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RESEARCH ARTICLE

Elevated Concentrations of Liver Enzymes and Ferritin Identify a New Phenotype of Insulin Resistance: Effect of Weight Loss After Gastric Banding

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Abstract
Background Several studies have associated elevated liver enzymes (LFTs), obesity, and type 2 diabetes (T2DM), and a link has been established between insulin resistance (IR) and elevated ferritin concentrations. We examined the relationship between LFTs, ferritin, and IR in morbid obese subjects and the effect of weight loss after bariatric surgery.
Methods We measured liver enzymes, ferritin, insulin resistance, and glucose tolerance (by OGTT) in 159 morbid obese subjects (BMI=44.4±0.4 kg/m^2) at baseline, 6 months and 1 year after laparoscopic-adjustable-gastric banding (LAGB). Subjects were divided in two groups: increased LFTs (ALT>30; AST/ALT<1) vs. normal LFTs.
Results A large proportion of morbid obese subjects had increased LFTs (44%) which were associated with increased IR and ferritin, suggesting potential liver disease. A majority of the morbidly obese with increased LFTs, IGT, and T2DM, were male and had almost double ferritin concentrations, strongly correlated with ALT (r=0.43, p< 0.0001). Both ferritin and ALT correlated with waist circumference and IR. One year after, LAGB glucose tolerance improved, LFTs and IR were reduced; ferritin did not change significantly, but was still correlated with IR.
Conclusions Ferritin may be an additional useful marker for more severe hepatic IR.

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Introduction

Obesity and elevated liver enzymes are both independent risk factors for the development of type 2 diabetes (T2DM) [1, 2] and fatty liver disease (FLD) [3]. FLD refers to a spectrum of disorders ranging from simple hepatic steatosis to more severe manifestations, including non-alcoholic steato-hepatitis (NASH), which can progress to fibrosis, cirrhosis, and liver failure [4, 5] and it is associated to insulin resistance and to all the features of the metabolic syndrome, such as dyslipidemia, hypertension, and hyperglycemia [6–8]. Thus, it is not surprising that the great majority of obese subjects have FLD [3, 9, 10].

Recently, a link has been established between insulin resistance and ferritin [5]. It is unclear whether elevated

ferritin may simply be another marker of insulin resistance or whether elevated ferritin concentrations identify iron stores that may contribute to the pathogenesis of altered metabolic states. A recent study has suggested that body iron contributes to excess oxidative stress already at non-iron overload concentrations [11]. Moreover, serum ferritin has been identified as an important and independent predictor of the development of diabetes [12] and high concentrations of ferritin, together with low oral glucose insulin sensitivity, have been identified as independent markers of fibrosis in NASH [13]. It has been hypothesized that iron could be an important cofactor in the pathogenesis and progression of some cases of NASH [13] since FLD subjects have increased hepatic fatty acid oxidation, and increased production of ROS [14]. In a large cohort of NASH patients, 21.1% had hyper-ferritinemia while only 7.4% had signs of peripheral iron overload and 9% had signs of hepatic iron overload [13].

Considering the close relationship between obesity, insulin resistance and development of FLD, we studied their association with hepatic profile and ferritin concentrations. Since bariatric surgery-weight loss is associated with reduced insulin resistance, restored glucose tolerance [15, 16], reduced hepatic steatosis, and improved liver enzymes [9, 10] we repeated the analyses after laparoscopic gastric banding surgery to evaluate the impact of weight loss on the association between hepatic profile, ferritin concentrations, and insulin resistance.

Methods

Subjects

We analyzed, retrospectively, clinical and metabolic parameters in 159 morbidly obese patients (60% NGT; 22% IGT; 18% T2DM) consecutively recruited at the Istituto Clinico Sant'Ambrogio, Ospedale San Paolo and Ospedale San Raffaele, Milano, Italy (BMI=44.4+0.4 kg/m^2). Eligibility criteria have been described previously in detail [16, 17].

In all subjects, BMI, OGTT, and liver enzymes were measured (ALT and AST). Subjects were considered to have high liver function test (LFT) if ALT>30 and ratio of AST/ALT<1. Measurements of ferritin, transferring, and iron content were available in 127 subjects. High Ferritin values (within normal range) were considered when subjects where in the upper tertile, (i.e., >100 ng/ml in women and >200 ng/ml in men).

