ORIGINAL ARTICLE

Effects of body fat reduction on plasma adipocyte fatty acid-binding protein concentration in obese patients with type 1 diabetes mellitus

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Abstract

OBJECTIVES: The influence of body fat reduction on adipocyte fatty acid-binding protein (A-FABP) in obese patients with type 1 diabetes mellitus (T1DM) was investigated to examine whether it relates to the etiopathogenesis of insulin resistance (IR) and obesity.

METHODS: We studied 14 obese patients with T1DM and IR (42.6±9.4 years, BMI 32.4±2.1 kg/m^2) and 13 non-obese control patients with T1DM (36.9±13.9 years, BMI 22.6±2.1 kg/m^2). Plasma FABP was measured by ELISA and plasma free fatty acids (FFA) were measured spectrophotometrically before weight reduction, immediately after 7 days of fasting and after 21 days on a low-calorie diet. The control group was studied only after overnight fasting. Body composition was examined using bioimpedance spectroscopy. The means ± SD, T-test, one-way ANOVA and Spearman's correlation were used for statistical evaluation.

RESULTS: All patients tolerated the period of fasting. Obese T1DM patients lost 6.1±1.1 kg. There was a significant decrease in body mass index and body fat measured 21 days after weight reduction ($p<0.05$). Plasma FABP and FFA concentrations in obese T1DM patients before weight reduction were significantly higher than in controls, further increased significantly after fasting ($p<0.05$) and were restored thereafter. Significant positive correlations between FABP and FFA and between FABP and BMI ($p<0.05$) were found.

CONCLUSION: Increased plasma FABP indicates insulin resistance in obese patients with T1DM. Weight reduction in T1DM patients is associated with a desirable decrease of body fat and transiently increased FABP. This increase might be a temporary adaptation of metabolism to non-stress fasting.

Abbreviations:
A-FABP - adipocyte FABP
BMI - body mass index
FABPs - fatty acid-binding proteins
FFA - free fatty acids
IR - insulin resistance
M - glucose disposal
PPAR - peroxosimal proliferator activating receptor
R - Pearson's correlation coefficient
T1DM - type 1 diabetes mellitus

INTRODUCTION

Traditionally, the phenotype of type 1 diabetes (T1DM) is normal weight or underweight; however, there is evidence that this may be changing and that the prevalence of obesity in T1DM patients is growing (Libman *et al.* 2003). Although insulin deficiency is the main pathogenic factor in T1DM, obese subjects with T1DM can also be insulin resistant (Pambianco *et al.* 2007), and the pathogenesis of their micro- and macrovascular complications is now attributed partly to insulin resistance (Kilpatrick *et al.* 2007, Thorn *et al.* 2005, Orchard *et al.* 2003) as well as classical factors, such as metabolic control, body mass index, smoking, dyslipidemia, and hypertension.

The pathophysiological link between obesity and insulin resistance remains to be elucidated, but several recent studies indicate a central role for adipose tissue (Ferroni *et al.* 2004). Adipocytes produce and release several obesity-related bioactive substances that show inflammatory and/or immunomodulatory properties (Aldhahi & Hamdy 2003) and act as true hormones responsible for the regulation of energy intake and expenditure (Mora & Pressin 2002), triggering diabetes.

Cytoplasmic fatty acid-binding proteins (FABPs) are members of a multigenic protein family that is known for the ability to bind with high affinity to hydrophobic ligands such as saturated and unsaturated long-chain fatty acids, eicosanoids and related compounds (bile acids or retinoids). The nine family members have between 20 and 70% identity in their amino acid sequences. Adipocyte FABP (A-FABP or FABP4 or aP2) is a 15 kDa lipid-binding protein that is highly expressed in a differentiation-dependent fashion in adipocytes and is critical in the regulation of the biological function of these cells. It is one of the most abundant cytoplasmic proteins in mature adipocytes (Maeda *et al.* 2005), and a significant proportion of this protein is also released into the bloodstream (Xu *et al.* 2006). Its expression is transcriptionally controlled during adipocyte differentiation and is regulated by peroxisome proliferator–activated receptor-γ agonists, insulin and fatty acids (Maeda 2007). Although its biological role is not yet well understood, its function has been correlated to insulin sensitivity, lipid metabolism and inflammation (Makowski & Hotamisligil 2004). Recent human clinical studies have proposed serum A-FABP level as a plasma biomarker of insulin resistance in metabolic syndrome (Xu *et al.* 2007; Stejskal & Karpisek 2006) and type 2 diabetes (Tso *et al.* 2007) in both in Asian and Caucasian populations. In type 1 diabetes, serum A-FABP levels have been studied in a non-obese, insulin-resistant Asian population (Hsu *et al.* 2011) who developed IR despite looking phenotypically lean. The nature of IR and its association with A-FABP and body fat reduction have not been examined in obese T1DM patients.

