Serum biomarkers of inflammation and adiposity in the LABS cohort: associations with metabolic disease and surgical outcomes

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Abstract

Background—The utility of serum biomarkers related to inflammation and adiposity as predictors of metabolic disease prevalence and outcomes after bariatric surgery are not well-defined.

Methods—Associations between pre- and post-operative serum levels of four biomarkers (C-reactive protein (CRP), cystatin C (CC), leptin, ghrelin) with baseline measures of adiposity and metabolic disease prevalence (asthma, diabetes, sleep apnea), and weight loss and metabolic disease remission after bariatric surgery were studied in the Longitudinal Assessment of Bariatric Surgery (LABS) cohort.

Results—Baseline CRP levels were positively associated with the odds of asthma but not diabetes or sleep apnea; baseline CC levels were positively associated with asthma, diabetes, and

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CONFLICTS OF INTEREST

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sleep apnea; baseline leptin levels were positively associated with asthma and negatively associated with diabetes and sleep apnea; baseline ghrelin levels were negatively associated with diabetes and sleep apnea. Increased weight loss was associated with increased baseline levels of leptin and CRP and decreased baseline levels of CC. Remission of diabetes and asthma was not associated with baseline levels of any biomarker. A higher likelihood of asthma remission was associated with a greater decrease in leptin levels, and a higher likelihood of diabetes remission was predicted by a lesser decrease in CC. Bariatric surgery was associated with decreased post-operative CC, CRP, and leptin levels, and increased post-operative ghrelin levels.

Conclusion—This is the largest study to date of serum biomarkers of inflammation and adiposity in a bariatric surgery cohort. Biomarker levels correlate with metabolic disease prevalence prior to bariatric surgery, and with weight loss but not metabolic disease remission after surgery. Bariatric surgery regulates serum biomarker levels in a manner consistent with anti-inflammatory and compensatory orexigenic effects. These data contribute to our understanding of the mechanisms underlying the biologic effects of bariatric surgery.

Keywords

LABS; leptin; ghrelin; cystatin-C; C-reactive peptide; obesity; bariatric surgery

INTRODUCTION

Serum biomarkers related to obesity and metabolic disease have been shown to predict disease and mortality in several studies.[1,2] In addition to potential diagnostic utility, an understanding of the association of biomarkers with obesity and outcomes after bariatric surgery will enhance an understanding of mechanisms underlying metabolic disease. The majority of studiesof serum biomarkers in surgical cohorts are small, consisting of <100 subjects. In the Longitudinal Assessment of Bariatric Surgery (LABS), bariatric surgery patients underwent preoperative and postoperative measurements of four serum biomarkers: C-reactive protein (CRP), an acute phase response protein and inflammatory marker;[1] cystatin C (CC), a cysteine protease inhibitor associated with renal function and inflammation;[3,4] and leptin and ghrelin, satiety and hunger factors with broad immunoregulatory and metabolic functions.[5]

The goal of this study was to investigate associations of serum biomarker levels with adiposity, baseline metabolic disease prevalence, and clinical outcomes in the LABS cohort. We hypothesized that baseline levels of CRP, CC, and leptin would associate positively, and ghrelin level would associate negatively with adiposity and metabolic disease prevalence, and with surgery-induced weight loss and metabolic disease remission.

SUBJECTS, METHODS

LABS, and its sub-study LABS-2, is a multicenter, observational cohort designed to assess safety and efficacy of bariatric surgery. Institutional Review Boards at each center approved protocols and informed consent. LABS-2 enrolled and completed baseline studies in 2,458 participants >=18 years old seeking a first bariatric operation between 2006-2009 among 10 geographically diverse centers.

Fasting serum levels of four biomarkers (CRP, CC, leptin, ghrelin) were available at baseline (pre-surgery) for 2,014, 2,355, 1,985, and 1,985 participants. Blood samples were collected at individual sites, transported to a central laboratory and assayed in batch. CRP and CC were measured using a Siemens Dade Behring BN II Nephelometer (Siemens Inc., Munich, Germany). Leptin and ghrelin (total) levels were determined using radioimmunoassay kits (EMD Millipore Inc., St Charles, MO, USA). Postoperative ghrelin levels were assessed only at years 1 and 2 due to resource limitations.

Five measures of adiposity were studied: fat mass (FM, kg), percent body fat (%BF), weight (lbs), body mass index (BMI, kg/m^2), and waist circumference (WC, cm). Weight, weight loss, and BMI were measured as reported.[6] %BF was measured using a Tanita-TBF bioelectrical impedance scale (Tanita Inc., Arlington Heights, IL, USA).[7]

Baseline prevalence of type 2 diabetes (DM), obstructive sleep apnea (OSA), and asthma were studied. DM was defined as HbA1c ≥ 6.5% (or if HbA1c not available, fasting blood glucose (FBG) ≥ 126mg/dL) or diabetes medication use, excepting subjects on metformin but no other diabetes medications who did not self-report diabetes, with HbA1c<6.5% (or FBG<126mg/dL) and a diagnosis of polycystic ovarian syndrome. OSA was defined by apnea-hypopnea index ≥ 5 from diagnostic polysomnogram within the prior year, or if polysomnogram was absent, via the validated Berlin sleep assessment questionnaire.[8] Baseline asthma was self-reported. Postoperative remission of DM and asthma were studied; OSA remission was not studied as LABS collected these data based on patient self-reporting, which has been shown to be inaccurate for OSA.[9,10] DM remission was defined as no hypoglycemic medication use and HbA1c<6.5% or FBG<126mg/dl. Asthma remission was self-reported, demonstrated to be accurate for asthma.[11]

