

Clinical Study

Long-Term Effects of Metformin and Lifestyle Modification on Nonalcoholic Fatty Liver Disease Obese Adolescents

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Objective. To assess the long-term effects of metformin in combination with lifestyle intervention and its association between insulin levels and the degree of steatosis at ultrasonography (US) in obese adolescents. *Methods.* Thirty-five postpubertal obese boys were randomized into two groups: one receiving metformin in combination with a multidisciplinary lifestyle intervention versus a placebo group, which also received the same intervention. The visceral, subcutaneous fat and degree of steatosis were measured by ultrasonography. Fasting blood samples were collected to analyze glucose, insulin, insulin resistance, and aminotransferases. Repeated ANOVA measures were used to compare changes over time and between groups, and Spearman's correlations were used to identify an association between insulin and the degree of steatosis at US. *Results.* There was a positive correlation between the degree of steatosis at US with insulin concentrations and HOMA-IR. Long-term therapy plus metformin significantly reduced body weight, body mass index, insulin, HOMA-IR, and visceral fat. *Conclusions.* Metformin was more effective than the placebo in improving clinical parameters associated with obesity and steatosis.

1. Introduction

Nonalcoholic Fatty Liver Disease (NAFLD) affects 10% to 39% of the world population, 50% of diabetic patients, 57% to 74% of obese people, and up to 90% of the people with morbid obesity. Its prevalence in obese adolescents is between 22.5% and 52.8% [1, 2]. When not appropriately treated, NAFLD can progress to cirrhosis [1, 2]. Thus, treatment of NAFLD is a relevant issue in clinical hepatology, and several therapeutic approaches have been tested in uncontrolled and controlled pilot studies [3].

Obesity and type 2 diabetes are considered the most powerful predisposing risk factors for the development of

severe manifestations of NAFLD [1]. It is important to note that adipose tissue is recently considered endocrine organ strictly related to several comorbidities. The expansion of visceral fat is a determinant risk factor for the development of NAFLD in obese individuals [4]. Moreover, a new study reported that the extent of hepatic inflammation and fibrosis is augmented incrementally with increases in visceral fat. For each 1% increase in visceral fat, the odds ratios for increasing liver inflammation and fibrosis were 2.4 and 3.5, respectively. Visceral fat remained an independent predictor of advanced steatohepatitis and fibrosis even when the model controlled for insulin resistance and hepatic steatosis [5].

Considering the relation between obesity and NAFLD, an adequate treatment must include strategies to reduce all Metabolic Syndrome predictor factors, including gradual body mass reduction and efforts to control blood glucose and lipid levels [6]. A cornerstone of the strategy to treat obese adolescents with NAFLD is a long-term multidisciplinary intervention that utilizes exercise, clinical, psychological, and nutritional therapies. Although an optimal management program for NAFLD in obesity and insulin resistance has not been established, some strategies are based on hypoglycemic and triglyceride-lowering agents and on lifestyle intervention through a low fat diet, vitamin E supplementation, and exercise [7].

Metformin is a well-established oral hypoglycemic agent in the treatment of adults with type 2 diabetes mellitus and other conditions of insulin resistance. It acts by decreasing hyperinsulinemia and improving hepatic insulin resistance [8]. In pediatric obese patients with insulin resistance, metformin has also been shown to promote weight loss [9, 10]. It was observed that short-term use of metformin is well tolerated by obese children and that it has a beneficial effect on Body Mass Index (BMI) and cardiovascular autonomic control as well as a trend toward the improvement of insulin sensitivity [11]. Thus, long-term treatment with metformin may provide a means to ameliorate the metabolic profile of obese adolescents.

The role of metformin in the reduction of insulin resistance and visceral in the pediatric NAFLD obese population has not been adequately studied. Therefore, the main aim of this study was to assess the effect of metformin in obese adolescents with NAFLD who participated in a multidisciplinary lifestyle modification program. Furthermore, it aimed to identify the association between insulin resistance and the degree of steatosis at US in obese patients.

