

Endoplasmic Reticulum Stress Is Reduced in Tissues of Obese Subjects After Weight Loss

Margaret F. Gregor,¹ Ling Yang,¹ Elisa Fabbrini,² B. Selma Mohammed,² J. Christopher Eagon,² Gökhan S. Hotamisligil,¹ and Samuel Klein²

OBJECTIVE—Obesity is associated with insulin resistance and type 2 diabetes, although the mechanisms linking these pathologies remain undetermined. Recent studies in rodent models revealed endoplasmic reticulum (ER) stress in adipose and liver tissues and demonstrated that ER stress could cause insulin resistance. Therefore, we tested whether these stress pathways were also present in obese human subjects and/or regulated by weight loss.

RESEARCH DESIGN AND METHODS—Eleven obese men and women (BMI 51.3 ± 3.0 kg/m²) were studied before and 1 year after gastric bypass (GBP) surgery. We examined systemic insulin sensitivity using hyperinsulinemic-euglycemic clamp studies before and after surgery and collected subcutaneous adipose and liver tissues to examine ER stress markers.

RESULTS—Subjects lost $39 \pm 9\%$ body wt at 1 year after GBP surgery ($P < 0.001$), which was associated with a marked improvement in hepatic, skeletal muscle, and adipose tissue insulin sensitivity. Markers of ER stress in adipose tissue significantly decreased with weight loss. Specifically, glucose-regulated protein 78 (Grp78) and spliced X-box binding protein-1 (sXBP-1) mRNA levels were reduced, as were phosphorylated elongation initiation factor 2 α (eIF2 α) and stress kinase c-Jun NH₂-terminal kinase 1 (JNK1) (all P values <0.05). Liver sections from a subset of subjects showed intense staining for Grp78 and phosphorylated eIF2 α before surgery, which was reduced in post-GBP sections.

CONCLUSIONS—This study presents important evidence that ER stress pathways are present in selected tissues of obese humans and that these signals are regulated by marked weight loss and metabolic improvement. Hence, this suggests the possibility of a relationship between obesity-related ER stress and metabolic dysfunction in obese humans. *Diabetes* 58:693–700, 2009

Increased adiposity is associated with a group of chronic metabolic disorders, including insulin resistance, type 2 diabetes, and nonalcoholic fatty liver disease (1). The prevalence of this cluster of abnormalities has increased significantly in the past few decades following the marked rise of obesity worldwide (2,3). The

From the ¹Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, Massachusetts; and the ²Center for Human Nutrition, Washington University School of Medicine, St. Louis, Missouri.

Corresponding author: Samuel Klein, sklein@wustl.edu, or Gökhan S. Hotamisligil, ghotamis@hsph.harvard.edu.

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M.F.G. and L.Y. contributed equally to this work.

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mechanisms responsible for the emergence of these disorders have been an intense area of investigation. In the past decade, it was recognized and established that chronic inflammatory and stress responses are a central feature of obesity, insulin resistance, and type 2 diabetes and contribute to the metabolic imbalance (4). However, the pathways and mechanisms giving rise to the chronic inflammatory responses remain to be elucidated.

Recently, we have identified endoplasmic reticulum (ER) dysfunction as a significant contributor to the development of experimental insulin resistance in obese tissues (5). The ER is a critical intracellular organelle that coordinates the synthesis, folding, and trafficking of proteins. All transmembrane and secreted proteins pass through the ER en route to cellular destinations; proteins that fail to fold into proper structures are removed from the ER through degradation. Under stress conditions, unfolded proteins accumulate in the ER and initiate an adaptive response known as the unfolded protein response (UPR). The UPR is initiated by three ER transmembrane sensors [namely PKR-like ER-regulated kinase (PERK), inositol-requiring enzyme-1 (IRE-1), and activating transcription factor-6 (ATF-6)], which activate an adaptive response that results in cessation of protein translation and transcriptional increase in protein-folding chaperones and ER-associated degradation genes (6). If the stress is too severe, the UPR may also induce cellular apoptosis through several different mechanisms involving the various branches of the UPR. In mouse models of obesity, ER stress is present in liver and adipose tissues, as evidenced by increased activity of both the IRE-1 and PERK branches (7). The UPR is also able to induce activation of the c-Jun NH₂-terminal kinase (JNK) pathway and thereby inhibit insulin signaling through the subsequent phosphorylation and/or degradation of IRS1 (7–9). Data obtained from mouse models demonstrate that genetically compromised ER folding capacity induces ER stress, activates JNK, and leads to whole-body insulin resistance in the mouse (7). In contrast, enhancing ER capacity in obese mice through the use of chemical or molecular chaperones relieves ER stress in adipose tissue and liver, reduces intrahepatic fat accumulation, and restores glucose homeostasis (10).

Although ER stress is associated with obesity and metabolic dysfunction in rodent models, the importance of ER stress in the pathogenesis of obesity-related metabolic disease and the potential regulation of ER function by weight loss in human subjects is not known. Therefore, the purpose of the present study was to evaluate the effect of marked weight loss induced by gastric bypass (GBP) surgery on insulin sensitivity and ER stress in key metabolic tissues in obese human subjects. A euglycemic-hyperinsulinemic clamp procedure, in conjunction with stable isotopically labeled tracer infusions, was used to assess insulin action in liver, skeletal muscle, and adipose

TABLE 1
Metabolic characteristics of subjects before and 1 year after gastric bypass surgery

	Before surgery	After surgery
n (men/women)	10 (2/8)	10 (2/8)
Age	40.3 ± 2.3	41.3 ± 2.3
Weight (kg)	143.5 ± 9.44	87.6 ± 5.9*
BMI (kg/m ²)	51.3 ± 3.0	31.4 ± 2.3*
Glucose (mg/dl)	98.5 ± 4.8	84.2 ± 2.2†
Insulin (mU/l)	18.7 ± 3.1	4.9 ± 0.8*
HOMA-IR	5.3 ± 0.9	1.6 ± 0.5†
Intrahepatic fat content (%‡)	19.5 ± 5.2	1.9 ± 2.1*
Systolic blood pressure (mm/Hg)	132 ± 7	108 ± 5*
Diastolic blood pressure (mm/Hg)	74 ± 3	63 ± 4†
Total cholesterol (mg/dl)	160 ± 13	161 ± 9
LDL cholesterol (mg/dl)	85 ± 10	79 ± 6
HDL cholesterol (mg/dl)	48 ± 4	68 ± 5§
Triglyceride (mg/dl)	135 ± 19	72 ± 9†

Data are means ± SE. Value significantly different from corresponding Before Surgery value; **P* < 0.001; †*P* < 0.05; §*P* < 0.01. ‡Data from four subjects. HOMA-IR, homeostasis model assessment of insulin resistance.

tissue; and UPR markers from among the three response branches were evaluated in liver and adipose tissue samples obtained before and 1 year after GBP surgery.