Operations included Roux-en-Y gastric bypass (RYGB), laparoscopic adjustable gastric band (LAGB), sleeve gastrectomy, and biliopancreatic diversion/duodenal switch. We focused on RYBG and LABG categories and combined sleeve gastrectomy and biliopancreatic diversion/duodenal switch into an "other procedures" category, as there were too few of these procedures to draw meaningful inferences. Twenty LAGB subjects (0.8% of entire study population, 3.3% of LAGB population) underwent conversion to RYGB (n=17) or sleeve gastrectomy (n=3) within the 3-year study period, 1 at 1.1 year, 9 between 2-2.4 years, and 10 between 2.5-2.9 years. These subjects were excluded from analyses comparing changes in serum biomarker levels between RYGB and LAGB (Results, section 4). Distributions of serum biomarker levels were skewed, prompting use of log-transformation for statistical analysis. Associations of biomarker levels with baseline measures of adiposity were estimated using least squares regression of adiposity measures (dependent variable) on biomarker levels, and inference performed using F- or t-tests as appropriate. Associations of baseline demographic and clinical predictors including serum biomarkers with baseline metabolic disease prevalence were analyzed using logistic regression (presence/absence of disease as outcome), and inference performed using Chi-squared and Z-tests. Associations between weight loss (predictor) and longitudinal changes in log serum biomarker levels (outcome) were estimated using generalized linear mixed effect models under normal likelihood having an autoregressive covariance pattern, and inference performed using F- or t-tests. Associations were additionally adjusted for baseline biomarker levels and baseline

weight. Models with cystatin C were adjusted for baseline and post-surgery serum creatinine levels. Missing outcomes were assumed missing at random with an ignorable missing data mechanism, under which the inference remains unbiased.

Disease remission was investigated using Cox proportional hazards models, with monotonic missing patterns treated as censored (non-informative), intermittent missing patterns imputed with last observation carried forward, and inference performed using Chi-squared tests. When investigating associations between post-surgery biomarkers or weight change and metabolic disease remission, biomarkers or weight over time were used as time-dependent covariates in the Cox model. Confidence intervals were constructed using the Wald method. In all models, analysis of ghrelin levels over time (both as covariate or as outcome) was limited to 24 months. When 36-month change in weight loss or biomarkers other than ghrelin levels were studied, ghrelin levels were excluded from covariate adjustment. All tests were two-sided; statistical significance was defined as $p < 0.050$. Statistical analyses and graphics were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

1. Participants, baseline biomarker distributions, surgery outcomes

Participants were mostly Caucasian (86.2%), female (78.6%), non-Hispanic (95.2%), married (63.8%), and employed (68.7%), with less than college-level education (63.6%).[12] Median age was 46 years; median BMI was 45.7kg/m^2 for women, 46.9kg/m^2 for men. A total of 2,458 subjects were studied: 1,770 subjects underwent RYGB (199 open, 1571 laparoscopic), 610 underwent LAGB, and 78 underwent "other procedures" (59 sleeve gastrectomy, 19 biliopancreatic diversion/duodenal switch). Median and interquartile range (IQR) serum levels of CRP, CC, leptin, and ghrelin were respectively: 0.7 (0.4:1.3)mg/L, 0.9 (0.8:1.0)mg/L, 56.8 (42.6:73.6)ng/ml, and 714.7 (602.1:873.5)pg/ml (Supplemental Figure 1).

Mean weight loss at 12, 24, and 36 months after surgery was 39kg (95%CI:38-40), 40(95% CI:39-40), and 37kg (95%CI:36-38kg), respectively. RYGBP pouch size and limb length data were collected and did not differ significantly among centers or correlate with weight loss.[13] At 12, 24, and 36 months after surgery, weight loss was 10.4kg (95%CI:9.4, 11.5) more for with RYGB than LAGB ($p < 0.001$), adjusting for baseline weight, and baseline and postoperative serum creatinine, and biomarker levels. For asthma there were 284 subjects and 195 remissions. For DM there were 352 subjects and 239 remissions. Remission rates in all patients at 36-months for DM and asthma were 62% and 69% respectively. Independent of weight loss, RYGB was associated with a 2.7-fold higher likelihood of DM remission than LAGB when adjusting for baseline weight, follow-up weight loss, and baseline and follow-up biomarker levels. The type of surgical procedure was not associated with asthma remission.

2. Baseline biomarker levels are associated with baseline adiposity and metabolic disease

Baseline serum levels of CRP, CC, and leptin were positively, and ghrelin levels were negatively associated with adiposity measures. For example, each half-log increase in CRP levels were associated with 2.0kg increase in fat mass, 0.7% increase in %BF, 2.17kg increase in body weight, and 1.02-unit increase in BMI (p<0.001 for all); a half-log increase in CC was associated with 8.9kg increase in fat mass, 1.5% increase in %BF, 13.5kg increase in body weight, and 4.8-unit increase in BMI (p<0.001). Positive associations with measures of adiposity were also observed for leptin levels. Ghrelin levels were negatively associated with adiposity measures; for each half-log increase in ghrelin level, fat mass, body weight, and BMI were decreased by 5.0kg, 11.2kg, and 2.5kg/m^2 respectively. When adjusted for other biomarker levels (all four biomarkers included as covariates), these associations remained significant. (Table 1A/Supplemental Figure 2A).

Associations between baseline levels of CRP, CC, leptin, and ghrelin and baseline metabolic disease prevalence were studied adjusting for baseline weight; analysis of CC additionally adjusted for baseline creatinine levels. The odds of asthma, DM, and OSA were higher for participants with higher levels of CC. For example, a half-log increase in CC was associated with 1.6-, 1.8-, and 1.4-fold increases in the odds of asthma, DM, and OSA, respectively (p<0.001). CRP had significant positive association with asthma (OR=1.1, 95%CI:1.04-1.17, per half-log CRP), but not with DM or OSA. Leptin showed significant positive association with asthma (OR=1.21, 95%CI:1.08-1.36, per half-log leptin) but a negative association with DM (OR=0.72, 95%CI:0.65-0.80, per half-log leptin) and OSA (OR=0.81, 95%CI: 0.73-0.90, per half-log leptin). Ghrelin levels were negatively associated with DM (OR=0.69, 95%CI:0.58-0.82, per half-log ghrelin) and OSA (OR=0.83, 95%CI:0.71-0.97 per half-log ghrelin). (Table 1B/Supplemental Figure 2B).

