

Biochemical parameters response to weight loss in patients with non-alcoholic steatohepatitis.

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Abstract

Background: Non-alcoholic steatohepatitis (NASH) is a chronic liver disease that is capable of progressing to end-stage liver disease, but generally has a benign course. Non-alcoholic steatohepatitis (NASH) is a growing public health problem with no approved therapy. NASH projected to be the leading cause of liver transplantation in the United States by 2020. Obesity, non-insulin-dependent diabetes mellitus and hyperlipidaemia are the most common associations of the disease. Global prevalence of NASH is 10-24% amongst general population but increases to 25-75% in obese diabetic individuals.

Objective: There is an urgent need for efficient therapeutic options as there is still no approved medication. The aim of this study was to detect changes in biochemical parameters including insulin resistance, cytokines, blood lipid profile and liver enzymes following weight loss in patients with non-alcoholic steatohepatitis.

Materials and methods: One hundred obese patients with NASH, their age between 35-50 years, body mass index (BMI) from 30 to 35 Kg/m² were included in the study in two subgroups; the first group (A) received moderate aerobic exercise training in addition to diet regimen, where the second group (B) received no treatment intervention.

Results: The mean values of leptin, TNF- α , IL6, IL8, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Homeostasis Model Assessment-Insulin Resistance- index (HOMA-IR), Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-c), Triglycerides (TG) and BMI were significantly decreased in group (A), where the mean value of Adiponectin and High Density Lipoprotein Cholesterol (HDL-c) were significantly increased, while there were no significant changes in group (B). Also, there was a significant difference between both groups at the end of the study.

Conclusion: Weight loss modulates insulin resistance, adiponectin, leptin, inflammatory cytokine levels and markers of hepatic function in patients with nonalcoholic steatohepatitis.

Keywords: Weight loss, insulin resistance, cytokines, liver enzymes, nonalcoholic steatohepatitis.

DOI: <http://dx.doi.org/10.4314/ahs.v16i1.32>

Cite as: Abd El-Kader SM, Al-Shreef FM, Al-Jiffri OH. Biochemical parameters response to weight loss in patients with non-alcoholic steatohepatitis. *Afri Health Sci.* 2016;16(1): 242-249. <http://dx.doi.org/10.4314/ahs.v16i1.32>

Introduction

Non-alcoholic steatohepatitis (NASH) is a pathological condition characterized by accumulation of lipids in the liver of non-alcoholic individuals and consequent oxidative stress leading to cirrhosis of liver in the long run¹. Incidence of NASH in USA is 30% of the adult population². Even, in Asian countries, number of individuals

being diagnosed with NASH is on the rise³. The clinical importance of NASH is due to its potential to progress to liver cirrhosis, hepatocarcinoma and liver failure⁴. Non-alcoholic steatohepatitis is a growing public health problem with no approved therapy and projected to be the leading cause of liver transplantation in the United States by 2020⁵.

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Obese individuals are at maximum risk of developing NASH⁶ and a strong positive correlation exists between insulin resistance (IR) and development of NASH as obesity, non-insulin-dependent diabetes mellitus and hyperlipidaemia are the most common associations of the disease. Persons with central obesity are at particular risk⁷. The prevalence of NASH is estimated to be as high as 40-100% in obese adults worldwide⁸.

Non alcoholic steatohepatitis is characterized by diffuse fatty acid infiltration and inflammation. The exact cause of NASH is unclear, but it is increasingly becoming more evident that the disease is much more common than was previously thought⁴. Inflammatory cytokines, IL-6, TNF- α and IL-8, and oxidative stress markers were significantly higher in the non-alcoholic steatohepatitis patients⁹.

The pathogenesis of NASH includes insulin resistance, increased inflammation, tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6) and increased oxidative damage¹⁰⁻¹². Moreover, leptin and adiponectin have been implicated in the pathogenesis of both non-alcoholic steatohepatitis and insulin resistance¹³. Tumor necrosis factor- α and other TNF-induced cytokines such as interleukin-6 and interleukin-8 levels were higher in animals with NASH. This is likely related to the progression of NASH in liver cirrhosis¹⁴.

