Research Article

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Effects of glucagon-like peptide-1 on glucagon secretion in patients with non-alcoholic fatty liver disease

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Background & Aims: We evaluated the glucagon-suppressive effect of glucagon-like peptide-1 (GLP-1) and its potential effects on endogenous glucose production and whole body lipolysis in non-diabetic patients with non-alcoholic fatty liver disease (NAFLD).

Methods: On two separate days, 10 non-diabetic patients with liver biopsy-verified NAFLD (NAFLD activity score 2.5 ± 1.0) and 10 matched controls underwent 2 h intravenous infusions of GLP-1 ($0.8\ pmol \times kg^{-1} \times min^{-1}$) and placebo. Since GLP-1-mediated glucagon suppression has been shown to be glucose-dependent, plasma glucose was clamped at fasting level during the first hour, and then raised and clamped at 'postprandial level' (fasting plasma glucose level plus 3 mmol/L) for the remaining hour. We evaluated relative plasma levels of glucagon, endogenous glucose production and whole body lipolysis rates with stable isotopes and respiratory quotient using indirect calorimetry.

Results: Compared to controls, patients with NAFLD were insulin resistant (homeostasis model assessment ($HOMA_{IR}$): 3.8 ± 2.2 *vs.* 1.6 ± 1.5, $p = 0.003$) and had fasting hyperglucagonaemia (7.5 ± 5.3 *vs.* 5.8 ± 1.5 mmol/L, $p = 0.045$). Similar relative glucagon suppression was seen in both groups during GLP-1 infusion at fasting (−97 ± 75 *vs.* −93 ± 41 $pmol/L \times min^{-1}$ $p = 0.566$) and 'postprandial' plasma glucose levels (−108 ± 101 *vs.* −97 ± 53 $pmol/L \times min^{-1}$, $p = 0.196$). Increased insulinotropic effect of GLP-1 was observed in NAFLD patients. No effect of GLP-1 on endogenous glucose production was observed in any of the groups.

Conclusions: Patients with NAFLD exhibited fasting hyperglucagonaemia, but intact GLP-1-mediated glucagon suppression independently of plasma glucose concentrations. Preserved glucagonostatic effect and increased insulinotropic effects of GLP-1 in NAFLD may be important to maintain normoglycaemia in these patients.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the industrialised world [1]. The term NAFLD covers a wide spectrum ranging from hepatic steatosis to non-alcoholic steatohepatitis, which entails a risk of cirrhosis and hepatocellular carcinoma [2]. NAFLD and type 2 diabetes share common features like insulin resistance and dyslipidaemia [3] and up to 70% of obese patients with type 2 diabetes have NAFLD [4].

In type 2 diabetes, fasting hyperglucagonaemia and impaired postprandial glucagon suppression contribute to hyperglycaemia [5] – most likely due to increased hepatic glucose production [6]. Non-diabetic patients with NAFLD also have hyperglucagonaemia [7] and exhibit impaired suppression of hepatic glucose production [3]. Although evidence suggests that hyperglucagonaemia may be part of the pathophysiology of NAFLD [8], the link between the two remains unclear [9].

The regulation of glucagon secretion involves the gut hormone, glucagon-like peptide-1 (GLP-1). GLP-1 is secreted from the gastrointestinal tract in response to meal ingestion and triggers insulin secretion and inhibits glucagon secretion at glucose levels above basal [10,11]. Patients with type 2 diabetes have impaired beta cell sensitivity to GLP-1 (insulin secretion) [12], but preserved alpha cell response (glucagon suppression) to GLP-1 [13]. Incretin physiology has been intensely studied in patients with type 2 diabetes, but no studies have investigated the role of GLP-1 in glucose tolerant individuals with NAFLD. Hyperglucagonaemia in NAFLD could reflect impaired regulation of glucagon by GLP-1. We studied the effects of physiological

Keywords: Non-alcoholic fatty liver disease (NAFLD); Glucagon-like peptide-1 (GLP-1); Glucagon; Endogenous glucose production; Lipolysis; Energy metabolism.

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Abbreviations: AUC, area under the curve; BMI, body mass index; GLP-1, glucagon-like peptide-1; $HOMA_{IR}$, homeostasis model assessment; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; Ra, rate of appearance; Rd, rate of disappearance.

doses of GLP-1 on glucagon secretion at two different plasma glucose levels (fasting and 'postprandial').

