST and in particular increased ALT in fructose fed rats is in line with the previous suggestion, where ALT is suggested to be not only an indicator of liver injury due to steatosis but also an early indicator for impaired insulin signaling and insulin resistance (Chang, et al., 2007).

The significant decrease in total protein may be attributed to insulin resistance and decreased influx of amino acids (Richter, et al., 2010).

Also, the macroscopic yellow appearance of liver, increased liver body weight ratio and the histological marked fatty infiltration, microvesicular, macrovesicular steatosis, and higher steatotic score in fructose fed rats (group II) all, indicate success of fructose in induction of fatty liver. High fructose diet in a recent study was associated with higher hepatic TG and increase liver/body weight ratio and increased microvesicular as well as macrovesicular steatosis compared to other types of foods (Kawasaki, et al., 2009). The distribution of macrovesicular steatosis in the perportal area of hepatocytes in this study was similar to a previous study with 10 days fructose diet. It was documented that the main portion of fructose reaching the portal circulation was taken up by perportal hepatocyte (Burns, et al., 2000). Traditionally, excessive intra-hepatic triglycerides or steatosis, has been defined chemically when intrahepatic TG content exceeds 5% of liver volume or liver weight, or histologically when 5% of hepatocytes contain visible intracellular triglycerides (Korenblat, et al., 2008). In this study hepatic TG represented nearly 6.8% of the liver weight and the steatosis score was high.

On the other hand electric muscle stimulated group showed more food consumption but with lesser weight gain which may be explained by the increased energy expenditure by contracting muscle up to 100 times (Gaesser, et al., 1975).

Also, electric muscle stimulation in this study demonstrated decreased retroperitoneal fat bud. In our previous study transcutaneous electric muscle stimulation reduced both, TG in muscle and total fat pad weights and elevated serum FFA indicating higher rate of lipolysis (EL-Kafoury, et al., 2004). This effect is likely the result of the lipolytic effect of nor-epinephrine released by electrically stimulated sympathetic neurons and bound to β1, β2, and β3-adenoreceptors in the innervations territory, which stimulate the fat cells (Dodt, et al., 2000). The abdominal fat has been reported to have rich sympathetic innervations (Boutcher, et al., 2011).

Electrical muscle stimulation resulted in reduced glucose, insulin, as well as insulin resistance score compared to fructose fed sedentary rats but they still having higher values than that of control. This may indicate partial improvement in insulin resistance.

However, electric stimulation in this study is associated with more spread of steatosis reaching zones very near to central vein and marked inflammation. Inflammatory reaction could be detected whether histologically or dictated from increased hepatic tissue MDA content and reduced GSH content.

In addition, electrical muscle stimulation associated with increased hepatic TG content and increased liver weight/bodyweight ratio though non-significantly or it did not reduce them. Thereafter, it could be suggested that exercise in this model improved peripheral insulin resistance rather than hepatic insulin resistance. In other study, swimming exercise 1h/day 5 day/week in fructose fed rats partially but significantly corrected peripheral insulin resistance but not hepatic insulin resistance (Teixeira-Lemos, et al., 2011).

Also, this modality of exercise may be suggested to be an exhaustive modality as it is 30% powerful (Windisch, et al., 1998; Rana, et al., 2009). Endurance exercise where reported before to increase blood flow in the working muscles while it decrease the blood flow in the liver and portal vein which causes damage of the hepatocytes and swelling of the mitochondria in the percentral hepatocytes with increased inflammatory cell infiltration when the exercise stop, a condition explained by ischemic reperfusion injury and free radicals release in liver (Praphatsorna, et al., 2010).

This suggestion may be supported by the histological percentral affection of hepatocytes and more increase in liver enzymes and MDA content even though non-significantly. Also the significant decreased hepatic tissue GSH may indicate exhaustion to meet some sort of oxidative stress.

Although we did not measure free fatty acids in this study, but it could be suggested that in electrically stimulated muscle the rate of lipolysis may increase as it was observed in our previous study where muscle...
TG was decreased while plasma free fatty acids was increased (EL-Kafoury, et al., 2004). Also, fructose in diet reported to increase the release of non esterified fatty acids from adipose tissue as its estirfication in the adipose tissue require glucose, resulting in increased plasma free fatty acids (Mayes P A, 1993; Vrána, et al., 1974). hus, fructose diet and electric muscle stimulation in this study may have additive effects increasing the rate of lipolysis to higher value and may provide the liver with high fatty acid influx and hence steatosis.

On the other hand it is reasonable to ask why these fatty acids were not oxidized by the contracting muscle instead of being directed to liver. In a previous study it was suggested that with 30min of low to moderate intensity exercise in addition to the increased availability of FFAs, the transport of FFAs away from the adipose tissue and toward the exercising muscle increased (Achten, et al., 2004).

Also it is a must to ask why the contracting muscle did not signal to liver to increase the liberation of energy and increasing its TG lipolysis. Previously a comparison of changes in liver and muscle adenine nucleotide concentrations showed that the energy status of the liver is much more sensitive to changes in metabolic demand than that of the muscle (Camacho, et al., 2006).

To answer these questions the increased TG in the liver with EMS of lower abdominal muscle may be explained on basis of a mismatch between lipolysis and fatty acid oxidation resulting in increased free fatty acids influx to the liver. The increased fatty acids influx may be due to: (1) the locality of exercising muscles overlying adipose tissue in abdomen where the latter is easily to affect the liver. it was reported previously that the metabolic products of visceral adipose tissue, including non esterified free fatty acids and glycerol, reach the liver directly and thus could exert disproportionately greater effects on hepatic metabolism (The portal drainage hypothesis) (Shi H, 2007). (Nelson, et al., 1999) The size of the contracting muscle may be not so diffuse in this model compared to other exercise modalities (running or swimming) and so it could not consume more energy. (Kahn, et al., 2000) Fructose diet in the presence of electrical stimulation has an additive effect in increasing FFA and if normal diet instead of fructose was given with exercise it may provide better effect on liver.

**Conclusion:**

This type of exercise may improve insulin resistance partially and body shaping as detected in reduced retroperitoneal body fat. However it may be beneficial in a state of normal liver but it is application on lower abdominal muscle may be harmful in condition of fatty liver in particular with uncontrolled sugary food intake (in fruits).

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**Abbreviations:** Homeostasis Model Assessment Score (HMAS), Malondialdehyde (MDA).

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