In many cases, NASH causes asymptomatic elevation of plasma aspartate transaminase (AST) and alanine transaminase (ALT). However, ALT is most closely related to liver fat accumulation, and it has been used as an indicator of NASH¹⁵. Moreover, insulin resistance plays a major role in the development of NASH¹⁶. However, serum concentrations of tumor necrosis factor-alpha (TNF- α) are related to the severity of insulin resistance and to central adiposity¹⁷.

The aim of this study was to detect changes in biochemical parameters including TNF- α , IL-6, IL-8, Leptin, Adiponectin, Insulin resistance, ALT, AST, TC, LDL-c, TG and HDL-c following weight loss in patients with nonalcoholic steatohepatitis.

Subjects, materials and methods

Subjects

One hundred obese nonalcoholic steatohepatitis patients of both sexes (50 males & 50 females) were selected from the Internal Medicine Department at King Abdul Aziz University Hospital and other Hospitals at Jeddah area. Their age was between 45-58 years, the body mass index (BMI) ranged from 30 to 35 Kg/m². Subjects with a history of smoking, cardiovascular disease, alcohol abuse problems, hepatic (i.e. infectious and viral disease) or renal disease were excluded from the study. Each patient underwent abdominal US (Siemens Antares equipment with CH4-1 MHz transducer; Siemens Medical, Erlangen, Germany) in a fasting state.

The presence of hepatic steatosis was assessed independently by radiologists according to findings such as hepatorenal contrast, blurring of the vascular wall, and profound attenuation of the diaphragm. According to the findings of ultrasound examination patient group with NASH was divided into three; as minimal, moderate and marked subgroup parallel to the increase in echogenicity¹⁸. The subjects were randomly allocated to the experimental group or control group. Each subject was given an envelope containing two cards and was instructed to blindly draw one card on each occasion. This study was single blinded: the person undertaking the assessment and data analysis was unaware of the group of each patient while the subjects and their therapists were not blinded to the treatment group. Patients were included into 2 equal groups; the first group (A) received aerobic exercise training in addition to diet regimen for 3 months, where the second group (B) received no treatment intervention. The study was approved by the Ethics Committee of King Abdulaziz University Hospital, Jeddah, Saudi Arabia and all patients signed informed consent for participation in this study.

Equipment

- 1) Treadmill (Track master 400E, gas fitness system, England) was used in performance of walking exercise.
- 2) Commercial kits (Roche Diagnostics, Geneve, Switzerland) were used to measure serum ALT, AST levels; however commercial kits (Siemens Healthcare Diagnostics, Deerfield, USA) were used to measure IL-6, IL-8 and TNF- α level. Also, kits (Bioclin, Quibasa, Belo Horizonte, MG, Brazil) were used for measurements of Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index for insulin sensitivity. Also, Commercial kits (Randox, Tokyo, Japan) with K2EDTA were used to measure leptin, adiponectin, total cholesterol, triacylglycerol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol.
- 3) Weight and height scale (JENIX DS 102, Dongsang, South Korea) was used to measure weight and height to calculate the body mass index (BMI). Body mass index was calculated by dividing the weight in kilograms by the square of the height in meters (Kg/m²). According to the WHO classification, a BMI of <18.5 Kg/m² is under weight, 18.5-24.9 Kg/m² is normal 25-29.9 Kg/m² is overweight. A BMI of > 30 Kg/m² is classified as obese and this group was further divided into moderate obesity (30-34.9 Kg/m²), severe obesity (35-39.9 Kg/m²) and very severe obesity (\geq 40 Kg/m²).