Metabolic measurements All metabolic tests were performed in the morning (0800 h) after a 10 to 12-h overnight fast. For the OGTT, timed blood samples were collected for the measurement of plasma glucose and insulin concentrations. Plasma glucose was measured by the glucose oxidase reaction (Beckman Glucose Analyzer, Fullerton, CA, USA). Plasma insulin concentrations were measured by RIA using specific kits (Linco Research, St Louis, MO, USA). Serum transaminases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]), serum lipid

Table 1 Clinical and metabolic characteristics of the population before LAGB

	Normal liver enzymes at baseline (n=89)	High liver enzymes at baseline (n=70)	$p_{\text{Liver enzyme}}$
Gender (F/M) [% of M]	83/6 [7%]	49/21 [30%]	<0.0001
[%NGT/IGT /T2DM]	[% 65/20/15]	[% 55/24/21]	0.003
Age (years)	42±1	41±1	ns
BMI (kg/m^2)	43.6 ±0.6	44.6±0.8	ns
Waist (cm)	119±2	126±2	0.002
HbA1c(%)	6.2±0.1	6.5±0.2	ns
Fasting plasma glucose (mM)	5.9±0.2	6.3±0.3	ns
Fasting plasma insulin (mU/l)	15 [10–20]	18 [11–26]	0.02
Plasma total chol (mg/dl)	199[178–232]	202[179–226]	ns
Plasma HDL chol (mg/dl)	49[41–56]	43 [37–53]	0.02
Plasma triglycerides (mg/dl)	121[92–159]	125 [89–202]	ns
AST (U/l)	18 [16–21]	30 [23–41]	<0.0001
ALT (U/l)	21 [17–25]	43 [36–58]	<0.0001
Ferritin (ng/ml)	45 [21–90]	101 [47–174]	<0.0001
Transferrin (mg/ml)	2.7±0.1	2.5±0.1	ns
Serum Iron (μg/dl)	76±3	88±4	0.02
Fibrinogen (mg/dl)	413±9	365±10	0.0007
Peripheral IS (OGIS)	7.0±0.2	6.4±0.2	0.02
Peripheral IS (Matsuda)	3.6±0.2	2.9±0.2	0.03
Hepatic IS (QUICKI)	316±3	308±3	0.02

profile, ferritin, transferring, and iron content were determined by standard laboratory methods (normal ranges, transferrin 200–400 mg/dl, ferritin women, 8–140 ng/ml, men, 30–400 ng/ml).

Peripheral insulin sensitivity (IS) was assessed (1) by the OGIS method [18], which estimates IS expressed as glucose clearance during a euglycemic insulin clamp from the plasma glucose and insulin profiles measured during the OGTT; (2) by calculating IS as the Matsuda index [19] which is an index of insulin resistance based on glucose and insulin profiles during OGTT. These estimates have been validated against the euglycemic insulin clamp technique in normal, obese, and diabetic subjects [20]. We also calculated the QUICKI index [21] which can be considered an index of hepatic insulin sensitivity [22] although it also correlates with the euglycemic insulin clamp [21]. Since HGP is the primary determinant of the fasting plasma glucose concentration [23], and the fasting plasma insulin concentration is the primary regulator of hepatic glucose production [24], the product of fasting plasma glucose and FPI primarily reflects hepatic insulin resistance.

Statistical analysis Data were given as the means±SEM for normally distributed variables, while were expressed as median and interquartile range (in square brackets) when they had a skew distribution. Group differences according to obesity and LFT were analyzed by two-way ANOVA using log-transformed values of non-normally distributed variables. The contribution of multiple factors to ALT and ferritin concentrations was assessed by multivariate analysis and partial correlation were reported.

Results

Baseline Study

Subjects were divided in two groups: normal or increased LFTs (defined as AST/ALT<1 and/or ALT≥30 U/l). At the time of enrolment, 44% of the subjects had increased liver enzymes. High LFTs were associated with higher prevalence of IGT (24% vs. 20%) and T2DM (21% vs. 15%) and with male gender (Table 1). Subjects with elevated liver enzymes showed increased waist circumference, fasting plasma insulin, HbA1c, and reduced HDL and insulin sensitivity (Table 1).