2. Methods

2.1. Subjects. Obese adolescent boys were recruited for this study from the Multidisciplinary Obesity Intervention Program outpatient clinic of the Federal University of São Paulo—Paulista Medicine School—between January and February of 2008. The boys included in the study were between 15 and 19 years old, presented with primary obesity (body mass index >95th percentile of the CDC reference growth charts) [12], and had reached the initial postpubertal Tanner stage five [13]. The initial cohort of the present study that met the above criteria included 80 individuals. Subsequently, 35 obese boys who had a positive ultrasound diagnosis of NAFLD were selected and randomized into two groups: lifestyle modification plus metformin ($n = 21$) and lifestyle modification plus placebo ($n = 14$). The adolescents and their families signed an agreement to participate, on a voluntary basis, in a long-term multidisciplinary therapy. All subjects performed an ergometric test before starting the program and had the approval of a physician. The exclusion criteria were an identified genetic disease, metabolic or endocrine disease, chronic alcohol consumption (>20 g/day) [14], and previous drug utilization or other causes of chronic liver disease.

All patients had normal age-specific growth and sexual development, and none had marked hirsutism or severe acne.

This study was carried out in accordance with the principles of the Helsinki declaration and was formally approved by the Ethical Committee of the Federal University of São Paulo—Paulista Medicine School (no. 0135/04). Informed consent was obtained from all subjects and/or their parents.

2.2. Protocol. After being diagnosed with NAFLD diagnosis, obese male adolescents were submitted to a long-term multidisciplinary therapy (12 months) that aimed at promoting changes in their sedentary lifestyles and nutritional habits. The basic requirement for participation was the motivation to follow the therapy, presenting high attendance (>75%) at the sessions.

The multidisciplinary therapy consisted of aerobic exercise, nutritional, psychological assistance and clinical management of obesity, and NAFLD. The short-term intervention corresponded to 6 months and the long-term intervention to 12 months. This model of multidisciplinary therapy was suggested by the World Health Organization (WHO) [15]. This kind of treatment attempts a more integrated overview of the key role of these emerging strategies for therapeutic intervention. The program consisted of four physicians, two dietitians, four physiologists, and one psychologist.

Subjects were medically screened, had their pubertal stage assessed, and had their anthropometrics profile measured (height, weight, and body mass index). A blood sample was collected and analyzed for glucose and insulin concentrations, as well as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl aminotransferase (GGT). Ultrasound and ergometric tests were performed. The patients were randomized to the metformin-treated group (500 mg twice daily) according to Freemark and Bursey [9] and to the placebo group. The results were compared at baseline and after short- and long-term interventions. To remove any influence of diurnal variations, the procedures were scheduled for the same time of day and at least 15 hours after the last training session. Thereafter, all 35 obese male adolescents with a diagnosis of NAFLD started the multidisciplinary long-term weight loss therapy. Two placebo-treated subjects and four metformin-treated subjects withdrew from the study within the first 12 weeks for reasons unrelated to drug toxicity or complications of the trial. Their laboratory results were not included in the final analysis.

3. Measurements

3.1. Anthropometric Measurements and Body Composition. Wearing light clothes and no shoes, subjects were weighed to the nearest 0.1 kg on a Filizola scale. Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer (Sanny, model ES 2030). BMI was calculated as body weight (kg) divided by height (m) squared (kg/m^2).

3.2. Serum Analysis. Blood samples were collected in the outpatient clinic around 08:00 h after an overnight fast. Insulin resistance was assessed by the homeostasis model assessment insulin resistance index (HOMA-IR). HOMA-IR was calculated using the fasting blood glucose (FBG) and the immunoreactive insulin (I): $[\text{FBG (mg/dl)} \times \text{I (mU/l)}]/405$. The HOMA-IR data were analyzed according to reference values described by Schwimmer et al. [14].

3.3. Hepatic Steatosis and Measurements of Visceral and Subcutaneous Adipose Tissues. All abdominal ultrasonographic procedures and measurements of visceral and subcutaneous fat tissue and fatty liver were performed before and after the intervention in a double-blinded manner by the same specialized physician. A 3.5 MHz multifrequency transducer (broad band) was used for the diagnosis. This procedure permits a reduced margin of risk for misclassification. The intraexamination coefficient of variation for US was 0.8%. This method was previously standardized for obese adolescents [2, 4].

Ultrasonic measurements of intraabdominal, “visceral”, and subcutaneous fat were taken. Ultrasound-determined subcutaneous fat was defined as the distance between the skin and external face of the rectoabdominal muscle. Visceral fat was defined as the distance between the internal face of the same muscle and the anterior wall of the aorta [16].

The definition of ultrasonic fatty liver was based on previously reported diagnostic criteria. Detected liver steatosis was classified as degree 1 (liver attenuation slightly less than spleen), degree 2 (more pronounced difference between liver and spleen and intrahepatic vessels not seen or slightly higher attenuation than liver), or degree 3 (markedly reduced liver attenuation with sharp contrast between liver and intrahepatic vessels) [17].