3. Surgery-induced weight loss is associated with changes in biomarker levels

To estimate associations between surgery-induced weight loss and changes in serum biomarker levels, we modeled post-surgery changes in serum biomarker levels (as dependent variables) on baseline and post-surgery weight (as independent variables) while adjusting for baseline biomarker levels and surgical procedure. Surgery-induced weight loss was associated with decreased levels of CRP, CC, and leptin, and increased levels of ghrelin (Figure 1, Table 2A). Adjusted for baseline log CRP, surgical procedure, and follow-up weights, mean decrease in CRP was 0.32 log mg/L higher for each 20kg increase in baseline weight, while decrease in CRP levels at follow-up was 0.60 log mg/L higher for each 20kg decrease in weight at follow-up adjusted for baseline weight, baseline CRP, and surgical procedure (p<0.001). Adjusted for baseline log leptin, surgical procedure, and weights at follow-up time-points, mean decrease in follow-up leptin was 0.55 log ng/ml higher for each 20kg increase in baseline weight, and 0.83 log ng/ml greater for each 20kg decrease in weight at follow-up (p<0.001). Mean increase in ghrelin was 0.08 log pg/ml higher for each 20kg increase in baseline weight adjusted for baseline log leptin, surgical procedure, and follow-up weights, and 0.15 log pg/ml higher for each 20kg decrease in weight at follow-up adjusting for baseline log ghrelin, surgical procedure, and baseline weight (p<0.001). The relationship between baseline and follow-up weight with mean decrease in log CC was mixed. At the 12-month visit, lower baseline weight was associated with decreasing log CC,

while at 24- and 36-months, higher baseline weight was associated with decreasing log CC. Conversely, at the 12-month visit, higher follow-up weight was associated with lower log CC, while at 24- and 36-month visits lower follow-up weight was associated with lower log CC (Figure 1, Table 2A). These findings demonstrate that higher baseline weight and greater surgery-induced weight loss are associated with greater decreases in CRP, CC, and leptin levels, and greater increase in ghrelin levels.

4. Changes in biomarker levels are procedure-specific

To determine if changes in serum biomarker levels differed among surgical procedures independent of weight loss, changes in biomarker levels (outcome) at month 36 (month 24 for ghrelin) were modeled as a function of surgical procedure, baseline biomarker level, serum creatinine (for CC analysis only), and baseline and follow-up weights. Adjusting for baseline weight as a covariate permitted controlling for differences in starting weight. RYGB resulted in 0.63mg/L greater declines in log CRP than LAGB (p<0.010). Mean decline in log CC was 0.037mg/L higher with RYGB compared to LAGB (p<0.010). Although log leptin decreased significantly over time, there was no statistically significant difference in the average decrease in log leptin between surgical procedures. Ghrelin levels increased after RYGB and LAGB (mean increase 0.13pg/ml higher in LAGB than RYGB, p<0.001) (Figure 2/Table 2B, C). These observations demonstrate that RYGB, compared to LAGB, is associated with greater decreases in CRP and CC levels, and lesser increase in serum ghrelin levels, independent of weight loss. Changes in leptin levels, in contrast, were similar among procedures.

5. Associations of biomarker levels with surgical outcomes

We studied associations of baseline weight with weight loss, and metabolic disease remission independent of weight loss. Mean weight loss at 12- and 24-month visits was 6.7kg greater for each 20kg increase in baseline weight, adjusting for serum creatinine and serum biomarkers (both baseline and postoperative levels), and type of surgery (p<0.001). Similar relationships were observed at 36 months (Figure 3/Table 3). In contrast, baseline weight was not associated with likelihood of remission of any metabolic disease, adjusting for serum creatinine, serum biomarkers (baseline and follow-up levels), and surgical procedure (data not shown).

We next studied associations of baseline biomarker levels with weight loss or metabolic disease remission, adjusting for baseline weight, serum creatinine, remaining serum biomarkers (baseline and follow-up levels), and surgery type. Mean weight loss at 12- and 24-month visits was 6.9kg higher for each 0.5 log ng/ml increase in baseline leptin (p<0.001), 1.4kg higher for each 0.5 log mg/L decrease in baseline CC (p=0.02), and 1.1kg higher for each 0.5 mg/L increase in baseline log CRP (p<0.001). Similar relationships were observed at the 36-month visit. Mean weight loss at 12- and 24-month visits was 2.1kg higher for each 0.5 log pg/ml decrease in baseline ghrelin (p<0.001); (Figure 3/Table 3). Baseline serum biomarker levels did not predict remission of DM or asthma (data not shown). These data demonstrate that higher baseline leptin and CRP levels and lower baseline CC and ghrelin levels predict greater weight loss after surgery, but baseline levels of these biomarkers do not predict metabolic disease remission.

Finally, we studied associations of changes in follow-up biomarker levels with disease remission at each postoperative visit while adjusting for baseline and follow-up biomarker levels and weights, and surgery type, and, when studying CC, baseline creatinine. The instantaneous (hazard) rate of asthma remission increased multiplicatively by 25.8% (p=0.004) for each 0.5-unit decrease in log leptin at follow-up. The instantaneous rate of DM remission increased by 73.8% (p=0.020) for each 0.5-unit increase in follow-up log CC. These data demonstrate that a higher likelihood of asthma remission was associated with a greater decrease (i.e. lower follow-up level) in leptin levels, independent of weight loss, and a higher likelihood of DM remission was predicted by a lesser decrease (i.e. higher follow-up level) in CC levels, independent of weight loss.