RESEARCH DESIGN AND METHODS

Subjects and GBP surgery. Eleven obese subjects (mean BMI 51.3 ± 3.0 kg/m²; two men and nine women) who were scheduled to undergo GBP surgery participated in this study. All subjects completed a comprehensive medical evaluation, which included a detailed history and physical examination, routine blood tests, and a 12-lead electrocardiogram. No subjects had diabetes, had other metabolic diseases, or were taking medications that affect insulin action or glucose and fatty acid metabolism. All subjects gave their written informed consent before participating in this study, which was approved by the Human Research Protection Office and the Center for Applied Research Sciences Advisory Committee of Washington University School of Medicine.

All GBP procedures were performed by the same surgeon (J.C.E.) using standard surgical techniques. All patients had an open GBP procedure, which involved constructing a small (~20 ml) proximal gastric pouch by stapling across the stomach. A 150-cm Roux-Y limb was constructed by transecting the jejunum 30 cm distal to the ligament of Treitz and creating a jejunojejunostomy 150 cm distal to the transection.

Metabolic studies and sample analyses. Insulin sensitivity was examined by using the hyperinsulinemic-euglycemic clamp procedure in 10 of the 11 subjects before and 1 year after GBP surgery. Subjects were admitted to the Intensive Research Unit at Washington University School of Medicine on the evening before the clamp procedure. The following morning, after subjects fasted overnight, a one-stage hyperinsulinemic-euglycemic clamp procedure was performed; [6,6-²H₂]glucose and [2,2-²H₂]palmitate were infused for 6 h (0- to 3-h basal period followed by 3- to 6-h insulin infusion at a rate of 15 mU · m⁻² body surface area [BSA] · min⁻¹, initiated with a priming dose of 60 mU · m⁻² BSA · min⁻¹ for 5 min and then 30 mU · m⁻² BSA · min⁻¹ for 5 min. Euglycemia was maintained at a blood glucose concentration of ~5.6 mmol/l (100 mg/dl) throughout the clamp procedure by infusing 20% dextrose enriched to 2.5% with [6,6-²H₂]glucose. Blood samples were obtained before beginning the tracer infusion to determine background plasma glucose and palmitate tracer-to-tracee ratios and every 10 min during the final 30 min of the basal period and of the clamp procedure to determine glucose and insulin concentrations and substrate kinetics. Plasma insulin was determined by a radioimmunoassay and plasma glucose and palmitate tracer-to-tracee ratios were determined by using gas chromatography-mass spectroscopy (MSD 5973 system with capillary column; Hewlett-Packard, Palo Alto, CA), as previously described (11,12). Glucose and palmitate kinetics were determined by using Steele's equation for steady-state conditions (13,14), and hepatic insulin sensitivity was determined by the reciprocal of the hepatic insulin resistance index [10,000/(basal glucose rate of appearance (*R_a*) in μmol · kg⁻¹ · min⁻¹ × basal insulin concentration in mU/l)], as previously described (15,16).

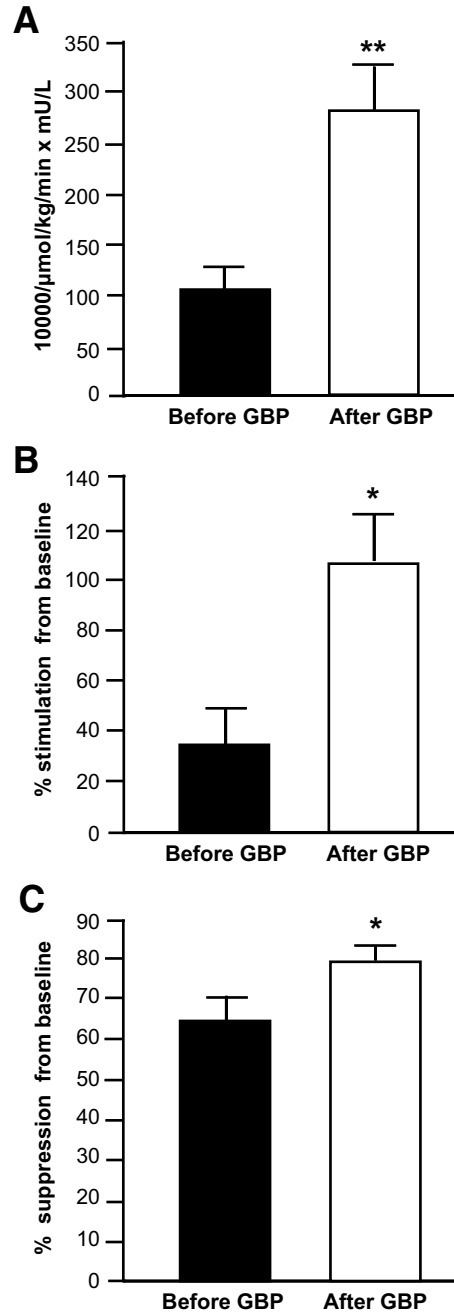


FIG. 1. Alterations in systemic insulin sensitivity in GBP subjects. Hepatic insulin sensitivity index, calculated as the reciprocal of the product of basal glucose production and insulin concentration (A); insulin-stimulated glucose disposal, an index of skeletal muscle insulin sensitivity assessed as the relative increase in glucose uptake during insulin infusion (B); and insulin-mediated suppression of palmitate *R_a* into plasma, an index of adipose tissue insulin sensitivity assessed as the relative decrease in palmitate *R_a* during insulin infusion (C), in obese subjects before (■) and 1 year after (□) GBP surgery. *Value significantly different from the before GBP value; **P* < 0.01, ***P* < 0.001.

