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Original article

Sustained effects of a protein and lipid preload on glucose tolerance in type 2 diabetes patients☆

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Abstract

Background. – Small amounts of nutrients given as a 'preload' can reduce post-meal hyperglycaemic peaks in type 2 diabetes (T2D) patients by activating a number of mechanisms involved in glucose homoeostasis. This study was undertaken to ascertain whether this positive effect extends to the late absorptive phase and to identify the main mechanisms involved.

Material and methods. – Eight well-controlled T2D patients, aged 40–70 years, were randomized to consume a 'preload' of either water or non-glucidic nutrients (50 g of Parmesan cheese, one boiled egg) 30 min before a 300-min oral glucose tolerance test.

Results. – After the nutrient preload, significant reductions were observed in peak glucose (−49%; $P<0.02$), total plasma glucose (iAUC: −28%; $P<0.03$), exogenous glucose (iAUC: −30%; $P<0.03$) and insulin clearance (−28%; $P<0.04$), with enhancement of insulin secretion (iAUC: +22%; $P<0.003$). These effects were associated with higher plasma levels of GLP-1 (iAUC: +463%; $P<0.002$), GIP (iAUC: +152%; $P<0.0003$) and glucagon (iAUC: +144%; $P<0.0002$).

Conclusion. – In T2D patients, a protein and lipid preload improves glucose tolerance throughout the whole post-absorptive phase mainly by reducing the appearance of oral glucose, and improving both beta-cell function and insulin bioavailability.

Keywords: Beta-cell function; Glucose absorption; Insulin secretion *in vivo*; Nutrient preload; Type 2 diabetes

1. Introduction

In the natural course of type 2 diabetes (T2D), postprandial hyperglycaemia is an early event that usually precedes elevation of fasting plasma glucose [1]. It also represents a major determinant of overall glycaemic control (as assessed by glycated haemoglobin) [2,3], and is a strong predictor of cardiovascular complications and all-cause mortality [4]. On the basis of this evidence, a great effort was made to optimize non-pharmacological treatment of postprandial hyperglycaemia for both the prevention and treatment of T2D [5,6]. A promising strategy to this end is to exploit the ability of nutrients to improve glucose tolerance, especially when ingested before carbohydrate intakes (as a 'preload'), through modulation of a number of mechanisms involved in glucose homoeostasis [7–9]. In T2D patients, a small amount of olive oil ingested 30 min before carbohydrate intake was able to delay the postprandial rise of plasma glucose by slowing down gastric-emptying [7], while a whey protein preload was able to blunt glycaemic excursions mainly by enhancing the glucose-induced insulin response [8,9]. As different mechanisms are involved, the hypoglycaemic effects of both lipids and proteins are expected to work in synergy when the two nutrients are given together. Indeed, it was recently

Abbreviations: FFM, fat-free mass; FPG, fasting plasma glucose; FPI, fasting plasma insulin; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; iAUC, incremental area under the curve; ISR, insulin secretion rate; ISR@FPG, insulin secretion rate at fasting plasma glucose concentration; OGIS, oral glucose insulin sensitivity; T2D, type 2 diabetes.

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found that a small, mixed, non-glucidic meal given 30 min before a 75-g oral glucose load was able to attenuate the 120-min glycaemic excursions following glucose ingestion in individuals with either normal or impaired glucose tolerance, or full-blown T2D [10]. The improvement was proportional to the degree of impairment of glucose tolerance, thus suggesting that the mechanisms involved, including a delay in glucose absorption and an improvement in beta-cell function, are particularly effective for the control of glucose homoeostasis in T2D. As the 'postprandial phase', particularly in T2D, can last for up to 5 h [2], the duration (2–3 h) of most of the previous studies represents a limitation that cannot preclude the idea that a delayed glucose delivery might produce a smoothed, but also prolonged, hyperglycaemia. Moreover, evaluation of the main mechanisms or outcomes (plasma glucose concentration, hormonal responses, glucose kinetics, insulin sensitivity and clearance) will become more robust when made after plasma glucose has reached preload values.

