Original article

Effects of biliopancreatic diversion on diurnal leptin, insulin and free fatty acid levels

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Background: Free fatty acid (FFA) levels are raised in obesity as a consequence of increased production and reduced clearance. They may link obesity with insulin resistance. Bariatric surgery can result in considerable weight loss and reduced insulin resistance, but the mechanism of action is not well understood. Although drugs such as metformin that lower insulin resistance can contribute to weight loss, a better understanding of the links between obesity, weight loss and changes in insulin resistance might lead to new approaches to patient management.

Methods: Variations in circulating levels of leptin, insulin and FFAs over 24 h were studied in severely obese (body mass index over 40 kg/m^2) women before and 6 months after biliopancreatic diversion (BPD). Body composition was measured by dual-energy X-ray absorptiometry. A euglycaemic–hyperinsulinaemic clamp was used to assess insulin sensitivity. Levels of insulin, leptin and FFAs were measured every 20 min for 24 h. Pulsatile hormone and FFA analyses were performed.

Results: Among eight patients studied, insulin sensitivity more than doubled after BPD, from mean(s.d.) 39·78(7·74) to 96·66(27·01) mmol per kg fat-free mass per min, under plasma insulin concentrations of 102·29(9·60) and 93·61(9·95) μunits/ml respectively. The secretory patterns of leptin were significantly different from random but not statistically different before and after BPD, with the exception of the pulse height which was reduced after surgery. Both plasma insulin and FFA levels were significantly higher throughout the study day before BPD. Based on Granger statistical modelling, lowering of daily FFA levels was linked to decreased circulating leptin concentrations, which in turn were related to the lowering of daily insulin excursions. Multiple regression analysis indicated that FFA level was the only predictor of leptin level.

Conclusion: Lowering of circulating levels of FFAs after BPD may be responsible for the reduction in leptin secretion, which in turn can decrease circulating insulin levels.

Surgical relevance

Insulin resistance is a common feature of obesity and type II diabetes. These patients are also relatively insensitive to the biological effects of leptin, a satiety hormone produced mainly in subcutaneous fat.

Biliopancreatic diversion, a malabsorptive bariatric operation that drastically reduces circulating lipid levels, improves insulin resistance independently of weight loss. The mechanism of action, however, has still to be elucidated.

This study demonstrated that normalization of insulin sensitivity after bariatric surgery was associated with a reduction in 24-h free fatty acid concentrations and changes in the pattern of leptin peaks in plasma. Bariatric surgery improves the metabolic dysfunction of obesity, and this may be through a reduction in circulating free fatty acids and modification of leptin metabolism.

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Introduction

Insulin resistance is an independent risk factor for cardiovascular diseases and type II diabetes, and is often associated with obesity[1]. Obesity has reached epidemic proportions in many parts of the world. In the USA the number of overweight and obese individuals exceeds two-thirds of the adult population[2]. From the mechanistic point of view, free fatty acids (FFAs) have been implicated in the link between obesity and insulin resistance[3]. Circulating levels of FFAs are increased in obese subjects as a consequence of increased production and reduced clearance, along with reduced hormone-sensitive lipase activity and blunted stimulated lipolysis[4,5]. Raised FFA levels decrease the ability of insulin to suppress hepatic glucose output and to promote peripheral glucose uptake, which are major features of insulin resistance[6,7].

It is known that an acute increase in FFA levels during the infusion of heparinized triglyceride emulsions induces insulin resistance[8–10]. In contrast, overnight reduction of FFA levels with acipimox, a drug used to treat hypertriglyceridaemia, improves insulin resistance and glucose tolerance in obese subjects[11]. It seems that this nocturnal increase in FFA level is relevant to the development of insulin resistance and subsequent increase in plasma insulin levels, as shown by the effect of a high-fat diet in dogs[12]. On the other hand, insulin is a well known antilipolytic hormone, inhibiting systemic, leg and splanchnic palmitate release in a dose-dependent manner[13]. It is still not clear whether the improvement in insulin sensitivity that follows bariatric surgery can be driven by lowering circulating levels of FFA. If it can be shown that the reduction in plasma FFA levels is relevant in improving insulin resistance after biliopancreatic diversion (BPD), other types of bariatric surgery might then be modified to increase lipid malabsorption, such as increasing the length of the biliopancreatic limb in a gastric bypass.

