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ARTICLE

Longitudinal changes in pancreatic and adipocyte hormones following Roux-en-Y gastric bypass surgery

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Abstract
Aims/hypothesis Bariatric surgery is an effective treatment for severe obesity, as in addition to dramatic weight loss, co-morbidities such as type 2 diabetes are frequently resolved. Although altered gastrointestinal peptide hormone secretion and its relationship with post-surgical improvements in insulin sensitivity has been studied, much less is known about long-term changes in pancreatic and adipose tissue-derived hormones. Our objective was to conduct a comprehensive longitudinal investigation of the endocrine changes following Roux-en-Y gastric bypass surgery (RYGBP), focusing on pancreatic and adipocyte hormones and systemic markers of inflammation.
Methods Nineteen severely obese women (BMI 45.6± 1.6 kg/m^2) were studied prior to RYGBP, and at 1, 3, 6, and 12 months after RYGBP. Body composition was assessed before surgery and at 1 and 12 months.
Results Pre-surgical adiposity was correlated with circulating adipocyte hormones (leptin, visfatin) and inflammatory molecules (IL-6, high sensitivity C-reactive protein [hsCRP], monocyte chemoattractant protein-1). As expected, RYGBP reduced fat mass and fasting insulin and glucose concentrations. In addition, reductions of fasting pancreatic polypeptide (PP) and glucagon concentrations were observed at 1 and 3 months, respectively. In the 12 months following RYGBP, concentrations of most adipocyte hormones (leptin, acylation-stimulating hormone and visfatin, but not retinol-binding hormone-4) and inflammatory molecules (IL-6, hsCRP and soluble intracellular adhesion molecule-1) were significantly reduced. Reductions of insulin resistance (measured by homeostasis model assessment of insulin resistance) were independently associated with changes of glucagon, visfatin and PP. Pre-surgical HMW adiponectin concentrations independently predicted losses of body weight and fat mass.
Conclusions/interpretation These results suggest that pancreatic and adipocyte hormones may contribute to the long-term resolution of insulin resistance after RYGBP.

Keywords Clinical science · Cytokines · Gastro-entero pancreatic factors · Human · Insulin sensitivity and resistance · Other hormones · Other islet cells/hormones · Weight regulation and obesity

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Abbreviations

ASP	acylation-stimulating protein
CRP	C-reactive protein
GLP-1	glucagon-like peptide 1
HMW	high molecular weight
HOMA-IR	homeostasis model assessment of insulin resistance
hsCRP	high-sensitivity C-reactive protein
MCP-1	monocyte chemoattractant protein-1
PP	polypeptide
RBP4	retinol-binding protein-4
RYGBP	Roux-en-Y gastric bypass
sICAM-1	soluble intercellular adhesion molecule-1

Introduction

Bariatric surgery is the most effective method for producing weight loss in patients with severe obesity (BMI>40 kg/m^2). In addition to weight loss, the co-morbidities of severe obesity, particularly type 2 diabetes mellitus, insulin resistance, hyperlipidaemia, sleep apnoea and hypertension are frequently resolved following surgery [1]. Accordingly, the number of bariatric surgical procedures performed in the USA increased eightfold between 1998 and 2004 [2]. Similar increases have been reported in many other countries [3].

There is considerable interest in the physiological mechanisms by which bariatric surgery, in particular Roux-en-Y gastric bypass (RYGBP), resolves the co-morbidities of severe obesity, as understanding them could lead to the development of new (non-surgical) therapeutic strategies. Much of the research to date has focused on gastrointestinal hormones, such as ghrelin, glucagon-like peptide-1 (GLP-1) and peptide-YY, and their relationships with the weight loss, reduction of appetite and improved insulin sensitivity that follow RYGBP [4]. However, concomitant changes in pancreatic (glucagon, pancreatic polypeptide [PP] and amylin) and adipocyte-derived hormones have not been as intensively investigated.

Over the last two decades, adipose tissue has been recognised as an active source of hormones involved in energy homeostasis and substrate metabolism [5]. Adipocyte-derived hormones include leptin, adiponectin and acylation-stimulating protein (ASP), as well as the more recently identified visfatin (also known as PBEF/NAMPT) [6]. With the exception of adiponectin, circulating concentrations of these hormones are usually increased in obesity and insulin-resistant states, and decline during weight loss [7]. A role for adipocyte-derived retinol-binding protein-4 (RBP4) in systemic insulin resistance has also been recently proposed: in transgenic mice, adipose-specific GLUT4 knockout increased serum RBP4 concentrations, which in turn led to insulin resistance by impairing skeletal muscle insulin action and increasing hepatic glucose output [8].

Obesity is also characterised by the infiltration of macrophages into adipose tissue, promoting a state of chronic, low-grade inflammation [9]. Relative to lean, healthy individuals, obesity is associated with elevated concentrations of C-reactive protein (CRP) [10], IL-6 [11], monocyte chemoattractant protein-1 (MCP-1) [12] and soluble intercellular adhesion molecule-1 (sICAM-1) [13]. This inflammatory state is implicated in the development of many complications of severe obesity, in particular, atherosclerosis, insulin resistance and type 2 diabetes [14].