We hypothesized that increased A-FABP would be associated with insulin resistance in obese, Caucasian T1DM patients in parallel with abnormal fuel utilization. A reduction of body weight in obese T1DM patients might be a crucial step in reducing their insulin resistance, but this is difficult (Kemmer 1992). Given the pathophysiologic features and metabolic ambiguity of diabetic types, in this pilot study, we set out to characterize the clinical phenotype in obese and non-obese patients with type 1 diabetes during body fat reduction, conducted a direct comparative analysis of IR by hyperinsulinemic-euglycemic clamp, and compared these IR results to the novel IR biomarker A-FABP.

MATERIAL AND METHODS

Subjects

Fourteen obese, C-peptide–negative patients with T1DM (9M+5F, aged 42.6±9.4 years, BMI 32.4±2.1 kg/m^2) with a mean duration of diabetes of 19.6±12.5 years were included in the study. The mean glycated hemoglobin concentration was 7.9±1.1%. Four patients were on a basal/bolus subcutaneous insulin regimen, and 10 were on continuous subcutaneous insulin infusion therapy. Patients had no history of cardiovascular disease, chronic renal failure, or other chronic conditions that would preclude the ability to fast for 7 days. Six obese T1DM patients had history of nonproliferative retinopathy, four patients had history of sensitive neuropathy, and three patients had history of microalbuminuria. Arterial hypertension was present in 10 obese T1DM patients. Two obese T1DM patients had history of hyperlipidemia and were treated with lipid-lowering agents. For controls, we recruited 13 non-obese, C-peptide-negative patients with T1DM (8M+5F, aged 36.9±13.9 years, BMI 22.6±2.1 kg/m^2). The mean duration of diabetes among the non-obese T1DM patients was 11.8±6.0 years. The mean glycated hemoglobin concentration was 9.2±2.1%. Four non-obese patients were on a basal/bolus subcutaneous insulin regimen, and nine were on continuous subcutaneous insulin infusion therapy. Non-obese T1DM patients had no history of cardiovascular disease or chronic renal failure. Six non-obese T1DM patients had history of nonproliferative retinopathy, four non-obese T1DM patients had history of sensitive neuropathy, and three non-obese T1DM patients had history of microalbuminuria. Arterial hypertension was present in four non-obese T1DM patients. Three non-obese T1DM patients had history of hyperlipidemia and were treated

with lipid-lowering agents. There was no significant difference between the two groups of patients in age, duration of diabetes, or glycated hemoglobin. Written informed consent was obtained from all patients. The study was approved by the local ethics committee.