Subcutaneous abdominal adipose tissue samples were collected during basal postabsorptive conditions in all subjects by using a 14-gauge needle; samples were immediately frozen in liquid nitrogen. Liver tissue samples were obtained by needle biopsy during GBP surgery and by percutaneous needle biopsy 1 year after GBP surgery in four subjects and placed in formalin for further analyses.

mRNA isolation and quantitative PCR. Frozen adipose tissue samples were homogenized in TRIzol Reagent (Invitrogen) for total RNA isolation according to the manufacturer's protocol. cDNA synthesis was performed using 1 μg sample RNA reverse transcribed with the high-capacity cDNA

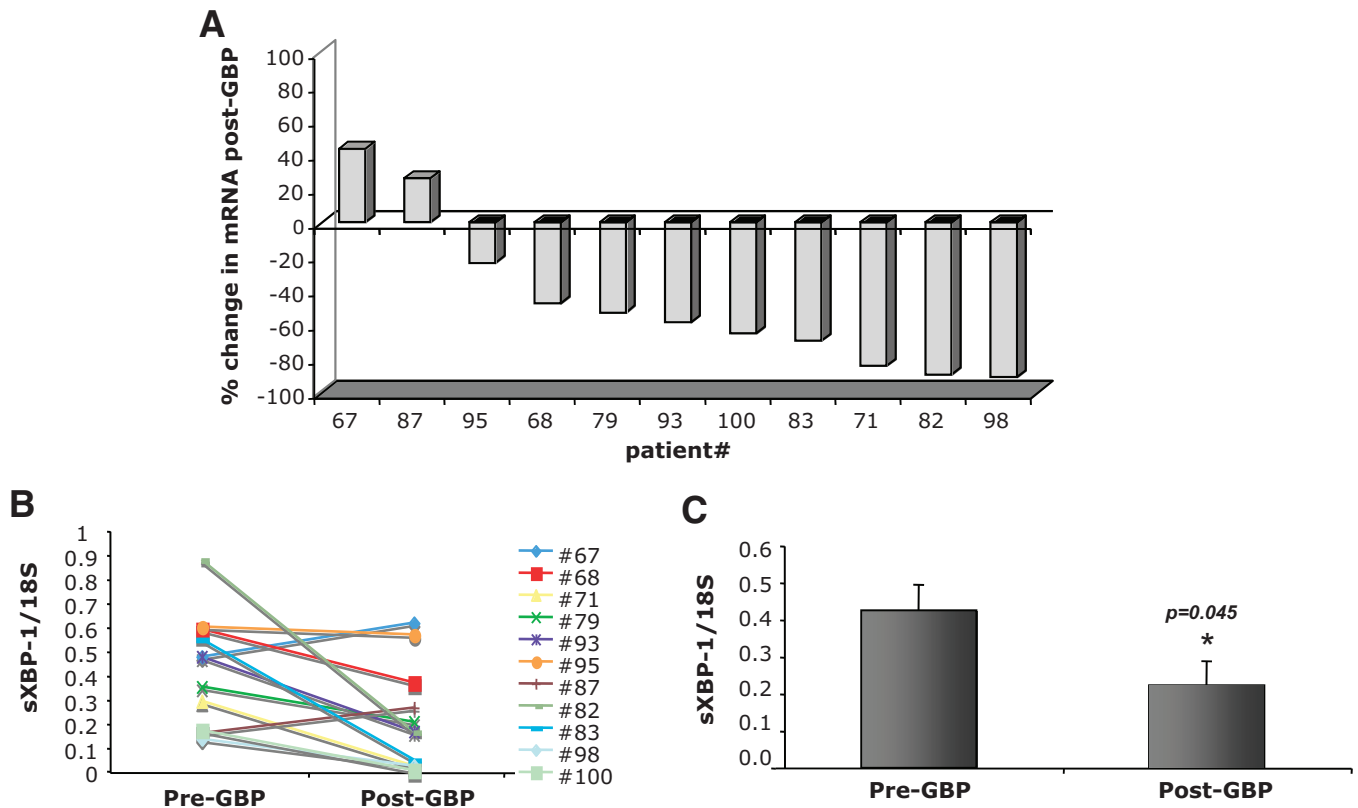


FIG. 2. Regulation of spliced XBP-1 mRNA in GBP subjects. Quantitative RT-PCR was performed on RNA isolated from adipose tissue of patients before and after GBP surgery, $n = 11$. **A:** Percent change in sXBP-1 mRNA from pre-GBP levels in each individual patient. **B:** Pre- and post-GBP surgery levels of sXBP-1 mRNA normalized to 18S ribosomal RNA. **C:** Average mRNA levels for sXBP-1 in pre and post-GBP surgery groups. Data indicate means \pm SE. * $P < 0.05$.

archive system (Applied Biosystems, Foster City, CA). Real-time, quantitative PCR was performed using SybrGreen reagent in the ABI 7300 real-time PCR system (Applied Biosystems) to determine the mRNA levels of the following genes in adipose tissue samples obtained before and after GBP surgery: 18S, C/EBP homologous protein (*CHOP*), spliced X-box binding protein-1 (*XBP-1*), glucose-regulated protein 78 (*Grp78*), and interleukin-6 (*IL-6*). Primer sequences used to detect these human genes are as follows: 18S forward, GTAACCGTTGAACCCATT; 18S reverse, CCATCCAATCGGTAGTAGCG; *Grp78* forward, CATCACGCCGTCCTATGTGCG; *Grp78* reverse, CGTCAAA-GACCGTGTCTCG; *sXBP* forward, GGTCTGCTGAGTCCGAGCAGG; *sXBP* reverse, GGGCTTGGTATATATGTGG; *CHOP* forward, GGAGAACCAG-GAAACGGAAAC; *CHOP* reverse, TCTCCTTCATGCGCTGCTT; *IL-6* forward, AAATTCGGTACATCCTCGACGG; and *IL-6* reverse, GGAAGGTTTCAGGTT-GTTTTCTGC.