Fat is a metabolically active tissue that synthesizes and secretes a large number of biologically active peptides, including leptin. Leptin is involved in fat storage, energy intake and energy expenditure[14]. Data on leptin after bariatric surgery are inconsistent, compared with those for most other gastrointestinal hormones. Some studies[15,16] have demonstrated significantly lower fasting leptin concentrations after Roux-en-Y gastric bypass compared with before surgery, whereas others[17–19] found no difference.

No studies have examined the temporal relationships between leptin, insulin and FFA levels after BPD. The aim of this study was, therefore, to measure diurnal variations in the circulating levels of leptin, insulin and FFA before and after malabsorptive bariatric surgery, in the hope that a better understanding of these relationships could modify existing approaches to bariatric surgery or stimulate novel pharmacological strategies.

Methods

The study group consisted of severely obese (body mass index (BMI) over 40 kg/m^2) women, investigated before and 6 months after BPD. All subjects were studied during the follicular phase of their menstrual cycle and were not taking oral contraceptives. None had impaired glucose tolerance, or other significant disease. At the time of the baseline study, all subjects were on an *ad libitum* diet. The nature and purpose of the investigation were explained to all subjects before they agreed to participate in the study, which complied with the guidelines of the hospital ethics committee. Subjects were studied randomly on two separate days, once for the assessment of hormone secretion during a standardized 24-h period and once for the determination of insulin sensitivity using the glucose clamp method.

The protocol was approved by the ethics committee of the Catholic University of Rome. All participants provided written informed consent to participate in the study. Additional written informed consent was obtained before the surgical procedures.

Biliopancreatic diversion

BPD consisted of about a 60 per cent distal gastric resection with stapled closure of the duodenal stump[20,21]. The residual volume of the stomach was approximately 300 ml. The small bowel was transected at 250 cm from the ileocaecal valve and the distal end anastomosed to the remaining stomach. The proximal end of the ileum, comprising the remaining small bowel carrying the biliopancreatic juice and excluded from food transit, was anastomosed in an end-to-side fashion to the bowel 50 cm proximal to the ileocaecal valve. Consequently, the total length of absorbing bowel was 250 cm, the final 50 cm of which represented the common channel, where ingested food and biliopancreatic juices were able to mix.

Body composition

Total body composition was determined by dual-energy X-ray absorptiometry using a Lunar Prodigy^{TM} whole-body scanner (GE Medical Systems, Madison, Wisconsin, USA). The subjects were scanned in light clothing lying flat on their back and with arms by their sides. Fat mass and fat-free mass were obtained in kilograms. Waist was measured over the unclothed abdomen at the navel. Two measurements were made and the mean (expressed in centimetres) was used for analyses.

Euglycaemic–hyperinsulinaemic clamping

Peripheral insulin sensitivity was evaluated by the euglycaemic–hyperinsulinaemic clamp method[22]. A cannula in a dorsal hand vein was used to sample arterialized venous blood and another in the antecubital fossa of the contralateral arm for infusions. Subjects rested in the supine position for at least 1 h, and one hand was warmed in a heated air box set at 60°C to obtain arterialized blood samples. Whole-body glucose uptake (*M* value) in micromoles per kilograms of fat-free mass per minute was determined during a primed constant infusion of insulin, at a rate of 6 pmol per min per kg. The fasting plasma glucose concentration was maintained throughout the insulin infusion by means of a variable glucose infusion and blood glucose determinations every 5 min. Whole-body peripheral glucose utilization was calculated during the last 40-min period of the steady-state insulin infusion.

Twenty-four-hour studies

During the study day, all subjects were assigned a diet with an energy content of 30 kcal per kg fat-free mass consisting of 55 per cent carbohydrate, 30 per cent fat and 15 per cent protein, shared between 20 per cent at breakfast, 40 per cent at lunch, 10 per cent as an afternoon snack and 30 per cent at dinner. The four meals prepared by a dietician using common foods included meat, fish, vegetables, bread and fruit. Food given and returned was weighed to the nearest gram on precision scales (KS-01; Rowenta, Berlin, Germany). Blood samples were drawn every 20 min from a central venous catheter for the measurement of glucose, insulin, leptin and FFA concentrations.

