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Apolipoprotein A-IV, a putative satiety/anti-atherogenic factor, rises after gastric bypass

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Abstract

Roux-en-Y gastric bypass surgery (RYGBP) leads to improvements in satiety and obesity-related co-morbidities. The mechanism(s) underlying these improvements are not known but may be revealed in part by discovery proteomics. Therefore, fasting plasma was collected from twelve subjects (mean BMI > 45) during RYGBP and a second procedure ~17 months later. Body weight, obesity-related co-morbidities and medication use were decreased following RYGBP. Mass spectrometry based proteomic analysis was performed on a subset of seven samples using isobaric isotope coded affinity tags (4 plex iTRAQ). Initial proteomic analysis (n=7) quantified and identified hundreds of plasma proteins. Manual inspection of the data revealed a 2.6 ± 0.5 fold increase in apolipoprotein A-IV (apo A-IV, Gene designation: APOA4), a ~46-kDa glycoprotein synthesized mainly in the bypassed small bowel and liver following RYGBP. The change in apoA-IV was significantly greater than other apolipoproteins. Immunoblot analysis of the full longitudinal sample set (n=12) indicated even higher increases (8.3 ± 0.2 fold) in apo A-IV. Thus iTRAQ may underestimate the changes in protein concentrations compared to Western blotting of apo A-IV. Apo A-IV inhibits gastric emptying and serves as a satiety factor whose synthesis and secretion are increased by the ingestion of dietary fat. It also possesses anti-inflammatory and anti-atherogenic properties. Based on these functions, we speculate changes in apo A-IV may contribute to weight loss as well as the improvements in inflammation and cardiovascular disease after RYGBP. In addition, the findings provide evidence validating the use of iTRAQ proteomics in discovery-based studies of post-RYGBP improvements in obesity-related medical co-morbidities.

Keywords

Gastric Bypass; GI Hormones; Apoproteins; Atherosclerosis; Food Intake

INTRODUCTION

Clinically severe obesity is commonly defined by a body mass index (BMI) greater than or equal to 40. Morbid obesity afflicts approximately 5% of the population and is associated with significant medical co-morbidities including: diabetes mellitus, hypertension, hyperlipidemia,

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and sleep apnea to name a few. Current medical therapies are only modestly effective in treating obesity and lack long-term durability (1). Only bariatric surgery has been demonstrated to be an effective and durable treatment for both the excess weight and medical co-morbidities associated with morbid obesity (e.g., 2,3-6).

RYGBP is the most common bariatric surgical procedure performed in the United States. The RYGBP procedure is associated with both rapid weight loss and improvement in medical co-morbidities (7,8). The commonly proposed mechanisms for weight loss following RYGBP include: 1) restriction of food intake by a small gastric pouch and narrow gastrojejunostomy; 2) a mild degree of malabsorption caused by intestinal bypass; and 3) abdominal discomfort or "dumping" caused by the ingestion of concentrated sweets (2,9,10). Despite the potential significance of these effects, the exact mechanisms of weight loss and improvement in obesity-related co-morbidities following RYGBP remain poorly understood. RYGBP patients frequently describe feeling "hungry all the time" prior to surgery and experience dramatic reductions in appetite immediately following the RYGBP procedure. Changes in how food tastes have also been reported (11). Additionally, certain obesity-related medical problems, like type 2 diabetes mellitus, improve dramatically after RYGBP, but prior to significant weight loss (12).

These observations and recent studies suggesting the gastric bypass surgery alters the secretion of gut-derived hormones have implicated the bypassed foregut as an important mechanism of weight loss and energy metabolism following RYGBP surgery (13,14). The bypassed segments of the distal stomach, duodenum and proximal jejunum constitute active endocrine zones in the gut influenced by changes in food intake. Growing evidence suggests that bypassing the foregut alters the hormonal output of the intestine affecting secretion of gut hormones which regulate appetite, satiety, weight loss, glucose homeostasis, and energy metabolism (15,16). To further test this hypothesis and characterize the biochemical changes associated with gastric bypass surgery we performed a prospective cohort study. Fasting plasma samples were collected from patients undergoing RYGBP at our institution. Some of these patients subsequently required a second surgical procedure to repair an incisional hernia or remove excess abdominal wall skin. At the time of the second procedure we collected additional plasma samples.

Longitudinal plasma samples from seven subjects underwent proteomic analysis using iTRAQ isotope coded affinity tags with a Matrix Assisted Laser Desorption/Ionization Mass Spectrometer (MALDI TOF-TOF). Manual inspection of the data indicated apo A-IV levels were significantly increased in the fasting plasma of the research subjects at the time of the second surgery. Apo A-IV is a gut-derived apolipoprotein secreted by the foregut in response to fat intake. It is posited to contribute to post-prandial satiety (17) and improve obesity-related cardiovascular disease and inflammation (18-22). We confirmed the changes in apo A-IV identified with iTRAQ by immunoblot analysis of the whole sample set. The results demonstrate the feasibility of discovery-based proteomic analysis as a technique for identifying potential mediators of weight loss and improvements in obesity co-morbidities. The potential significance of increased fasting apo A-IV levels as a mechanism of satiety and weight loss following RYGBP is discussed.

