Suppression of High Fat Diet-Induced Liver Cell Injury by Swim Exercise

Supresión de Lesión en Células de Hígado Inducida por Dieta Alta en Grasas con Ejercicio de Natación

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SUMMARY: The rapid rise in obesity, particularly among children is a major public health concern that adversely affects vital organs including the liver. We sought to investigate the effect of exercise on the healing of liver cells from damage induced by high fat diet (HFD) in a rat model of hepatic steatosis. Rats were randomly divided into four groups (n=6 in each group); control group fed on a low fat diet (LFD), LFD plus exercise group (LFD+EX), model group fed on HFD, and swim exercise treated group (HFD+EX). Training swim exercise started from the 11th week up until the end of week 15. Liver index and body mass index (BMI) were determined, and harvested liver tissues were examined using basic histological staining and visualised under light microscopy. In addition, collected blood samples were assayed for biomarkers of liver injury. Histological images from the model group showed accumulation of lipid droplets in the hepatocytes (steatosis) and damaged liver cells that were inhibited by swimming exercise. Compared to control groups, HFD caused an increase in BMI and liver weight but not in liver index. In addition, HFD significantly (p<0.05) increased liver injury biomarkers; high-sensitivity C-reactive protein (hsCRP) and alkaline phosphatase (ALP) that were effectively (p<0.05) decreased by swimming exercise. Furthermore, a negative correlation between these biomarkers and the antioxidant and anti-inflammatory protein adiponectin was observed. Thus, HFD-induced hepatic steatosis is treated by swim exercise.

KEY WORDS: Hepatic steatosis; Swim exercise; Liver injury; Animal model.

INTRODUCTION

Abdominal obesity is a criteria of the insulin resistance syndrome, also called metabolic syndrome characterised by insulin resistance, inflammation, oxidative stress, hypertension and dyslipidaemia which carries increased risk of type-2 diabetes, cardiovascular disease, non-alcoholic fatty liver disease and cancer (Kopelman, 2000; Eckel *et al*., 2005; Grattagliano *et al*., 2008). Hepatic steatosis is the hepatic component of metabolic syndrome characterised by accumulation of fat caused by dysfunction of fat metabolism in the liver (Benlhabib *et al*., 2004), which is histologically comparable to liver disease caused by alcohol consumption (Sakhuja, 2014). This can lead to, if not treated, more serious complications such as nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, liver failure and even hepatocellular carcinoma (Choi & Diehl, 2005; Paschos & Paletas, 2009). The majority of people with NASH and one-third of subjects with hepatic steatosis have high insulin resistance and low levels of adiponectin (Hui *et al*., 2004).

Adiponectin, C-reactive protein (CRP) and ALP are known to be involved in the pathology of different types of liver injuries including non-alcoholic fatty liver disease in animal models and humans (Matsuzawa *et al*., 2004; Kerner *et al*., 2005; Enocsson *et al*., 2009). The majority of CRP and ALP are produced by liver cells (Pepys & Hirschfield, 2003; Lilford *et al*., 2013), whereas adiponectin is secreted exclusively by the adipose tissue which plays an important role in glucose and lipid metabolism by increasing insulin sensitivity thus lowering blood sugar and enhancing fatty acid oxidation, acting as a antilipogenic hormone (Kraemer *et al*., 2003; Yamauchi *et al*., 2003).

The beneficial effects of exercise on metabolic and pathophysiological changes associated with obesity and the metabolic syndrome have been previously studied (Birrer & Sedaghat, 2003; Nagatomo *et al*., 2012). Treatment strategies for hepatic steatosis aim to decrease hepatic

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accumulation of fat, weight loss, improve insulin sensitivity, modify metabolic risk factors and protect the liver from oxidative stress (Chalasani *et al.*, 2012). Therefore, this study provides information about the effect of swim exercise training on HFD-induced hepatic steatosis. We focused on the histological integrity of the liver tissue with and without exercise and assessed three biomarkers that are known to be modulated in liver diseases.

MATERIAL AND METHOD

Animals. Sprague–Dawley rats (n=24) weighing 170-200 g were used in this study. All rats were bred and housed in the research centre of King Khalid University, college of medicine (Abha, Saudi Arabia), at temperature of 23 ± 1 °C and a 12h light: 12h dark cycle. All rats had free access to tap water and fed standard laboratory chow during the acclimatization period. All experimental procedures were approved by the medical research ethical committee at King Khalid University and accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. (NIH publication No. 85-23, revised 1996).