3.4. Clinical Therapy. The patients visited the endocrinologist once a month to record health and clinical parameters. Medical followup included initial medical history, physical examination, and appropriate tests following regular clinical surveillance. The endocrinologist also evaluated the possible collateral effects of metformin use.

3.5. Nutritional Therapy. Energy intake was set at the levels recommended by the dietary reference intake for subjects with low levels of physical activity of the same age and gender. They were encouraged to reduce their food intake and follow a specifically balanced diet, reducing saturated fats and refined sugars [18]. No drugs or antioxidants were recommended. Once a week, adolescents had a dietetics lesson (food pyramid, recordatory inquiry, weight loss diets, dietetic versus low calorie foods, fat and cholesterol, nutrition facts). Additionally, individual nutritional counseling was recommended when patients had problems making changes in their dietary consumption [19].

At the beginning and after the long-term multidisciplinary therapy, each adolescent filled in a three-day dietary record with the help of his/her parents. Portions were

measured in terms of familiar volume and size. The nutritionist taught the parents and the adolescents how to record food consumption. These dietary data were transferred to a computer by the same nutritionist, and the nutrient composition was analyzed by a PC program developed at the Federal University of São Paulo—Paulista Medicine School (Nutwin software, for windows, 1.5 version, 2002) —based on Western and local food tables. In addition, the parents were encouraged to call if they needed further information.

3.6. Exercise Therapy. During the multidisciplinary intervention, adolescents followed a personalized aerobic training program comprised of 60-minute sessions, three times a week (180 minutes/week) under the supervision of a physiologist. The program was developed according to the results of an initial oxygen uptake test for aerobic exercises (cycle ergometer and treadmill). The intensity was set at a workload corresponding to ventilatory threshold 1 (50% to 70% of oxygen uptake test). After 6 weeks, aerobic tests were performed to assess physical capacities and to individually adjust physical training intensity. During the aerobic sessions, the adolescents’ heart rate was continuously monitored. The exercise program was based on the American College of Sports Medicine Statement (2001) [20]. To assess improvement in cardiovascular function, the subjects performed the same exercise test protocol used at the beginning of the study after 6 weeks and again at the end of the short- and long-term therapies. The physiologists regularly participated in the training sessions to encourage lifestyle changes.

3.7. Psychological Therapy. Obesity has been associated with several psychological problems, such as depression, disturbances in body image, anxiety, and a lowered self-esteem. Validated questionnaires designed to assess these problems were used to diagnose psychological problems in the study participants. During the multidisciplinary intervention, the adolescents had weekly psychological orientation group sessions in which they discussed body image and alimentary disorders such as bulimia, anorexia nervosa, and binge eating and their signs, symptoms, and consequences for health. The relation between feelings and food and more familiar problems, such as alcoholism, and other topics were also discussed. An individual psychological therapy program was recommended when nutritional and behavioral alterations were found.

4. Data Analysis

All data were analyzed using STATISTICA version 6 for Windows with the significance level set at $P < .05$. All data were expressed as mean \pm SD unless otherwise stated. Distributional assumptions of quantitative variables were verified by the Kolmogorov-Smirnov test. Comparisons between groups and changes over time were made using the ANOVA for repeated measures, followed by post hoc comparison with a Tukey test. Spearman’s Correlations were made to identify a possible association between insulin resistance and the degree of steatosis at US in obese patients.

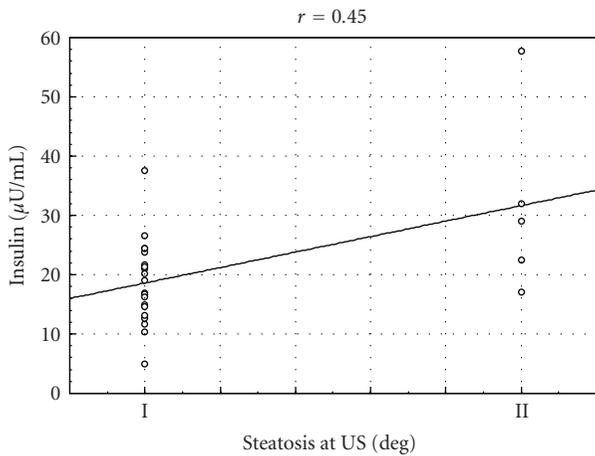


FIGURE 1: Correlation between degree of steatosis at US and Insulin levels at baseline conditions of adolescent obese boys.

