Atherosclerosis 212 (2010) 217–222

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Exenatide suppresses postprandial elevations in lipids and lipoproteins in individuals with impaired glucose tolerance and recent onset type 2 diabetes mellitus

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ARTICLE INFO

Article history:
Received 16 March 2010
Received in revised form 23 April 2010
Accepted 4 May 2010
Available online 25 May 2010

Keywords:
Exenatide
High-fat meal
Triglycerides
Remnant lipoproteins
NEFA
Apolipoprotein B-48
Apolipoprotein C-III

ABSTRACT

Objective: Chronic exenatide treatment in type 2 diabetes is associated with improved glucose control and fasting lipid levels, as well as weight loss. Less established is whether exenatide directly reduces postprandial lipid and lipoprotein levels without the reduction in body weight or fasting glucose and triglycerides levels that frequently occur with prolonged therapy. Therefore, the effect of a single injection of exenatide on postprandial lipids, remnant lipoproteins, and apolipoproteins was studied.
Methods: A double-blinded, randomized, placebo-controlled, crossover study was conducted in 35 subjects (31 men and 4 women) with impaired glucose tolerance ($n=20$) or recent onset type 2 diabetes ($n=15$). A single subcutaneous injection of exenatide (10 μg) or normal saline was administered just prior to a high-calorie, fat-enriched breakfast meal. Concentrations of triglycerides (TG), apolipoproteins B-48 and CIII, non-esterified fatty acids (NEFA), and remnant lipoprotein (RLP) cholesterol and TG in serum or plasma were measured prior to the injection and for up to 8 h postprandially.
Results: Exenatide markedly reduced postprandial elevation of TG, apolipoproteins B-48 and CIII, RLP-cholesterol and RLP-triglyceride (all $p<0.001$). Postprandial declines in NEFA were less pronounced but persisted longer with exenatide compared to placebo ($p<0.05$). These effects of exenatide were not affected either by glucose tolerance status or by treatment with statins.
Conclusion: These results demonstrate that exenatide acutely and profoundly inhibits postprandial excursions of proatherogenic lipids and lipoproteins and may offer additional cardiovascular risk reduction (NCT00974272).

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1. Introduction

Cardiovascular disease (CVD) is responsible for two-thirds of all deaths in patients with type 2 diabetes (T2D) [1]. Importantly, CVD risk increases before the onset of clinical diabetes and is believed closely associated with the presence of insulin resistance [2,3]. Characteristic metabolic abnormalities in insulin resistance include accentuated rises in postprandial glucose and triglycerides.

A growing body of evidence supports an important role for postprandial increases in blood glucose and lipids in the development of CVD. While post-challenge or postprandial glucose concentrations appear at least equivalent to fasting glucose values as predictors of CVD in individuals with or without T2D [4,5], non-fasting triglyceride concentrations have been found comparable or better predictors of CVD than fasting triglycerides, at least in predominantly non-diabetic populations [6,7]. The increased risk associated with non-fasting triglycerides may reflect the many proatherogenic lipids and lipoproteins generated from triglyceride-rich chylomicrons formed during the postprandial period [8]. Chylomicron metabolism generates localized increases in plasma fatty acids and remnant lipoproteins at the vessel wall and is associated with increased levels of apolipoproteins such as apolipoprotein C-III, each of which have been demonstrated to have proatherogenic or proinflammatory actions within the vessel wall [9–15].

Therefore, there is an interest in identifying therapies that would favorably influence postprandial concentrations of both glucose and lipids. Exenatide is a clinically approved diabetes medication with 53% homology to endogenous glucagon-like peptide 1 (GLP-1). As an incretin mimetic, it lowers glucose concentrations by several mechanisms and effectively lowers postprandial glucose [16]. Exenatide also appears to beneficially alter other CVD risk factors. Long-term therapy with exenatide is associated with significant improvements in systolic and diastolic blood pressure and decreased fasting triglycerides, as well as more modest changes in total, LDL and HDL cholesterol [17]. Chronic exenatide therapy has been associated with enhanced satiety and weight loss, and it has

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0021-9150/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.atherosclerosis.2010.05.028

been suspected that these effects may be responsible for improvement in several CVD risk factors, including reductions in fasting and postprandial triglycerides. However, recent studies provide support for more direct benefits of exenatide on postprandial lipid metabolism. In fact, continuous exenatide infusion and continual use of subcutaneous exenatide reduced postprandial elevations in triglyceride concentrations in patients with T2D [18,19]. It remains unknown if acute use of the typical clinical dose of exenatide reduces the multiple components of postprandial hyperlipidemia.

In the present study, we tested the effects of a single acute injection of exenatide to determine what direct benefits (in the absence of changes in satiety, weight loss and other chronic effects) this agent may have on increments in triglycerides, apolipoproteins, and cholesterol- and triglyceride-rich remnant particles, following a standardized fat-enriched meal challenge.

2. Research design and methods

The protocol was approved by the Institutional Review Board of the Phoenix VA Health Care System and all subjects provided written informed consent before participation. Eligible participants were adults between 35 and 70 years of age, with fasting triglyceride levels >1.6 and <5.6 mmol/l, and with impaired glucose tolerance (IGT) or diet controlled (HbA1c $\leq$ 7.5), recent onset ($\leq$3 years) T2D. The diagnosis of diabetes was based on a fasting blood glucose $\geq$7.8 mmol/l, a 2-h OGTT value of $\geq$11.1 mmol/l, and/or a medical history of T2D. Screening OGTT was not performed in those with known T2D. Participants receiving statins were required to be on a stable dose for at least 2 months prior to study enrollment. Individuals using lipid-lowering medications other than statins were excluded.