DISCUSSION

We demonstrate that baseline serum levels of CRP, CC, leptin, and ghrelin correlate with adiposity; that baseline CC correlates most strongly and consistently with baseline metabolic disease prevalence; that baseline leptin and CRP levels correlate directly and baseline CC and ghrelin levels indirectly with surgery-induced weight loss; and that surgery-induced weight loss leads to reductions in levels of CRP, CC, and leptin, and increased ghrelin levels, consistent with a beneficial effect of surgery on systemic inflammation and adiposity, changes that are greater with RYGB compared to LABG.

Baseline biomarker levels and adiposity

Baseline levels of CRP, CC, and leptin correlated positively and ghrelin correlated negatively with baseline measures of adiposity, consistent with prior data.[14,15] Leptin correlated directly with all measures of adiposity except for WC, for which a negative correlation was observed; leptin is secreted at higher levels by subcutaneous relative to visceral adipose tissue,[16,17] which may explain a weaker association with WC, a measure of visceral adiposity. Despite prior data linking CRP to obesity,[18-20] CRP correlated less strongly with adiposity than other markers, possibly reflecting higher BMI and metabolic disease prevalence in surgery patients relative to non-surgical populations in other studies, variables that may overwhelm the strength of associations of these clinical measures and CRP. These data suggest that CRP is not as tightly linked to adiposity in the obese surgical population. Multiple other data confirm our observation of direct relationships of leptin and CC levels, and an inverse relationship of serum ghrelin levels, with adiposity.[21-23]

Baseline biomarker levels and metabolic disease prevalence

Associations between baseline biomarker levels and disease prevalence were complex. CC levels associated directly with all three metabolic diseases, with higher odds ratios than other biomarkers, although this association lost significance for asthma in multivariate analysis. These findings are consistent with prior data demonstrating a positive relationship between serum CC levels and metabolic disease.[24-28] Ours is the first report of which we are aware that studies these associations specifically in an obese population.

Baseline CRP levels were less consistently associated with disease, being positively associated with asthma, but not with DM or OSA. Others have demonstrated positive

associations between CRP and OSA, asthma,[29] and DM,[30] in conflict with some of our data. These discrepancies may reflect unique features of the LABS patient population, which is characterized by high BMI, a preponderance of women, selection for surgical fitness, and preoperative weight loss and glucose homeostasis optimization. Our data suggest that CC has potential as a marker for metabolic disease to a greater extent than CRP in the bariatric surgery patient population.

Leptin and ghrelin levels were inversely associated with DM and OSA prevalence. Data regarding associations between leptin levels and DM are conflicting.[31-33] Leptin is expressed at higher levels by subcutaneous adipose tissue than visceral adipose tissue,[16,17] and subcutaneous adiposity may exert a protective effect with respect to metabolic disease risk,[34,35] observations that may explain the observed negative correlations of leptin with DM and OSA, especially in the predominantly female LABS cohort, who may have a higher subcutaneous adipose tissue mass than sex-balanced study populations. While less well-studied, a preponderance of data supports our findings of an indirect association of ghrelin levels with DM and OSA, reinforcing serum ghrelin as a marker for metabolic health.[36-38]

Leptin and ghrelin levels were associated indirectly with all metabolic diseases except asthma, for which a direct association with leptin and no association with ghrelin were observed. Conversely, CC levels were associated directly with all metabolic diseases except asthma. While causal relationships cannot be inferred, these findings suggest that relative to other metabolic diseases, asthma pathogenesis may be distinct and less directly related to mechanisms underlying alterations in serum biomarker levels in the context of obesity (e.g. inflammation, adiposity).[5]

The observed associations of biomarkers with metabolic disease prevalence suggest potential utility in predicting incident disease. Prospective studies in patients with evolving metabolic disease will be necessary to confirm this hypothesis, but our data reinforce CC as a candidate for such study.

Biomarkers and surgical outcomes

Baseline serum levels of CRP, leptin, and ghrelin correlated directly with the magnitude of weight loss, but the magnitudes of these associations were low, with only small amounts of weight loss predicted by relatively large changes in levels of CRP, leptin, and ghrelin. Thus while predictive, the biomarkers studied have relatively limited utility in predicting substantial weight loss after surgery.

Importantly, no baseline biomarker levels predicted remission of DM or asthma. In contrast to our data, a small (n=28) study links elevated baseline CRP levels and lower leptin levels with decreased rates of DM remission after surgery,[39] while another study (n=37) demonstrated no relationship between CRP or leptin levels and post-operative DM remission.[40] Another study (n=30) demonstrated an association between higher baseline CRP levels and lower post-operative HDL levels, suggesting that elevated CRP portends a worse metabolic response to surgery.[41] Notably, these investigators reported no association between baseline CRP levels and DM remission, consistent with our observations. Our study adds to this conflicting literature with the largest cohort to date in surgery patients, and

suggests that serum biomarkers have limited utility in predicting disease remission after surgery.