The objective of this study was to conduct a comprehensive longitudinal examination of the endocrine and metabolic changes following RYGBP, focusing on pancreatic and adipocyte hormones as well as inflammatory markers. Many previous studies of the endocrine effects of bariatric surgery have either been cross-sectional in design or have only examined a single time-point after surgery. We have previously reported that concentrations of high molecular weight (HMW) adiponectin increase following RYGBP, and that these increases were proportional to relative improvements in insulin resistance [15]. We now provide additional information pertaining to other adipocyte-derived hormones (leptin, ASP, visfatin, RBP4), inflammatory markers (IL-6, high-sensitivity CRP [hsCRP]) and pancreatic hormones (proinsulin, glucagon, PP and amylin) after RYGBP in these severely obese individuals.

Methods

Subjects Nineteen severely obese women (aged 40.6± 1.8 years, mean±SEM) underwent RYGBP surgery performed at the University of California, Davis Medical Center, or at Mercy San Juan Hospital in Sacramento, CA, USA. Although patients were encouraged to lose weight prior to surgery, their weight had been stable for the preceding 3 months. Fasting blood samples were collected prior to surgery, and at 1, 3, 6 and 12 months postoperatively. Body composition was assessed prior to surgery and at 1 and 12 months. The experimental protocol was approved by Institutional Review Board of UC Davis, and all participants provided written informed consent for participation in the study.

Anthropometric and biochemical measurements Weight, height, and waist and hip circumferences were measured using standard methods. Body composition was determined by air-displacement plethysmography (BodPod, Life Measurements, Concord, CA, USA).

All biochemical measurements were performed on fasting plasma samples, except measurements of RBP4 and visfatin, which were performed on fasting serum samples. Insulin, proinsulin, leptin, glucagon and cortisol were measured by radioimmunoassay (Linco, St Charles, MO, USA). Plasma glucose was measured with a YSI glucose analyser (Yellow Springs, OH, USA). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as previously described [16]. NEFA concentrations were measured with an enzymatic assay (Waco Chemicals, Richmond, VA, USA). RBP4 was measured by ELISA (Alpco, Salem, NH, USA). MCP-1 and sICAM-1 were measured by ELISA, and IL-6 was measured using a Quantikine HS ELISA (all from R&D Systems, Minneapolis, MN, USA). ASP and amylin were measured by ELISA (Linco). The amylin assay measured human amylin, deaminated amylin, the 1–20 fragment of amylin, but not reduced amylin. PP was measured by radioimmunoassay (Alpco). CRP was measured with an Immulite analyser and hsCRP reagents (Diagnostic Products, Los Angeles, CA, USA). Visfatin was measured by ELISA (Adipogen, Seoul, South Korea).

Statistical analysis Statistical analysis was performed using GraphPad Prism v. 4 (San Diego, CA, USA) and JMP Start Statistics (SAS, Cary, NC, USA). All continuous variables were assessed for normality using the Kolmogorov–Smirnov test. Comparisons between baseline and post-surgical values were made with one-way repeated measures ANOVA or the non-parametric Friedman test. Post-test comparisons were made with Bonferroni's multiple comparison test, with the pre-surgical values used as the reference. For sICAM-1 and MCP-1, comparisons between baseline and 12 months were made using paired *t* tests. Relationships between continuous variables were assessed by non-parametric Spearman rank correlation. Multivariate analyses were performed using forward stepwise multiple logistic regression, as described earlier [15]. The two-sided level of significance was $p<0.05$. All mean values are shown ±SEM.

Results

Baseline characteristics and changes of body weight and body composition following RYGBP Body composition and biochemical measurements in the 19 participants are shown in Table 1. At 1, 3, 6 and 12 months after RYGBP, average reductions in body weight were 10.3±0.4%, 17.8±1.0%, 24.7±1.0% and 31.9±1.5%, respectively. Weight loss was predominantly due to changes in fat mass, which was reduced by 11.7±0.8% (7.6±0.5 kg) at 1 month and 49.6±2.5% (32.7±2.4 kg) at 12 months. This was accompanied by a modest, but significant, decrease of fat-free mass: 8.7±0.9% (5.4±0.4 kg) at 1 month and 15.2±2.9% (9.2±1.7 kg) at 12 months. Waist circumference and WHR were significantly reduced at 1 and 6 months, respectively.

Plasma glucose, HOMA-IR and pancreatic hormones Three individuals presented with fasting hyperglycaemia (plasma glucose≥7.0 mmol/l) and another three with impaired fasting glucose (6.1–6.9 mmol/l) prior to surgery. After RYGBP, plasma glucose and insulin concentrations decreased progressively over the next 12 months (Fig. 1a,b).