Study design

Two days prior to the beginning of the fasting period, the patients were admitted to the diabetology ward and placed on a standardized diabetic diet containing 225 g of carbohydrates and a total of 7 400 kJ daily. One day prior to the beginning of fasting, we performed a hyperinsulinemic-euglycemic clamp. After the clamp, patients fasted for 7 days. During the fasting period, patients were only allowed to drink water or sugar-free beverages. Patients were supplemented with potassium (40 mmol/day), ascorbic acid (100 mg/day) and vitamin B complex (thiamine 15 mg/day, riboflavin 15 mg/day, pyridoxine 10 mg/day, niacin 50 mg/day). Patients received only a basal insulin dose. The dose of basal insulin was changed according to the glycemia. Glycemia during fasting was maintained at 5 mmol/l by adjustment of the basal insulin dose. There was no severe hypoglycemia in obese T1DM patients during the fasting period. There were seven mild episodes of symptomatic hypoglycemia in the group of obese T1DM patients during the fasting period, which were treated with 10–20 g of glucose in liquid form. To avoid the development of ketoacidosis, β-hydroxybutyrate concentration was measured together with glycemia using blood from a fingerprick test and analyzed by a bedside analyzer (Precision PCx, Abbott Laboratories, Abbot Park, USA). β-Hydroxybutyrate concentration increased significantly during fasting (0.16±0.24 on the first day versus 1.66±0.75 mmol/l on the seventh day, $p<0.001$). Fasting was accompanied by a transient elevation in serum uricemia ($p<0.001$). On the eighth day of the testing period, we repeated the hyperinsulinemic-euglycemic clamp, after which patients were placed on a standardized, low-calorie, diabetic diet containing 150 g of carbohydrates and a total of 5 000 kJ. Twenty-one days after the fasting period, patients were admitted to the diabetology ward again, and the third hyperinsulinemic-euglycemic clamp was performed. In the group of non-obese T1DM patients, only one hyperinsulinemic-euglycemic clamp was performed without any further intervention.

Body composition monitor

Bioimpedance spectroscopy (Fresenius Medical Care, Bad Homburg, Germany) was used to determine body composition. Total body fat mass (kg), relative fat mass (%), lean tissue mass (kg), and relative lean tissue mass (%) were measured.

Indirect calorimetry

We used a ventilated canopy system (VMAX, Sensormedics, Anaheim, USA) for indirect calorimetry. Gas exchange measurements were taken during a 30-minute basal period and during the final 30-minute period of the insulin infusion. Patients relaxed in the supine position for at least 15 min before each measurement, which was performed in a thermally comfortable environment for at least 30 min or until a steady state was reached. Subjects were familiarized with the canopy so that they did not fall if they were suffocating.

Hyperinsulinemic-euglycemic clamp

All studies were performed after an 8- to 10-hour overnight fast. The hyperinsulinemic-euglycemic clamp, lasting 6 hours, was conducted as previously described (deFronzo *et al.* 1979). Briefly, a Teflon cannula was inserted into the left antecubital vein to infuse all test substances. A second cannula was inserted in a retrograde fashion into a wrist vein of the same hand for blood sampling, and the hand was placed in a heated (45 °C) cover to achieve venous blood arterialization. A primed-continuous infusion of 10 mU/kg/min of Humulin R (Eli Lilly, Indianapolis, USA) was administered to maintain the plasma concentration of insulin. Decreases in serum potassium during insulin infusion were prevented by co-infusion of potassium chloride with glucose (60 mmol KCl/l of 20% glucose). Plasma glucose was maintained at 5 mmol/l during the clamp by continuous infusion of 20% glucose. Insulin action was estimated as glucose disposal (M) calculated between 320 and 360 minutes. IR was calculated as IR=1/M.

Analytical methods

Basic biochemical parameters characterizing the patients' overall condition were monitored: mineralogram, kidney function, urea, creatinine, uric acid, bilirubin, liver enzymes, and basic lipid metabolism (Modular Analytics, Roche, Basel, Switzerland). These parameters were monitored before and after 7 days of starvation and 21 days thereafter. Glycemia during hospitalization was determined by the glucose oxidase reaction by a bedside analyzer (Precision PCx). Immunoreactive insulin was determined by radioimmunoassay using an Insulin IRMA kit (Immunotech, Prague, Czech Republic). Glycated hemoglobin was measured using the liquid chromatography Variant Test (Bio-Rad Laboratories, Montreal, Canada). Free fatty acids were analyzed with a FFA-HR kit (Wako chemicals GmbH, Neuss, Germany) using a UV-VIS spectrophotometer (Shimadzu Pharma Spec 1700 UV Probe, Kyoto, Japan). Plasma A-FABP was analyzed by sandwich ELISA with a biotin-labeled antibody technique (BioVendor, Brno, Czech Republic).