MATERIALS AND METHODS

Study population and samples

Patients with morbid obesity who were considering RYGBP at our institution from December 2003 to present were asked to participate in a proteomics study approved by the Penn State College of Medicine Institutional Review Board. Written informed consent was obtained from all patients by the surgeon and/or his IRB approved designees. All patients met the criteria for

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surgical weight loss established by the NIH Consensus Conference in 1991 including: age > 18 years, body mass index (BMI) ≥ 40, medically-complicated obesity, failure of medical weight loss, and evaluation by a multidisciplinary team composed of medical, nutrition, psychiatry, and surgical specialists. Patients < 18 yrs of age or who were felt to be inappropriate for gastric bypass by the multidisciplinary team (e.g., psychiatric illness, excessive medical risk, etc.) or in cases where sample collections were not technically feasible were excluded. Study participants underwent open RYGBP by the same surgeon (RNC). Blood samples were obtained from patients following the induction of anesthesia when a 2nd intravenous catheter was placed. A two ounce divided gastric pouch was created from the intact proximal stomach using an Echelon 60 GIA stapler (Ethicon Corp, Cincinatti, OH). The jejunum was divided approximately 50 cm from the ligament of Trietz. A 100 cm or a 150 cm Roux limb was created and an enteroenterostomy to the biliopancreatic limb was performed. The Roux limb was brought in a retrocolic position and a two-layer hand sewn side to side gastrojejunostomy (12 mm diameter) was performed.

Patients were seen in follow up at 2 weeks, 3 months, 9 months after GBS, then annually or as needed thereafter. Patient weight, medication use, and active medical conditions were recorded in the medical record at follow up visits and stored in a clinical database. At the time of this study, twelve of the 174 patients who underwent RYGBP required a second surgical procedure (incisional hernia repair or panniculectomy). Blood samples were collected in EDTA vacutainers® (Beckman Dickson, Franklin Lakes, NJ) just prior to the first or second operation when the patients were fasting for at least 8 hours. These samples were placed on ice until they were centrifuged at 1500×G for 10 minutes. Plasma was isolated, frozen with liquid nitrogen and then stored at -80°C. Twelve patients had pre- and post-RYGBP plasma samples available for analysis. Standard clinical chemistry values were obtained from the subject's chart by the physician and plasma insulin was measured by RIA () n the Penn State GCRC as approved by the IRB and described in the consent form. HOMA values were calculated to estimate insulin resistance as previously described (23).

i-TRAQ Proteomics

For proteomic analysis, plasma samples were first "decomplexed" to remove the most abundant plasma proteins. 80 μL of plasma was passed over a Multiple Affinity Removal Spin Column (MARS column, Agilent, Santa Clara, CA) using elution buffers and conditions recommended by the manufacturer to remove six highly abundant proteins from the plasma: IgG, IgA, transferrin, albumin, haptoglobin, and antitrypsin. To confirm reproducible and consistent removal of the proteins in the pre and post surgery specimens, 5 μg of the plasma was electrophoresed on a 10% Tris-HCl Criterion gel (Hercules, CA) and silver stained with GE-Amersham's Plus One, protein stain (Piscataway, NJ). Next, 200 μg of post-MARS column protein was denatured and reduced using 2% SDS and 3.5mM tris(2-carboxyethyl)phosphine HCl. About 90% of that protein was then digested overnight with Promega Sequencing Grade trypsin in 0.5mM triethylammonium bicarbonate, pH 8.5. To confirm complete digestion, another 5 μg of protein was electrophoresed on a 10% Tris-HCl Criterion gel and silver stained. Then 100 μg of the trypsin digested peptide sample was dried and reconstituted with 20 μl iTRAQ Dissolution Buffer (0.5 M triethylammonium bicarbonate at pH 8.5 (Applied Biosystems, Foster City, CA). Peptides samples from each time point were labeled with one of four iTRAQ reagents. Each iTRAQ tag is named for a chemically identical reporter component on the molecule next to one of two collision-induced dissociation fragmentation sites on the molecule, and the fragment of the iTRAQ molecule which dissociates has a mass of either 114, 115, 116 or 117AMU. To compensate for the mass differences in this part of the iTRAQ molecule, another part of the molecule is designed with changes in amounts of stable isotopes, such that the overall chemical structure (and overall mass) of each iTRAQ molecule is both identical and isobaric (24). The peptides in individual tubes were labeled with one of

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four iTRAQ reagents and then the further reaction of the reagent was quenched according to the manufacturer's protocol. In a typical experiment, the tryptic peptides from protein collected from two patients, both before and several months after the bariatric surgery, were labeled with a different iTRAQ reagent (e.g., peptides from Pt1 before surgery-114 iTRAQ, Pt1 after surgery-115 iTRAQ, Pt2 before surgery 116-iTRAQ and Pt2 after surgery-117). The contents of the 4 iTRAQ labeled samples, pre-surgery and post-weight loss samples from 2 patients, were combined and separated by multidimensional liquid chromatography.

Multidimensional liquid chromatography was performed to separate the tryptic peptides prior to mass spectrometry analysis. The combined 4 iTRAQ-labeled samples were separated into 15 strong cation exchange fractions using a 4.6 × 250 mm PolySULFOETHYL Aspartamide Strong Cation exchange (SCX) column (PolyLC, Columbia, MD) with an ammonium formate gradient in 20% acetonitrile. The resulting SCX fractions were dried and resuspended in ultrapure water three times to remove all acetonitrile and ammonium formate. The peptides in these fractions were further separated on a LC-Tempo nanoflow and MALDI spotting system, using a Chromolith CapRod column C18 column (150 × 0.1 mm, Merck). Each of these chromatography runs yielded ~370 MALDI spots on a stainless steel MALDI target plate. Thirteen calibration spots were also added to each of the 15 resulting target plates.