Protein isolation, antibodies, and Western blotting. Frozen adipose tissue samples were homogenized in lysis buffer containing 25 mmol/l Tris-HCl (pH 7.4), 10 mmol/l Na_2VO_4 , 100 mmol/l NaF, 50 mmol/l $\text{Na}_2\text{P}_2\text{O}_7$, 10 mmol/l EGTA, 10 mmol/l EDTA, and 1% Nonidet P-40 with protease inhibitors (Sigma). After homogenization, the tissue lysate was centrifuged at 4,000 rpm for 15 min at 4°C followed by 14,000 rpm for 20 min at 4°C. Eighty micrograms total tissue protein was used for direct immunoblotting. Rabbit polyclonal anti-phosphorylated eukaryotic initiation factor 2 α (eIF2 α) antibody (Invitrogen) was used at 1:1,000 dilution; mouse monoclonal anti-phosphorylated JNK (Cell Signaling) was used at 1:1,000 dilution; rabbit polyclonal anti-calsestrin (Stressgen) was used at 1:1,000 dilution; β -tubulin (Santa Cruz) was used at 1:1,000 dilution. Horseradish peroxidase-linked anti-mouse or anti-rabbit secondary antibody (GE Healthcare) was used at 1:4,000 dilution.

Immunohistochemical examination of liver sections. Liver biopsy specimens were evaluated for steatosis and inflammation by hematoxylin-eosin (H-E) staining and for markers of ER stress by using immunohistochemistry. Human liver sections were subjected to routine deparaffinization/hydration process and blocked with goat serum albumin for 1 h at room temperature. Primary antibody incubation at 1:50 concentration was performed overnight at 4°C. After primary antibody incubation, sections were washed with PBS and incubated with secondary antibody at 1:200 dilution for 60 min. At the end of the secondary antibody incubation, sections were washed with PBS followed by application of mounting solution containing DAPI (Vector Lab). Secondary antibodies used in the current study were fluorescein isothiocyanate- or

Texas-red-conjugated anti-rabbit IgG (Santa Cruz). Negative controls included omission of the primary antibody. Cells were examined with a fluorescence microscope (Axio Observer; Carl Zeiss). Quantification of immunohistochemical staining was performed using Axiovision Software (Carl Zeiss) from images of liver tissue captured at random and is represented as relative fluorescence intensity.

Selection of UPR markers. We chose UPR markers from among the three branches that respond to ER stress. The transmembrane kinase PERK phosphorylates eIF2 α , leading to inhibition of general protein translation. As a result of alternative translation, the proapoptotic transcription factor CHOP is induced. IRE-1 activates the stress kinase JNK1 and also possesses endoribonuclease activity, which splices an intron from the mRNA of *XBP-1*, creating an activated transcription factor. Spliced XBP-1, along with active ATF-6, translocates to the nucleus and induces expression of protein chaperone genes. One well-known target of both of these transcription factors is the chaperone Grp78.

Statistical analyses. Insulin sensitivity and patient information datasets were normally distributed according to the Kolmogorov-Smirnov test, so that comparisons between subjects before and after weight loss were performed using parametric procedures. The statistical significance of the effect of insulin infusion on substrate metabolism before and after weight loss was analyzed by using the Student's *t* test for paired samples. Results are presented as means \pm SE. All reported *P* values are two sided, and a *P* value of <0.05 was considered to be statistically significant.

RESULTS

Study subject characteristics and insulin sensitivity. On average, subjects lost $\sim 40\%$ of their initial body weight at 1 year after GBP surgery (Table 1). Weight loss caused a marked decrease in plasma glucose and insulin concentrations and in intrahepatic fat content (Table 1). The hepatic insulin sensitivity index value was 160% greater 1 year after than before GBP surgery ($P < 0.01$) (Fig. 1A). Insulin infusion during the clamp procedure increased plasma insulin concentration from 18.7 ± 3.1 to 43.6 ± 4.2