Analytical methods

Analysis of plasma samples

Plasma samples were centrifuged immediately and stored at −80°C before analyses. Plasma glucose was measured by the glucose oxidase method (Beckman, Fullerton, California, USA). Plasma insulin was assayed by microparticle enzyme immunoassay (Abbott, Pasadena, California, USA) with a sensitivity limit of 1 μunit/ml. Plasma leptin concentrations were measured by a double antibody radioimmunoassay (Human Leptin RIA Kit; Linco Research, St Charles, Missouri, USA) with a sensitivity limit of 0·5 ng/ml. Plasma FFA level was assessed by a colorimetric method[23]. All samples were measured in duplicate.

Pulse analysis

To measure the positions, heights and widths of peaks in the 24-h time series of leptin, insulin and FFAs, the first derivative of the signals was smoothed, before looking for downward-going zero crossings. Only those zero crossings whose slope exceeded the slope threshold at a point where the original signal exceeded the amplitude threshold were taken. This procedure was employed to take account of the presence of random noise in the experimental signals. A least squares curve fitting of segments of the original unsmoothed signal in the vicinity of the zero crossing was used, so that the peak parameters extracted by curve fitting were not distorted.

Diurnal variability analysis

A semiparametric linear mixed-effects approach[24] was used to look for diurnal variations. The hormone or FFA time series were modelled using a simple harmonic function[25].

Cross-correlation analysis

The temporal relationship between the hormone and FFA profiles was quantified using cross-correlation analysis. Synchronicity of the pulses among the insulin, FFA and leptin time series was assessed independently by cross-correlation analysis and cross-approximate entropy. Cross-correlation analysis assesses the tendency of two time series to co-vary in the same or opposite directions simultaneously with either a positive or negative lag, and thus estimates the overall coordinated behaviour of the two series. Displacement of the first peak from lag zero in either direction is a direct measure of the phase difference between the reference and experimental periodicities. Cross-correlation was calculated after lagging the concentration time series of one hormone relative to the concentration time series of the other hormone or FFA.

Approximate entropy

The value of entropy is high (approximate to 1) when time is random, whereas it is low (approximate to 0) in deterministic time series. Entropy therefore gives information about the level of determinism or randomness of time series. Approximate entropy was applied to sequential measures, including hormone time series[26]. Details are provided elsewhere[27].

Granger causality test

The leptin, insulin and FFA data were fitted by vector autoregression followed by the Granger causality test[28], which implies a correlation between the current value of one variable and the past values of others.

Statistical analysis

Unless otherwise specified, data are expressed as mean(s.d.). Intragroup comparisons were performed

Table 1 Body mass index, body composition, glucose uptake and insulin concentration during the last 40 min of clamping

	Before BPD	After BPD
Body mass index (kg/m^2)	51·20(7·95)	38·94(8·34)‡
Fat mass (kg)	68·68(9·85)	45·02(13·01)‡
Free fat mass (kg)	68·47(21·26)	58·79(15·43)†
Waist circumference (cm)	141·12(17·07)	118·62(14·82)‡
M value (mmol per kg fat-free mass per min)*	39·78(7·74)	96·66(27·01)‡
Clamp insulin (μunits/ml)	102·29(9·60)	93·61(9·95)

Values are mean(s.d.). *Glucose uptake. BPD, biliopancreatic diversion. †$P < 0·020$, ‡$P < 0·010$ *versus* before BPD (Wilcoxon test).

by Wilcoxon test. Spearman's correlation coefficients were used to study relationships between plasma leptin and percentage body fat and with other variables. Multiple linear regression analyses with a stepwise forward procedure were used to determine predictors of the 24-h leptin concentrations. Significance was set at $P < 0·050$. All analyses were performed using STATA® version 10 (StataCorp LP, College Station, Texas, USA).

Results

Eight patients aged 34(4) years were studied. At 6 months after BPD, the patients had a large reduction in BMI, from a mean of 51·2 to 38·9 kg/m^2, related principally to fat mass loss but also to a significant decrease in fat-free mass (*Table 1*). Waist circumference was greatly reduced. Insulin sensitivity (*M* value) was more than doubled in spite of similar insulin levels during the last 40 min of the euglycaemic clamp (*Table 1*).