Effect of surgery on biomarkers

Literature studying changes in serum biomarker in response to bariatric surgery consists of small studies; a recent meta-analysis of >50 reports of serum CRP in bariatric surgery patients included only 3 studies >100 subjects, the largest of which studied 765 subjects.[42] In a recent systematic review of post-surgical ghrelin levels, the majority of studies included <35 subjects.[43] We study the largest patient cohort to date, with standardized methods and testing schedules, and demonstrate that surgery-induced weight loss was associated with reduced serum CRP, CC, and leptin levels, and increased ghrelin levels consistent with reduced inflammation and a compensatory orexigenic response. Previously published data confirm reductions in CRP in response to surgical[40-42,44-47] and non-surgical weight loss[48,49]. Notably, in one small study (n=37), the magnitude of CRP reduction correlated directly with extent of DM remission.[40] Data regarding the effect of surgery-induced weight loss on CC levels are conflicting, with some studies demonstrating decreased levels[50] while others show no change[44,53]. At least one study demonstrated no change in CC levels in response to diet-induced weight loss,[52] suggesting that surgical and non-surgical weight loss may have qualitatively different effects on CC. Previous data confirm reductions in leptin in response to surgical and non- surgical weight loss, consistent with loss of adipose tissue mass, the primary source of leptin. Prior case series and cohort studies of patients undergoing RYGB demonstrate considerable variation in ghrelin response,[23,43,53-55] which may reflect small patient numbers, variability in assay techniques, testing schedules, and/or surgical technique, or weight stability or lack thereof at the time of assay, as ghrelin levels are increased during negative energy balance. We assessed ghrelin levels 2 years after surgery, when most patients would be expected to be weight-stable. Given the large number of patients and standardized testing schedule and methods in LABS, we conclude that there is no evidence in this study that clinically important suppression of ghrelin in response to surgery contributes to weight loss. Rather, we observed a compensatory increase in ghrelin levels in response to surgically-induced weight loss. While underlying mechanisms are unclear, these findings suggest that compensatory ghrelin responses to bariatric surgery do not correlate linearly with weight loss, and support continued study of ghrelin as a potential bariatric surgery-mimetic. This possibility aside, the observed small but statistically significant greater increase in ghrelin associated with LAGB compared with RYGB suggests that gastric remnant exclusion may attenuate long-term compensatory responses of ghrelin to weight loss, possibly contributing to greater efficacy of RYGB.

The likelihood of asthma remission correlated directly with the magnitude of decrease in leptin, suggesting that weight reduction, for which leptin would be expected to serve as a marker, is a dominant driver of asthma remission. The likelihood of DM remission correlated indirectly with the magnitude of decrease in CC, which is counterintuitive given that CC is thought to be pro-inflammatory. This discordance speaks to complex relationships between CC, inflammation, and DM remission, elucidation of which will require further study. While causality cannot be determined, these observations suggest that changes in leptin and CC may influence asthma and DM remission.

Compared to LAGB, RYGB was associated with a 2.4-fold higher likelihood of DM remission and greater reductions in CRP and CC independent of weight loss, supporting prior data demonstrating disproportionate effects of RYGB on DM and systemic inflammation relative to other procedures.[42,56] Conversely, LAGB was associated with greater increases in ghrelin independent of weight loss compared to RYGB. These observations confirm that alterations in gastrointestinal anatomy differentially influence biomarker levels independent of weight loss. While causality and mechanism cannot be determined, it is tempting to speculate that the greater magnitude of changes in CRP and CC in RYGB may contribute to its greater efficacy in achieving DM remission independent of weight loss. No procedure-specific weight loss-independent differences in change in leptin levels were observed, consistent with leptin reductions being primarily related to reductions in adipose tissue mass.

Conclusion

This is the largest study to date describing associations of inflammatory and adiposity-related serum biomarkers with disease prevalence and outcomes in bariatric surgery patients. We demonstrate associations between baseline serum biomarker levels and baseline metabolic disease prevalence and surgery-induced weight loss. We also demonstrate changes in serum biomarker levels consistent with anti-inflammatory effects that may contribute to disease remission, and compensatory orexigenic effects despite significant weight loss that may contribute to procedure-specific differences in weight loss. Notably, baseline serum biomarker levels did not predict metabolic disease remission, suggesting that the complexity of disease remission cannot be reduced to biomarker levels. Caution must be exercised in extrapolating findings from large cohorts such as LABS to individual patients, as significant variability in biologic responses to surgery exist. Nonetheless, our findings provide insight intomechanisms underlying the effects of surgery on metabolic disease and weight loss.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

AW	adjudicated weight
BMI	body mass index
CRP	C-reactive protein

CC	cystatin C
DM	type 2 diabetes
FBG	fasting blood glucose
FM	fat mass
IQR	interquartile range
LABS	Longitudinal Assessment of Bariatric Surgery
LAGB	laparoscopic adjustable gastric banding
OSA	obstructive sleep apnea
%BF	percent body fat
RYGB	Roux-en-Y gastric bypass
WC	waist circumference