Experimental design. After one week of acclimatization to the laboratory environment, the rats were randomly divided into four groups (n=6 each). Animals in group 1 (LFD) were used as the control group and fed with standard laboratory chow for 15 weeks. Animals in group 2 (LFD+Ex) were fed a standard laboratory chow for 15 weeks and forced to swim from the 11th to the 15th week. The third group was fed a high-cholesterol and fructose diet (HFD) for 15 weeks (Aragno *et al.*, 2009). Animals in the fourth group (HFD+Ex) were fed with a HFD for 15 weeks and forced to swim from the 11th to the 15th week. Body weight, and food intake were recorded weekly (Collino *et al.*, 2010).

Calculation of the Body Mass Index (BMI). Lee index= cube root of body weight (g) / nose-to-anus length (cm) (Bernardis, 1970).

Exercise Program. Swimming was practiced in a cylindrical tank (120 cm diameter x 80 cm height) containing temperature-controlled water (30°-32°C). Rats were placed in the tank 3 days/week, for 1 hour (9:00 - 10:00 a.m). At the end of each exercise session, animals were dried and kept in a warm environment. Sedentary controls were restricted to cage activity. However, on the days of exercise, the sedentary animals were removed from their cages and kept in the container (previously cleaned and dried) for 1 hour, where the swimming sessions had taken place in order to handle stress. In order to minimize the acute effect of exercise, the trained animals were sacrificed 48 hours after the end of the final training session (de Lemos *et al.*, 2007). Food was removed from the animal cages the night before.

Biochemical measurements

Blood samples. At the end of experimental period, blood samples were collected by cardiac puncture under anesthesia (sodium thiopentone at 40 mg/kg body weight) after an overnight fast of 12 hours. These blood samples were collected without anticoagulant, left for 10 min, then centrifuged for 10 min at 4000 r/min to obtain serum, which was stored at –20 °C until further biochemical analysis for determination of serum adiponectin and hs-CRP Liver function was evaluated by assessing for serum ALP levels using an enzymatic kit (Randox Laboratories, UK) according to the manufacturer's instructions.

Determination of the serum Adiponectin and hs-CRP. Quantitative determination of serum adiponectin was performed using the mouse/rat adiponectin ELISA kit (B-Bridge international, Inc.), according to the manufacturer's instructions. ELISA kits were purchase to determine serum levels of high sensitive C-reactive protein (hsCRP, Cat. No. ERC1021-1) from ASSAYPRO, USA.

Histopathological Examination of the liver. Liver samples were taken from rats in different groups that were fixed in 10 % formal saline for one day, then washed with water. Ascending serial dilutions of ethyl alcohol were used for dehydration. Samples were cleared in xylene, then embedded in paraffin at 56 oC in a hot oven for 24 Hrs. Paraffin blocks were prepared and cut at a thickness of 4-6 microns. Sections are mounted on glass slides, deparaffinized and stained by hematoxylin and eosin (H&E) stains for histological examination through the light microscope . All liver biopsy specimens were examined. Scoring of necroinflammmation and fibrosis was performed using the criteria devised by Brunt & Tiniakos (2005) (Paschos & Paletas). NASH was diagnosed according to liver histology indicating steatosis (0–3) 1: < 33 % of lobules, 2: 33–66 % of lobules and 3: > 66 % of lobules with ballooning degeneration of hepatocytes (0–2) 1: mild or 2: marked, and lobular inflammation (0–3) based on observations of foci per 20x field: 1: 1–2 foci, 2: up to 4 foci, 3: > 4 foci with total score 0–8. Fibrosis was assessed as the following criteria: stage 1: zone 3 perivenular, perisinusoidal/pericellular fibrosis, focal or extensive, stage 2: as above with focal or extensive periportal fibrosis, stage 3: bridging fibrosis, focal or extensive, and stage 4: cirrhosis.

Statistical analysis. The data was expressed as mean ± standard deviation (SD). Data was processed and analyzed using the SPSS version 10.0 (SPSS, Inc., Chicago, Ill., USA). One-way ANOVA was done followed by Tukey's post hoc test. Pearson correlation statistical analysis was done for detection of a probable significance between two different parameters. Results were considered significant if $p \leq 0.05$.