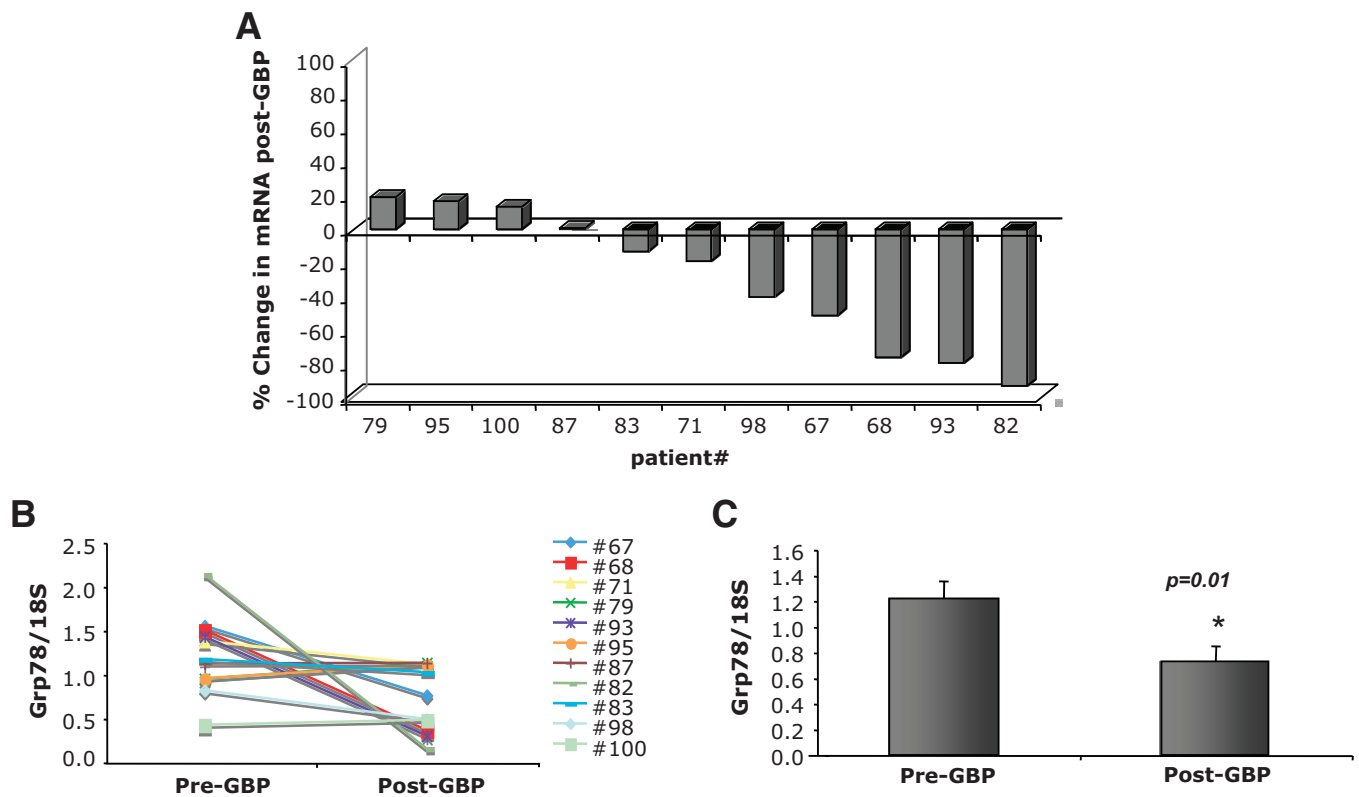


FIG. 3. Regulation of Grp78 mRNA in GBP subjects. Quantitative RT-PCR was performed on RNA isolated from adipose tissue of patients before and after GBP, $n = 11$. **A:** Percent change in Grp78 mRNA from pre-GBP levels in each individual patient. **B:** Pre- and post-GBP surgery levels of Grp78 mRNA normalized to 18S ribosomal RNA. **C:** Average mRNA levels for Grp78 in pre- and post-GBP groups. Data indicate mean \pm SE. * $P < 0.02$.

mU/l before and from 4.9 ± 0.8 to 27.0 ± 1.5 mU/l 1 year after GBP surgery. Plasma glucose concentrations during insulin infusion were 97.7 ± 1.7 and 99.6 ± 1.9 mg/dl during the clamp procedure performed before and after weight loss, respectively. Insulin infusion caused an increase in glucose R_d from $1,081 \pm 69$ to $1,418 \pm 108$ $\mu\text{mol}/\text{min}$ before GBP surgery and from 813 ± 57 to $1,666 \pm 171$ $\mu\text{mol}/\text{min}$ 1 year after GBP surgery. The increase in glucose R_d during insulin infusion was 200% greater after than before weight loss ($P < 0.01$) (Fig. 1B). Insulin infusion caused a decrease in palmitate R_a from 161 ± 13 $\mu\text{mol}/\text{min}$ to 61 ± 14 $\mu\text{mol}/\text{min}$ before GBP surgery and from 105 ± 9 to 21 ± 4 $\mu\text{mol}/\text{min}$ 1 year after GBP surgery. Insulin-mediated suppression of palmitate R_a was 23% greater 1 year after than before weight loss ($P < 0.01$) (Fig. 1C). Taken together, these data demonstrate marked metabolic improvement in subjects after weight reduction by GBP surgery.

Adipose tissue expression and regulation of UPR-related genes. The expression of UPR induced genes in subcutaneous abdominal adipose tissue was evaluated in all 11 subjects before and 1 year after GBP surgery. We first examined the splicing of the *XBP-1* mRNA, a critical transcription factor and indicator of ER stress and adaptive responses. After GBP, there was an average 47.5% reduction ($P = 0.04$) in spliced *XBP-1* mRNA compared with levels in pre-GBP tissues (Fig. 2C). When examined individually, *sXBP-1* levels were decreased in 9 of the 11 subjects (Fig. 2A and B). Next, we examined the expression of *GRP78* mRNA because this chaperone can be targeted by *sXBP-1* and also by ATF-6, the second transcription factor regulating the UPR. As seen in Fig. 3, the levels of *Grp78* mRNA were significantly decreased in

post-GBP tissues, with an average 40.1% reduction in mRNA post-GBP ($P = 0.01$). When examined individually, decreased *Grp78* gene expression was evident in 7 of 11 subjects (Fig. 3A and B), suggesting that its regulation may be greater in variability than *sXBP-1*. In contrast, adipose tissue *CHOP* expression was not significantly different between the samples collected before or after surgery (Fig. 4). Interestingly, in rodent models of obesity, we also did not detect a significant regulation of *CHOP* mRNA expression between lean and obese WAT (M.F.G., G.S.H., unpublished data). Adipose tissue *IL-6* expression was determined to compare the UPR changes to a well-established inflammatory alteration. Earlier studies have shown that *IL-6* expression is significantly and consistently downregulated in adipose tissue after weight loss (17–21). Consistent with these observations, *IL-6* mRNA levels were also significantly reduced in 8 of 11 subjects after GBP (Fig. 4).

Regulation of biochemical markers of ER stress in adipose and liver. The presence of tissue ER stress in obesity is best evaluated by the phosphorylation of PERK, IRE-1, or eIF2 α and stimulation of JNK activity, because these are more proximal measures of UPR activity (7). Because there are no antibodies that can detect the phosphorylated form of PERK or IRE-1 in humans yet, we first measured the phosphorylated form of eIF2 α in adipose tissue before and after GBP. Figure 5 reveals the presence of robust eIF2 α phosphorylation in adipose tissue in all obese subjects. Remarkably, there was a significant reduction in the phosphorylation of adipose tissue eIF2 α after GBP surgery, which was observed in all of the subjects studied ($P < 0.0001$). We also examined the activity of JNK, which can be activated through the IRE-1