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Matrix-assisted laser desorption/ionization tandem mass spectroscopy (MALDI TOF/TOF) was then used to identify peptides in each spot and to provide relative quantification of the 114, 115, 116 and 117 iTRAQ ions. Plate alignment, updated plate calibration and MS/MS default calibration for each plate was performed as each plate was inserted into an Applied Biosystems 4800 MALDI TOF TOF mass spectrometer. After these calibrations, MS spectra from 400 laser shots were acquired for each spot, then a data-dependent MS/MS spectra was acquired from the largest MS peak representing each of the unique peptide peaks observed across the 370 target spots across the entire plate. Combination of the MS/MS data from all 15 SCX fractions was used for a Paragon algorithm search (Protein Pilot 2.0 software, Applied Biosystems/MDS Sciex) against concatenated normal plus Decoy (randomized) databases (SwissProt, NCBI, etc.) constructed in our Mass Spec/Proteomics Core Facility. This search of concatenated normal and randomized database allowed estimation of the False Discovery or False Positive rate. We set the score limits for "positive Ids" to only accept IDs which had an aggregate False Discovery rate estimation of 5% or lower, as calculated by counting the accumulated number of protein IDs from the Decoy database (i.e., false hits) at any threshold score, and calculated the estimated false discovery rate (FDR) as (2 * number of decoy IDs)/ total IDs at that threshold score.

Immunoblot Analysis

100 μg of total plasma protein was separated on a 7.5% Tris-HCl Gel and transferred to PVDF membrane. The 46kDa apo A-IV protein was detected using an affinity purified polyclonal goat-anti-apo-A4 (Santa Cruz Biotechnology, Santa Cruz, CA) and enhanced chemiluminescence (ECL, GE-Amersham Piscataway, NJ) according to the manufacturer's protocol (Santa Cruz). Band intensity was quantified using NIH image and data were reported as relative intensity units.

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Statistical Analysis

Medical co-morbidities are presented as reported incidence amongst the cohort at each time point. Medications are reported as average number of medications the patients were taking for each listed indication at the time of each surgery.

Estimated changes in the relative amounts of each identified protein (pre- vs. post-operative amounts from the same patient) were calculated from changes using the mean of all iTRAQ

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reagent ratios. Each of the peptides identified from a specific protein is presented as a ratio, with a ratio of 1.0 representing "no significant change" for any particular peptide or protein between pre-operative and post-operative amounts. Each protein identified as "Changed" between pre- and post-operative conditions passed through four statistical filters: first, it had to have a paragon algorithm (Protein Pilot) confidence interval score of 95% or better ("Unused Score" of 1.3 or higher); second, it had to pass through the ProFound algorithm which identifies the minimum set of proteins that will account for all of the observed peptide data; third, it had to have an estimated False Discovery Rate of 5% or lower (based on the number of IDs selected from the Decoy (randomized) database at an identical or better ProteinPilot score); and fourth, the quantitative difference (the average ratio of the iTRAQ signals of all of the peptides coming from the particular protein) had to be significantly different from 1.0 as determined by the ProFound algorithm in ProteinPilot software. This final statistical test transforms the iTRAQ ratios into log(iTRAQ ratio) so that the resulting averages will correctly pass the inversion test, i.e., so that the result of averaging N iTRAQ 115:114 ratios will give the exact inverse of averaging the same data in its 114:115 ratio form, and thus tests the null hypothesis that the observed average ratio is not different than a ratio of 1.0, which in the case of ratio calculations represents no change. This is done by calculation of a p-value based on the log(average iTRAQ ratio) and the standard deviation of all of the individual peptide iTRAQ ratios contributing to that average iTRAQ ratio.

Ordinal data such as relative apo A-IV concentration, body weight, and BMI are reported as mean ± standard error of the mean (SE). Analysis of Western blot data (mean ± SE) was performed using paired *t*-test. Statistical significance was set at $P < 0.05$.

RESULTS

Research subject recruitment began in January 2003. Demographics of the complete longitudinal data set are shown in Table I. Eleven of the subjects in the present study were causasian (91%) and one was African American (9%). Notably 66% of the subjects were female (Table 1). At the time of the current communication, 174 subjects tissue and blood samples were entered into our tissue bank and 12 had second surgeries with plasma available for study. These subjects constituted 6% of the total subjects recruited. Subjects returned as needed for second surgical procedures with a mean time of approximately 19 months (Table 1). The demographics and rates of comorbid diseases of the subjects presented in this report, including diabetes, are comparable to those of the subjects in the entire tissue bank. Therefore, the subjects presented here can be assumed to be representative sample.

All subjects in this report had an open RYGBP for the first surgery and an elective incisional hernia repair for the second procedure. As seen in Table 1 the subjects lost an average of ~60 kg by the time of the second surgical procedure and the mean BMI had decreased from 55 to 34. Mean plasma glucose, plasma insulin and HOMA trended post post-RYGBP, but were not significantly decreased. This may be due to the relatively small sample size in the current study as reductions were seen in all patients and are consistent with reported findings from other groups (e.g., 3,5,6,8). Several subjects were being medically treated for their diabetes at the time of the second procedure, making these data difficult to interpret. In agreement with previous studies, there was a substantial reduction in the rates of diabetes. As seen in figure 1 the rate of diabetes decreased from 54.5% to 18% and the average number of diabetic medications decreased from 0.64 per patient to 0.36 (Fig 1). Substantial improvements were seen in hypertension, hyperlipidemia, sleep apnea, degenerative joint disease, venous stasis, respiratory disease, and gastroesophageal reflux disease (Fig 1). Post-RYGBP improvements in medical co-morbidities are reflected as reductions in medication use (Fig 1). Collectively these data are consistent with other reports of RYGBP as an effective treatment for weight loss and obesity-related medical comorbidities (4,5).