Table 1 Changes of body composition, hormones and markers of inflammation in severely obese women following RYGBP

Variable	Pre-operative	1 month	3 months	6 months	12 months	Overall *p* value
n	19	19	18	19	19	–
Body weight (kg)	126.4±3.6	113.4±3.3***	107.2±4.4***	95.4±3.3***	85.9±2.8***	<0.0001
BMI (kg/m^2)	45.6±1.6	41.0±1.5***	38.8±2.1***	34.5±1.5***	30.8±1.0***	<0.0001
Fat mass (kg)	65.5±2.7	58.2±2.5**	–	–	32.9±1.8***	<0.0001
Fat-free mass (kg)	60.9±2.0	55.2±1.6***	–	–	53.0±2.5***	<0.0001[a]
Waist circumference (cm)	130±2	119±3*	111±3***	104±2***	100±2***	<0.0001
WHR	0.95±0.03	0.90±0.02	0.89±0.02	0.87±0.02*	0.88±0.02**	0.046
HOMA-IR	7.4±1.0	4.0±0.5***	3.1±0.3***	2.6±0.2***	2.3±0.2***	<0.0001
Proinsulin:insulin ratio (%)	16.7±1.6	15.6±1.7	16.4±1.3	16.2±1.3	15.1±1.1	0.59
NEFA (mmol/l)	0.28±0.03	0.37±0.04	0.26±0.03	0.23±0.04	0.20±0.02	<0.0001
Cortisol (nmol/l)	232±22	265±28	262±25	295±30	257±25	0.10[a]
IL-6 (pg/ml)	4.16±0.50	3.88±0.33	–	–	2.11±0.20***	<0.0001[a]
hsCRP (mg/l)	12.6±2.1	9.5±1.4	7.4±2.0**	5.2±1.4***	2.8±0.7***	<0.0001
sICAM-1 (ng/ml)	243±9	–	–	–	216±9*	0.011
MCP-1 (pg/ml)	163±8	–	–	–	161±8	0.81

Data are shown as mean±SEM. Comparisons between baseline and each time-point were made using repeated measures one-way ANOVA, except where its non-parametric equivalent, the Friedman test was used[a]. Levels of significance at each time-point were calculated using Bonferroni's multiple comparison test.
*$p<0.05$, **$p<0.01$, ***$p<0.0001$

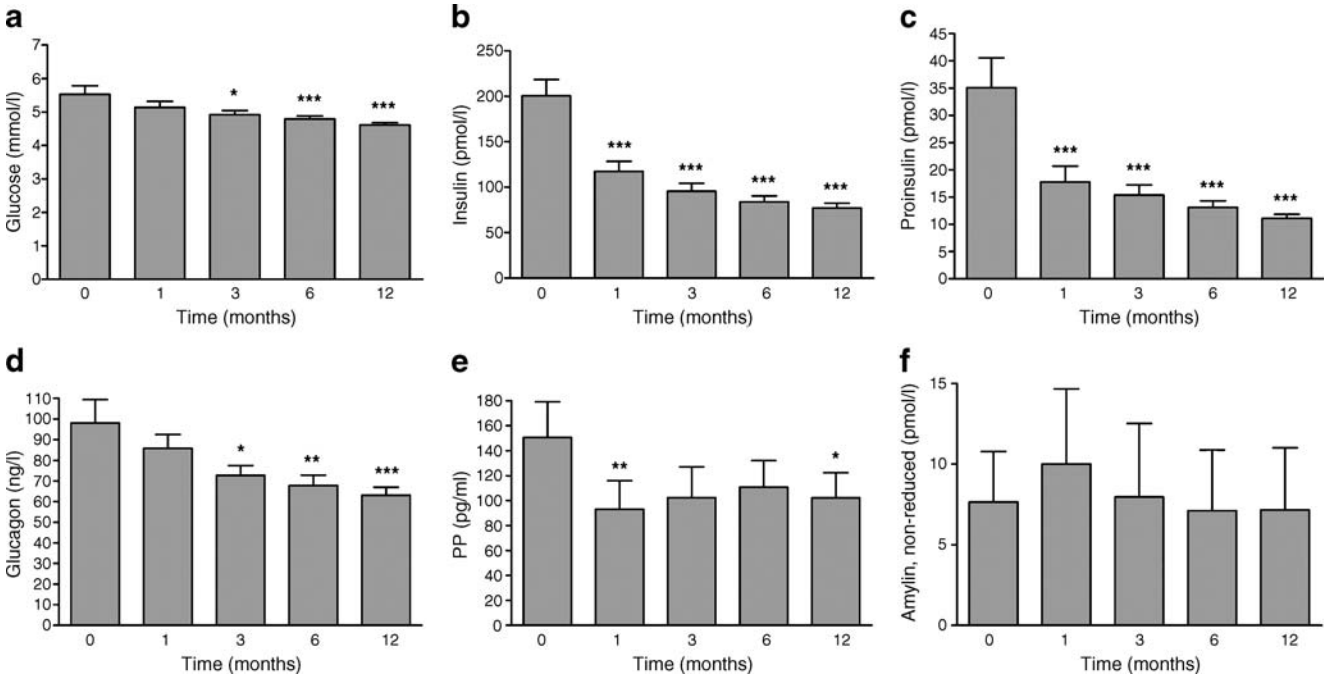

Fig. 1 Fasting plasma concentrations of glucose, insulin and other pancreatic hormones following RYGBP. Differences between time-points were determined by repeated measures one-way ANOVA. *$p<0.05$, **$p<0.01$ and ***$p<0.0001$ relative to pre-surgical values

After 1 month, fasting insulin concentrations had decreased by 34±8%, and glucose levels were normalised in all individuals. Accordingly, HOMA-IR was significantly reduced by 1 month and continued to decrease thereafter (Table 1). Plasma proinsulin concentrations decreased in parallel with changes of insulin (Fig. 1c), such that the proinsulin/insulin ratio (a marker of pancreatic beta cell dysfunction [17]) was unchanged following RYGBP (Table 1). When stratified by the presence or absence of pre-surgical hyperglycaemia, the proinsulin/insulin ratio did not change significantly in either group (data not shown). This contrasts with the reported improvement of dynamic beta cell function (accompanied by a reduced proinsulin/insulin ratio) in patients with weight loss induced by vertical gastric banding and jejunoileal bypass [18].