Patients and methods

The study protocol was approved by the scientific-ethical committee of the Capital region of Denmark (H-1-2012-153) and registered with the Danish Data Protection Agency (02009 GEH-2012-049). The study was conducted according to the principles of the Helsinki Declaration II. Written informed consent was obtained from all participants.

Outcome measures

The primary outcome measure was the plasma glucagon response (expressed as the incremental area under curve (AUC)) to an intravenous GLP-1 infusion compared to a saline (placebo) infusion. Secondary outcome measures were insulin responses, insulin secretion rates, amount of glucose needed to maintain plasma glucose, endogenous glucose production, lipolysis, respiratory quotient and resting energy expenditure during the infusions.

Participants

We included patients with histologically verified NAFLD and controls matched with regards to age, sex and body mass index (BMI) (Table 1). Patients were recruited from the outpatient clinic at the Department of Medicine, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark. The degree of steatosis in liver biopsies was graded according to the quantity of hepatic fat infiltration: mild (5–33% fat infiltration), moderate (33–66% fat infiltration) and severe (>66% fat infiltration) [14]. Non-alcoholic steatohepatitis (NASH) and fibrosis were graded according to the NAFLD activity score [14]. All participants were normal glucose tolerant according to a 75 g oral glucose tolerance test, with fasting plasma glucose <6.1 mmol/L and plasma glucose at 120 min <7.8 mmol/L. Exclusion criteria were: Weekly alcohol consumption of more than 7 units for women and 14 for men, treatment with steatogenic drugs three months prior to inclusion, anaemia, inflammatory bowel disease, gut resection (except appendectomy), creatinine >150 μmol/L, albuminuria and other chronic diseases. Healthy control subjects were without family history of diabetes, had no signs of liver disease based on patient history, biochemical measurements and ultrasonic assessment and had no other chronic diseases.

Table 1. Demographic, anthropometric and laboratory data.

	NAFLD	Controls	*p* value
N (male)	10 (6)	10 (5)	0.847
Age (years)	49 ± 26	55 ± 30	0.880
BMI (kg/m^2)	30 ± 8	28 ± 4	0.623
Waist circumference (cm)	105 ± 20	104 ± 7	0.775
Biochemistry			
Fasting plasma glucose (mmol/L)	5.6 ± 0.7	5.0 ± 0.3	0.023
HbA_{1c} (mmol/mol)	33 ± 4	33 ± 5	0.706
Alanine aminotransferase (U/L)	86 ± 105	23 ± 8	0.014
Aspartate aminotransferase (U/L)	61 ± 35	32 ± 9	0.002
Gamma glutamyltransferase (U/L)	167 ± 193	19 ± 10	0.006
Total cholesterol (mmol/L)	4.8 ± 1.9	5.1 ± 1	0.384
Low density lipoprotein cholesterol (mmol/L)	2.6 ± 1	3.1 ± 1.2	0.112
High density lipoprotein cholesterol (mmol/L)	0.9 ± 0.4	1.3 ± 0.6	0.227
Triglycerides (mmol/L)	1.7 ± 0.4	1.1 ± 1.1	0.121
$HOMA_{IR}$	3.8 ± 2.2	1.6 ± 1.5	0.003
Matsuda index	5.3 ± 2.3	11.9 ± 5.7	<0.001
NAFLD activity score	2.5 ± 1	-	

Data are presented as median ± interquartile range. NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; HbA_{1c}, glycated haemoglobin A_{1C}; $HOMA_{IR}$, the homeostasis model assessment.

Experimental procedure

Synthetic GLP-1 (PolyPeptide Laboratories, Strasbourg, France) was prepared for infusion by the Capital Region Pharmacy in Denmark. The peptide was dissolved in sterilised water containing 2% human albumin (Statens Serum Institute, Copenhagen, Denmark), subjected to sterile filtration, dispensed into vials and microbiologically tested. Stable isotopes [6,6-D_2]-glucose and [1,1,2,3-D5]-glycerol (Cambridge Isotope Laboratories Inc., Andover, Massachusetts, USA) were prepared as previously described [15]. An independent person generated an allocation sequence of GLP-1 or placebo by computer and prepared and blinded the infusions. The allocation sequence was revealed after the last experimental day.