Measurements

1. Laboratory analysis:

Venous blood samples were collected in polystyrene tubes after a 12-h fasting, by venipuncture of the antecubital vein while patients rested in a supine position. The blood samples were transported to a laboratory within 1 h and centrifuged at + 4 °C to remove serum (1000 = g for 10 min). Serum ALT, AST levels were assayed using a Cobas Integra 800 analyzer (Roche Diagnostics, Geneva, Switzerland). While IL-6 levels were measured using "Immulite 2000" immunassay analyzer (Siemens Healthcare Diagnostics, Deerfield, USA). However, interleukin-8 (IL-8) and TNF- α levels were analyzed with ELISA kits using ELISA microplate strip washer (ELX 50), and ELISA microplate reader (ELX 808; BioTek Instruments, USA). Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index for insulin sensitivity was computed following this equation: fasting glycemia (mmol/L) x fasting insulin(mIU/L)/22.5¹⁹ and was measured by the colorimetric enzymatic method using an automatic spectrophotometer and respective kits for analysis (Bioclin, Quibasa, Belo Horizonte, MG, Brazil). Human insulin has been measured with an insulin kit (Roche Diagnostics, Indianapolis, IN, USA) using a cobas immunoassay analyzer (Roche Diagnostics). Also, plasma sample with K2EDTA was collected after centrifugation (2000 \times g for 10 min at 4°C) and stored at -80°C to analyze leptin, adiponectin, total cholesterol, triacylglycerol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol. All analyses were carried out on a Hitachi 7170 Autoanalyser (Tokyo, Japan) or with commercial kits (Randox).

2. Evaluation of anthropometric parameters

All measurements were performed at pre-treatment and after three months at the end of the study. The patients were measured while wearing their undergarments and hospital gowns. Height was measured with a digital stadiometer to the nearest 0.1 cm (JENIX DS 102, Dong-sang, South Korea). Body weight was measured on a calibrated balance scale to the nearest 0.1 kg (HC4211, Cas Korea, South Korea), and BMI was calculated as $BMI = \text{Body weight} / (\text{Height})^2$. All measurements of TNF- α , IL-6, IL-8, leptin, adiponectin, HOMA-IR, ALT, AST, TC, LDL-c, TG, HDL-c and BMI were taken before the starting of the study and after three months at the end of the study.

Procedures

Following the previous evaluation, all patients were divided randomly into the following groups:

1. The training group: Fifty nonalcoholic steatohepatitis patients of both sexes received aerobic exercise training program and diet regimen. The aerobic treadmill-based training program (Track master 400E, gas fitness system, England) was at 65 % to 75 % of the maximum heart rate (HRmax) achieved according to a modified Bruce protocol. After an initial, 5-minute warm-up phase performed on the treadmill at a low load, each endurance training session lasted 30 minutes and ended with 5-minute recovery and relaxation phase according to recommendation of American College of Sports Medicine regarding the aerobic exercise application²⁰, three sessions / week (i.e. a total of 36 sessions over a 3-month period). Also, the interview-based food survey was performed by dieticians to specify previous food habits and possible anomalies in dietary behavior. The prescribed low calorie diet was balanced, with 15% as protein, 30 to 35% as fat and 50 to 55% as carbohydrate, on average, in order to provide about 1200 Kilo-calories/day for three months²¹.

2. The control group: Fifty non-alcoholic steatohepatitis patients of both sexes were asked to maintain their ordinary current life style.

Statistical analysis

The mean values of TNF- α , IL-6, IL-8, leptin, adiponectin, HOMA-IR, ALT, AST, TC, LDL-c, TG, HDL-c and BMI obtained before and after three months in both groups were compared using paired "t" test. Independent "t" test was used for the comparison between the two groups ($P < 0.05$). Pearson's product moment correlation coefficients (r) were applied to examine the degree of correlation between changes in BMI, ALT and AST levels.

Results

One hundred NASH patients completed the screening evaluation and underwent randomization. The baseline characteristics of the patients who underwent randomization are shown in Table (1). Most patients (70%) were men. Fifty patients were assigned to aerobic exercise and diet regimen ($n = 50$; 34 males and 16 females) and control group ($n = 50$, 36 males and 14 females). None of the baseline characteristics differed significantly between the two groups as listed in table (1).