Fasting glucagon, PP and amylin concentrations were also measured. In these severely obese women, fasting plasma glucagon concentrations at baseline (98±11 ng/l) were twice as high as those we have previously reported in normal weight and overweight women, using the same assay [19, 20]. Glucagon concentrations decreased following RYGBP, being reduced by 22±8% at 3 months, 26±5% at 6 and, 31±4% at 12 months (Fig. 1d). Plasma PP concentrations were significantly decreased at 1 (−27±10%) and 12 months (−17±13%), but not at 3 or 6 months (Fig. 1e). Amylin concentrations did not change after RYGBP (Fig. 1f).

Adipocyte hormones and inflammatory molecules As expected, leptin concentrations prior to surgery were proportional to body weight, BMI and fat mass (data not shown). Concentrations of other adipocyte hormones and inflammatory markers (visfatin, IL-6, MCP-1 and hsCRP) were also correlated with body adiposity, but not with fasting insulin concentrations or HOMA-IR (Table 2). Visfatin concentrations were also positively correlated with fat-free mass. Concentrations of RBP4, ASP and sICAM-1 were not significantly associated with measures of body composition or insulin resistance at baseline.

Concentrations of most adipocyte hormones decreased significantly 1 month after RYGBP (Fig. 2). Reductions of leptin (−36±3%), visfatin (−20±6%), RBP4 (−28±6%) and ASP (−18±7%) were observed. At 12 months, similarly, concentrations of leptin and visfatin were reduced by 64±4% and 41±8%, respectively. However, concentrations of ASP and RBP4 at twelve months were not statistically different from pre-surgical values.

RYGBP also led to a progressive reduction of some, but not all markers of inflammation (Table 1). IL-6 concentrations were unchanged at 1 month after RYGBP, but were reduced by 43±7% at 12 months ($p<0.001$). hsCRP concentrations were reduced by 46±7% and 74±7% at 3 and 12 months, respectively. Concentrations of sICAM-1 were reduced by 10±4% after 12 months, while MCP-1 concentrations did not change significantly (Table 1).

Table 2 Significant correlations between continuous variables at baseline and at 12 months

Variable 1	Variable 2	*r*	*p* value
Significant correlations at baseline			
Visfatin	Body weight	0.65	0.0028
	Waist circumference	0.50	0.035
	Fat-free mass	0.55	0.015
IL-6	Body weight	0.59	0.007
	BMI	0.67	0.002
	Fat mass	0.70	0.0009
hsCRP	Body weight	0.55	0.015
	Waist circumference	0.51	0.030
	Fat mass	0.55	0.014
MCP-1	BMI	0.60	0.007
Significant correlations at 12 month follow-up			
IL-6	WHR	0.48	0.036
	Insulin	0.67	0.0017
	HOMA-IR	0.59	0.0074
sICAM-1	Glucose	−0.48	0.036

Before RYGBP and at 12 month follow-up, hormones and inflammatory markers were tested for correlation with measures of body composition (weight, BMI, fat mass, lean mass, waist circumference, WHR), fasting glucose and insulin concentrations, and insulin resistance (HOMA-IR) by non-parametric Spearman rank. The level of significance was $p<0.05$

At 12 months, the relationship between leptin concentrations and adiposity remained significant (data not shown), while leptin concentrations were also positively correlated with fasting insulin ($r=0.55$, $p=0.015$) and HOMA-IR ($r=0.48$, $p=0.039$). IL-6 concentrations were positively correlated with WHR, fasting insulin and HOMA-IR, and sICAM-1 concentrations were negatively correlated with fasting glucose.

Hormones independently associated with changes of body weight and body composition In our previous report [15], we found that changes of HMW adiponectin were associated with the changes in fat mass (Δfat mass) in the 12 months following RYGBP, independently of age and initial BMI. Using this model, we found that Δfat mass was also related to changes of leptin (model 1, Table 3). This model accounted for 71% of the variance in Δfat mass.

We next tested which baseline variables were predictive of Δfat mass. Of the hormones studied, only baseline HMW adiponectin concentrations predicted Δfat mass, independently of age and BMI at baseline (model 2, Table 3 and Fig. 3a). Similar results were obtained when we examined proportional changes of fat mass; however, the models explained less of the overall variance (data not shown).