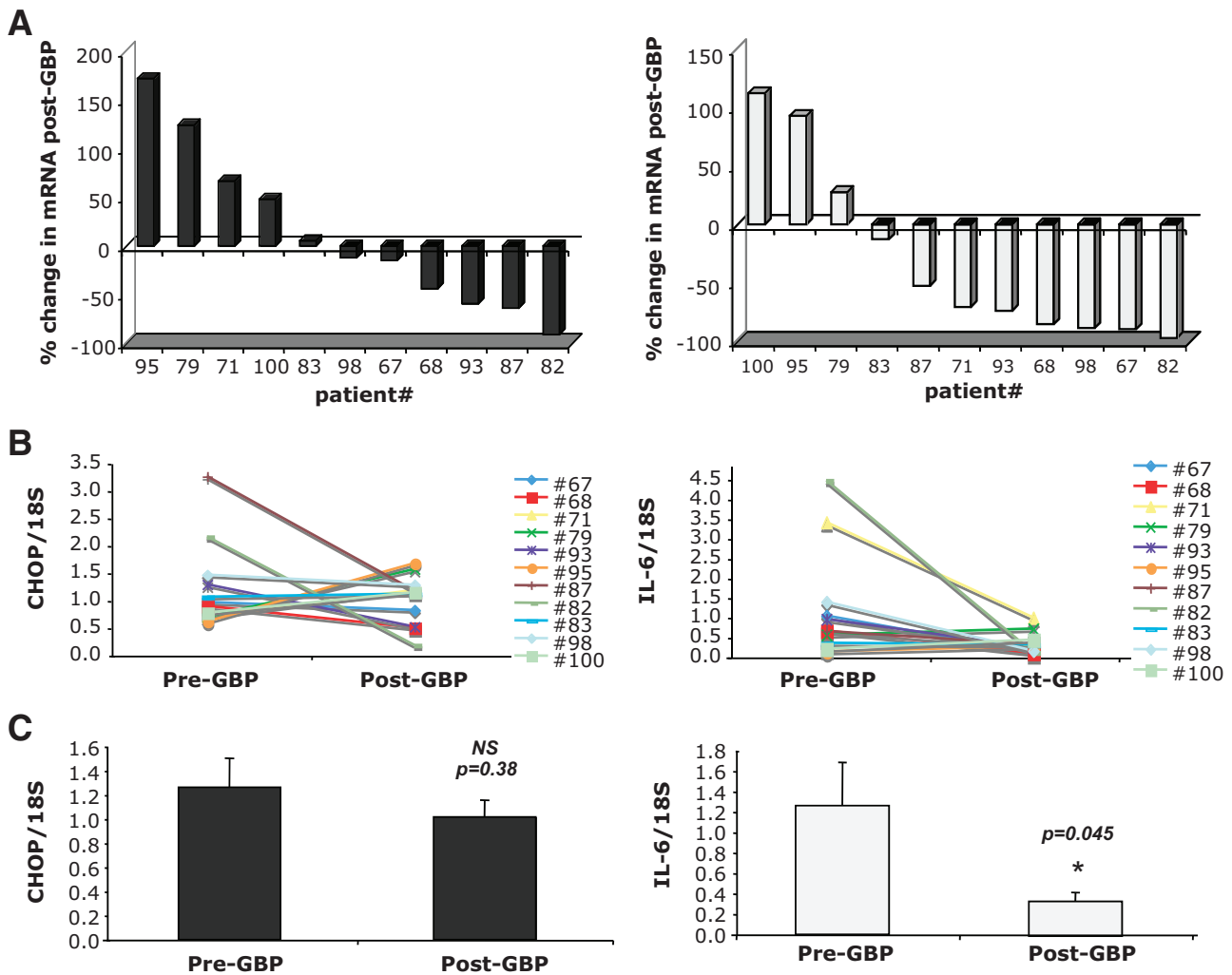


FIG. 4. CHOP (left panel) and IL-6 (right panel) mRNA levels in GBP subjects. Quantitative RT-PCR was performed on RNA isolated from adipose tissue of patients before and after GBP, $n = 11$. **A:** Percent change in CHOP and IL-6 mRNA from pre-GBP levels in each individual patient. **B:** Pre- and post-GBP levels of CHOP and IL-6 mRNA normalized to 18S ribosomal RNA. **C:** Average mRNA levels for CHOP and IL-6 in pre- and post-GBP groups. Data indicate means \pm SE. * $P < 0.05$.

branch of the UPR during ER stress. JNK activation, as determined by phosphorylation of the JNK1 isoform, was detectable in the obese subjects before GBP surgery. As shown in Fig. 5C, JNK phosphorylation also significantly decreased in adipose tissue after GBP surgery. Taken together, these observations indicate alleviation of ER stress in adipose tissue after substantial weight loss.

In a subgroup of four subjects, liver biopsy samples were obtained before and 1 year after GBP surgery. H-E staining of the liver sections demonstrated a 90% decrease in intrahepatic triglyceride content after GBP surgery (Table 1; Fig. 6A). In this limited number of samples, we were also able to examine the effects of weight loss on eIF2 α phosphorylation and *GRP78* protein levels by immunohistochemistry. Immunostaining with an anti-phospho-eIF2 α antibody revealed strong immunoreactivity in liver sections obtained before GBP (Fig. 6). Similarly, immunostaining with an antibody against the ER chaperone *GRP78* revealed strong staining in the obese subjects before GBP. Particularly, areas surrounding the lipid droplets revealed intense staining. In the samples obtained after weight loss, immunoreactivity against both phospho-eIF2 α and *GRP78* was reduced either in general intensity or number of areas. In some cases, the signal was reduced markedly (Fig. 6; supplemental Fig. 1, available in an

online appendix at <http://dx.doi.org/10.2337/db08-1220>), and in others, it was reduced partially and with residual and scattered signal. Although variable among subjects, these results indicate that similar to adipose tissue, obesity in humans may also be associated with ER stress in liver tissue, which is ameliorated upon weight loss.

DISCUSSION

Recent data from studies conducted in obese rodent models have demonstrated that ER stress and the activation of related stress signaling pathways may be an important mechanism underlying insulin resistance and type 2 diabetes (5). The results of the present study demonstrate that in obese humans, ER stress is present in adipose and liver tissues and is highly regulated by weight loss induced by GBP surgery.