RESULTS

Swimming exercises reduce the gain in BMI and liver weight, but not liver index. Table I shows data on body weight, BMI, liver weight and liver index (percentage of the ratio of liver weight to body weight) of rats sacrificed after 15 weeks. Compared to control groups, LFD and LFD+EX, rats on HFD showed a significant increase in body weight (gain 54.5 % ±4.8, p< 0.05), BMI (p<0.05), and liver weight (p<0.05), but no significant increase in liver index (p>0.05) was observed. Swim exercise training for five weeks prior to the sacrifice day (HFD+EX) significantly decreased the percentage of weight gain (p<0.05), BMI (p<0.05), and liver weight (p<0.05) compared to HFD group, but again had no significant decrease in liver index (p>0.05). In addition, swim exercise for the control rats significantly (p<0.05) reduced body weight but not for BMI, liver weight and liver index.

Swim exercise training inhibits HFD-induced liver injury biomarkers. Tissue injury biomarkers such as ALP and hs-CRP, are known to be increased in liver damage (Pepys & Hirschfield; Al Akwaa *et al.*, 2011). We investigated whether swim exercise can suppress the release of these biomarkers in our model of hepatic steatosis. HFD caused a three-fold increase in the blood level of hs-CRP and about 30 % increase in ALP level compared to control groups that were significantly inhibited by swim exercise (Fig. 1A and 1B; p<0.05) to levels comparable to the control group. The relative degree of inhibition of these enzymes by swim exercise training was hs-CRP > ALP.

Negative regulation between adiponectin and hs-CRP and ALP. Adiponectin is reported to have decreased in fatty liver (Polyzos *et al.*, 2010) and there is a negative correlation between circulating TNF-α and adiponectin, and serum TNF-α and adiponectin receptors in the liver in a rat model of NASH: a complicated type of NAFLD (Liu *et al.*, 2011). Therefore, we tested the hypothesis that adiponectin is negatively correlated with both hs-CRP and ALP. Adiponectin was measured in all rat groups and compared with the data for hs-CRP and ALP. As shown in Figure 2A and 2B, a negative correlation was shown between adiponectin and these biomarkers; adiponectin versushs-CRP (r = - 0.7767) (p<0.05), and adiponectin versus ALP (r = - 0.8072) (p<0.05).

Swim exercise training treats steatosis and liver damage induced by HFD. We investigated the effect of swim exercise on the treatment of hepatic steatosis. Liver sections obtained from sacrificed rats after 15 weeks were examined by light microscopy after staining with H&E (Fig. 3). Compared to control groups; control and control + exercise (Figs. 3A and 3B) showed intact hepatic lobule, rats fed on HFD (Fig. 3C) showed lipid droplets (steatosis), abnormal hepatocytes with atrophic nuclei, vaculated sinusoids, and the central vein is infiltrated with cells. Exposure of a group of rats to swimming exercises (Fig. 3D) substantially treated hepatic lobule injury; as shown by normal hepatocytes, central vein and sinusoids, but still few lipid droplets can be seen. In addition, histopathological scoring (Fig. 3E) of the liver biopsy showed that the scoring significantly (p<0.05) increased in the HFD group as compared to both control groups. On the other hand, HFD+EX group showed a significant (p<0.05) reduction in the histopathological scoring versus the HFD group.

Table I. Effects of swim exercise on body weight, Body mass index (BMI), liver weight, and liver index in all experimental groups of rats. Values are expressed as Mean ± SD for 6 rats in each group. Values were considered significantly different at P < 0.05. a: Significant in comparison to control (LFD); b:Significant in comparison to control+exercise; c: Significant in comparison to HFD.

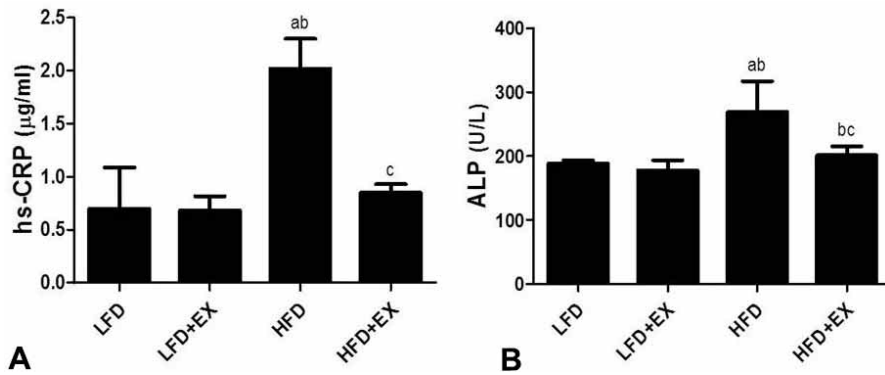|	Final body weight (gm)	Weight gain (%)	BMI (gm/cm^2)	Liver weight (gm)	Liver index (%)
LFD	333.8±4.3	31±4.5	0.60±0.04	10.8±0.48	3.24±0.17
LFD + Exercise	314.9±3.2[a]	21.2±3.69 [a]	0.58±0.03	10.9±0.45	3.43±0.11
HFD	398.5±7.9[a b]	54.5±4.8 [a b]	0.72±0.05 [a b]	13±0.22 [a b]	3.26±0.08
HFD + Exercise	350±6.4 [a b c]	36.27± 4.08 [a b c]	0.65± 0.02 [a b c]	11.65±0.40 [c]	3.33±0.15