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Mass spectrometry based proteomic analysis was performed on a subset of samples (n=7) using isobaric isotope coded affinity tags (4 plex iTRAQ), followed by 2-D liquid chromatography (SCX LC/C18 nano-LC), matrix-assisted laser desorption/ionization mass spectroscopy (MALDI TOF/TOF) of the fractions for identification and quantification of the reporter iTRAQ ions. Pilot studies indicated more proteins were quantifiable when all four iTRAQ ions were present. This is felt to be due in part to the increase in the concentrations of each of the peptides available. Under these conditions the iTRAQ assay identified hundreds of plasma proteins when processed as described in Methods. Interestingly much higher identification numbers have been possible when tissue samples have been analyzed in our laboratory. The reasons for the lower number with plasma samples are unclear at this time, but may reflect the continued presence in plasma of a small number of highly abundant proteins which are not removed by the MARS column procedure. Because of this, after seven iTRAQ analyses were completed, sample processing was halted to evaluate recently released multiple affinity removal systems and a newer iTRAQ reagent with eight reporter ions (8-plex).

Manual inspection of the initial data sets revealed consistent changes in the relative expression of apo A-IV when samples pre- and post-RYGBP patients were compared. These changes were 2-3 fold on average based on the peak heights of the reporter ions (Fig 2). Each patient served as their own control in these comparisons. The increase in the apo A-IV in follow up measurements was greater than apparent changes in other apolipoproteins detected with this technique. Notably, no changes were observed in apolipoprotein A, apolipoprotein A2, apolipoprotein B, apolipoproteins C1-4, apolipoprotein-E, apolipoprotein H, or apolipoprotein M. Small, but significant increases in apolipoprotein A1 and D were observed, as well as reductions in apolipoprotein D (Fig. 2).

We chose to confirm and verify the change in apo A-IV by Western blot analysis in all of our longitudinal plasma samples. In this case, plasma samples were not processed over the MARS column. As seen in Fig 3, Western blotting with a commercial apoA-IV antibody detected a 46kDa protein that was significantly increased after RYGBP. Compared to pre-RYGBP samples, apoA-IV was increased 15 fold on average in different subjects after RYGBP. All subjects started with relatively low fasting apo A-IV level prior to RYGBP and had a marked increase n fasting apo A-IV levels after their surgical weight loss. The magnitude of observed change seen in the Western Blots is thus far greater than that seen in the iTRAQ assay. Thus at least for apoA-IV, iTRAQ seems to underestimate the changes in protein compared to Western blotting.

DISCUSSION

In this study we used the iTRAQ proteomic assay to analyze paired fasting plasma samples from human subjects prior to and after RYGBP surgery. All of the subjects in the present study had improvements in body weight, BMI, glucose homeostasis and medical co-morbidities comparable to those described elsewhere, making data from this cohort generalizable (2,3,6, 8,25,26). Additionally, the subjects presented in this study were similar in comorbidites and outcome to the rest of the entire cohort making it unlike the changes in apo A-IV observed were the result of a selection bias. Analysis of proteomic data revealed rises in apo A-IV, a protein with anti-inflammatory and anti-atherogenic properties that has also been identified as a satiety factor. The increase in apo A-IV was higher on average than other apolipoproteins in our proteomic analysis. Increases in apo A-IV were confirmed by immunoblot analysis, with larger magnitude changes demonstrated using the Western blot technique. Interestingly, the magnitude of change in apo A-IV levels was not predictive of the resolution of diabetes. However given the limited sample size and the fact that insulin resistance improved in all subject, even those whose diabetes did not resolve, it is difficult to determine a true correlation.

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Of note, mortality is decreased by 40% in obese subjects after RYGBP, especially cardiovascular-related deaths (2). Post-operative changes in apolipoproteins resulting in a less atherogenic phenotype may contribute to these improvements in cardiovascular disease. Indeed, several changes in apolipoproteins were observed after RGYP in our study. For example, the iTRAQ proteomic studies indicated increases in the plasma Apo A1/Apo A2 ratio after RYGBP. Increased apo A1/A2 ratios following RYGBP were previously described using other analytical methods (25,27) and thus provide additional proof of concept that the iTRAQ technique accurately identifies relative changes in protein abundance. Increases in the A1/A2 ratio are posited to reflect rises in plasma high-density apolipoprotein fraction 2 (HDL2) that contribute to improvements in the atherogenic profile of patients following RYGBP (25,27).

ApoD was also significantly increased. This lipocalin family protein is widely expressed at a higher levels in omental as opposed to subcutaneous fat (28). While its physiological role has not been definitively determined, apo D appears to be involved in cellular transport of small hydrophobic ligands, such as progesterone, cholesterol, bilirubin and free fatty acids (29,30). Notably, APOD polymorphisms are associated with obesity and hyperinsulinemia (31), factors that improve after RYGBP as APOD became elevated.

In contrast to other apolipoproteins, ApoL3 concentrations were significantly reduced (32). This protein is structurally related to BCL-2 and has been posited to be involved in apoptosis or necrosis. The protein is found in the plasma and cytoplasm; it may affect the movement of lipids or lipophilic ligands to organelles, or form an anionic channel. The gene is expressed in endothelial cells of most organs and is most highly expressed in immune cells where it is strongly induced by TNF and interferon (33). Since ApoL3 appears to mediate inflammatory responses, its observed decline might also be involved in the reduction of systematic inflammatory responses that occur after RYGBP.