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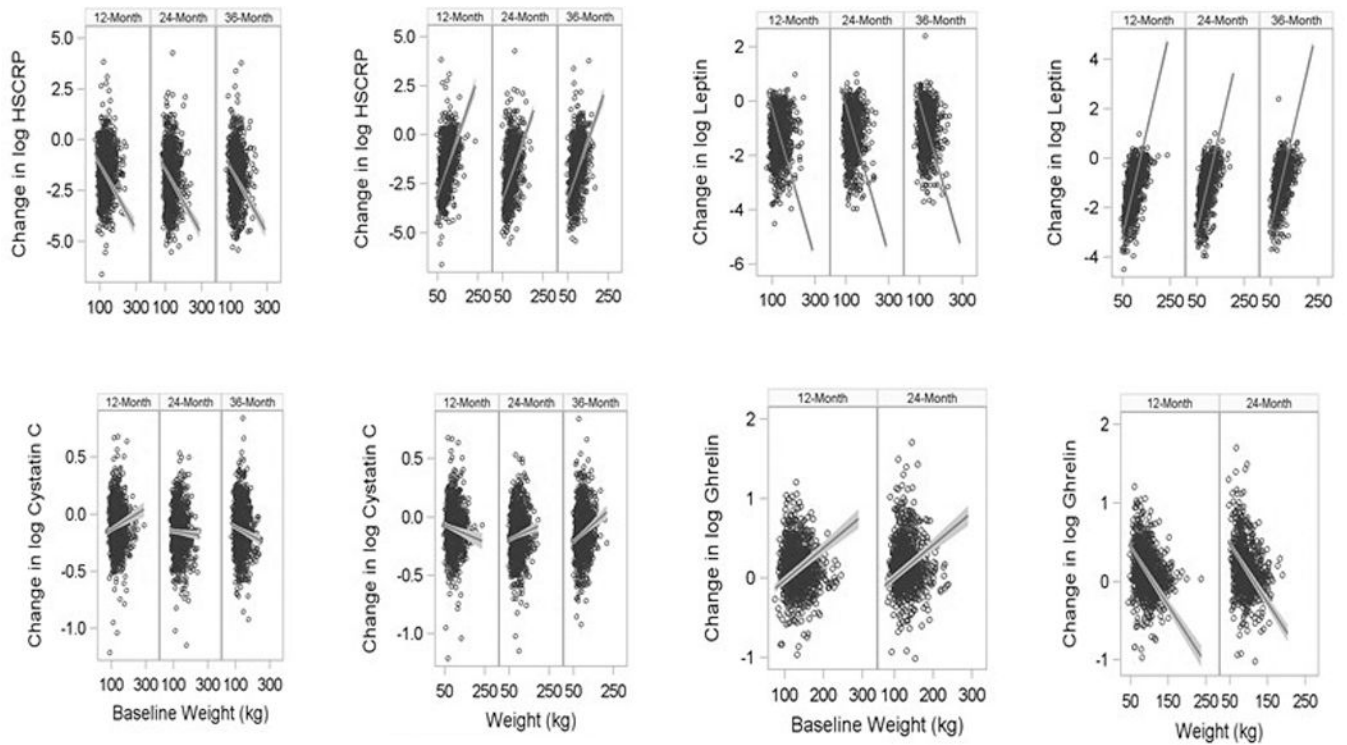
Figure 1. Associations of weight with change in serum biomarkers
Scatter plots with estimated regression lines. Shaded areas represent 95% confidence intervals. Estimated mean change in log serum biomarkers at 12-, 24-, and 26-month visits, controlling for surgery group, respective baseline log serum biomarker level, and baseline and follow-up weight. The model for log Cystatin C also controls for baseline and follow-up creatinine. Variables not plotted are set to their mean values, surgery=RYGB.
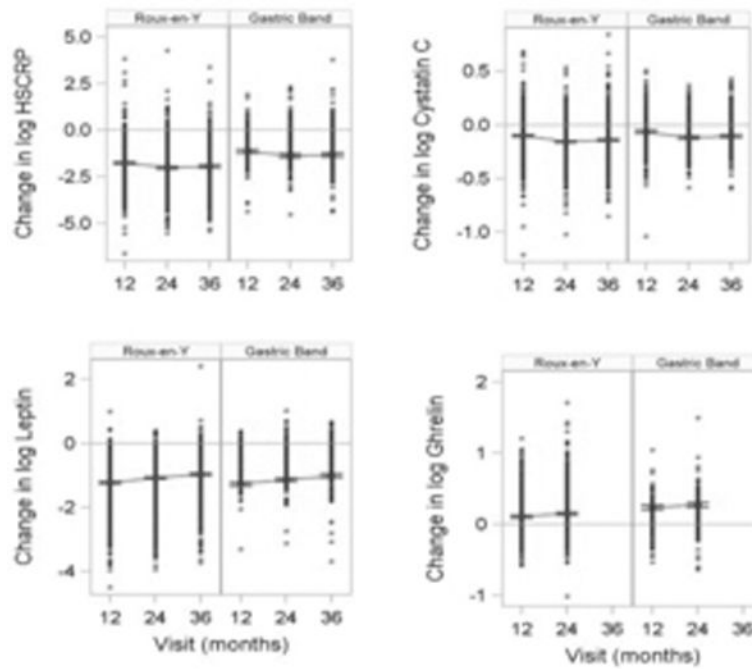
Figure 2. Change in serum biomarker levels after surgery
Estimated mean change in log serum biomarkers at 12-, 24-, and 26-month visit for each surgery group, controlling for respective baseline log serum biomarker level and baseline and follow-up weight. The model for log Cystatin C also controls for baseline and follow-up creatinine. Variables not plotted are set to their mean values, surgery=RYGB. The blue dots plot the observed data. The red dots plot the mean change in weight, connected by red lines to aid in comparison. The red bars represent 95% confidence intervals.
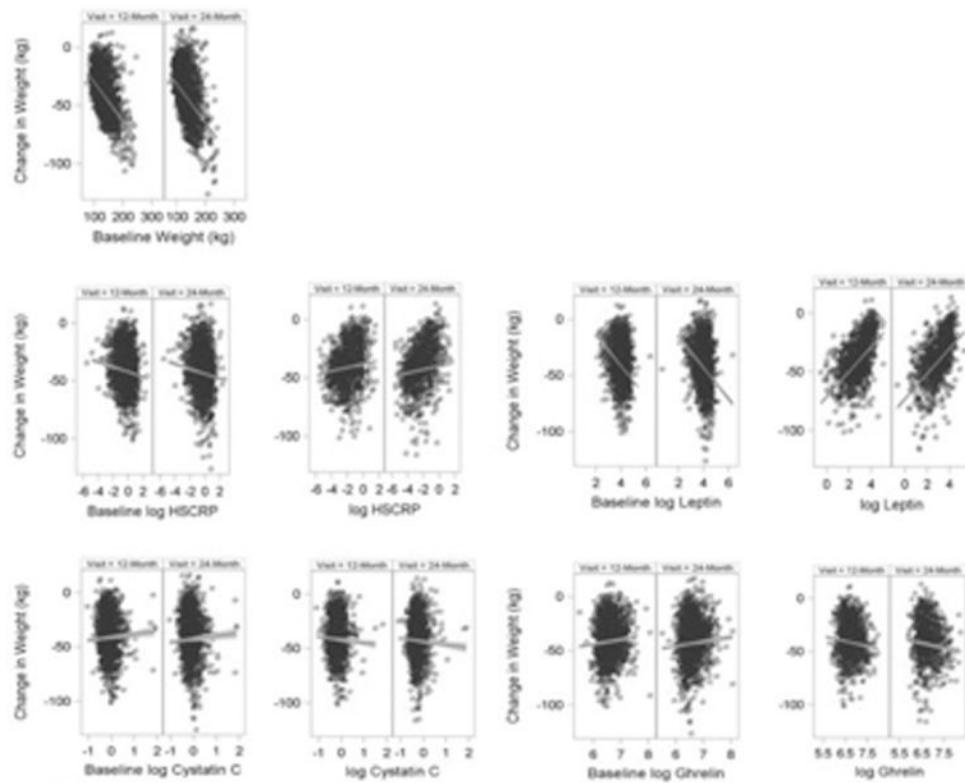
Figure 3. Associations of baseline and interval serum biomarker levels with weight loss
Scatter plots with estimated regression lines. Shaded areas represent 95% confidence
intervals. Estimated mean change in weight for a given change in baseline weight (top),
baseline log serum biomarker level (left graph in each pair of graphs for each biomarker), or
interval log serum biomarker level (right graph in each pair of graphs for each biomarker),
while controlling for surgery group, baseline weight, and other serum biomarkers and
creatinine at baseline and follow-up. Variables not plotted are set to their mean values. Data
presented is for RYGB surgery group only. Serum biomarkers in log scale. Ghrelin total was
not collected at the 36-month visit.