Table (1): Mean value of demographic data and health state for patients in both groups.

	Mean +SD		P-value
	NASH	Control	
Age (year)	50.78± 5.34	51.12± 5.86	0.098
Gender ratio (male/female)	34/16	36/14	0.271
Weight (kg)	93.24 ± 6.51	92.85± 6.22	0.152
Height (cm)	173.11± 7.82	172.35 ± 6.95	0.084
BMI (kg/m ²)	32.35± 2.54	31.76± 2.92	0.093
Waist–hip ratio	0.97± 0.15	0.96 ± 0.06	0.125
Systolic BP, mm Hg	141.26± 12.56	140.87 ± 11.93	0.157
Diastolic BP, mm Hg	89.31 ±8.64	88.75 ± 10.21	0.162
HbA1C, %	5.28 ± 0.96	5.6 ± 0.84	0.063
Bilirubin level, mol/L	13.83 ± 3.41	14.25 ± 4.3	0.921
Albumin level, g/L	43.57± 2.73	42.78 ± 3.11	0.073

BMI: Body mass index; BP: Blood pressure; HbA1c: Glycylated hemoglobin

Further, a significant decrease ($P < 0.05$) in the anthropometric measurements including BMI was observed with the lifestyle intervention group of NASH pa-

tients who received dietary and exercise therapy for 3 months, compared to NASH patients of control group (see Table 2).

Table (2): Mean value and significance of BMI, TNF- α , IL-6, IL-8, ALT, AST,TC,LDL-c, HDL-c, TG, HOMA-IR in group (A) before and after treatment.

	Mean +SD		P-value
	Before	After	
BMI (kg/m ²)	32.35± 2.54	29.15± 2.48*	0.0035
TNF- α (pg/mL)	12.76 ± 2.21	8.21 ± 2.35*	0.0083
IL-6 (pg/mL)	5.26 ± 1.52	3.40 ± 1.17*	0.0077
IL-8 (pg/mL)	18.53 ± 3.78	15.16 ± 3.35*	0.0056
Leptin Ng/ml	41.64 ± 5.24	36.68 ±4.76*	0.0043
Adiponectin (μ g/mL)	10.51 ± 3.73	14.96 ± 3.54*	0.0034
ALT (U/L)	46.32 ± 4.65	34.65± 4.13*	0.0035
AST (U/L)	44.71 ± 5.53	35.22± 4.97*	0.0023
TC (mg/dl)	196.11±12.42	171.63 ±11.04*	0.0019
LDL-c (mg/dl)	136.52 ±10.84	118.31 ± 11.15*	0.0024
TG (mg/dl)	157.14 ±11.06	124.17 ±10.43*	0.0018
HDL-c (mg/dl)	34.65 ±2.87	38.11 ±2.32*	0.0037
HOMA-IR	4.92 ± 2.36	2.95 ± 1.98*	0.0026

BMI: Body mass index; TNF- α : tumor necrosis factor – alpha; IL-6: Interleukin-6; IL-8: Interleukin-8; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TC: Total cholesterol; LDL-c: Low density lipoprotein cholesterol; HDL-c: High density lipoprotein cholesterol; TG: Triglyceride; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance Index; * Significant level ($p < 0.05$).

While at the same time, HDL-C level was somewhat reduced among the control group of NASH patients. On the other hand, the LDL-C level decreased among lifestyle intervention group, whereas an increasing trend was observed for the control group of NASH patients, as LDL-C considered to be bad cholesterol with levels of less than 130 normally recommended. The TC of

lifestyle intervention group could be maintained very close to its recommended level of 200 throughout the treatment duration, while TC was found to be slightly increased among the control NASH patients which might indicate a potential risk for atherosclerosis development by accumulation of cells containing excess lipids within the arterial wall that are closely related to NASH development(see Table 2 and 3).

Table (3): Mean value and significance of BMI, TNF- α , IL-6, IL-8, ALT, AST, TC, LDL-c, HDL-c, TG, HOMA-IR in group (B) before and after the study.