Statistical methods

The numerical data were tested according to distribution. If a normal distribution was detected, the T-test and ANOVA were used. If the distribution of data was not normal, non-parametric tests were applied: the

Mann Whitney test and ANOVA on ranks. For correlation analysis, Pearson's correlation coefficient (R) and the associated p value were calculated. The statistical significance was determined based on a probability level of less than 0.05. The statistical evaluation was performed using SigmaStat (Systat Software, Chicago IL, USA). All data are expressed as the means ± SD unless otherwise indicated.

RESULTS

Clinical characteristics of the obese T1DM and non-obese T1DM patients are summarized in Table 1. The clinical phenotype of obese T1DM patients was characterized by increased BMI and waist circumference in comparison to the non-obese T1DM subjects (p<0.001). All obese T1DM patients tolerated the period of weight reduction, lost 6.1±1.1 kg of body weight and 7.3±2.9 cm in waist circumference after weight reduction and maintained this reduction in body weight and waist circumference after 21 days on the low-calorie diet. The mean basal insulin dose on the seventh day of fasting and the daily insulin dose after 21 days on the low-calorie diet remained lower than before weight reduction (p<0.001). There was a significant difference in body fat between obese T1DM and non-obese T1DM (p<0.001) and a significant decrease in body fat after weight reduction and after 21 days on the low-calorie diet in obese T1DM patients (p<0.05), as measured by bioimpedance spectroscopy. IR as estimated by 1/M during hyperinsulinemic-euglycemic clamp was significantly increased in obese T1DM patients in comparison to non-obese T1DM subjects (p<0.05). A reduction of glucose disposal after weight reduction was caused by a significant reduction of glucose oxidation (p<0.001). Glucose disposal measured after 21 days on the low-calorie diet returned to baseline.

Plasma FABP and FFA in obese T1DM patients before weight reduction were significantly higher than in non-obese T1DM patients, further increased significantly after starvation (p<0.05) and returned to the basal values after 21 days on the low-calorie diet (Table 2).

Significant positive correlations between FABP and FFA concentrations and between FABP concentration and BMI (p<0.05) before and after weight reduction were found (Figure 1). Moreover, FABP significantly correlated with insulin resistance (IR=1/M) during hyperinsulinemic-euglycemic clamp (p=0.002).

DISCUSSION

This study provides a comparative analysis of the clinical phenotype of type 1 diabetic subjects with regard to obesity and insulin resistance. This study confirms that type 1 diabetes in obese subjects shares many physical characteristics with previous data in insulin-resistant obese individuals with type 2 diabetes or metabolic syndrome but retains differences fundamental to their respective pathophysiologies. In spite of having a different type of diabetes, obese T1DM patients with increased BMI, body fat and waist circumference share

Tab. 1. Clinical characteristics of the obese T1DM and non-obese T1DM patients.

Obese T1DM (n=14)	Before fasting	After 7 days of fasting	21 days after the fasting period	Non-obese T1DM (n=13)
Body mass index (kg/m^2)	32.34±2.07#	30.41±1.82**	30.42±1.92**	22.65±2.07
Waist circumference (cm)	105.46±9.3#	98.21±7.86**	98.0±7.76**	80.70±7.5
Daily insulin dose (units)	49.01±13.44	14.22±8.86**(a)	30.42±1.92**	52.02±15.36
Daily insulin dose kg of body weight (units/kg)	0.51±0.13#	0.39±0.09**	0.40±0.07**	0.76±0.22
Body fat (kg)	36.89± 4.77#	32.99±5.46*	33.32± 5.53 *	13.55± 5.29
Glucose disposal (mg/min/kg)	9.69±1.48#	6.78±1.21**	9.31±1.16	12.02±2.16

**p<0.001, before fasting vs. the indicated time in obese T1DM subjects (ANOVA RM).
*p<0.05, before fasting vs. the indicated time in obese T1DM subjects (ANOVA RM).
#p<0.05 between obese T1DM subjects before fasting and non-obese T1DM (t-test).
(a) Basal insulin dose on the seventh day of fasting.

Tab. 2. FABP and FFA in the obese T1DM and non-obese T1DM patients.