GBP surgery is the most commonly performed bariatric surgical procedure in the U.S. and worldwide and is an effective approach for achieving weight loss in obese patients (1). Weight loss induced by GBP surgery improves or completely resolves most obesity-related medical complications and increases survival (22,23). We chose to investigate ER stress in patients who had GBP surgery so that comparisons could be made within the same individ-

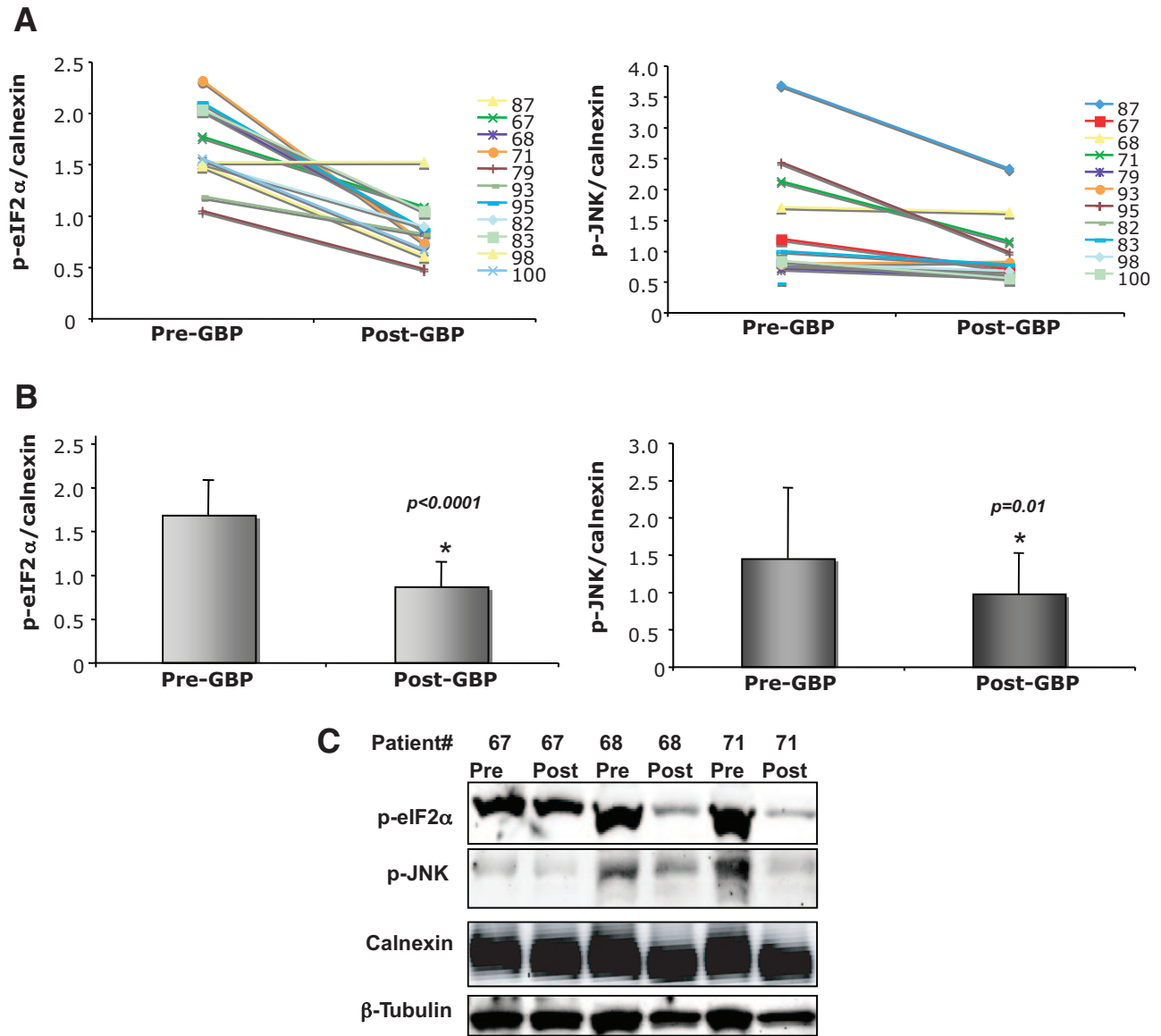


FIG. 5. Adipose tissue *p-eIF2α* and *p-JNK* levels after GBP surgery. Protein expression levels of *p-eIF2α* and *p-JNK* in adipose tissue from patients before and after GBP were detected by Western blot assay, followed by densitometric analysis. **A:** Pre- and post-GBP levels of *p-eIF2α* and *p-JNK* normalized to calnexin. **B:** Average levels of *p-eIF2α* and *p-JNK* in pre- and post-GBP groups. **C:** Immunoblot analyses of *p-eIF2α* and *p-JNK* expression levels in patients 67, 68, and 71 before and after GBP. Data indicate means ± SE. **P* < 0.05. (Please see <http://dx.doi.org/10.2337/db08-1220> for a high-quality digital representation of this figure.)

ual before and after the metabolic improvements of extreme weight loss. This approach has several important strengths. First, each individual served as his/her own control, which removes confounding variables that may not necessarily be accounted for if the control group comprised a different cohort. Second, by measuring change over time within an individual, we are able to capture the state of the ER in flux. This is important given the highly dynamic nature of the ER, which adjusts its adaptive capacity to the condition of the cells and to environmental and intrinsic stress signals, such as alterations in metabolism and body weight. Given the fluctuating environment of the cell and the adaptive responses of the ER, a solitary UPR molecule may not a reliable indicator of ER functional capacity. Therefore, we measured multiple markers of ER stress to assess the different UPR pathways, ranging from proximal biochemical activities of the sensors, to their substrates, to the downstream transcriptional outcomes of target gene expression.