The apolipoprotein most affected by gastric bypass in our study however was apo A-IV. This circulating ~46kDa apolipoprotein was first described in the 1970s and since the 1990s various groups have put forth data demonstrating it to be a satiety factor whose release s stimulated by consumption of dietary fat (reviewed in 17,34). Thus our observation that apo A-IV levels are increased after RYGBP seems quite logical as these patients are known to have an augmented sensation of satiety. Surprisingly, apo A-IV is most highly expressed in the duodenum and the first part of the small intestine, the parts of the gastrointestinal (GI) tract bypassed by the RYGBP procedure (35). However, intestinal secretion of apo A-IV is also stimulated by an ileal factor, probably peptide tyrosine-tyrosine (PYY), which is increased following RYGBP (16,36-38). The results of our study are unable to ascertain whether the observed increases in apo A-IV arise from changes in gut or hepatic secretion after RYGBP surgery. While liver apo A-IV expression is moderate compared to duodenal cells, the mass of the liver far greater and it is not known which tissue contributes most to the plasma concentration of the protein.

The evidence that apo A-IV is a GI peptide regulating satiety includes the observation that ingested fat produces a rapid induction and secretion of apo A-IV. The secreted protein is initially associated with chylomicrons in the lacteal, but then a significant fraction disassociates from these particles in the plasma (nevertheless some still circulates in association with HDL, for review see 38). Rapid rises in apo A-IV after fat ingestion and the disassociation from the lipid particles are consistent with an involvement in the short-term regulation of satiety. Apo A-IV protein has been detected in the fenestrated part of the hypothalamus and the arcuate nucleus, a region of the brain which responds to circulating factors regulating energy expenditure and food intake. Cortical injection of apo A-IV and its antiserum demonstrate a direct role of apo A-IV in satiety (17).

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Interestingly, apo A-IV is frequently elevated in rodent models of obesity and obese humans (39-41) and polymorphisms are associated with elevated BMI (42). However the apo A-IV system in the central nervous system (CNS) appears to be down regulated by high fat feeding. Therefore, apo A-IV resistance could explain this observation and may represent a significant factor as is known to occur with leptin in obesity, which also regulates apo A-IV secretion (43). It has been demonstrated that intravenous injection of apo A-IV into rats causes a reduction in food intake (44). In another study apo A-IV potentiated cholecystokinin-mediated regulation of appetite, although intravenous injection of apo A-IV alone had no effect on food intake (45). Disappointingly, significant changes in satiety or body weight were observed in apo A-IV knock out mice out to 5 months (46,47). This observation is potentially explained by the complex, multifactorial regulation of food intake.

Apo A-IV has been postulated to have other roles, however, such as reducing the host susceptibility to atherogenesis by modulating cholesterol metabolism and transport. In support of this idea, both apo AIV and apo AI were able to promote so called reverse cholesterol transport in vitro (48) and this has been validated in several relevant cell types and transgenic mice over-expressing human apo-AIV(for review see ref 49). In this context, apo A-IV is found at 3-7 fold higher concentrations in the interstitial fluid relative to canine or rodent plasma (49). In peripheral lymph, apo A-IV and apo A-I (both increased in plasma after RYGB) are found in unnamed discoidal-shaped cholesterol/phospholipid/lipoprotein particles that are increased by dietary cholesterol (50). Reverse cholesterol efflux is thought to be anti-atherogenic. Thus Apo A-IV KO mouse had lower plasma HDL, frequently called “good” cholesterol. Transgenic expression of human Apo A-IV was found to strongly reduce atherosclerotic lesions in mice (for review see ref 49). Similarly a human polymorphism of apo A-IV was associated with increased progression of arterial calcification in type 1 diabetic patients (51). Chronic administration of human apo A-IV to mice susceptible to atherosclerosis caused diminished lesions and decreased the secretion of proinflammatory cytokines in response to LPS (21). These anti-inflammatory, antiatherogenic effects are consistent with the beneficial changes observed following RYGBP and the rises in apo A-IV we observed in this population.

In conclusion, we report increased plasma apo-AIV concentrations in plasma of subjects following RYGBP for morbid obesity. Because of its putative role as a satiety factor, anti-atherogenic apolipoprotein or both, it is reasonable to suspect it plays a positive role in the improved health benefits after RYGBP. This study adds another name to the list of GI hormones whose plasma concentrations rise after RYGBP, including PYY and GLP-1 (16). Further studies are needed to examine the time course of changes in apo A-IV after surgery. Notably changes in insulin sensitivity and GI peptides following RYGBP typically occur within days to weeks. Whether elevations in apo A-IV also occur after gastric banding and sleeve gastrectomy or only after bariatric procedures which bypass the foregut like biliopancreatic diversion and RYGBP is unclear at this time. Additional studies will be required to address these and other important questions regarding the mechanisms of improved health following bariatric surgical procedures.

