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Effect of caloric restriction with or without n-3 polyunsaturated fatty acids on insulin sensitivity in obese subjects: A randomized placebo controlled trial☆

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ABSTRACT

Background: Caloric restriction and n-3 polyunsaturated fatty acid (PUFA) supplementation protect from some of the metabolic complications. The aim of this study was to assess the influence of a low calorie diet with or without n-3 PUFA supplementation on glucose dependent insulinotropic polypeptide (GIP) output and insulin sensitivity markers in obese subjects.
Methods: Obese, non-diabetic subjects (BMI 30–40 kg/m^2) and aged 25–65 yr. were put on low calorie diet (1200–1500 kcal/day) supplemented with either 1.8 g/day n-3 PUFA (DHA/EPA, 5:1) ($n = 24$) or placebo capsules ($n = 24$) for three months in a randomized placebo controlled trial. Insulin resistance markers and GIP levels were analysed from samples obtained at fasting and during an oral glucose tolerance test (OGTT).
Results: Caloric restriction with n-3 PUFA led to a decrease of insulin resistance index (HOMA-IR) and a significant reduction of insulin output as well as decreased GIP secretion during the OGTT. These effects were not seen with caloric restriction alone. Changes in GIP output were inversely associated with changes in red blood cell EPA content whereas fasting GIP level positively correlated with HOMA-IR index. Blood triglyceride level was lowered by caloric restriction with a greater effect when n-3 PUFA were included and correlated positively with fasting GIP level.
Conclusions: Three months of caloric restriction with DHA + EPA supplementation exerts beneficial effects on insulin resistance, GIP and triglycerides.
General significance: Combining caloric restriction and n-3 PUFA improves insulin sensitivity, which may be related to a decrease of GIP levels.

1. Introduction

Obesity and its associated disorders (impaired insulin sensitivity, hyperinsulinemia, dyslipidemia, hypertension) often lead to type 2 diabetes with increased risk for cardiovascular disease [1–3]. Obesity contributes to type 2 diabetes development by affecting glucose and lipid homeostasis, which depends on the balance between insulin sensitivity and insulin secretion [4]. Incretins account for 50–70% of insulin secretion after meals or an oral glucose load [5]. Glucose-dependent insulinotropic polypeptide (GIP) is released from enteroendocrine K cells in response to oral ingestion of fat or glucose [6] and stimulates insulin secretion from pancreatic β-cells in a glucose-dependent manner [7]. Transduction of its biological effects involves stimulation of G protein-coupled receptors (GPCR) [8], for example GPR 120 [9].

Adequate insulin sensitivity and insulin secretion can be restored by achieving appropriate body mass through adopting a healthy diet, energy restriction, and physical activity [10–11]. A diet rich in omega-3 (n-3) polyunsaturated fatty acids (PUFA) reduces risk for obesity complications by influencing lipid metabolism, inflammation, coagulation and

Abbreviations: AUC, area under curve; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GIP, glucose dependent insulinotropic polypeptide; IGI, insulinogenic index; NEFA, non esterified fatty acids; OGIS, oral glucose insulin sensitivity index; OGTT, oral glucose tolerance test; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acids.

☆ This trial was registered at isrctn.com as ISRCTN11445521.

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atherogenesis [12–14]. Increasing intake of n-3 fatty acids leads to changes in cell membrane phospholipid fatty acid composition, which affects cell and tissue function through alterations in the properties of membranes, altered cell signalling pathways, and modified gene expression profiles [15–18]. Docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) are considered the most biologically active n-3 PUFA [19–20].

Beneficial effects of n-3 PUFA on glucose homeostasis have been documented in animal models of obesity and metabolic syndrome [21–22]. Improvement in glucose tolerance after n-3 PUFA supplementation in obese humans has been less frequently reported and seems to be related to the presence of obesity complications [23–26]. Whether GIP is involved in the effect of n-3 PUFA on the insulin response to a glucose load has not been reported in human studies. Thus, it is not clear if n-3 PUFA affect the incretin system in humans with obesity and prediabetes.

The aim of this study was to investigate the effects of caloric restriction with or without consumption of n-3 PUFA (DHA + EPA) on GIP, insulin release and metabolic variables at fasting and during an OGTT in obese, non-diabetic subjects.

2. Materials and methods

2.1. Subjects

Obese subjects (BMI 30–40 kg/m^2) aged 25–65 years were recruited from the Out-Patient Clinic of Obesity and Lipid Disorders and the Department of Metabolic Disorders, Jagiellonian University Medical College, Krakow, Poland. Exclusion criteria included: diabetes or other endocrine disorders, chronic inflammatory diseases, and kidney or liver dysfunction. Subjects participating in the study had not taken lipid-lowering or anti-inflammatory drugs or supplements containing vitamins A, C, or E, β-carotene or PUFA. Fish consumption was not allowed during the study period.