Variables related to the change of body weight over the 12 months (Δbody weight) were also studied. As for Δfat mass, changes of both leptin and HMW adiponectin were

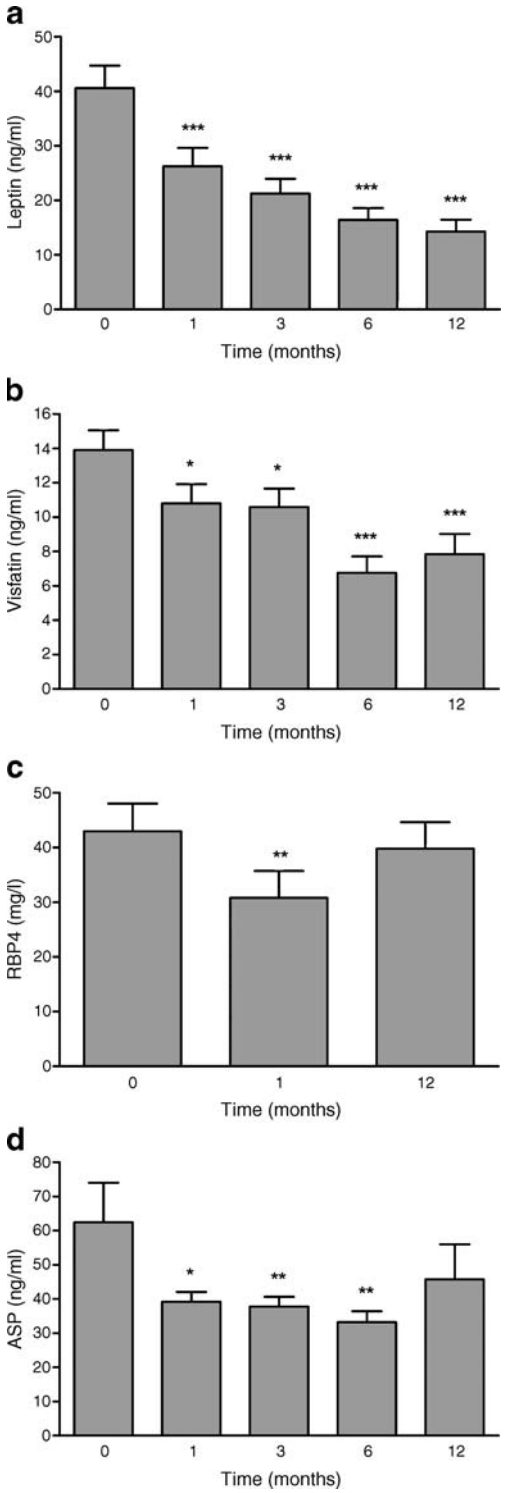

Fig. 2 Fasting plasma concentrations of adipocyte hormones following RYGBP. Differences between time-points were determined by repeated measures one-way ANOVA. *$p<0.05$, **$p<0.01$, and ***$p<0.0001$ relative to pre-surgical values

Table 3 Multivariate analyses of variables associated with RYGBP-induced changes of body weight, fat mass, fasting insulin and HOMA-IR

Model	*n*	Dependent variable	Independent variable	ß coefficient	SE	*p* value	Model r^2 (adj)
1	19	ΔFat mass (kg)	Age	0.432	0.194	0.043	0.71
	0–12 months		Initial BMI	−0.237	0.222	0.30	
			ΔLeptin	0.349	0.111	0.0070	
			ΔHMW Ad	−3.786	1.217	0.0077	
2	19	ΔFat mass (kg)	Age	0.287	0.219	0.21	0.49
	0–12 months		Initial BMI	−0.728	0.246	0.0098	
			Baseline HMW Ad	−4.695	1.721	0.016	
3	19	ΔWeight (kg)	Age	0.357	0.235	0.15	0.64
	0–12 months		ΔLeptin	0.478	0.120	0.0012	
			ΔHMW Ad	−3.740	1.459	0.022	
4	19	ΔWeight (kg)	Age	−0.070	0.246	0.78	0.58
	0–12 months		Baseline HMW Ad	−7.604	1.958	0.0017	
			Baseline hsCRP	−0.628	0.229	0.016	
			Baseline ghrelin	0.082	0.038	0.047	
5	19	ΔFasting insulin	Age	0.601	1.789	0.74	0.47
	0–12 months		ΔVisfatin	7.429	2.348	0.0069	
			ΔGlucagon	0.846	0.325	0.021	
			ΔPP	2.273	0.938	0.030	
6	18	ΔHOMA-IR	Age	−0.187	0.092	0.062	0.42
	0–12 months		ΔGlucagon	0.072	0.020	0.0031	
			ΔVisfatin	0.343	0.147	0.035	
7	18	%ΔHOMA-IR	Age	0.732	0.321	0.040	0.59
	0–12 months		ΔHMW Ad	−8.995	2.511	0.0033	
			ΔNEFA	−76.77	21.547	0.0035	
			ΔIL-6	−2.391	1.139	0.056	

Stepwise multiple logistic regression was performed using the least squares method, as described by Swarbrick et al. [15].
Ad, adiponectin; r^2 (adj), adjusted proportion of the variance accounted for by the model

independently associated with Δbody weight (model 3, Table 3). ΔBody weight was also independently predicted by baseline concentrations of HMW adiponectin (Fig. 3b), hsCRP (Fig. 3c) and ghrelin (model 4, Table 3).

Changes of each of the hormones measured here were also tested for association with the changes of fat-free mass after surgery. None of these were correlated with Δfat-free mass (data not shown).

Hormones independently associated with changes of fasting insulin and HOMA-IR In all participants, changes of fasting insulin concentrations between baseline and 12 months (Δinsulin) were significantly and independently associated with changes of visfatin (p=0.0069) (Fig. 3d), glucagon (p=0.021) and PP (p=0.030; model 5, Table 3). This model accounted for 47% of the variation in Δinsulin. Due to the non-linear relationship between Δglucagon and Δinsulin, the proportional (%) changes were examined and these were significantly correlated (r=0.56, p=0.013; Fig. 3e).

