Research Article

Diabetes Mellitus, Nonalcoholic Fatty Liver Disease, and Conjugated Linoleic Acid (Omega 6): What Is the Link?

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Background and Objective. Type 2 DM and obesity are the coming epidemics and their association with NAFLD is well established; essential fatty acids are vital for body health yet the body cannot make them; 2 essential fatty acids are especially important: linoleic (omega-6) and alpha-linoleic (omega-3) acids; they can be considered as “bioactive lipids” and serve as functional foods.

Methods. 50 type 2 Egyptian diabetic patients controlled on oral hypoglycemic drugs together with 20 age- and sex-matched healthy participants were enrolled in the study; all were subjected to complete history taking, BMI, fasting plasma glucose, HOMA-IR, ALT, AST, GGT, urea and creatinine, total lipid profile, hepatitis markers including hepatitis B surface antigen and hepatitis C virus antibodies, conjugated linoleic fatty acid “CLA,” and abdominal ultrasound for grading of NAFLD.

Results. Our study in Egyptian diabetics with NAFLD revealed a low level of serum CLA compared to healthy control; such deficiency was more marked with advanced grades of NAFLD; lowest levels were observed in those with severe steatosis (NASH) with definite association between CLA and obesity.

Conclusion. Insulin resistance is the main link between NAFLD, diabetes, and obesity. Conjugated linoleic acid (CLA) has a role in fat deposition in the liver and in development and improvement of insulin resistance. Fatty food had a documented role in the pathogenesis of obesity and diabetes but it can also be the cure.

1. Introduction

Some important food components cannot be made by the body and have to be supplied from outside, and they are vital for human health and are so-called “essential”; of those 2, main polyunsaturated fatty acids are getting much attention: linoleic acid (omega-6) and alpha-linoleic acid (omega-3) can modulate metabolism and alter body weight so they can be considered as bioactive lipids and can serve as functional food [1].

Conjugated linoleic acid (CLA) refers to a heterogeneous group of geometric and constitutional isomers of linoleic acid. Up to 28 forms are known differing from each other in their double-bond arrangement; of them, “c9,t11” and “t10,c12” are especially important [1].

CLA is a natural trans fat present in many healthy foods, e.g., animal food sources mainly ruminant animals such as cows, sheep, and goats with greatly variable amounts depending on the animals’ feeding pattern; e.g., grass-fed cow dairy and beef have 300-500% higher CLA content (mainly c9,t12) compared to grain-fed cows [2], while industrial trans fats found mainly in chemically treated vegetable oils, e.g., sunflower oils, are harmful and supplements are mainly rich in t10,c12 isomer of CLA that does not have the same health effects as this taken from natural foods [3].

The liver is the main organ concerned with energy homeostasis converting excess glucose into fatty acids (FA) which later export as triglycerides (TG) [4]. It is also an important target for CLA effects regardless of its physiological condition.

Nonalcoholic fatty liver disease (NAFLD) is an important health concern mainly due to its high global prevalence (20-30% of adult population) especially in subjects having insulin resistance and metabolic syndrome [5]. Hepatic lipids in the form of triglycerides primarily accumulate in the liver [6] with multiple potential steps involved in the progression of...
NAFLD including increased circulating fatty acid uptake [7], enhanced de novo lipogenesis [8], diminished rate of FA oxidation [9], or reduced secretion [10].

NAFLD is a major cause of chronic liver disease and it encompasses a spectrum from simple steatosis to steatohepatitis, fibrosis, or cirrhosis. The mechanisms involved in the occurrence of NAFLD and its progression are probably due to the metabolic profile expressed within the context of a genetic predisposition and is associated with higher energy intake [11].

The metabolic syndrome is a cluster of alterations associated with an increased risk for the development of cardiovascular diseases and diabetes. NAFLD patients have more than a feature of the metabolic syndrome, and now they are considered the hepatic component of metabolic syndrome. Several scientific advances in understanding the association between NAFLD and MS have identified insulin resistance as the key aspect in the pathophysiology of both diseases. In the multiparallel hit theory of NAFLD pathogenesis, IR was described to be central in the predisposition of hepatocytes to be susceptible to other multiple pathogenetic factors [12].

Despite the absence of specific guidelines for treatment of NAFLD, most recommendations advise body weight reduction [13]; the dietary fatty acid composition is crucial in NAFLD development as 15% of hepatic TG comes from the diet [14]; in this regard, bioactive lipid is important.

1.1. Objective. The aim of the present study is to find out a relation between obesity, insulin resistance, and conjugated linoleic fatty acid in type 2 diabetic patients with variable degrees of NAFLD severity.