Each participant was studied twice within 1–3 weeks in a randomized, double-blinded, placebo-controlled, crossover design. Both sessions commenced between 7:00 and 10:00 AM after an overnight fast. An indwelling catheter was placed in the antecubital vein and subjects rested in a recumbent position for at least 30 min before a baseline blood sample was taken. A masked injection of exenatide (10 μg, Byetta®, Amylin Pharmaceuticals, San Diego, CA) or an equivalent volume of normal saline placebo was then administered subcutaneously in the lower abdominal quadrants. Immediately following the injection, subjects were asked to consume (within 15 min) a standardized solid breakfast meal consisted of egg, muffin, pancakes and oatmeal fortified with palm stearin. The caloric content of the meal was 2511 kJ/m^2 of body surface area distributed as 45% fat [60% saturated and 40% unsaturated], 40% carbohydrate and 15% protein. Blood samples were collected at 2, 4, 6 and 8 h following the injection.

Upon sampling, blood specimens were centrifuged at 3500 rpm and 4 °C for 15 min and aliquots of serum or plasma were stored at −80 °C until analyzed. Serum concentrations of glucose, insulin, LDL cholesterol, total apolipoprotein B and triglycerides (TG) were measured in the clinical laboratory of the Phoenix VA Health Care System using standard automated clinical chemistry methods on an Architect autosampler (Abbott Laboratories, Abbott Park, IL). Non-esterified fatty acid (NEFA) concentration in serum was measured with a colorimetric assay (Polymedco, Valhalla, NY) run on an Architect autosampler. Serum concentrations of apolipoprotein B-48 (ApoB48) were measured by ELISA (Polymedco). Plasma apolipoprotein C-III (ApoCIII) was measured by an automated colorimetric immunoassay run on a Poly-Chem autosampler (Polymedco).

Remnant-like particles (RLP) were isolated from plasma using an FDA-approved immunoaffinity assay (Polymedco). Briefly, plasma aliquots (5 μL) were added to standardized portions of buffered immunoseparation gel suspension (300 μL) and subjected to gentle agitation via a steel bead driven by a Photal J-100A magnetic RLP mixer for 2 h. The gel containing the non-remnant lipoproteins was then allowed to sediment by gravity for 15 min and the supernatants (containing the remnant-like particles) were collected. Remnant-like particle cholesterol (RLP-C) was measured in these supernatants by standard clinical chemistry methods run on an Architect autosampler (cholesterol oxidase assay); remnant-like particle triglycerides (RLP-TG) were also measured in these preparations using a standard triglyceride assay (Polymedco).

Statistical analyses were performed using software from the SAS Institute (version 9.2; Cary, NC, USA). The effect of exenatide was tested by repeated measures analysis of covariance (ANCOVA) using the PROC MIXED procedure, accounting for the crossover study design and potential confounders including glucose tolerance status (IGT or T2D) or use of statins. An interaction term between the effect of study drug (exenatide vs. placebo) and time post-meal was included in the models to directly test whether exenatide therapy significantly modified the postprandial time course of the study outcomes of interest. Post hoc tests, corrected for multiple comparisons, were used to test the differences from pre-meal values and between exenatide and placebo for each postprandial time point. The variables not normally distributed were $\log_{10}$ transformed to approximate normal distribution. Two-tailed p values less than 0.05 were considered to be statistically significant.

3. Results

Baseline clinical characteristics of the study group are shown in Table 1. The study participants were predominantly Caucasian males, reflecting the Phoenix VA population. Because inclusion criteria favored selection of participants with insulin resistance, subjects tended to be overweight or obese, and had high normal systolic but normal diastolic blood pressure. Three participants had a history of recent onset T2D. Twelve other individuals were found during a screening OGTT to have fasting or 2-h plasma glucose concentrations consistent with T2D. The remaining 20 subjects were found to have IGT during the screening OGTT.

The average caloric content of the test meal was 5384 kJ. The only adverse effect was transient mild or moderate nausea (no emesis) after ingestion of the study meal, which, as expected, occurred more frequently with exenatide (n = 16) than with placebo (n = 3). All but 5 subjects ingested their entire meal on both occasions; 4 of these participants ingested lower amounts during the placebo phase.

Administration of exenatide significantly modified the rise (Drug effect) and/or the time course (Drug × Time interaction)

Table 1
Clinical characteristics of study participants.

Variable	n = 35	Range
Ethnicity (% Caucasians)	85	–
Gender (M/F)	31/4	–
Age (years)	61 (58–64)	42–68
BMI (kg/m^2)	33.1 ± 4.9	26–44
SBP (mm Hg)	135 ± 13	116–163
DBP (mm Hg)	74 ± 9	57–95
Anti-hypertensive use (%)	46	–
Fasting glucose (mmol/l)	5.6 (5.3–6.4)	4.7–8.6
2-h glucose (mmol/l)[a]	10.4 ± 2.1	7.7–15.8
IGT/T2D	20/15	–
Hemoglobin A1c (%)	6.2 ± 0.5	4.8–7.5
Triglycerides (mmol/l)	2.6 ± 0.9	1.0–4.4
Total cholesterol (mmol/l)	4.7 ± 0.8	2.8–6.6
HDL cholesterol (mmol/l)	1.0 ± 0.2	0.54–1.58
LDL cholesterol (mmol/l)	2.5 ± 0.8	1.2–4.4
Statin use (%)	57	–

Data are represented as means ± SD if normally distributed or median (interquartile range) if non-normally distributed.

[a] OGTT was performed in 33 participants.