Ferritin concentration was found almost double (although within normal ranges) in subjects with high LFTs compared with those with normal LFTs (101 vs. 45 ng/ml, $p<0.0001$), that also had increased serum iron (88±4 vs. 76±3, $p=0.02$), while no difference was found for transferrin (2.5±0.1 vs. 2.7±0.1 mg/dl, p=ns). On the other hand, both hemoglobin and hematocrit were similar in the two groups and within normal ranges (13.1±0.2 vs. 13.8±0.2, $p<0.05$; 38.9±0.5 vs. 40.3±0.6, p=ns, respectively).

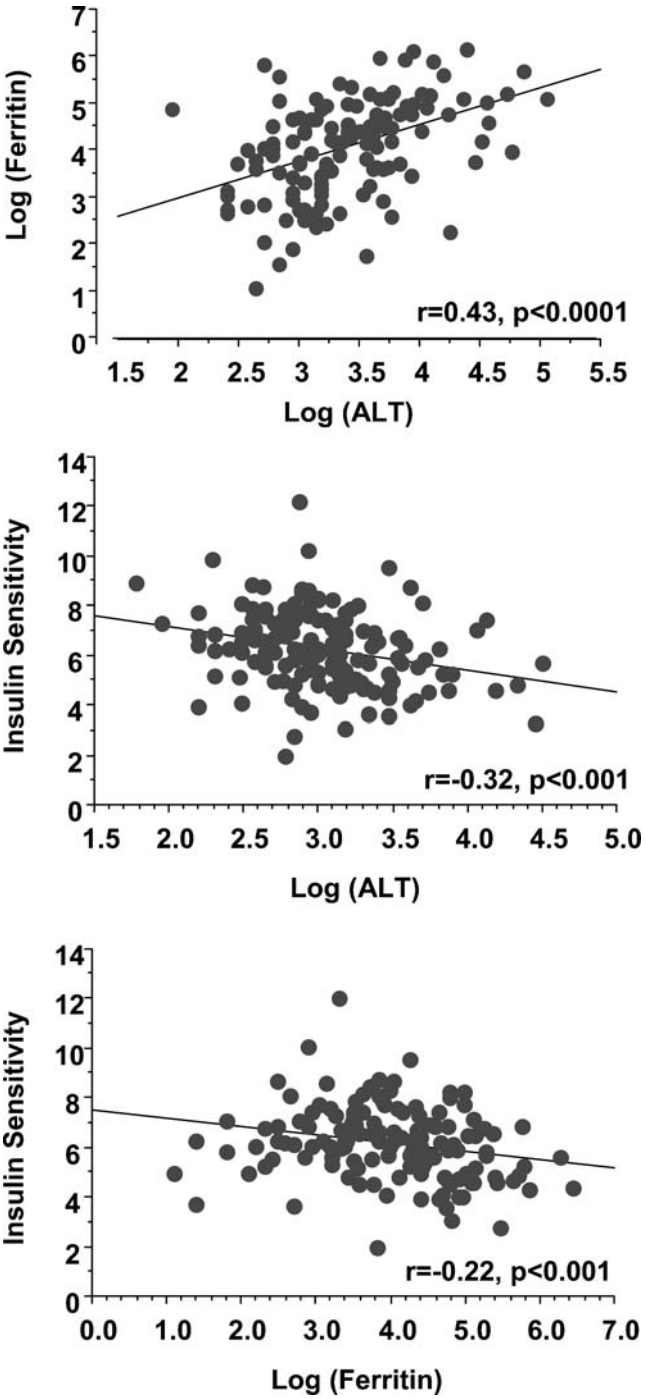

Fig. 1 *Top panel* correlations between ferritin and ALT concentrations. Insulin sensitivity (calculated using OGIS) as a function of ALT (*central panel*) and Ferritin (*bottom panel*) concentrations