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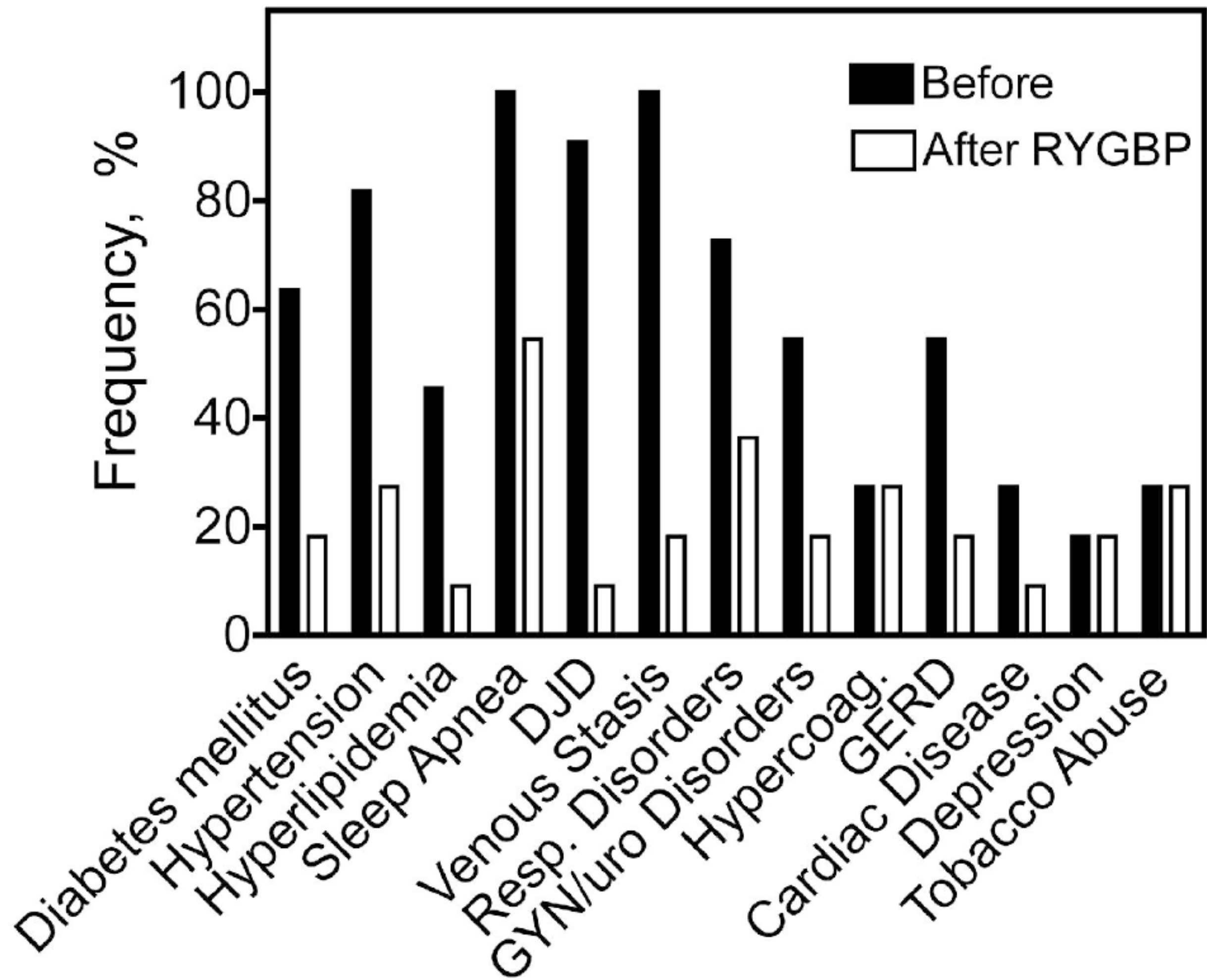

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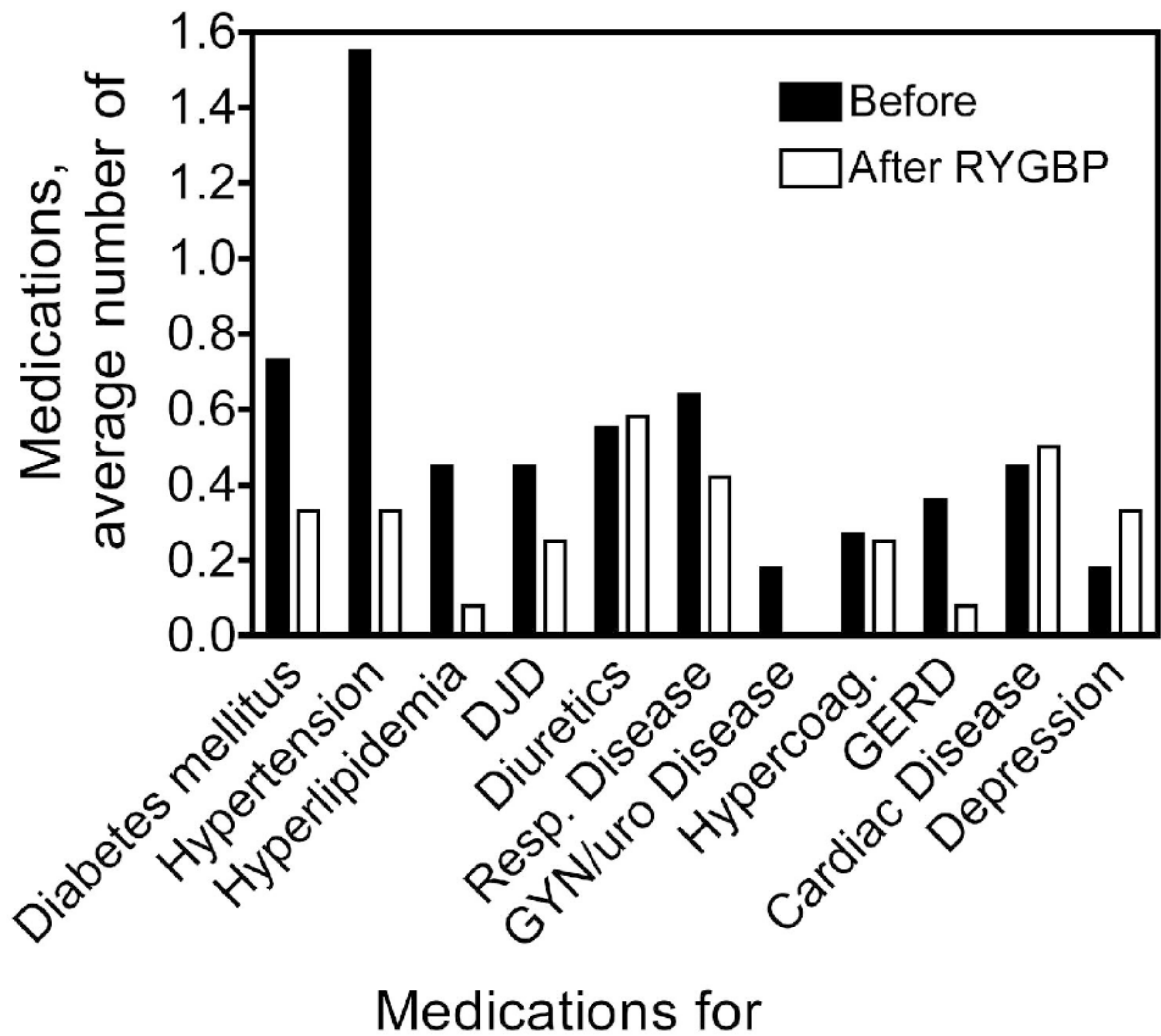