2. Patients and Methods

A total of 50 type 2 diabetic patients, 25 normal weight, and 25 obese (36 females and 14 males) with NAFLD were enrolled in this prospective study from December 2015 to November 2016. They were recruited from Diabetes and Hepatology outpatient clinics of Kasr El Ainy Hospital. Their age ranged from 29 to 57 years.

Twenty healthy, normal-weight volunteers who were age- and sex-matched were included as a control group. An informed consent was obtained from all participants prior to enrolment.

The diagnosis of NAFLD was based on ultrasonographic finding of a bright liver, which was defined and graded as a diffuse hyperechoic echotexture (bright liver) (grade 1), increased liver echotexture compared to the kidney (grade 2), vascular blurring (grade 3), and deep attenuation (grade 4) [15].

The study was conducted with appropriate approval by the Ethics Committee of Cairo University (N-17-2015) in accordance with the ethical guidelines of the Declaration of Helsinki [16].

2.1. Inclusion Criteria. For diabetic patients with NAFLD, all were controlled on oral hypoglycemic medications, age above 18 years, normal and overweight (BMI > 25 kg/m²), and a bright liver on abdominal ultrasound with or without elevated liver enzymes.

2.2. Exclusion Criteria. Patients were excluded from the study if one of the following criteria was present: any liver disease other than NAFLD such as hepatitis B or C, autoimmune hepatitis, alpha-one antitrypsin deficiency or Wilson’s disease, alcohol consumption, history of drug intake (such as use of amiodarone, corticosteroids, tamoxifen, methotrexate, and oral contraceptives), pregnancy, hypertension, thyroid disease, malignancy, history of taking any omega-6 or CLA capsules, and decompensated liver disease [17]. Any subjects with evidence of local or systemic infection on physical examination were also excluded from the study.

2.3. Selection of Control. In all healthy control volunteers, the absence of any current or past liver disease was established based on the presence of normal liver function tests and the presence of a normal abdominal ultrasound.

2.4. Methodology. All participants included in the study were subjected to the following: detailed history taking, complete clinical examination including anthropometric evaluation (weight, height, and body mass index (BMI) were calculated). BMI between 25 and 29.9 kg/m² and ≥30 kg/m² were defined as overweight and obesity, respectively.

Laboratory investigations include fasting plasma glucose “oxidase method,” ELISA for conjugated linoleic fatty acid “CLA,” fasting insulin level, calculation of HOMA to determine insulin resistance (American formula: fasting glucose mg/dl × fasting insulin μU/ml /405) [18], liver function tests, serum transaminases (ALT, AST), GGT, urea and creatinine, total lipid profile, and hepatitis markers including hepatitis B surface antigen and hepatitis C virus antibodies.

Abdominal ultrasonography was performed for all subjects using a Toshiba Aplio XV scanner equipped with a broadband 3.5 MHz curved array probe to assess the presence of liver steatosis (bright liver) and by a single operator to avoid interobserver variability. Patients were examined after at least 8 hours of fasting and were examined in the supine, right, and left lateral positions.

2.5. Statistical Methods. Data were statistically analyzed using the General Linear Model (GLM) procedure of SAS software (SAS Institute, version 8.2, 2001) and Excel 2010. Differences between groups were evaluated by one way ANOVA. The significant differences between group means were tested by the Duncan multiple range test. The data were presented in mean ± SE. Level of significance was set at <0.05.

3. Results

The anthropometric and laboratory data of all enrolled patients and control are shown in Table 1; diabetics have statistically significant lower CLA levels (13.25 ± 0.66) compared to control (9.78 ± 1.34), with markedly low levels in obese diabetics (6.13 ± 0.57).

According to results of abdominal ultrasound examination, diabetic patients were classified into 4 groups according to the grade of NAFLD: grade 1 steatosis (n.4), grade
II steatosis (n.16), grade III steatosis (n.18), and grade IV steatosis (n.12).

All diabetic subjects with different grades of NAFLD were compared as regards their BMI and different laboratory values, and comparative data are illustrated in Table 2, and data are presented graphically in Figure 1.

All patients with grade I steatosis had normal BMI, and all grade IV patients were obese (high BMI) as they represent the extremes; therefore, no comparison—as regards laboratory data including their level of CLA—was done among these two grades. While in those with grades II and III NAFLD, some of the patients had normal BMI while the others were obese so a comparison was done in those patients with grades II and III steatosis. Data of patients with grade II NAFLD after being classified according to their BMI is illustrated in Table 3.

### 4. Discussion

Type 2 diabetes is a major health concern with an expected doubling of the number of patients in the next few years; insulin resistance plays an important role in the pathogenesis of type 2 diabetes [19].