Obese T1DM (n=14)	Before fasting	After 7 days of fasting	21 days after the fasting period	Non-obese T1DM (n=13)
FABP (ng/ml)	18.5±6.15#	29.2±18.3*	18.9±9.5	6.3±2.74
FFA (mmol/l)	0.51±0.27#	1.03±0.42*	0.47±0.21	0.25±0.17

*p<0.05, before fasting vs. the indicated time in obese T1DM subjects (ANOVA RM).
#p<0.05 between obese T1DM subjects before fasting and non-obese T1DM (t-test).

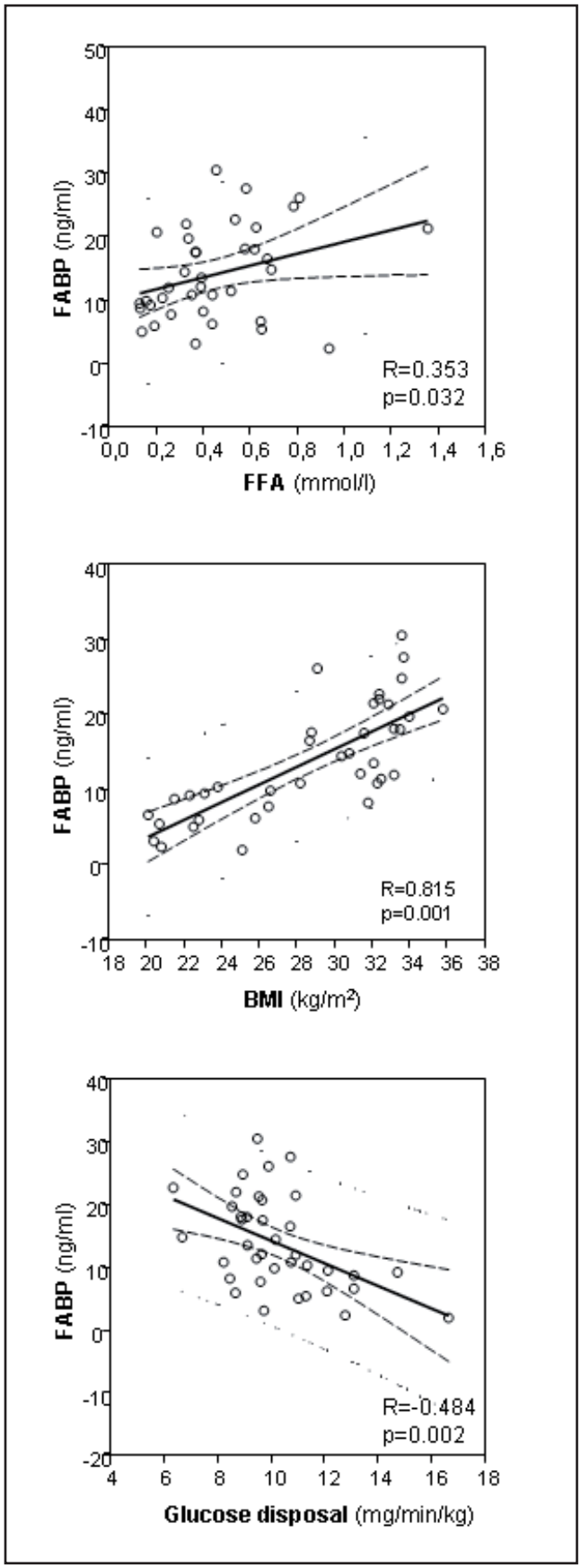

Fig. 1. Relationships between adipocyte fatty acid binding protein (FABP) in obese patients with type 1 diabetes mellitus and free fatty acid (FFA), body mass index (BMI) and glucose disposal. Circles = type 1 diabetes group.

features of insulin resistance with type 2 diabetes. A-FABP was identified as having a strong correlation to IR.

