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Associations between erythrocyte membrane fatty acid compositions and insulin resistance in obese adolescents

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ABSTRACT

Background/objective: Cytokines released from the adipose tissue and fatty acids (FAs) derived from lipolysis or uptake of fats go in to competition with glucose to be uptaken from the liver leads to insulin resistance (IR). We aimed to show the associations among serum lipid profile, FA compositions and IR.
Methods: Anthropometrical measurements, biochemical parameters and erythrocyte membrane (EM) FA levels of 95 obese adolescents (41 with IR) and 40 healthy controls were compared.
Results: LDL-C, fasting insulin levels, HOMA-IR were significantly higher and HDL-C levels were significantly lower in obese patients than in controls ($p = 0.013$, $p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively). EM C 24:0, C 16:1 ω7 and C 22:1 ω9 FA levels were significantly higher, while C 20:5 ω3 (EPA) levels were significantly lower in obese subjects than in controls ($p < 0.001$, $p = 0.018$, $p < 0.001$, $p = 0.043$ and $p < 0.001$, respectively). Moreover, when obese subjects divided into two groups according to the presence of IR; EM C 16:1 ω7 levels were still significantly higher and EPA levels were still significantly lower in both obese subjects with and without IR compared to controls ($p < 0.001$ for both).
Conclusion: Saturated FA intake should be decreased because of its role in the development of obesity and IR, and ω-3 group FA intake should be increased.

1. Introduction

The rising prevalence of childhood obesity poses a major public health challenge both in developed and developing countries by increasing the burden of chronic non-communicable diseases (Lakshman et al., 2012). Obesity rates have arisen three fold or more in various regions of the world, since 1980. The prevalence rates of childhood and adolescent obesity also increased and suggest that it may be increasing at a faster rate than adult obesity, with major implications for the population's future (Controlling the Global Obesity Epidemic, 2003). Although there is no study in our country, which have been conducted to investigate obesity prevalence and affecting factors in children, it is reported in some studies carried out in various cities that the rate of overweight in preschool children is between 4 and 13%, whereas rate of obese children is between 9 and 27% (Calısır and Karacam, 2011; Dündar et al., 2000). These results demonstrate that overweight and obesity are significant growing public health problems in children which are threatening their physical and mental health.

Many of the metabolic and cardiovascular complications of obesity are already present during childhood and are closely related to the presence of insulin resistance (IR) which is the most common abnormality of obesity (Lee et al., 2006). Such IR does not appear to be confined to the glucoregulatory actions of insulin, because elevated levels of fasting free fatty acid (FFA), that occur in plasma as a result of lipolysis in adipose tissue or when plasma triacylglycerols are taken into tissues, have also been reported in obese and non-obese adolescents despite hyperinsulinemia (Caprio et al., 1989). In a study by Roden et al. it was suggested that increased concentrations of FFA induce IR through inhibition of glucose transport/phosphorylation activity (Roden et al., 1996a).

Increase in circulating FFA in obese humans is largely attributed to increased lipolysis of basal adipose tissue. However, antilipolytic effect of the insulin may also be impaired in obesity. Regardless of

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the exact mechanisms involved, increased FFA is associated with insulin resistance in obese individuals (Mook et al., 2004). The exact mechanism of FFA induced IR has not been fully elucidated. However, Randle et al. (1963) have originally proposed that the insulin resistant properties of fatty acids (FAs) were mediated through direct inhibition of glucose metabolism. Overall, researchers have suggested that increased FFA induces peripheral IR and stimulates an initial hyperinsulinemic state, but as type 2 diabetes mellitus (T2DM) develops, β-cell dysfunction occurs and insulin secretion can no longer compensate for elevated FFA and thus hyperglycemia becomes apparent.

Typically, plasma or serum samples are more commonly analyzed for assessment of an individual's FA status because the FA composition of plasma reflects recent dietary fat intake (Rise et al., 2007). However, levels of FAs in plasma are subject to multiple different dietary influences. The plasma FA profile may be determined not only by the time elapsed between the ingestion of fat-containing foods, but also by the type of dietary lipid ingested (Blau et al., 2008). And because RBCs have a rather long lifespan (~120 days), the FA profile is considered a better indicator of long-term FA intake compared to the intermediate lifespan (3 weeks–3 months) of platelet or plasma lipids (Rise et al., 2007; Sun et al., 2007). In this respect, the red cells are more stable, as it is generally believed that these cells keep their FA distribution throughout their life.

In the present study, we aimed to determine and compare the serum lipid profile and FA compositions of erythrocyte membrane (EM) in obese adolescents with and without IR and healthy controls by gas chromatography–flame ionisation detector (GC–FID).

2. Methods

This study was approved by the local ethics committee of Gülhane Military Medical Academy, which was conducted according to the Helsinki II Declaration.

2.1. Study population

Ninety five obese adolescents and age and gender matched 40 healthy participants between 8 and 18 years old and Tanner stages between 2 and 5 were included in the current study.