Circulating leptin concentrations were higher before BPD throughout the 24-h period (24-h mean 51·94(9·21) ng/ml before *versus* 15·04(4·80) ng/ml after BPD; $P = 0·012$) (*Table 2*). Circulating levels of leptin, insulin and FFA had significant pulsatility, with diurnal and ultradian rhythms, before and after BPD (*Table 2*, *Figs 1–3*).

When evaluated by approximate entropy, the secretory patterns of leptin were significantly different from random, although the normalized means were not statistically different before and after surgery (0·781(0·039) *versus* 0·754(0·040) ng/ml. Leptin concentrations had similar pulse frequencies, interpeak intervals and relative increments before and after surgery, although the pulse height was significantly lower after BPD ($P = 0·017$) (*Table 2*). Circulating plasma leptin levels showed a diurnal pattern, with a nadir occurring in the late afternoon before BPD and in the early afternoon after BPD, and a peak at around 22.00 hours before and 04.00 hours after BPD (*Fig. 1*).

Insulin levels and hormonal excursions were much higher before than after BPD, throughout the entire day (*Fig. 2*). Insulin pulse frequency and interpeak interval were significantly increased after BPD, whereas the pulse height was reduced threefold (*Table 2*).

Plasma FFA levels throughout the day were significantly higher before than after BPD ($P < 0·010$) (*Table 2*, *Fig. 3*).

A strong positive cross-correlation between plasma FFA and leptin was observed, with FFA preceding leptin release by 20–40 min. A negative cross-correlation was observed between leptin and insulin, whereby the leptin time series led the insulin time series by 20–60 min.

A significant relationship was found between mean plasma leptin and insulin concentrations ($R^2 = –0·68$, $P < 0·001$), and between FFA and leptin levels ($R^2 = 0·48$, $P < 0·001$), after BPD. After BPD, a significant inverse relationship was found between insulin and FFA ($R^2 = –0·28$, $P < 0·001$).

Spearman's ρ coefficients between leptin concentration and insulin sensitivity (*M* values), insulin level and insulin sensitivity, and FFA concentration and insulin sensitivity were –0·76 ($P = 0·028$), 0·74 ($P = 0·037$) and –0·97 ($P < 0·001$) respectively. When these variables were entered into a linear multiple regression analysis ($R^2 = 0·46$, $P = 0·010$), FFA concentration was the only significant predictor of leptin level (where leptin level = –148·78, FFA level = +51·48). Insulin sensitivity (coefficient –0·073, $P = 0·871$) and insulin concentration (coefficient 0·43, $P = 0·230$) were excluded from this equation.

Table 2 Twenty-four-hour dynamics of leptin, insulin and free fatty acids before and after biliopancreatic diversion

	Leptin (ng/ml)		Insulin (μunits/ml)		Free fatty acids (mmol/l)	
	Before BPD	After BPD	Before BPD	After BPD	Before BPD	After BPD
Mean hormone concentration	51·94(9·21)	15·04(4·80)†	54·84(6·51)	10·03(1·48)‡	496·75(18·23)	244·90(7·71)‡
Pulse frequency (per 24 h)	17·25(1·06)	19·12(0·44)	16·37(0·42)	19·25(0·41)*	17·75(0·62)	17·63(0·42)
Interpeak interval (min)	70·15(4·12)	79·68(1·84)	68·23(1·75)	80·21(1·72)†	73·96(2·58)	73·44(1·75)
Pulse height concentration	10·56(2·73)	4·85(2·56)†	47·59(13·83)	13·90(1·72)†	148·23(23·09)	123·68(15·89)
Relative increment (%)	5·01(1·19)	2·45(0·72)	47·65(21·18)	45·25(16·71)	44·26(17·47)	13·69(2·06)
Valley concentration	6·31(1·99)	2·94(0·78)	25·37(8·25)	5·72(1·21)†	211·94(38·91)	94·05(24·84)

Values are mean(s.d.). BPD, biliopancreatic diversion. *$P < 0·050$, †$P < 0·020$, ‡$P < 0·010$ *versus* before BPD (Wilcoxon test).