Table 2 Clinical and metabolic changes after LAGB

	Normal liver enzymes at baseline (n=89)			High liver enzymes at baseline (n=70)		
	Basal	6 months	12 months	Basal	6 months	12 months
Weight (kg)	113±2	96±2*	93±2*	122±3	105±2*	102±2*
BMI (kg/m^2)	43.6 ±0.6	37.0±0.6*	36.2±0.6*	44.6±0.8	38.3±0.8*	37.2±0.7*
Waist (cm)	119±2	106±1*	104±1*	126±2	113±2*	111±1*
HbA1c (%)	6.2±0.1	5.8±0.1*	5.6±0.1*	6.5±0.2	5.9±0.1*	5.8±0.1*
Fasting plasma glucose (mM)	5.9±0.2	5.3±0.1*	5.2±0.1*	6.3±0.3	5.2±0.2*	5.5±0.3*
Fasting plasma insulin (mU/l)	15 [10–20]	9 [7–12]*	9 [6–12]*	18 [11–26]	10 [7–16]*	10 [6–14]*
Plasma HDL chol (mg/dl)	49[41–56]	51 [44–60]*	55 [46–63]*	43 [37–53]	47 [39–55]*	49 [42–60]*
AST (U/l)	18 [16–21]	16 [14–19]*	16 [14–19]*	30 [23–41]	21 [17–26]*	21 [17–26]*
ALT (U/l)	21 [17–25]	16[13–21]*	18 [14–21]*	43 [36–58]	26 [21.31]*	24 [20–32]*
Ferritin (ng/ml)	45 [21–90]	38 [11–81]*	34 [17–73]*	101 [47–174]	72 [34–150]*	108 [56–156]
Peripheral IS (OGIS)	7.0±0.2	8.6±0.2*	9.0±0.2*	6.4±0.2	7.8±0.2*	8.2±0.2*
Peripheral IS (Matsuda)	3.6±0.2	5.8±0.3*	6.4±0.3*	2.9±0.2	5.5±0.8*	6.0±0.6*
Hepatic IS (QUICKI)	316±3	341±2*	346±3*	308±3	335±5*	342±4*

*p<0.05 vs. baseline (two-way ANOVA for repeated measurements)

Before surgery, increased ALT values were significantly correlated with larger waist circumference (r=0.33), increased HbA1c (r=0.20) and insulin concentrations (r=0.29) and reduced insulin sensitivity measured either as QUICKI, OGIS, or Matsuda index (r=−0.28, r=−0.32 and r=−0.30, respectively, all p<0.05). Ferritin correlated positively with waist circumference (r=0.26), HbA1c (r=0.22) and ALT concentrations (r=0.43) and negatively with insulin sensitivity measured either as QUICKI, OGIS index (r=−0.20, r=−0.30 and r=−0.22, respectively, all p<0.05). We observed a strong relationship between ALT and ferritin, even after accounting for age, gender, BMI, and insulin sensitivity (Fig. 1). In a multiple regression model, insulin sensitivity (OGIS) correlated independently with ALT (partial r=−0.39, p=0.0002), after accounting for age/gender/BMI (explaining 28% of OGIS variability).

Follow-up Study after LAGB

One year after surgery, mean weight loss was 21±1 kg, BMI was 36.6±0.4 kg/m^2 and waist circumference decreased by 15.2±0.8 cm. After LAGB both groups decreased liver enzymes and 68% of the subjects with high LFTs before surgery had normal LFTs. Both LFT groups improved their OGTT profiles with a reduction in plasma glucose and insulin concentrations and improved both peripheral and hepatic insulin sensitivity; most of the improvement was achieved during the first 6 months (Table 2).The number of subjects that restored normal glucose tolerance increased independently of changes in liver enzymes (79% in the group with normal LFTs and 75% in the group that after 1 year still had high LFTs, Fig. 2). However, subjects that started with increased LFTs remained more insulin resistant than subjects with normal LFTs (Table 2).

A slight decrease in ferritin concentration was observed only in the normal LFT group (Table 2), but the association between insulin resistance and ferritin concentration (r=−0.20, p=0.02) was still present (Fig. 3). We observed that subjects that after 1 year remained IGT or T2D had higher ferritin concentrations (92 [112] vs. 49 [80] ng/ml, p=<0.01). The changes in insulin sensitivity observed 1 year after LAGB were strongly correlated with