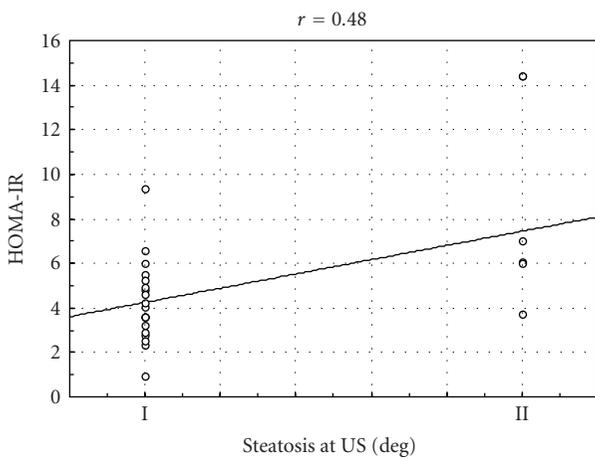


FIGURE 2: Correlation between degree of steatosis at US and HOMA-IR at baseline conditions of adolescent obese boys.

5. Results

At baseline, all patients presented with degree I to II liver steatosis. After long-term therapy, the prevalence of NAFLD was decreased similarly in both groups, from 100% to 46.2% and 50% for metformin-treated and placebo-treated groups, respectively. Indeed, there was a positive correlation between the degree of steatosis at US with insulin concentrations (Spearman's $r = 0.45$) and HOMA-IR (Spearman's $r = 0.48$) (Figures 1 and 2).

At baseline, the two groups did not differ significantly in all measures analyzed. After short- and long-term therapies, the metformin-treated group presented a significant reduction in body weight, BMI, insulin concentration, HOMA-IR, and visceral fat ($P < .05$ measured by two-way ANOVA for repeated measures). On the other hand, in the placebo-treated group, the reduction in body weight and visceral fat after long-term therapy was not significant. The mean values of serum hepatic transaminases did not show differences in either group at any time point (Table 1).

After one year of multidisciplinary therapy, all patients in the placebo group presented altered HOMA-IR (>2.0) according to Schwimmer et al. criteria [14], and two of them had normalized ALT enzyme levels. Indeed, in the metformin group, three individuals had normalized HOMA-IR, and five had normalized ALT values.

6. Discussion

The pathogenesis of NAFLD is still not clear; however, there is evidence that insulin resistance and visceral fat play an important role in this disease [21]. According to Park et al. [22], patients with central adiposity and insulin resistance have a higher risk of NAFLD, reinforcing the strict association between these two parameters. In fact, it was observed that both groups analyzed had altered HOMA-IR and insulin levels at baseline. Indeed, a positive correlation between the degree of steatosis at US with insulin concentrations and HOMA-IR was shown, corroborating the findings of previous studies [21, 22] (Figures 1 and 2).

Given the close relations between the metabolic syndrome and NAFLD, an adequate treatment must be included for all risk factors associated with obesity [23]. In this way, metformin use can be an important strategy to control insulin sensitivity in patients with NAFLD.

The mechanism by which metformin improves the clinical parameters of NAFLD might involve the effect of the drug on the inhibition of intestinal glucose absorption, on the reduction of hepatic glucose production, and on the improvement of insulin sensitivity in peripheral target tissues [24, 25].

In the present investigation, only the metformin-treated group presented a significant reduction in body mass, BMI, insulin concentration, HOMA-IR, and visceral fat after short- and long-term interventions (Table 1). Although we did not observe a statistical decrease in all parameters in the placebo group, this group did have a significant reduction in visceral fat tissue. These findings suggest that multidisciplinary therapy in combination with metformin is important to improve these variables, as well as ALT enzyme levels, which are clearly correlated with the development of NAFLD [26, 27].

Our data corroborated with those of Fu et al. [24], suggesting that metformin therapy and lifestyle modification improve parameters indicative of Metabolic Syndrome and NAFLD. A study with obese adolescents demonstrated that insulin resistance and the associated compensatory hyperinsulinemia are usually associated with obesity, and it can be considered an initial factor of Metabolic Syndrome development [28].

The effects of metformin on NAFLD were recently compared with a lipid and calorie-restricted diet in a 6-month, open label, randomized study. The mean changes in insulin, insulin resistance (HOMA-IR), and aminotransferase concentrations were greater in the experimental arm than in the group given only dietary advice [29].