The high lipolytic activity during massive weight loss after seven days of fasting in our obese T1DM patients who had IR before the weight reduction program was associated with significant increases of both FABP and FFA. In previous studies in obese T1DM subjects, adipose tissue expression of A-FABP has been correlated to circulating free fatty acid concentrations, which might explain part of the insulin-resistant environment observed in obesity (Fisher *et al.* 2002). In fact, A-FABP physically interacts with hormone-sensitive lipase and stimulates its activity, promoting adipose tissue lipolysis (Shen *et al.* 2001). Cytoplasmic A-FABP in adipocytes may help to prevent the toxicity of nonesterified fatty acids for the cell, and its expression is reduced in adipose tissue after weight loss in morbid obesity (Fisher *et al.* 2002). However, these changes may vary over time. The obese T1DM patients in our study showed significant increases in FFA and FABP after seven days of fasting. Engl *et al.* (2008), who observed increased serum A-FABP after the first 3 months of weight loss in obese woman, during which the patients had maximum weight loss, suggested that the amount of serum A-FABP is an indicator of weight change due to lipolysis. A-FABP in our group of obese T1DM subjects decreased but remained elevated compared to control T1DM subjects. Mobilization of stored fat is mediated by lipolytic enzymes. Adipose triglyceride lipase is the predominant enzyme that performs the initial step in triglyceride hydrolysis and therefore seems to play a pivotal role in the lipolytic catabolism of stored fat in adipose tissue (Zimmermann *et al.* 2004). Hormone-sensitive lipase also hydrolyzes triglycerides and, with a higher specificity, diglycerides (Zimmermann *et al.* 2004). Cytoplasmic A-FABP increases the hydrolytic activity of hormone-sensitive lipase, and both together constitute a functionally important lipolytic complex (Shen *et al.* 2001; Carmen & Victor 2006). During weight maintenance and an IR state, it is reasonable to assume that the increased circulating fraction of A-FABP indicates paradoxical lipolytic activity due to the lack of inhibition of lipolysis by insulin (Arner 2002).

The obese T1DM patients in our study showed significant increases in FFA and FABP after seven days of fasting, which paralleled a temporary decrease in insulin sensitivity caused by the reduction of glucose oxidation. This decline in glucose oxidation reflects a metabolic adaptation to non-stress fasting and is in agreement with previous publications (Awad *et al.* 2009; Duška *et al.* 2005; Féry & Balasse 1994). Interestingly, in previous studies no correlation between serum FFA and A-FABP was observed after 6-month weight loss (Haider *et al.* 2007) or after up to 1 year of weight reduction (Simón *et al.* 2009). There is no obvious explanation for these differences, but on the one hand, they could be due to

the different study populations (those studies did not include T1DM patients treated by insulin), or on the other hand, they could be explained by a recent publication arguing that circulating FFA level may also derivate due to lipolysis of plasma lipoproteins (Engl *et al.* 2008).

The major limitation of this study is the small number of patients (14 obese patients with T1DM and IR and 13 non-obese control patients with T1DM). However, our patients were investigated four times during the observation period of 21 days to obtain a detailed impression of the A-FABP serum levels during pronounced weight loss. Second, although our data suggest that insulin resistance is associated with increased plasma A-FABP, the study was observational, and we cannot completely exclude other explanations for our results, such as confounding factors not properly controlled. The concentrations of A-FABP and FFA were measured up to the 21st day of the experimental protocol but no longer, which might have underestimated the association between the investigated factors and insulin resistance. Additionally, we cannot exclude the presence of any genetic variations in the A-FABP gene associated with the concentrations of A-FABP and FFA, although the contribution of these variants might be small compared to other clinical features, such as BMI (Stejskal *et al.* 2008). A-FABP gene polymorphism has a highly significant effect on intramuscular fat content in swine (Gao *et al.* 2011) and chicken (Ye *et al.* 2010). In nondiabetic Hispanic and non-Hispanic white males, the polymorphisms FABP4-376 and PPARγ Pro12Ala work together to influence a biologic pathway affecting insulin sensitivity and body composition (Damcot *et al.* 2004).

We conclude that increased plasma FABP indicates insulin resistance in obese patients with T1DM. Body weight reduction in T1DM patients using a low-calorie diet provides a safe and effective method to decrease body fat. This method is associated with transiently increased FABP, which might be a temporary adaptation of metabolism to non-stress fasting.

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Conflict of interest

There is no conflict of interest.

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