Fig. 1. HFD-induced biomarkers of liver injury, is inhibited by swim exercise. Blood levels of hs-CRP (A) and ALP (B) were measured in 4 groups of rats; Control, Control+exercise (Control+EX), HCFD, and HCFD+exercise (HCFD+EX) groups after 15 weeks. Results represent the mean (±SD); n=6 for each group. Experiments were performed in triplicate. All shown p values are significant (P<0.05). a: Significant in comparison to control (LFD); b:Significant in comparison to control+exercise; c: Significant in comparison to HFD.
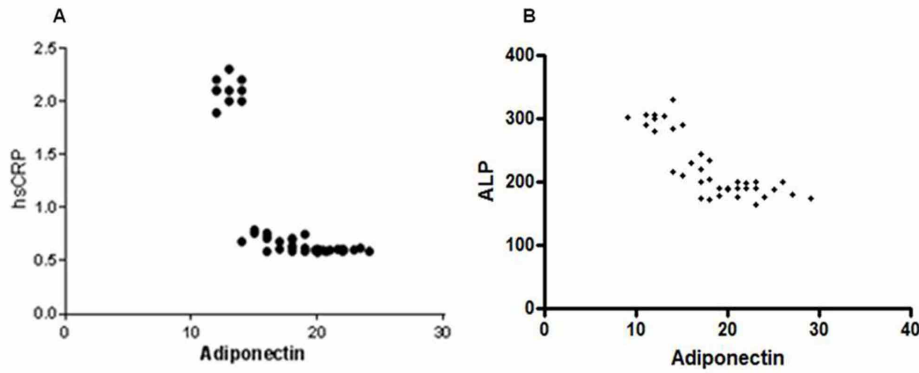

Fig. 2. Negative correlation between adiponectin and hs-CRP and ALP. Serum adiponectin levels were measured in all groups of rats after 15 weeks and the relationship between adiponectin and hs-CRP is shown in (A) and with ALP is shown in (B).
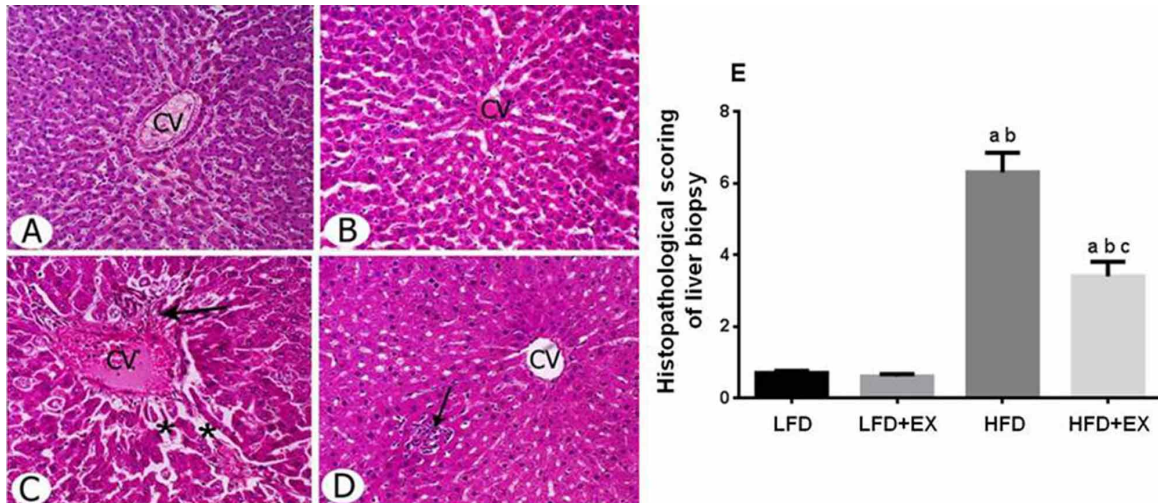