	Mean +SD		P-value
	Before	After	
BMI (kg/m²)	31.76 \pm 2.92	31.98 \pm 2.87	0.126
TNF- α (pg/mL)	12.98 \pm 2.37	13.54 \pm 2.56	0.114
IL-6 (pg/mL)	5.76 \pm 1.71	5.98 \pm 1.95	0.137
IL-8 (pg/mL)	18.85 \pm 3.26	19.23 \pm 3.57	0.210
Leptin Ng/ml	41.57 \pm 5.13	42.11 \pm 4.92	0.251
Adiponectin (μg/mL)	10.36 \pm 3.15	10.85 \pm 2.97	0.134
ALT (U/L)	46.53 \pm 4.27	46.94 \pm 4.62	0.116
AST (U/L)	44.80 \pm 5.53	45.17 \pm 5.21	0.131
TC (mg/dl)	195.83 \pm 11.54	196.85 \pm 10.88	0.315
LDL-c (mg/dl)	137.11 \pm 10.16	137.92 \pm 10.73	0.271
TG (mg/dl)	158.45 \pm 11.32	159.26 \pm 11.17	0.127
HDL-c (mg/dl)	35.21 \pm 2.33	34.85 \pm 2.62	0.113
HOMA-IR	4.48 \pm 2.17	4.75 \pm 2.15	0.254

BMI: Body mass index; TNF- α : tumor necrosis factor – alpha; IL-6: Interleukin-6; IL-8: Interleukin-8; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TC: Total cholesterol; LDL-c: Low density lipoprotein cholesterol; HDL-c: High density lipoprotein cholesterol; TG: Triglyceride; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance Index.

Considerable changes were observed in the serum lipid profiles of the NASH patients within the lifestyle intervention group of NASH patients; the results are shown in Table 4. The concentration of TG in the blood that usually provides energy for different metabolic processes was effectively controlled among the lifestyle inter-

vention group, whereas the TG level was higher for the control group even after 3 months of dietary and exercise therapy. An increase in HDL-C level was observed in lifestyle intervention group, which is a good indicator of the improvement in NASH patients because its high level seems to be associated with low incidence of coronary heart diseases.

Table (4): Table (2): Mean value and significance of BMI, TNF- α , IL-6, IL-8, ALT, AST, TC, LDL-c, HDL-c, TG, HOMA-IR in group (A) and group (B) after the study.

	Mean +SD		P-value
	Group (A)	Group (B)	
BMI (kg/m²)	29.15 \pm 2.48*	31.98 \pm 2.87	0.0046
TNF- α (pg/mL)	8.21 \pm 2.35*	13.54 \pm 2.56	0.0091
IL-6 (pg/mL)	3.40 \pm 1.17*	5.98 \pm 1.95	0.0083
IL-8 (pg/mL)	15.16 \pm 3.35*	19.23 \pm 3.57	0.0057
Leptin Ng/ml	36.68 \pm 4.76*	42.11 \pm 4.92	0.0052
Adiponectin (μg/mL)	14.96 \pm 3.54*	10.85 \pm 2.97	0.0038
ALT (U/L)	34.65 \pm 4.13*	46.94 \pm 4.62	0.0044
AST (U/L)	35.22 \pm 4.97*	45.17 \pm 5.21	0.0038
TC (mg/dl)	171.63 \pm 11.04*	196.85 \pm 10.88	0.0026
LDL-c (mg/dl)	118.31 \pm 11.15*	137.92 \pm 10.73	0.0029
TG (mg/dl)	124.17 \pm 10.43*	159.26 \pm 11.17	0.0022
HDL-c (mg/dl)	38.11 \pm 2.32*	34.85 \pm 2.62	0.0051
HOMA-IR	2.95 \pm 1.98*	4.75 \pm 2.15	0.0063

BMI: Body mass index; TNF- α : tumor necrosis factor – alpha; IL-6: Interleukin-6; IL-8: Interleukin-8; ALT: Alanine amino transferase; AST: Aspartate amino transferase; TC: Total cholesterol; LDL-c: Low density lipoprotein cholesterol; HDL-c: High density lipoprotein cholesterol; TG: Triglyceride; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance Index; * Significant level ($p < 0.05$).