Our data clearly demonstrate a significant regulation of ER stress upon weight loss, as evidenced by the suppression of biochemical indicators of ER stress such as eIF2α and JNK phosphorylation, and downregulation of UPR transcriptional arms via spliced XBP-1 and Grp78. Given a relatively small patient number, we observed remarkably consistent effects on ER stress in post-GBP tissues. It is interesting that despite marked suppression of eIF2α phosphorylation in adipose tissue upon weight loss, there was no change in the levels of *CHOP* mRNA expression, which is generally considered to be downstream of eIF2α phosphorylation. It is possible that *CHOP* is regulated through other signals and does not respond to changes in body weight. In fact, in our recent work in rodent models comparing adipose tissue samples obtained from genetic and dietary models of obesity with lean controls subjects, we did not observe any change in the expression levels of *CHOP* mRNA (unpublished results). Nonetheless, our find-

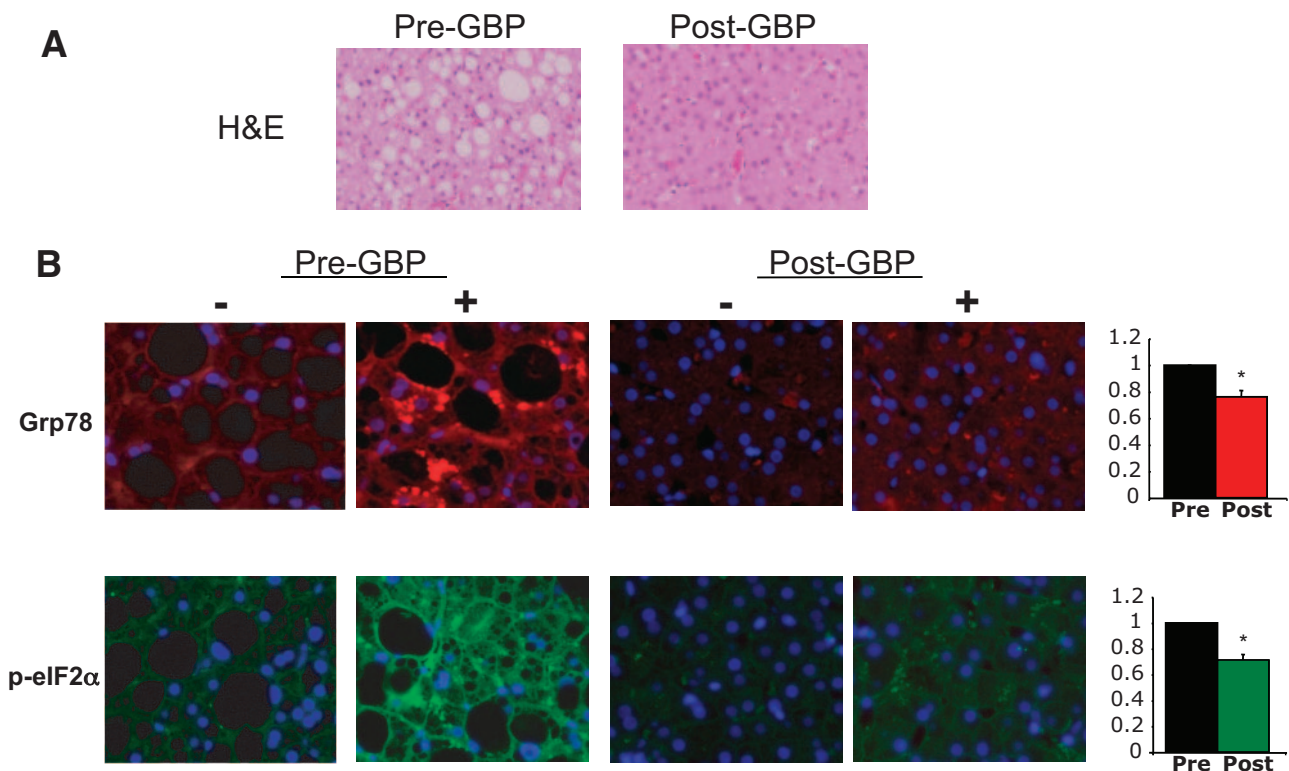


FIG. 6. Liver histology and *p-eIF2 α* and Grp78 immunoreactivity after GBP. Liver tissue sections from patient 67 obtained during and after GBP surgery were stained with H-E or with antibodies against *p-eIF2 α* (green) or Grp78 (red). Nuclei are stained with DAPI (blue). The *top panel* illustrates severe steatosis in the pre-GBP sample and marked reduction after GBP. Staining is particularly dense around the lipid droplets (black in the stained samples). Quantification of staining is presented as relative intensity of fluorescence in pre- and post-GBP sections. **P* < 0.01. (Please see <http://dx.doi.org/10.2337/db08-1220> for a high-quality digital representation of this figure.)

ings suggest that all branches of the UPR are potentially regulated by obesity and weight loss in humans.

The mechanism responsible for weight loss-induced reduction in ER stress in our subjects is not known. Potential factors may include the decrease in adipose and liver triglyceride content or the decrease in systemic plasma insulin and glucose concentrations. Although it is unclear how these factors influence ER stress, data from *in vitro* studies suggest that inflammation, reactive oxygen species, saturated fatty acids, or hypoxia could also be involved. These signals have all been shown to compromise ER folding capacity and/or induce ER stress *in vitro* and have also been described in obese, insulin-resistant tissues (5). Nonetheless, the *in vivo* physiological mechanisms/causes remain unknown.

Two recent studies utilizing lean and obese cohorts have demonstrated regulation of ER stress markers. Boden et al. (24) reported an increase in ER stress proteins and gene expression in subcutaneous adipose tissue in obese individuals compared with lean. A second study measured ER stress markers within a range of BMIs and found a significant positive correlation with increasing BMI (25). These data support the hypothesis that obesity leads to increased ER stress in adipose tissue and complements our findings of the effect of weight loss on tissue stress.

Interestingly, recent studies have also provided evidence that ER stress pathways are activated in pancreatic islets in type 2 diabetes and in atherosclerotic vascular lesions (26,27). Although this is a relatively young field, these observations have raised the possibility that ER dysfunction and the resulting stress responses may be important components of chronic metabolic diseases in a broader sense. The extent to which these pathways will be

relevant to human metabolic diseases, particularly obesity and type 2 diabetes, remains a critical but unanswered question.

In summary, the results from the present study demonstrate that human obesity is associated with insulin resistance and ER stress in both adipose tissue and the liver. Weight loss is associated with decreased ER stress and improved insulin sensitivity, suggesting the possibility that the ER is involved in regulating insulin action in obese humans. These findings, while correlative, are consistent with data from studies conducted in rodent models, which have found that obesity is associated with ER stress and that ameliorating ER stress improves insulin action. Therefore, the ER could be an important new target for treating the metabolic complications of obesity.

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