Table 1A

Associations between baseline serum biomarker levels and baseline measures of adiposity

Dependent variable	Biomarker	Unadjusted*			Adjusted**		
		Estimate***	95% CI	p-value	Estimate***	95% CI	p-value
Fat Mass (kg)	C-reactive protein	1.95	(1.52, 2.39)	<0.0001	1.36	(0.95,1.78)	<0.0001
	Cystatin C	8.86	(7.04, 10.68)	<0.0001	3.94	(2.01,5.87)	<0.0001
	Leptin	6.49	(5.64, 7.34)	<0.0001	5.56	(4.67,6.45)	<0.0001
	Ghrelin	−4.95	(−6.30, −3.60)	<0.0001	−5.51	(−6.75, −4.26)	<0.0001
Percent Body Fat	C-reactive protein	0.70	(0.55, 0.85)	<0.0001	0.40	(0.26,0.54)	<0.0001
	Cystatin C	1.47	(0.82, 2.12)	<0.0001	−0.86	(−1.51, −0.22)	0.009
	Leptin	3.00	(2.72, 3.28)	<0.0001	3.03	(2.73,3.33)	<0.0001
	Ghrelin	0.33	(−0.14, 0.81)	0.170	0.11	(−0.31,0.53)	0.604
Weight (kg)	C-reactive protein	2.17	(1.54, 2.79)	<0.0001	1.74	(1.13, 2.35)	<0.0001
	Cystatin C	13.46	(11.01, 15.92)	<0.0001	9.99	(7.30, 12.69)	<0.0001
	Leptin	4.96	(3.70, 6.23)	<0.0001	3.00	(1.69, 4.29)	<0.0001
	Ghrelin	−11.2	(−13.15, −9.30)	<0.0001	−12.00	(−13.87, −10.13)	<0.0001
BMI (kg/m^2)	C-reactive protein	1.02	(0.85, 1.20)	<0.0001	0.69	(0.53,0.85)	<0.0001
	Cystatin C	4.82	(4.12, 5.51)	<0.0001	2.32	(1.60,3.04)	<0.0001
	Leptin	3.30	(2.97, 3.64)	<0.0001	2.83	(2.48,3.18)	<0.0001
	Ghrelin	−2.49	(−3.05, −1.94)	<0.0001	−2.78	(−3.27, −2.28)	<0.0001
Waist circumference (cm)	C-reactive protein	1.06	(0.66, 1.45)	<0.0001	0.90	(0.52,1.27)	<0.0001
	Cystatin C	9.69	(8.09, 11.28)	<0.0001	8.70	(6.95,10.44)	<0.0001
	Leptin	2.04	(1.24, 2.84)	<0.0001	0.40	(−0.40,1.21)	0.326
	Ghrelin	−7.78	(−8.98, −6.58)	<0.0001	−8.11	(−9.26, −6.96)	<0.0001

Table 1B

Associations between baseline serum marker levels and baseline metabolic disease prevalence