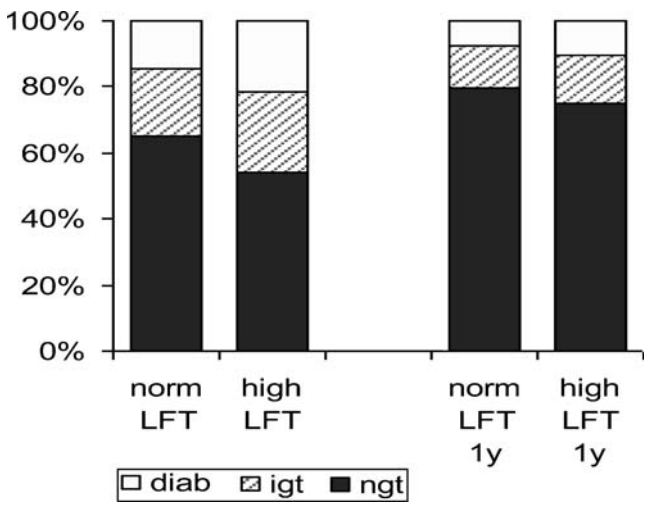

Fig. 2 Prevalence of NGT, IGT, and T2DM in the morbid obese group before and 1 year after LAGB surgery

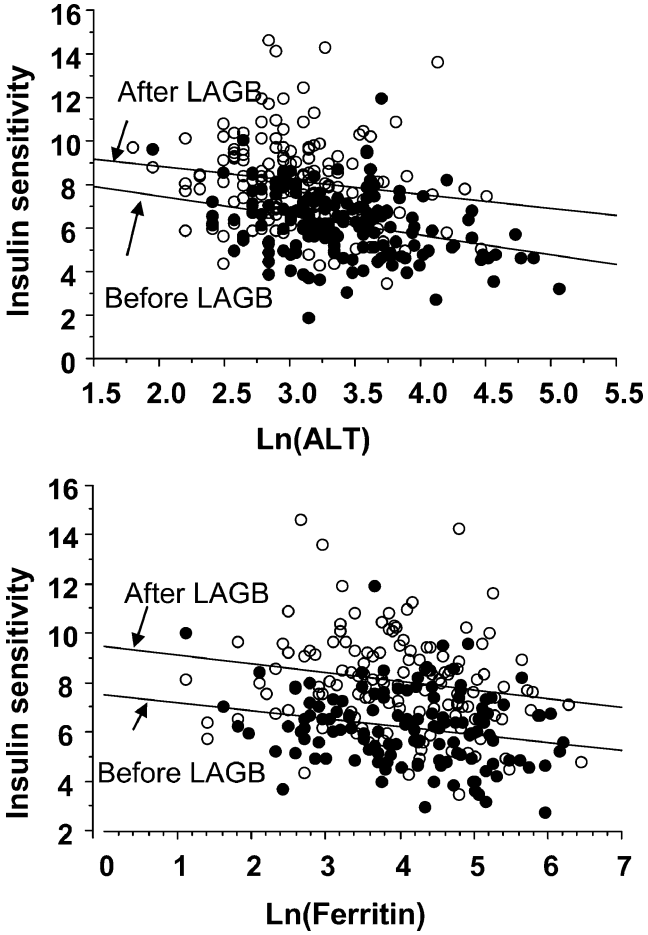

Fig. 3 *Top panel* correlations between insulin sensitivity (calculated using OGIS) and ALT concentrations before (*black circles*) and after (*white circles*) LAGB. *Bottom panel* correlations between insulin sensitivity (calculated using OGIS) and ferritin concentrations before (*black circles*) and after (*white circles*) LAGB

the improvement in BMI, body weight, and waist circumference (r=−0.48, r=−0.46 and r=0.39, all $p<$ 0.0001) but not with the changes in ALT or ferritin (Table 3).

Discussion

The last decade has witnessed an epidemic increase in the incidence of obesity [25], a major risk factor for the development of fatty liver disease (FLD). Several studies have associated elevated liver enzymes, obesity, and type 2 diabetes mellitus (T2DM) [2, 26, 27] and it has been estimated that FLD occurs in >30% in obese subjects [3] and in >60% of morbidly obese patients [9]. Recently, a link has been established also between insulin resistance and ferritin concentrations [5, 28]. We, thus, examined the relationship between alanine aminotransferase (ALT), serum ferritin concentrations and insulin resistance in a group of morbid obese subjects and the effect of weight loss after bariatric surgery on these parameters. We found that subjects with increased liver function tests (LFTs) had values of ferritin almost double of those with normal LFTs although still within the normal range. A strong correlation was observed between plasma ALT and ferritin concentrations, and between these two variables and increased body mass index and insulin resistance. After bariatric surgery there was a reduction in liver enzymes and insulin resistance. Although the changes in ferritin concentrations were mild, the correlation between ferritin and insulin resistance was still present indicating ferritin concentration as an additional useful marker for more severe (hepatic) insulin resistance.