2.2. Study design and intervention

This clinical trial was randomized, double-blind placebo-controlled parallel and single center. The trial was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and with the Good Clinical Practise guidelines. The trial was approved by Bioethics Committee of the Jagiellonian University in Cracow (written consent, opinion No. KBET/82/B/2009) and all subjects gave written informed consent. The trial was conducted at the Department of Clinical Biochemistry, Jagiellonian University, Medical College, Krakow, Poland. The trial was registered at isrctn.com as ISRCTN11445521.

Recruited subjects were randomly assigned to the n-3 PUFA or the placebo group by using minimalization software balanced for age and sex.

Obese subjects (BMI 30–40 kg/m2) underwent an adaptation period of two weeks. During this time they were advised by a dietician on how to apply an isocaloric diet containing 2300–2400 kcal/d according to individual caloric requirement. The diet contained 57% of energy from carbohydrates, 30% from fat and 13% from protein. After the adaptation period, subjects were advised to use a low calorie diet. The caloric value of the low calorie diet was 1200 kcal/d for women and 1500 kcal/d for men and 60% of energy was provided from carbohydrates (with low glycemic index), 15% from protein and 25% from fat. Subjects were randomly assigned to receive placebo (corn oil) capsules (CR placebo) or n-3 PUFA (EPAX 1050TG; EPAX, Norway) capsules (CR + n-3). Subjects consumed 3 capsules per day for 3 months. Three n-3 PUFA capsules contained 1.8 g DHA + EPA in a ratio of 5:1. Both capsule types were identical in size, shape and appearance and contained 4 mg of vitamin E per capsule. Subjects were advised to swallow their capsules after a meal.

All subjects were given written and verbal instructions by a dietician about the preparation of a caloric restricted diet and consumption of capsules. Body composition changes including fat content, muscle mass and water content were measured by Tanita Body Composition Analyser BC-418 (Tanita, Japan). Hip and waist circumferences, diet and supplementation compliance, and nutritional habits were assessed.

Anthropometric measurements (BMI, hip and waist circumference, systolic and diastolic blood pressure, adipose tissue content) were performed at baseline (after the two week adaptation period) and at the end of three months intervention and after a 12 h overnight fast. An oral glucose tolerance test (OGTT; 75 g glucose load) was performed according to WHO guidelines. At fasting and every 30 min up to 2 h venous blood was collected for measurement of serum glucose, insulin, GIP, non-esterified fatty acids (NEFA), total cholesterol, HDL cholesterol and triglycerides. Additionally, at fasting baseline and at the end of treatment venous blood was collected for determination of plasma phosphatidylcholine (PC) composition and fatty acid content of erythrocytes' membranes (see below). All samples were centrifuged at 1000 x g for 15 min at 4 °C. Serum, plasma and erythrocytes' samples were aliquoted and stored at −80 °C until analysis. Sample size was calculated to 25 per group to detect a 50% change in insulin sensitivity markers at a P value <0.05 with a power of 80%.

2.3. Compliance

Counting the number of returned capsules during the follow up visits was used to assess compliance to capsules intake. In addition, the fatty acid composition of plasma phosphatidylcholine (PC), and of erythrocytes (RBC) was determined by gas chromatography using methods described previously [27].

Because of concerns about compliance to n-3 PUFA capsules in the n-3 PUFA group and contamination in the placebo group (i.e. intake of n-3 PUFA capsules) it was decided before hand to use a cut-off of a 20% increase in "omega-3 index", the sum of EPA + DHA in erythrocytes, to identify compliers in the n-3 PUFA group and violators in the placebo group. Using the threshold of a 20% increase in omega-3 index resulted in retention of data for 24 subjects in the n-3 PUFA group and 24 subjects in the placebo group.

2.4. Biochemical measurements

Subjects were instructed to avoid strenuous exercise and alcohol consumption the day before blood collection. Plasma glucose, total cholesterol, HDL-cholesterol and triglycerides were assayed by automated, enzymatic colorimetric methods (ELITech Clinical Systems, France). The intra and inter-assay variability coefficients were as follows: 2.3% and 3.5% (glucose), 1.4% and 3.4% (triglycerides), 1.4% and 3.8% (total cholesterol), 2.1% and 2.8% (HDL-cholesterol), respectively. LDL-cholesterol was calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the Friedewald formula.

Non esterified fatty acids (NEFA) concentration was measured immediately in non-frozen plasma by enzymatic quantitative colorimetric method (Roche Diagnostics GmbH, Germany).

Insulin was determined by an immunoradiometric method (DIAsource ImmunoAssays, Belgium) and read using a gamma counter (LKB Instruments). Within and between-run imprecision CVs were 2.1% and 6.5%, respectively.

GIP was measured using ELISA (EMD Millipore, St. Charles, MO, USA). Within-run CV was 6.1% and between-run CV 8.8%. The limit of detection was 8.2 pg./ml.

2.5. Presentation of results

Area under concentration time curve (AUC) for glucose, insulin, GIP, triglycerides and NEFA during the OGTT was calculated by the trapezoidal method [28]. B-cell function was assessed by the ratio of the insulin to glucose AUC (AUC_I/AUC_G) and the insulinogenic index (IGI). IGI is a