Dependent variable	Biomarker	Unadjusted[*]			Adjusted[**]		
		OR[***]	95% CI	p-value	OR[***]	95% CI	p-value
Asthma	C-reactive protein	1.10	(1.04, 1.17)	<0.001	1.08	(1.02, 1.15)	0.007
	Cystatin C	1.56	(1.22, 1.99)	<0.001	1.27	(0.97, 1.66)	0.080
	Leptin	1.21	(1.08, 1.36)	0.001	1.15	(1.01, 1.31)	0.029
	Ghrelin	0.96	(0.81, 1.15)	0.680	0.93	(0.77, 1.11)	0.420
Diabetes	C-reactive protein	0.96	(0.91, 1.01)	0.100	1.00	(0.95, 1.06)	0.900
	Cystatin C	1.81	(1.47, 2.24)	<0.001	2.48	(1.93, 3.20)	<0.001
	Leptin	0.72	(0.65, 0.80)	<0.001	0.63	(0.56, 0.71)	<0.001
	Ghrelin	0.69	(0.58, 0.82)	<0.001	0.68	(0.57, 0.81)	<0.001
CVD	C-reactive protein	0.90	(0.82, 0.97)	0.010	0.91	(0.83, 0.99)	0.034
	Cystatin C	3.21	(2.32, 4.45)	<0.001	4.10	(2.80, 6.00)	<0.001
	Leptin	0.84	(0.71, 0.99)	0.040	0.69	(0.58, 0.82)	<0.001
	Ghrelin	0.88	(0.66, 1.17)	0.370	0.80	(0.60, 1.08)	0.140
Sleep Apnea	C-reactive protein	0.97	(0.92, 1.02)	0.210	1.00	(0.95, 1.05)	0.890
	Cystatin C	1.41	(1.14, 1.73)	0.001	1.69	(1.34, 2.13)	<0.001
	Leptin	0.81	(0.73, 0.90)	<0.001	0.75	(0.67, 0.84)	<0.001
	Ghrelin	0.83	(0.71, 0.97)	0.019	0.83	(0.70, 0.97)	0.022

[*] Adjusted for only baseline weight; models including CC also included serum creatinine;

[**] Adjusted for other three biomarkers, baseline weight, and serum creatinine;

[***] Estimate is the regression coefficient in Table 3A, and odds ratio (OR) in Table 3B per half log

Table 2A

Change in serum biomarker level in response to weight loss

	Predictor	Coefficient	95% CI	p-value
C-reactive protein (log mg/L)	Baseline weight	−0.32[*]	(−0.37, −0.27)	<0.001
	Followup weight	0.60[**]	(0.54, 0.66)	<0.001
Cystatin C (log mg/L)	Baseline weight	−0.016	(−0.025, −0.008)	<0.001
	Followup weight	0.030	(0.021, 0.040)	<0.001
Leptin (log ng/mL)	Baseline weight	−0.55	(−0.57, −0.52)	<0.001
	Followup weight	0.83	(0.80, 0.87)	<0.001
Ghrelin (log pg/mL)	Baseline weight	0.082	(0.065, 0.099)	<0.001
	Followup weight	−0.15	(−0.17, −0.13)	<0.001

Coefficients are change in log serum biomarker level over post-surgery visits for each 20kg change in either baseline or follow-up weights, adjusting for surgical procedure and baseline serum biomarker level.

[*] Negative coefficients for baseline weight indicates a positive association with decline in biomarker levels. For example, each 20kg additional weight is associated with a 0.32 log mg/L decline in CRP

[**] Positive coefficient for follow-up weight indicates positive relationship with weight loss and decline in biomarkers. For example, each 20kg additional decline in weight at follow-up, is associated with a 0.6 log mg/L decline in CRP.

Table 2B

Change in serum biomarker levels in response to surgical procedures

Effect	RYGB			LAGB		
	Est	95% CI	p-value	Est	95% CI	p-value
C-reactive protein (log mg/L)	−1.95	(−2.02, −1.89)	<.001	−1.32	(−1.43, −1.22)	<0.001
Cystatin C (log mg/L)	−0.26	(−0.29, −0.23)	<.001	−0.22	(−0.25, −0.19)	<0.001
Leptin (log ng/ml)	−0.97	(−1.01, −0.94)	<.001	−1.02	(−1.08, −0.96)	<0.001
Ghrelin(log pg/mL)	0.15	(0.13, 0.16)	<.001	0.27	(0.24, 0.30)	<0.001

Coefficients (Est.): estimated change in serum biomarker levels from baseline to 36-month visit with baseline and follow-up weight at mean values. Values for ghrelin are change from baseline at 24-month visit.

Table 2C

Differences in change in serum biomarker levels between surgical procedures

Effect	LAGB vs RYGB		
	Est	95% CI	p-value
C-reactive protein (log mg/L)	0.63	(0.52, 0.74)	<0.001
Cystatin C (log mg/L)	0.037	(0.021, 0.053)	<0.001
Leptin (log ng/ml)	−0.046	(−0.109, 0.016)	0.150
Ghrelin (log pg/ml)	0.13	(0.09, 0.16)	<0.001

*Coefficients: Estimated difference of change from baseline to 36-month visit adjusting for baseline and follow-up weight, and baseline serum biomarker. Values for ghrelin are change from baseline to 24-month visit. A positive coefficient indicates greater decline for the latter group, e.g., mean decline in CRP was 0.63 log mg/L higher for RYGB compared to LAGB.

Table 3

Associations of weight loss with baseline weight, and baseline, follow-up serum biomarker levels

Coefficients (Est.): estimated change in weight for a 20kg increase in baseline weight (top line) or a 0.5-unit increase in baseline or follow-up log serum biomarker; these estimates represent the slopes depicted in Figure 6.

Effect	Est	95% CI	p-value
Weight (Baseline) kg	−6.66	(−7.00, −6.32)	<0.001
C-reactive protein (Baseline) log mg/L	−1.07	(−1.31, −0.83)	<0.001
C-reactive protein (Follow-up) log mg/L	0.74	(0.60, 0.89)	<0.001
Cystatin C (Baseline) log mg/L	1.42	(0.20, 2.64)	0.020
Cystatin C (Follow-up) log mg/L	−1.41	(−2.43, −0.39)	0.007
Leptin (Baseline) log ng/ml	−6.92	(−7.47, −6.37)	<0.001
Leptin (Follow-up) log ng/ml	5.35	(5.10, 5.60)	<0.001
Ghrelin (Baseline) log pg/ml	2.08	(1.19, 2.98)	<0.001
Ghrelin (Follow-up) log pg/ml	−2.41	(−3.05, −1.77)	<0.001