A previous randomized, controlled trial of metformin in an obese pediatric population with insulin resistance demonstrated that metformin therapy is safe and well

TABLE 1: Characteristics of adolescent obese boys measured at baseline, 6 months, and 1 year after multidisciplinary lifestyle program associated with metformin therapy.

	Placebo group			Metformin group		
	Basal	6 months	1 year	Basal	6 months	1 year
BM (wt)	114.20 ± 10.7	110.9 ± 8.56	112.22 ± 5.68	105.42 ± 8.00	98.65 ± 9.37 [#]	97.39 ± 10.41 [†]
BMI (wt/ht ²)	37.02 ± 3.15	36.69 ± 2.89	37.23 ± 2.83	37.24 ± 3.44	34.05 ± 4.01 [#]	34.39 ± 4.60 [†]
Glucose (mg/dl)	91.75 ± 7.47	93.25 ± 8.08	96 ± 6.48	93.58 ± 7.30	92.28 ± 7.73	96 ± 8.97
Insulin (μU/mL)	18.77 ± 6.71	19.73 ± 6.84	19.2 ± 5.30	22.35 ± 11.82	16.52 ± 9.7 [#]	15.83 ± 10.34 [†]
HOMA-IR	4.28 ± 1.66	4.53 ± 1.56	4.57 ± 1.39	5.23 ± 3.1	3.83 ± 2.73 [#]	3.81 ± 2.62 [†]
Visceral (cm)	4.87 ± 1.52	5.37 ± 1.85	3.55 ± 1.80	4.90 ± 1.04	3.72 ± 1.24 [#]	4.00 ± 1.07 [†]
Sub (cm)	3.29 ± 0.83	3.20 ± 0.87	3.46 ± 0.08	3.17 ± 0.60	2.86 ± 0.74	2.69 ± 0.96
AST(U/L)	28.37 ± 14.38	26.75 ± 9.40	33 ± 16.71	33.52 ± 10.13	26.78 ± 6.80	28.77 ± 11.99
ALT (U/L)	52.12 ± 24.93	48.25 ± 17.36	57.25 ± 38.01	52.05 ± 26.89	39.64 ± 16.35	41.11 ± 12.48
GGT (U/L)	27.62 ± 7.19	28 ± 5.70	24.25 ± 7.50	33.52 ± 6.69	29.35 ± 10.35	31.44 ± 14.37

Reference normal values to Glucose (60–110 mg/dL), Insulin (<20 μU/mL), HOMA-IR (<2.0), AST (10–40 U/L), ALT (10–35 U/L), and GGT (17–30 U/L) [14]

Placebo group versus Metformin group on the same occasion $P \leq .05$ (ANOVA for repeated measures),

[#] Basal versus 6 months for the same group $P \leq .05$ (ANOVA for repeated measures),

[†] Basal versus 1 year for the same group $P \leq .05$ (ANOVA for repeated measures),

6 months versus 1 year for the same group $P \leq .05$ (ANOVA for repeated measures).

BM (Body mass), BMI (Body Mass Index), HOMA-IR (homeostasis model assessment insulin resistance index), Sub (Subcutaneous adipose tissue), AST (aspartate aminotransferase), ALT (alanine aminotransferase), and GGT (Gamma-glutamyl transferase).

tolerated. Indeed, the patients had improved insulin sensitivity with metformin, similar to data verified in the present investigation [18].

Other relevant findings in this study were that short-term lifestyle intervention plus metformin was efficient to promote a significant improvement in most of the parameters analyzed, and the long-term intervention improves NAFLD control. Similar results were observed by Nadeau et al. [30], suggesting that taking metformin in combination with making lifestyle changes is important to NAFLD management and that lifestyle improvement by itself may not be as effective. However, both treated groups showed an important decrease in the prevalence of NAFLD.

A recent study indicates that metformin may have antiinflammatory and lipolytic effects mediated through adipocytokines, suggesting that researchers should consider these effects in new studies [24]. Further clinical trial investigations involving a larger population, a placebo-control group, a double-blind format, and a long-term followup of metformin treatment are needed to improve our findings.

The lack of a histological endpoint is a limitation of this study; however, we used the degree of steatosis observed at US diagnosis and the hepatic transaminases as a noninvasive score of liver damage in NAFLD.

In conclusion, taking metformin in combination with lifestyle intervention was more effective than intervention alone to promote an improvement in insulin resistance, visceral adiposity, and other commonly observed clinical parameters.

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