To ascertain whether or not the positive effect of a nutrient preload on glucose tolerance is sustained throughout the whole postprandial phase in T2D patients, and also to investigate the main mechanisms involved, the present study evaluated total, exogenous and endogenous plasma glucose, insulin secretion, insulin clearance and sensitivity, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) and glucagon for 300 min after a 75-g oral glucose load, following the ingestion of either a water or a small, non-glucidic preload.

2. Materials and methods

2.1. Subjects

Eight well-controlled early-diagnosed T2D patients were enrolled in the study. Inclusion criteria were age 40–70 years, body mass index (BMI) 20–35 kg/m^2, glycated haemoglobin (HbA_{1c}) 48–58 mmol/mol with either diet alone or metformin with/without a short-acting sulphonylurea. None of the patients had diseases other than diabetes, nor were they taking any other drugs that could potentially interfere with carbohydrate absorption and metabolism.

The study protocol was approved by our institutional ethics committee, and all volunteers provided their written informed consent before inclusion in the study.

2.2. Study design

This was a randomized, open-label, crossover clinical trial. Volunteers were studied on two days separated by a period of 2–4 weeks, during which time they were asked to keep their diet, physical activity and pretest evening meal as similar as possible. Blood glucose-lowering drugs were suspended 48 h before the test. On each test day, patients were admitted to our clinical research unit at 0800 h after an overnight fast (12 h), and stayed in bed in a semi-upright sitting position throughout the study. A 20-gauge polyethylene cannula was inserted into a wrist vein for blood sampling, and the patient's hand and forearm were kept wrapped in a heated blanket to achieve arterialization of venous blood.

At the first clinical visit, participants were randomized by a coin toss to consume a preload of either 500 mL of water (water preload) or a small, non-glucidic mixed meal, consisting of 50 g of Parmesan cheese and one small boiled egg (23 g of protein, 17 g of fat and 2 g of carbohydrate for a total of ~1000 kJ), followed by 300 mL of water (nutrient preload). The preloads were consumed over 5 min (between −30 and −25 min) after two fasting blood samples were taken; the preloads also had the same volumes, the difference being in the amount of water ingested, to exclude the influence of mechanical dilatation of the stomach on gastric-emptying and neurovagal activation [8,11,12]. At 30 min after preload ingestion, all participants consumed an oral glucose drink consisting of 147 mL of a 50% glucose solution (wt/vol), enriched with 1.5 g of U-[13C]glucose (Spectra 2000, Roma, Italy) to evaluate the contribution of exogenous glucose to total plasma glucose concentrations. Timed arterialized blood samples were collected throughout the test to measure plasma glucose, insulin, C-peptide, GLP-1, GIP, glucagon and U-[13C]glucose concentrations.

2.3. Analytical procedures

Plasma glucose was measured immediately by the glucose oxidase technique (Glucose Analyzer II, Beckman Coulter, Brea, CA, USA). Blood samples were centrifuged for 15 min (3000 g at 4 °C), frozen at −20 °C and analyzed within 30 days of collection. Insulin and C-peptide measurements were performed by electrochemiluminescence, using a Cobas e411 analyzer (Roche Diagnostics, Indianapolis, IN, USA). Plasma GLP-1 and GIP were assessed by multiplex immunoassays (MILLIPLEX® MAP assay kits, Merck KGaA, Darmstadt, Germany). Plasma glucagon was measured by enzyme-linked immunosorbent assay (ELISA; Mercodia AB, Uppsala, Sweden). U-[13C]glucose was assessed by gas chromatography–mass spectrometry, as previously described [13].

2.4. Mathematical modelling

Insulin secretion rate (ISR) was estimated by a C-peptide deconvolution method [14]. Beta-cell function parameters were calculated by an insulin secretion and glucose concentration model, as detailed elsewhere [15–18]. Briefly, this model describes insulin secretion as the sum of three components. The first represents the dependence of insulin secretion on the absolute glucose concentration and is characterized by a quasi-linear dose–response function relating the two variables. This function is described by the slope and intercept of the line within the observed glucose range, termed 'beta-cell glucose sensitivity' and 'ISR at fasting plasma glucose' (ISR@FPG), respectively. The individual dose–response function was not fixed throughout the test, but modulated by all the physiological processes that acutely modify insulin secretion (antecedent hyperglycaemia, gastrointestinal hormones, neurotransmitters, non-glucose substrates) and are taken into account by a time-dependent factor