NAFLD nowadays is ranked as the commonest chronic liver disease as its prevalence is rising: estimated 20-30% in the general population, and can reach up to 70-90% in obese and diabetic patients [20]. It has a strong association with...
many conditions; most commonly obesity, type 2 diabetes, insulin resistance, dyslipidemia, and hypertension, and it is considered to be "hepatic manifestation of metabolic syndrome" [21].

And despite that no specific guidelines exist concerning the treatment of NAFLD, yet most recommendations were aimed at treating associated conditions such as obesity by weight reduction and diabetes by glycemic control and lipid control [22]. In this regard, modulating body weight and metabolism through functional foods as bioactive lipids may be important. A specific group of polyunsaturated FA collectively known as conjugated linoleic acid (CLA) has been suggested to have an effect on regulating energy metabolism.

This polyunsaturated conjugated fatty acid is a naturally present trans fat found mainly in dairy products (milk, cheese, yogurt, and cream) and meat, veal, and lamb from ruminant grass-fed animals. It is formed as an intermediate during isomerization of dietary linoleic acid by the action of bacteria in the digestive tract of ruminant animals [23], and it is formed mainly of cis-9,trans-11 CLA (>80%) with small amounts of (t10,c12) CLA and other isomers [24].

**Figure 1:** Comparison between BMI, HOMA-IR, and CLA of all enrolled subjects according to their grade of steatosis. BMI: body mass index; CLA: conjugated linoleic acid.

**Table 3:** Comparison between laboratory data of subjects with grade II NAFLD after being classified according to their BMI into those with normal BMI and with high BMI.

| Parameter     | Diabetics with normal BMI (n.5) | Diabetics with high BMI (n.11) | P value |
|---------------|---------------------------------|--------------------------------|---------|
| BMI (kg/m²)   | 24.21 ± 0.14                    | 30.8 ± 0.75                    | <0.0001** |
| AST (IU/l)    | 21.10 ± 1.36                    | 34.30 ± 1.62                   | <0.0001** |
| ALT (IU/l)    | 33.60 ± 2.34                    | 55.6 ± 3.31                    | <0.0001** |
| T. Chol (mg/dl)| 214.10 ± 5.24                   | 225.20 ± 7.42                  | <0.0056** |
| HDL (mg/dl)   | 18.1 ± 0.61                     | 16.10 ± 0.43                   | <0.04500* |
| LDL (mg/dl)   | 163.2 ± 3.69                    | 164.2 ± 2.61                   | <0.2135 |
| TG (mg/dl)    | 177.6 ± 13.05                   | 221.8 ± 14.45                  | <0.0001** |
| FBG (mg/dl)   | 199.8 ± 13.36                   | 228.80 ± 14.89                 | <0.1174 |
| Insulin (µIU/ml)| 7.62 ± 0.34                     | 7.10 ± 0.42                    | 0.2875 |
| HOMA-IR       | 3.78 ± 0.26                     | 3.92 ± 0.32                    | <0.4188 |
| CLA (ng/ml)   | 8.42 ± 0.58                     | 7.92 ± 0.65                    | <0.3569 |

**Table 4:** Comparison between laboratory data of studied diabetics with grade III NAFLD.

| Parameter     | Diabetics with normal BMI (n.8) | Diabetics with high BMI (n.10) | P value |
|---------------|---------------------------------|--------------------------------|---------|
| BMI (kg/m²)   | 24.21 ± 0.14                    | 30.9 ± 0.83                    | <0.0001** |
| AST (IU/l)    | 20.80 ± 0.84                    | 33.80 ± 1.18                   | <0.0001** |
| ALT (IU/l)    | 34.70 ± 1.91                    | 53.60 ± 2.69                   | <0.0001** |
| T. Chol (mg/dl)| 224.4 ± 5.17                    | 243.3 ± 7.32                   | <0.0188* |
| HDL (mg/dl)   | 15.1 ± 0.45                     | 13.9 ± 0.64                    | <0.0806 |
| LDL (mg/dl)   | 107.7 ± 3.39                    | 177.8 ± 5.64                   | <0.2244 |
| TG (mg/dl)    | 194.7 ± 12.31                   | 260.3 ± 15.41                  | <0.0001** |
| FBG (mg/dl)   | 251.6 ± 14.6                    | 266.7 ± 17.32                  | <0.4933 |
| Insulin (µIU/ml)| 7.34 ± 0.28                     | 6.46 ± 0.42                    | 0.2976 |
| HOMA-IR       | 4.73 ± 0.28                     | 3.95 ± 0.38                    | <0.0681 |
| CLA (ng/ml)   | 7.78 ± 0.26                     | 5.92 ± 0.37                    | <0.0002** |