In the lifestyle intervention group of NASH patients, the values of leptin, TNF- α , IL6, IL8 and HOMA-IR were considerably reduced to significant levels over the period of therapy. While, the levels of these parameters were continuously increased in the control group of NASH patients. Also, as is evident from Tables 2, 3 and

4, the lifestyle intervention group of NASH patients had significant change in levels of AST and ALT from baseline, however ; there was a progressive rise in AST and ALT levels for the control group NASH patients. Moreover, there was a positive relationship between weight changes and levels of AST and ALT in both groups(Tables 5 and 6).

Table (5): The Pearson’s correlation coefficients test values and the relationship between the BMI, ALT, AST in group (A).

Test	Pearson’s value	Test Pearson’s value Relationship to body mass index
Alanine aminotransferase	0.82	Strong direct relationship
Aspartate aminotransferase	0.81	Strong direct relationship

BMI : Body mass index ; ALT :Alanine aminotransferase ; AST : Aspartate aminotransferase

Table (6): The Pearson’s correlation coefficients test values and the relationship between theBMI, ALT, AST in group (B).

Test	Pearson’s value	Test Pearson’s value Relationship to body mass index
Alanine aminotransferase	0.83	Strong direct relationship
Aspartate aminotransferase	0.80	Strong direct relationship

BMI : Body mass index ; ALT :Alanineaminotransferase ; AST : Aspartate aminotransferase

Discussion

The aim of this study was to detect changes in biochemical parameters following weight loss in patients with non-alcoholic steatohepatitis. The mean values of leptin, TNF- α , IL6, IL8, ALT, AST, HOMA-IR, TC, LDL-c, TG and BMI were significantly decreased in group (A), where the mean value of adiponectin and HDL-c were significantly increased, while there were no significant changes in group (B). Also; there was a significant difference between both groups at the end of the study, these findings are supported and agreed by several previous studies.

There is a growing concern for NASH as the next big therapeutic challenge in hepatology because of the possible evolution of NASH toward end-stage liver disease, as well as the need for liver transplantation. The novel of this study is that although exercise and diet improvement may reduce the overall magnanimity of in-

sulin resistance, hyperlipidemia and abnormal cytokine metabolism, there has been only limited research on the effects of weight reduction as the sole intervention on these abnormal biochemical parameters in individuals with NASH. However, the limitation of this study is no recording of the histological changes to the treatment intervention

Results of our study was confirmed with Dandona et al. who reported that weight loss reduces TNF- α in the obese²². Also, Sandoval and Davis approved that patients who had bariatric surgery gained reduction in IL-6 concentration and improved insulin sensitivity in parallel to weight loss²³. However, Chu and colleagues confirmed that serum leptin level was reduced after weight reduction program²⁴. Also, Arita and co-authors found that exercise training resulted in both reduction in serum leptin and improved leptin sensitivity²⁵. Ruche and McDonald confirmed that weight loss induces adiponectin synthesis²⁶.

Ueno and colleagues conducted their study of weight reducing program on thirty-five obese NIDDM patients for twelve weeks (diet restriction and exercise) and confirmed that improved serum leptin, lipoprotein profile, insulin sensitivity and glucose control were associated with weight loss²⁷. Tilg and Kaser in their previous study on twenty-five obese patients with fatty liver on three months of restricted diet and exercise, concluded that weight reduction was associated with reduction in serum levels of aminotransferase, albumin, total cholesterol and fasting blood glucose²⁸.

Conclusion

Weight loss modulates insulin resistance, adiponectin, leptin, inflammatory cytokines and markers of hepatic function in patients with non-alcoholic steatohepatitis.

Acknowledgment

This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant no.(324/290/1434).The authors, therefore, acknowledge with thanks DSR technical and financial support.

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