Relationships between the changes of hormones and changes of HOMA-IR over the 12 months were also examined. Analyses were limited to participants in whom HOMA-IR improved by >15% at 12 months after surgery [15], which yielded 18 participants. As for Δinsulin, the absolute changes of HOMA-IR over the 12 months were associated with changes of glucagon and visfatin (model 6, Table 3).

The final model (model 7, Table 3) examined relative changes of HOMA-IR, to account for the substantial absolute improvement of insulin sensitivity that occurred in the patients with hyperglycaemia at baseline. As previously described [15], the proportional improvement in HOMA-IR (%ΔHOMA-IR) over the 12 months was related to age, ΔNEFA (Fig. 3f) and ΔHMW adiponectin (model 7, Table 3). In this model, the change of IL-6 concentrations also tended to be independently associated with %ΔHOMA-IR (p=0.056; Fig. 3g). This model explained 59% of the variation in %ΔHOMA-IR.

Discussion

This study investigated the longitudinal changes of pancreatic and adipocyte hormones following RYGBP in severely obese women. Markers of inflammation were also examined. While previous studies have reported changes of several of these variables following bariatric surgery, we believe that this study is the first longitudinal

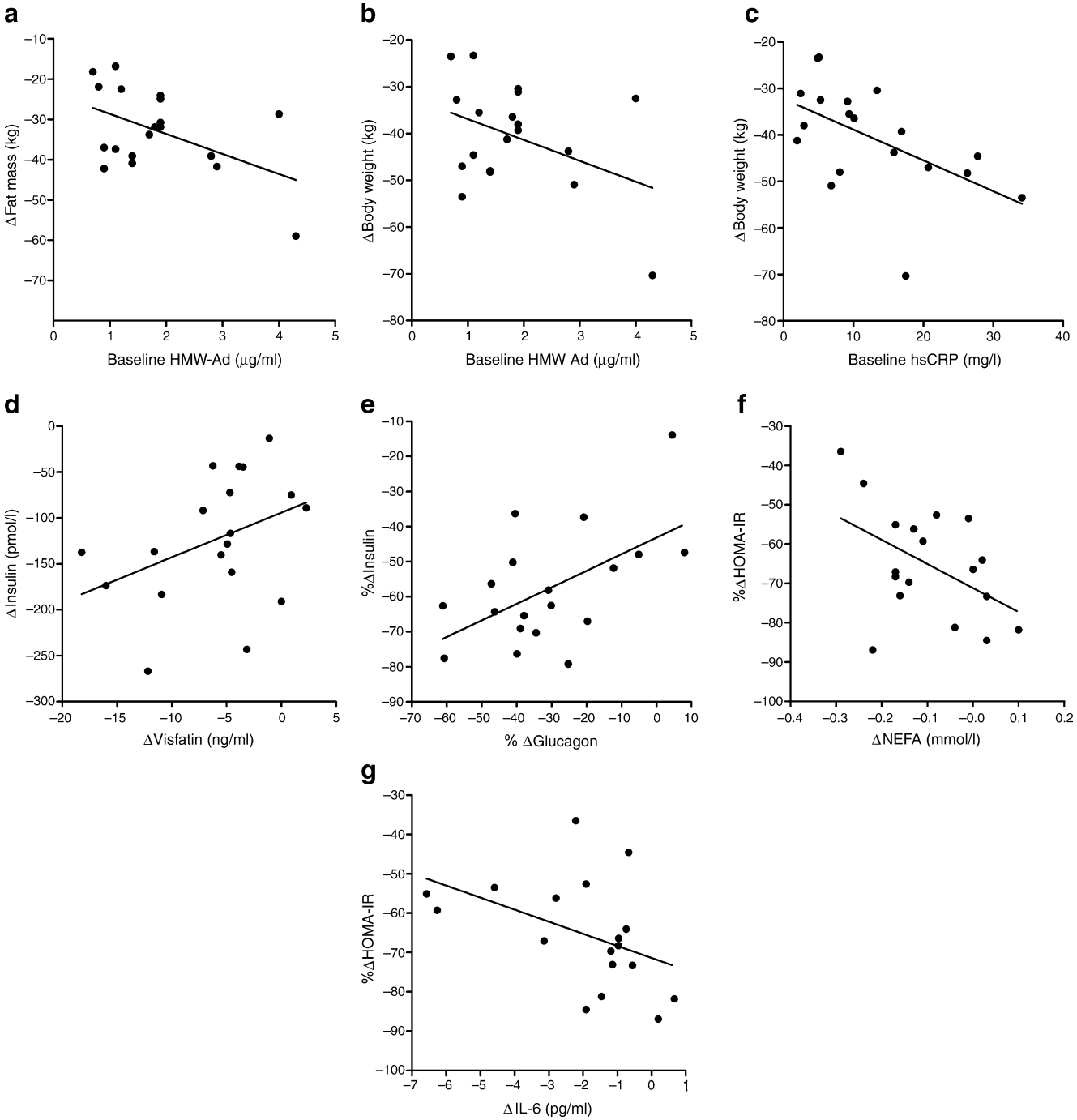

Fig. 3 Significant independent predictors of absolute and proportional changes of body weight, fat mass, fasting insulin concentrations and HOMA-IR. **a** Baseline HMW adiponectin (Ad) concentrations predicted Δfat mass, r=–0.50, p=0.016 (model 2, Table 3) and **b** Δbody weight, r=–0.40, p=0.0017 (model 4). **c** Baseline hsCRP concentrations were also associated with Δbody weight following RYGBP, r=−0.55, p=0.016 (model 4). **d** ΔVisfatin and Δinsulin were significantly correlated, r=0.39, p=0.0069 (model 5). **e** Proportional changes of glucagon and insulin were positively correlated with each other (r= 0.56, p=0.013). Independent relationships between changes of **f** NEFA (r=–0.48, p=0.0035) and **g** IL-6 (r=–0.44, p=0.056) with the relative improvement of HOMA-IR were also observed