Children were eligible if they were obese but not receiving an ongoing weight management program. Exclusion criteria included a known endocrine or chromosomal cause for obesity, major health and developmental conditions, usage of any medication and/or nutritional supplement particularly including ω-3 drugs and fish oil. Therewithal, subjects without a history of familial dyslipidemia and/or type 2 diabetes mellitus (T2DM), usage of ω-3 drugs and fish oil, and with body mass index (BMI) <85th percentile were included in the study as control group.

2.2. Clinical examination

The participants underwent routine medical history, physical examination including anthropometry and laboratory assessments. Measurement of height was performed with a fixed stadiometer (Harpender stadiometer) sensitive to 0.1 cm, and weight was performed twice with a digital scale sensitive to 0.1 kg and arithmetic mean was used. All anthropometric measurements were taken by the same trained technician following a standard protocol (Ferrario et al., 1995). Body mass index (BMI, kg/m^2) was calculated as weight in kilograms divided by height in squared meters. Based on the obtained values, the body mass index standard deviation score (BMI SDS) was calculated, expressing the BMI of the examined child by the number of standard deviations from the mean value for age and sex in the Turkish population. All subjects were evaluated according to the 2000 Centers for Disease Control and Prevention (CDC) Growth Charts, which are widely used in clinical practice. Those with a BMI ≥95th percentile were considered obese (Centers for Disease Control and Prevention Growth Charts (CDC), 2014). Tanner scale was used for assessing pubertal stage, and stages II–V were considered as puberty (Tanner, 1962).

2.3. Laboratory measurement

Fasting blood samples were obtained from the antecubital vein, and serum samples were separated for the analysis of the biochemical parameters without freezing. Fasting plasma glucose (FPG), triglyceride (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) levels were measured by enzymatic colorimetric methods with an Olympus AU2700 (Beckman Coulter, USA) auto analyzer using commercially available reagents. Low-density lipoprotein cholesterol (LDL-C) was calculated by the Fridewald formula (LDL-C = TC − (TG/5 + HDL-C)). Fasting serum insulin levels were measured by an ADVIA Centaur assay (Siemens Medical Solutions Diagnostics, Tokyo, Japan) with a sensitivity of 0.5 μU/L, and with the intra-assay and inter-assay CV of 4.6% and 5.9%, respectively. The homeostasis model of assessment-insulin resistance (HOMA-IR) was calculated according to the formula of fasting glucose (mg/dL) *x* fasting insulin (μU/mL)/405 (Matthews et al., 1985).

2.4. Erythrocyte suspension collection and analysis

A minimum of 50 μL of plasma or red-cell haemolysate was needed. Venous blood samples were taken to K_2-EDTA-containing tubes. Hematocrit levels for all samples were determined with ABX Pentra XL 80 (Horiba Medical, USA). Afterwards, the erythrocytes were separated from the plasma by centrifugation (3000 rpm, 1500 × *g*, for 10 min) and washed with an equal volume of saline. Following the removal of the saline, the cells were resuspended with saline to Hematocrit of 45%. These erythrocyte suspensions were stored in a freshly-butyl hydroxy toluene (BHT)-treated eppendorf vials at −80 °C.

Erythrocyte suspensions were thawed at 4 °C before the study. EM FAs measurement process was performed according to the method defined by Blau et al. (2008) and modified by Sertoglu et al. (2014). Linearity of our method was obtained between 2.5 μg/mL and 1000 μg/mL (r^2 = 0.9579–0.9973), recovery rate was 87–107%, LOD was 0.3–4.2 μg/mL and the reproducibility was between 4.2% and 10.4%.

TRACE GC Ultra Gas Chromatograph with FID was used (Thermo Scientific™, USA) with SPTM-2560 capillary column, (100m × 0.25 mm × 0.2 μm, Catalog no. 23362-U) for the measurement of saturated fatty acids (SFAs) (C 14:0 (myristic acid-MA), C 16:0 (palmitic acid-PA), C 18:0 (stearic acid-SA), C 20:0 (arachidic acid), C 22:0 (behenic acid), C 24:0 (lignoceric acid)), monounsaturated fatty acids (MUFAs) (C 16:1 ω7 (palmitoleic acid), C 18:1 ω9 (oleic acid-OA), C 22:1 ω9 (erucic acid), C 24:1 ω9 (nervonic acid)), ω-3 PUFAs (C 20:5 ω3 (eicosapentaenoic acid-EPA), C 22:6 ω3 (docosahexaenoic acid-DHA)), ω-6 PUFAs (C 18:2 ω6 (linoleic acid-LA), C 20:4 ω6 (arachidonic acid-AA), C 20:3 ω6 (eicosatrienoic acid)) of EM.

2.5. Statistics

Statistical analyses were made using the Statistical Package for Social Science v 15.0 software (SPSS, Chicago, IL., USA). Anthropometric and biochemical features were showed as categorical or continuous variables. Comparisons between the groups were made