This polyunsaturated conjugated fatty acid is a naturally present trans fat found mainly in dairy products (milk, cheese, yogurt, and cream) and meat, veal, and lamb from ruminant grass-fed animals. It is formed as an intermediate during isomerization of dietary linoleic acid by the action of bacteria in the digestive tract of ruminant animals [23], and it is formed mainly of cis-9,trans-11 CLA (>80%) with small amounts of (t10,c12) CLA and other isomers [24].
The main organ concerned with energy homeostasis is the liver, It is also an important target for CLA effects that differ according to the type of isomer; e.g., in animals, trans-10,cis-12 caused increased lipid accumulation and hepatic steatosis but neither trans-10,cis-12 nor cis-9,trans-11 CLA has an effect on atherosclerosis [25]; both isomers promote insulin sensitivity by reducing adipose inflammation [26], and both also protect against oxidative stress and enhance hepatic mitochondrial function decreasing insulin resistance in rats by both isomers [27].

The relation between type 2 diabetes and NAFLD is well established; there is an increased risk of NAFLD among patients with type 2 diabetes and despite as previously mentioned that the pathogenesis of NAFLD is not fully understood, yet the most accepted theory is "multiple hit theory," and the first hit being insulin resistance as the most important contributing factor with obesity as the main insulin-resistant state causing inflammatory state and altering lipid metabolism [28, 29].

On the other hand, NAFLD also can act as a risk factor for development of diabetes; a study evaluated subjects with NAFLD and control subjects for 11 years; none of them has diabetes at baseline; those with NAFLD were more likely to develop metabolic syndrome and diabetes [30], and when those patients develop overt diabetes in the setting of preceding obesity and insulin resistance, it was found that diabetes acts as an independent risk factor for the progression of NAFLD [31].

So obesity and insulin resistance are both initiating and stimulating risk factors for the development and progression of NAFLD, hence the importance of dietary modifications and functional foods as a tool in prevention and management.

Conjugated linoleic acid (CLA) is a natural polyunsaturated fatty acid. It is the most common omega 6 fatty acid widely used for slimming; conjugated refers to the arrangement of double bonds in the fatty acid molecule (mainly cis and trans), and the number refers to the placement of such bonds in the chain [32]; there are nearly 28 different isomers.

CLA is a natural trans fat present in many healthy foods, e.g., animal food sources mainly ruminant animals such as cows, sheep, and goats with greatly variable amounts depending on the animals' feeding pattern; e.g., grass-fed cow dairy and beef have 300-500% higher CLA content (mainly cis and trans), and the number refers to the placement of such bonds in the chain [32]; there are nearly 28 different isomers.

Concerning the type of isomer given, cis-9,trans-11 CLA (which is the type mostly present in natural food) was shown to promote insulin sensitivity by reducing adipose inflammation. It mainly enhances hepatic mitochondrial function and conveys protection against oxidative stress through action on mitochondrial antioxidant enzymes increasing their activities while of the different CLA isomers, trans-10,cis-12 CLA (which is taken in supplement) causes increased lipid accumulation mainly through reduction of adiponectin and leptin leading to hepatic steatosis [42].

5. Conclusion and Limitation and Recommendations

(i) The study is a cross-sectional study with a small number of patients in the subgroups due to the cost

(ii) The study is a cross-sectional study with a small number of patients in the subgroups due to the cost

(iii) The study is a cross-sectional study with a small number of patients in the subgroups due to the cost

(iv) The study is a cross-sectional study with a small number of patients in the subgroups due to the cost

(v) The study is a cross-sectional study with a small number of patients in the subgroups due to the cost
of kits; it revealed a low level of serum CLA compared to healthy control; such deficiency was more marked with advanced grades of NAFLD with a definite association between CLA and obesity

(ii) We recommend with this documented deficiency a study on a large scale of patients to document such deficiency and to try supplementation with CLA to prove causality not only an association; mainly in consumption of foods naturally enriched with CLA, we recommend against artificial supplements due to possible harmful effects

(iii) Listed here are some of the functional diet-rich sources with CLA as we recommend incorporating them in the typical Egyptian diet so as to modulate the level of CLA: namely, grass-fed beef, whole milk cheddar cheese from grass-fed cows, and whole milk from grass-fed cows and lamb.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The abstract was accepted as a poster presentation in the Endocrine Society 2018.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors’ Contributions

Mona Hegazy innovated the idea and aim of the work. Mona and Hanan collected the patients and applied the methodology. Laila performed the laboratory workup. Naglaa collected the data, wrote the manuscript, and managed all the submission issues. Hala did the statistics and helped in manuscript writing.

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