Fig. 3. Swim exercise treats liver lobules injured by HFD. H&E stained images (x400) of harvested tissues obtained after 15 weeks from the liver of rats are visualised using light microscopy. (A). Control group. (B). Control+EX group. (C). HFD group. (D). HFD +EX group. Note that arrows point to the fat droplets and asterisks point to dilated sinusoids. Abbreviations: H, hepatocyte; N, nucleous; CV, central vein. (E). Degree of liver damage in rats fed on HFD compared with controls and swim exercise treated group. All shown p values are significant (P<0.05). a: Significant in comparison to control (LFD); b:Significant in comparison to control+exercise; c: Significant in comparison to HFD.

DISCUSSION

The principal finding of this study was that liver injury induced by hepatic steatosis caused by HFD can be treated using a nonconventional treatment method, swimming exercise, which substantially restored the normal architecture of the liver cells. This conclusion is supported by the data indicating that swim exercise markedly prevented damages occurred to liver lobules after 15 weeks in rats fed on HFD (Fig. 3). In addition, swim exercise significantly inhibited biomarkers of tissue injury like hs-CRP and ALP (Fig. 1). Furthermore, our data that point to a significant reduction in body weight, BMI, and liver weight following swimming exercises (Table I) also supports our conclusion mentioned above.

Our data shown in this report that pointed to the elevation of ALP and hs-CRP (Fig. 1) and depression of adiponectin (Fig. 2) are in agreement with previous work suggesting that these biomarkers are indicative of liver injury including hepatic steatosis (Chidambaram & Carani Venkatraman, 2010; Leite *et al*., 2013). In addition, other work that drew a negative correlation between serum adiponectin and TNF-α in a rat model of NASH, a complicated type of NAFLD (Liu *et al*.) in principal, is in agreement with our data shown in Figure 2 which points to a negative correlation between adiponectin and hs-CRP and ALP.

Our histopathology data (Fig. 3) confirmed the development of hepatic steatosis, 15 weeks post feeding rats on HFD (Fig. 1C), showed a substantial destruction of the liver lobule. Interestingly, there is no specific drug of choice to treat this disease and its complications, and liver transplant is the only available treatment if progress of the disease leads to a liver failure (Said, 2013; Zezos & Renner, 2014). Here, we used a nonconventional method, swim exersise to treat steatosis in rats and demonstrated its effectiveness, which may offer therapeutic potential in humans.

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RESUMEN: El aumento de la obesidad, especialmente entre los niños, es un problema importante en la salud pública que afecta negativamente los órganos vitales, incluyendo el hígado. En este estudio se investigó el efecto del ejercicio en la curación de las células del hígado y el daño inducido por la dieta alta en grasas (HFD) en un modelo de rata de esteatosis hepática. Las ratas se dividieron aleatoriamente en cuatro grupos (n = 6 en cada grupo); grupo control, alimentado con una dieta baja en grasas (LFD); grupo de ejercicio LFD más (LFD + EX); grupo modelo alimentado con HFD; y grupo tratado con ejercicio de natación (HFD + EX). El entrenamiento con ejercicio de natación comenzó a partir de la semana 11 hasta el final de la semana 15. Se determinaron el índice hepático y el índice de masa corporal (IMC). Los tejidos hepáticos recolectados se examinaron mediante tinción histológica básica y se visualizaron con microscopía óptica. Además, se analizaron las muestras de sangre recogidas para identificar biomarcadores de lesión hepática. Las imágenes histológicas del grupo modelo mostraron acumulación de gotitas de lípidos en los hepatocitos (esteatosis) y células hepáticas dañadas que fueron inhibidas por el ejercicio de natación. En comparación con los grupos control, HFD causó un aumento en el IMC y el peso del hígado, pero no en el índice de hígado. Además, HFD aumentó significativamente (p <0.05) los biomarcadores de lesiones hepáticas; la proteína C reactiva de alta sensibilidad (hsCRP) y la fosfatasa alcalina (ALP) disminuyeron efectivamente (p <0.05) con el ejercicio de natación. Además, se observó una correlación negativa entre estos biomarcadores y la proteína antioxidante y antiinflamatoria adiponectina. Por lo tanto, la esteatosis hepática inducida por HFD puede ser tratada mediante el ejercicio de natación.

PALABRAS CLAVE: Esteatosis hepática; Ejercicio de natación; Lesión hepática; Modelo animal.

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