Fig 1.
Obesity Co-morbidities and medications used by our subjects before and after bariatric surgery

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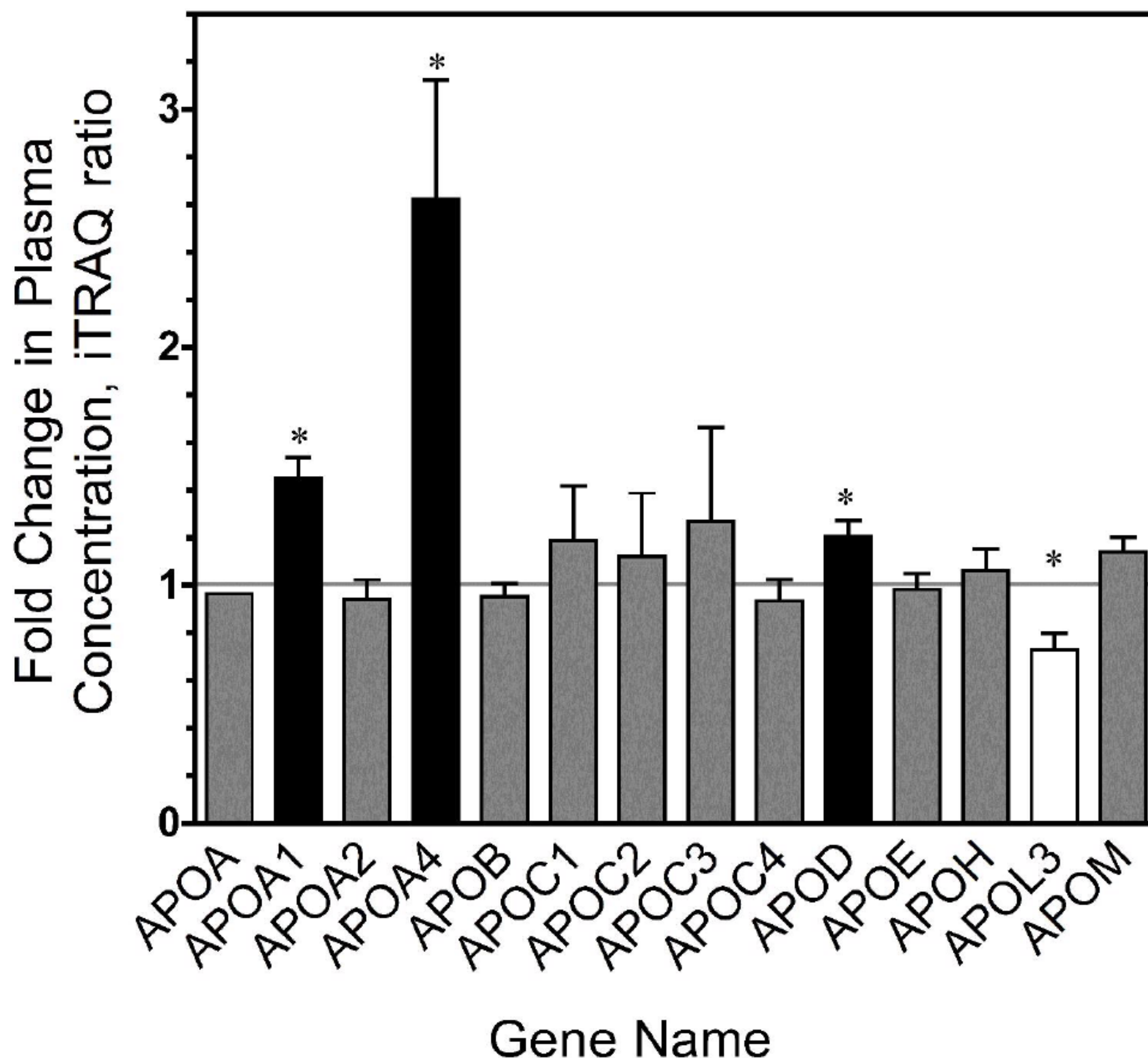

Fig 2. Relative changes in plasma apolipoprotein concentrations of iTRAQ labeled peptides

Plasma from subjects before and ~19 months after GBS was depleted of major proteins by affinity chromatography and digested with trypsin. The resulting peptides were labeled with one of four iTRAQ reagents. The relative concentration of the "before" and "after" peptides and the identification of the peptides was determined after two dimensional liquid chromatography and automated mass spectrometry of the fractions as described in the text. The graph shows apolipoproteins detected in seven of the twelve subject samples analyzed by proteomics as described in Methods. A ratio of one indicates no change after bariatric surgery while a number greater than 1 in the iTRAQ ratio indicates a rise in the plasma concentration and a value less than one indicates a reduction in concentration. Below each bar is the gene name for the apolipoprotein. An asterisk indicates a significant difference in the ratios averaged from the seven subjects.