Fig. 1 Time course of leptin concentration measured for 24 h before and after biliopancreatic diversion (BPD) in each subject

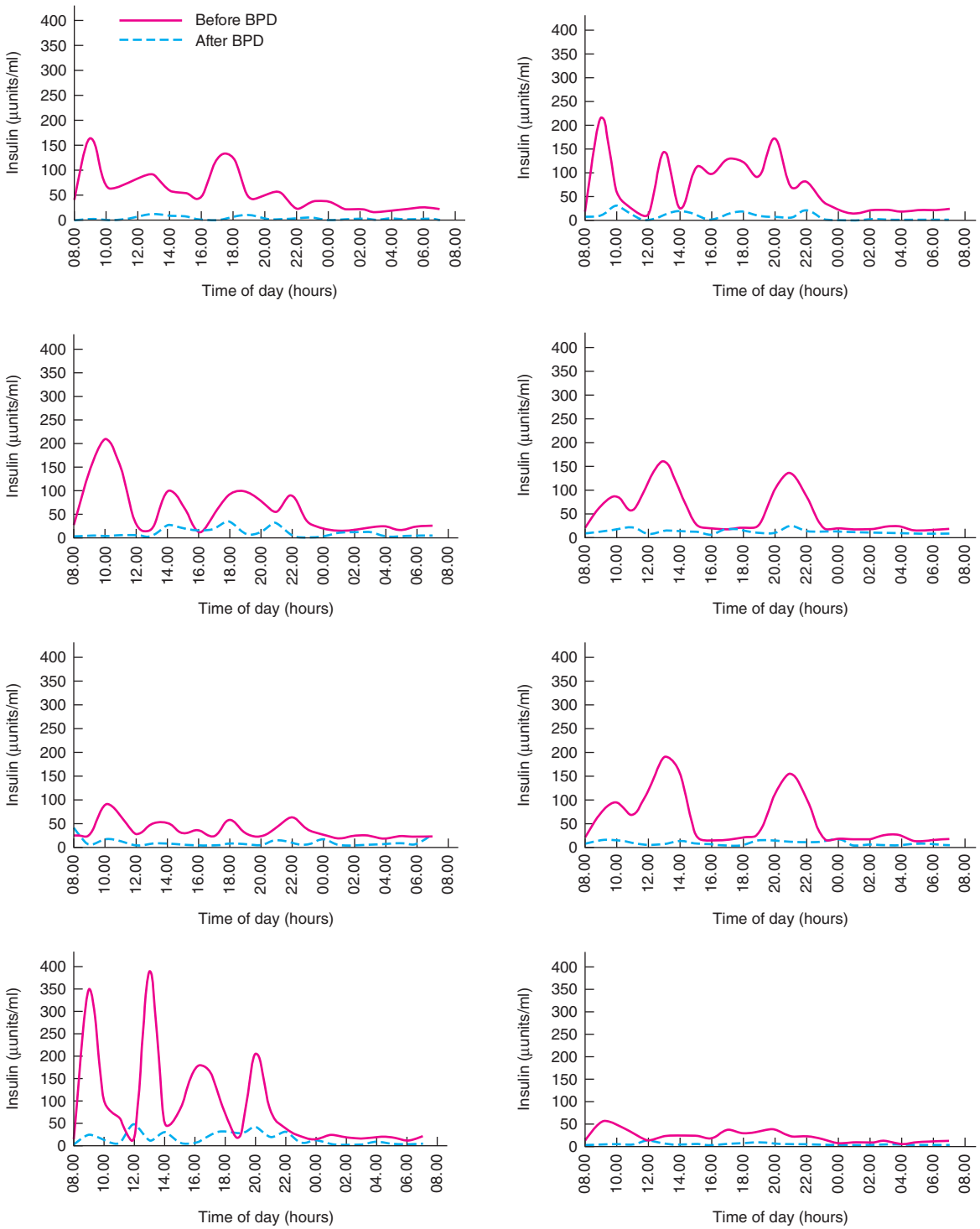

Fig. 2 Time course of insulin concentration measured for 24 h before and after biliopancreatic diversion (BPD) in each subject