examination to be comprehensive in scope, due to the number of measurements performed and the frequency of follow-up examinations. A further strength of the present study was that changes of body composition were assessed. Its main limitations were that the sample size was limited to only 19 individuals, all of whom were women, and that we were not able to study responses to a standardised meal. It was also not possible to assess insulin sensitivity by FSIVGTT or euglycaemic clamps in this study.

The first important new finding was that the elevated fasting plasma glucagon concentrations in these severely obese participants were markedly reduced after RYGBP. This observation may be of physiological significance, as changes of fasting glucagon concentrations were significantly and independently associated with the changes of fasting insulin at 12 months after surgery. This relationship was present when proportional changes of glucagon and insulin were examined. In multivariate analysis, changes of glucagon concentrations were independently related to the improvements of HOMA-IR following RYGBP. Previous studies of fasting glucagon concentrations before and after RYGBP have reported no change either at 12 weeks [21] or 6 to 9 months after surgery [22]. This discrepancy may be due to the limited time course of the former study; and in the second study, a closer examination of the data actually suggests a 28% reduction of 'pancreatic' glucagon levels in the six patients in which both pre- and post-surgical samples were measured.

Glucagon plays a key physiological role in the maintenance of fasting and postprandial glucose concentrations by stimulating hepatic glucose production (reviewed in Dunning and Gerich [23]). Inappropriately elevated plasma glucagon concentrations have been reported in humans with diabetes [24]. Future studies employing more direct methods for determining insulin sensitivity and hepatic glucose production will be required to determine the specific role of glucagon in the resolution of insulin resistance after RYGBP.

Although we did not have an opportunity to examine meal-induced gastrointestinal hormone responses in the present study, there is a growing consensus that changes in their secretion are likely to contribute to improvement of insulin sensitivity after RYGBP, and may do so prior to significant weight loss [25]. For example, postprandial GLP-1 levels increase as early as 2 days after RYGBP [26]; and in addition to its potential effects to stimulate insulin secretion, GLP-1 has actions to suppress endogenous glucose production, promote glucose uptake, and importantly, to reduce glucagon secretion [27]. Interestingly, our results suggest that the decrease in fasting glucagon concentrations may be specific for RYGBP, as another group has reported that they did not change in 13 patients who lost 25% of body weight after vertical gastric banding or jejunoileal bypass [18]. We did measure fasting total GLP-1 concentrations in the present study; however, no significant changes were observed (data not shown). Nonetheless, it is likely that postprandial GLP-1 secretion was increased, and it is possible that repeated intermittent increases of GLP-1 in the postprandial period contributed to a sustained decrease of alpha cell function and reduced pancreatic glucagon release.

Reduced fasting PP concentrations were also observed after RYGBP. While this has been reported in one study [28], another reported no change of PP after surgery [22]. This discrepancy may be due to small sample sizes or differences in surgical technique. PP is released from pancreatic F cells in response to food intake, gastrointestinal hormones and neuropeptides. Its most important regulator, however, is cholinergic vagal stimulation. Consequently, plasma PP concentrations are useful as a marker of pancreatic parasympathetic activity [29]. Our results suggest that this declines shortly after RYGBP, consistent with reductions of insulin and glucagon, since the secretion of these hormones is also regulated by the parasympathetic input to the islet [30]. These results suggest at least a partial loss of the vagal input to the pancreas following RYGBP.

The second major finding was that RYGBP reduced concentrations of most of the adipocyte hormones and inflammatory molecules studied: leptin, hsCRP, ASP, IL-6 and sICAM-1.

Visfatin is a secreted protein produced at high levels in visceral fat, and its plasma concentration is correlated with visceral adiposity [6]. Accordingly, plasma concentrations of visfatin are higher in humans with metabolic syndrome than in normal weight control individuals [31]. Visfatin was initially reported to have insulin-mimetic and adipogenic actions [6], but these results were not supported by a follow-up study [32]. However, in addition to reduced circulating visfatin concentrations, female mice heterozygous for visfatin deficiency exhibit impaired glucose tolerance, and islets isolated from these mice have reduced glucose-stimulated insulin secretion [32]. To date, studies examining changes in visfatin with weight loss have reported conflicting results: one study reported decreased visfatin following RYGBP [33], while a subsequent study reported an increase [34]. These discrepancies may be partially explained by the use of a C-terminal assay for visfatin that has recently been shown to lack specificity [35]. Employing an ELISA that instead detects full-length visfatin [35], we found: (1) visfatin concentrations were positively associated with body weight and waist circumference prior to surgery; (2) visfatin decreased progressively after RYGBP; and (3) reductions of visfatin were independently associated with the changes of fasting insulin concentrations and HOMA-IR (Fig. 3d and Table 3). These results are consistent with the hypothesis that visfatin is over-produced in severe obesity, possibly to compensate for insulin resistance by either increasing adipose mass or augmenting glucose-stimulated insulin secretion. It follows that the need for such a mechanism would be reduced in the weight-reduced state.