Participants were instructed to maintain a regular diet and avoid alcohol and excessive eating for three days prior to each experimental day. Participants arrived at the research centre after an overnight (10 h) fast having avoided strenuous physical activity from the day before. They were placed in a recumbent position and had an intravenous catheter inserted into a cubital vein. The forearm was placed in a heating box (55 °C) throughout the experiment for collection of arterialised blood samples. Another intravenous catheter was inserted into a contra-lateral cubital vein for glucose, stable isotope and hormone infusions. Two hours prior to study start (−120 min), a primed constant infusion of [6,6-D_2] glucose (priming bolus 17.6 μmol × kg^{-1}; continuous infusion rate of 0.6 μmol × kg^{-1} × min^{-1}) and [1,1,2,3-D5] glycerol (priming bolus 3.0 μmol × kg^{-1}; continuous infusion rate of 0.1 μmol × kg^{-1} × min^{-1}) was initiated. At study start (0 min) either a GLP-1 (0.8 pmol × kg^{-1} × min^{-1}) or placebo were initiated and kept until the end of the experiment.

Plasma glucose was measured every 5 min and clamped for two 60 min periods using a continuous infusion of sterile 20% (w/v) glucose. For the first period (0 to 60 min) plasma glucose was clamped at the fasting level and for the second period (60 to 120 min) at a 'postprandial' level (fasting level plus 3 mmol/L) using a bolus injection of sterile 50% (w/v) glucose during 3-5 min (3 mmol/L × 35 (mg glucose × 1 mmol/L^{-1} × body weight (kg)$^{-1}$)) and clamped to the end of the experiment (65 to 120 min).

Arterialised blood was drawn regularly at 15 min intervals from −30 to 120 min. Blood was distributed into chilled tubes containing EDTA and dipeptidyl-peptidase-4 inhibitor (valine-pyrrolidide, 0.01 mmol/L) for analyses of glucagon, total GLP-1, enriched [6,6-D_2] glucose, [1,1,2,3-D5] glycerol and dry tubes for insulin and C-peptide. All tubes were immediately cooled on ice and centrifuged (1,200 g for 20 min at 4 °C). Plasma for analysis of glucagon, GLP-1, [6,6-D_2] glucose and [1,1,2,3-D5] glycerol enrichment analyses was stored at −20 °C. Serum for insulin and C-peptide analyses was stored at −80 °C. For bedside measurement of plasma glucose, blood was collected in fluoride tubes and centrifuged (7.400 g) immediately for one min at room temperature. Urine was collected after each experimental day and stored at −20 °C until analysis for nitrogen.

Energy expenditure (O_2/CO_2 exchange) was measured by indirect calorimetry using a continuous open-circuit system with a facemask (CCM Express, Medgraphichs Corp, St. Paul, Minnesota, USA with Breezesuite software) at basal (−10 to 0 min) and during the last 10 min of each glucose clamp interval (50 to 60 min and 110 to 120 min).

Biochemical analysis

Plasma glucose concentrations were measured using a glucose analyser (Yellow Springs Instrument YSI 2300 STAT plus analyser; YSI Inc., Yellow Springs, Ohio, USA). Concentrations of serum insulin, C-peptide, glucagon and GLP-1 were measured as previously described [16–18]. Stable isotopes of glucose and glycerol were measured using liquid chromatography-mass spectrometry as previously described [19]. Urine nitrogen was measured using the vitros-slide method (Ortho-Clinical Diagnostics, Johnson & Johnson, Birkerød, Denmark) using the Vitros 5.1 FS analyser.

Calculations and statistical analysis

Glucose and glycerol rate of appearance (Ra) and the rate of disappearance (Rd) were calculated from the change in glucose enrichment at basal (−30 to 0 min) at fasting (30 to 60 min) and at postprandial glucose level (90 to 120 min for glycerol and 105 to 120 min for glucose). We used a single compartment, fixed volume, non-steady state single pool model of Steele [20] as modified for use with stable isotopes [21] and a pool fraction of 200 ml × kg^{-1} for glucose and 570 ml × kg^{-1} for glycerol. The respiratory quotient and resting energy expenditure were calculated from gas exchange and urinary nitrogen excretion as previously described [22].