Serum ferritin is a widely used marker of total body iron status in epidemiological studies and has been shown to closely reflect body iron stores [29]. A syndrome of liver iron overload was proposed [30], following an observation that there was a higher prevalence of metabolic disorders among patients with high ferritin, normal transferrin saturation, and normal transferrin without genetic hemochromatosis than among patients with genetic hemochromatosis; both groups had similar ferritin levels. The liver iron overload syndrome shares features with the metabolic or insulin resistance syndrome. The mechanisms of such an

Table 3 Matrix of Pearson's correlation coefficients between changes observed after bariatric surgery

	Δ Body weight	Δ BMI	Δ Waist	Δ Insulin sensitivity	Δ QUICKI	Δ ALT[a]	Δ Ferritin[a]	Δ Triglyceride[a]
Δ Body weight	–	0.98*	0.82*	−0.46*	−0.16*	0.22	0.20	0.16
Δ BMI	0.98*	–	0.81*	−0.48*	−0.19*	0.21	0.17	0.14
Δ waist	0.82*	0.81*	–	−0.39*	−0.19*	0.19	0.26*	0.21*
Δ insulin sensitivity	−0.46*	−0.48*	−0.39*	–	0.26*	−0.05	−0.09	−0.10
Δ QUICKI	−0.16*	−0.19*	−0.19*	0.26*	–	0.12	−0.18	−0.08
Δ ALT[a]	0.22	0.21	0.19	−0.05	0.12	–	0.13	0.16
Δ Ferritin[a]	0.20	0.17	0.26*	−0.09	−0.18	0.13	–	0.19
Δ Triglyceride[a]	0.16*	0.14	0.21*	−0.10	−0.08	0.16	0.19	–

*$p<0.05$

[a] Only subjects while elevated basal LFTs were included in this analyses

association have not been identified, and it has been hypothesized that the hyperinsulinemia of the metabolic syndrome could be related to an accumulation of iron in the liver [31] and thus involved in hepatic damage [14]. FLD subjects have increased hepatic fatty acid oxidation and increased production of ROS [32]. With regard to this, iron is a transition metal capable of causing oxidative tissue damage by catalyzing the formation of free radicals [33]. On the other hand, ferritin could be simply another independent marker of insulin resistance [13]. In our group of subjects, before bariatric surgery, ferritin concentrations were increased proportionally to ALT concentrations, although, in general, within normal ranges and similar in NGT, IGT, and T2DM. A positive correlation was observed between ferritin plasma concentrations and insulin resistance, consistently with the recently proposed link between iron load and insulin resistance [5, 28]. Bariatric surgery induced weight loss has been shown to restore normal glucose tolerance in T2DM and to improve hepatic function [9] and also in this study liver enzymes decreased after LAGB. After surgery, however, we did not observe a significant decrease in plasma ferritin concentrations despite the improvement in hepatic function and insulin resistance. However, the correlations between ferritin, ALT, and insulin resistance remained suggesting that ferritin may simply identify a new phenotype of insulin resistance. Consistent with this, a recent prospective study showed that the incidence of the metabolic syndrome after 6 years was more than four-fold higher in subjects with ferritin and transferrin values above the tertile compared with participants with values below the bottom tertile thresholds [28].

In conclusion, our results show in obese subjects have increased plasma ferritin concentrations that are associated with insulin resistance and elevated liver function tests suggesting potential liver disease. Weight loss, achieved with laparoscopic gastric banding, reduced liver enzymes and insulin resistance, but it did not significantly change ferritin that remained correlated with insulin resistance. Thus, it is tempting to hypothesize that the dyed increased liver enzymes and ferritin concentrations could be an additional useful marker for hepatic insulin resistance and greater cardiovascular disease risk.

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