The role of the pro-inflammatory cytokine IL-6 in the insulin resistance associated with human obesity remains a subject of debate, as it has been reported to exert some

insulin-sensitising effects in both healthy human individuals and those with type 2 diabetes [36]. Nonetheless, circulating IL-6 concentrations have been reported to be two- to fourfold higher in obese, type 2 diabetic patients than in normal weight individuals [37] and to decrease with weight loss [38]. In the present study, there were strong positive correlations between circulating IL-6 concentrations and measures of body adiposity prior to RYGBP. Furthermore, IL-6 concentrations were reduced by 43±7% 12 months after surgery. At this time, IL-6 concentrations were positively correlated with central adiposity, fasting insulin and HOMA-IR. Last, there was a trend (p=0.056) for a relationship between the changes of IL-6 and the relative improvement in HOMA-IR, independent of age and changes of NEFA and HMW adiponectin (Table 3 and Fig. 3g). As proposed previously [38], in obesity, adipose tissue is likely to be a major contributor to elevated IL-6 concentrations, and the underlying stimulus for its production is removed by weight loss. In the weight-reduced state, subsequently, the relationship between IL-6 concentrations and insulin resistance is re-established.

Changes in RBP4 were also examined in the present study, at 1 and 12 months after RYGBP. Serum RBP4 concentrations are elevated in overweight/obese humans and positively correlated with BMI and insulin resistance [39]. In the present study, however, we found no evidence linking RBP4 with RYGBP-induced improvements in insulin sensitivity. Although RBP4 concentrations decreased by nearly 30% 1 month after surgery, at 12 months there was no significant decrease relative to pre-surgical levels, despite marked weight loss and improved insulin resistance.

Studies of RBP4 concentrations during weight loss have shown significant reductions occur with weight loss of >5%. One study [40] reported that serum RBP4 concentrations in severely obese humans decreased by ~26% at 6 months after gastric banding, when participants had lost 13% of their initial weight. In patients undergoing vertical banded gastroplasty, a similar reduction of RBP4 concentrations (~20% after 1 month) was reported, although it was not stated how much weight the patients lost [41]. In healthy, insulin-sensitive menopausal women, however, weight loss of 5% over 13 weeks, achieved by exercise and reduction of energy intake by 2,510 kJ/day (600 kcal/day), did not change serum RBP4 concentrations, despite a 20% decrease of fasting insulin [42]. We also measured RBP4 concentrations in 14 healthy women before and after a 7 day period of energy restriction to 2,635 kJ/day (630 kcal/day) [43], during which the women lost 4% of their body weight and plasma insulin concentrations decreased by 46%. There was no significant change of fasting RBP4 concentrations (before, 46.2±5.9 mg/l; after, 40.9±4.9 mg/l; p=0.33).

Moreover, there is some evidence to suggest that weight loss-mediated reductions in RBP4 concentrations may not be sustained in the long-term (12 months). Similar results to ours have been reported recently in a study of obese women consuming a very low energy diet (3,350 kJ [800 kcal/day]) for 4 weeks: a ~7% reduction in body weight was associated with a 15–20% decrease in plasma RBP4 concentrations [44]. Interestingly, an additional 3% weight loss in these patients, achieved by a low-energy diet for 8 weeks followed by a weight-maintaining diet for 12–16 weeks, resulted in a modest increase of RBP4 concentrations, although they were still reduced compared with initial levels. This and other evidence [45] suggests that changes in RBP4 concentrations may be more indicative of energy intake than changes in body weight per se. It should also be noted that adipose tissue is not the sole source of RBP4, as it is most highly produced in liver, with adipose tissue levels being about 20% of hepatic levels [46].

Last, as we previously reported for total adiponectin concentrations [7], pre-surgical concentrations of HMW adiponectin independently predicted weight loss following RYGBP, with higher concentrations associated with greater weight loss. Pre-operative HMW adiponectin concentrations also predicted loss of fat mass. Adiponectin stimulates fatty acid oxidation in liver and skeletal muscle [47], so it is reasonable to hypothesise that a higher initial concentration could enhance fat oxidation during negative energy balance; however, this hypothesis will need to be addressed in future studies.

The present study was designed to be a comprehensive investigation of the longitudinal endocrine and metabolic changes following RYGBP in severely obese women, with an emphasis on pancreatic and adipocyte hormones and markers of inflammation. While the contribution of gastrointestinal hormones to improvements of insulin resistance after RYGBP has been more extensively studied, the present findings suggest that hormones from the pancreas (glucagon and PP in particular) and adipose tissue (visfatin and HMW adiponectin) may contribute to the resolution of insulin resistance in the long term. Future studies, involving larger numbers of patients of both sexes as well as mechanistic studies, will be required to fully determine the contributions of each of these hormones. Accordingly, appropriate enhancement or blocking of the effects of specific hormones may lead to the development of new, non-surgical approaches for medical management of the co-morbidities associated with severe obesity.

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