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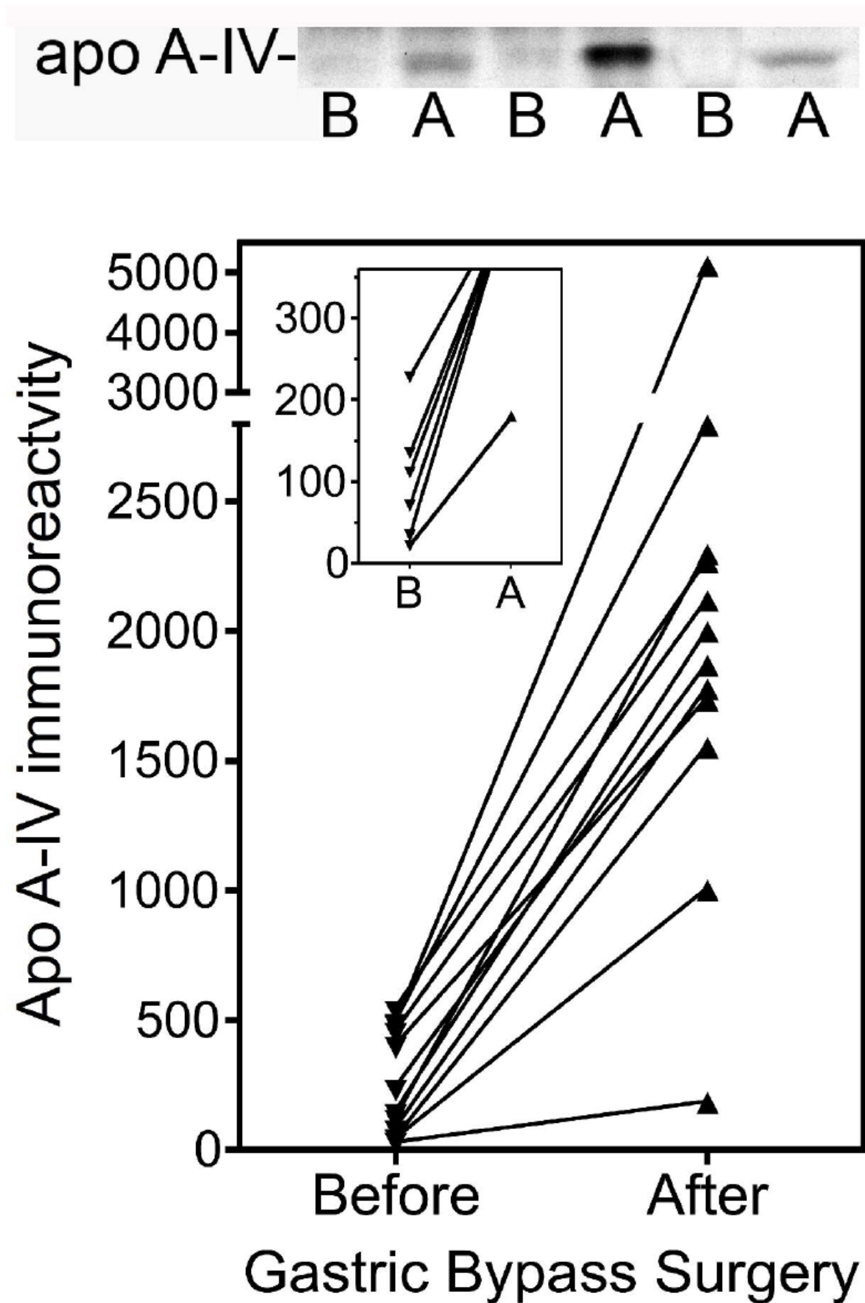

Fig 3. Relative changes in plasma apo A-IV concentration after RYGBP

The relative changes in plasma apo A-IV (46 kDA) immunoreactivity was determined by Western blotting. The top panel shows the representative results for three subject plasma samples before (A) and after (A) GBS. The bottom panel shows the changes in each of the individual patients by densitometry. Changes in apo A-IV were found to be significantly different after surgery based on a Student's paired t-test ($p<0.05$).

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Table I

Human Subject Demographics

Subject Parameter	First Surgery "Before"	Second Surgery "After"
Gender (M:F)	(4:8)	(4:8)
Age at Surgery (years)	48.1 ± 2.6	49.7 ± 2.7
Weight (kg)	163 ± 9.6	103.3 ± 6.7
Weight Loss (kg)		59.6 ± 5.0
BMI (kg/m^2)	55.1 ± 3.6	34.55 ± 2.3
Roux Length 150cm/100cm	6/6	6/6
Time Between Surgery (Months)		19.2 ±2.51
Glucose (mg/dl)	140 ± 19	119 ±19
Insulin ng/ml	18.6 ± 6.3	12.2 ± 7.2
HOMA	7.8 ± 4.3	3.5 ± 1.8