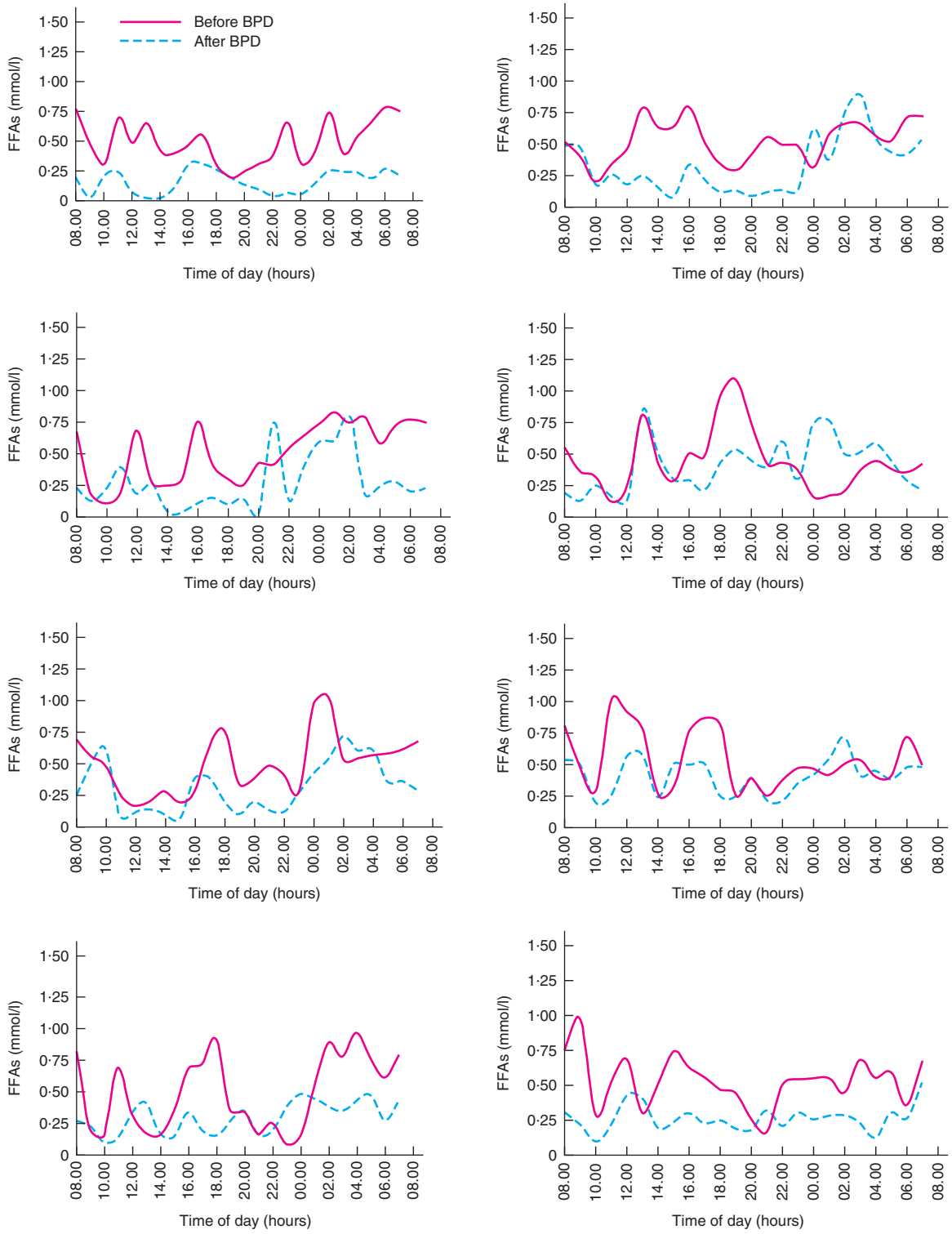

Fig. 3 Time course of free fatty acid (FFA) concentration measured for 24 h before and after biliopancreatic diversion (BPD) in each subject

The Granger causality test predicted that FFAs influenced daily leptin levels and that daily leptin levels influenced 24-h insulin concentrations.

Discussion

The major finding of this study was that the 24-h circulating levels of leptin had pulsatile patterns both before and after BPD that were related to FFA levels. Approximate entropy indicated low values of entropy for day and night leptin levels, suggesting a certain degree of regularity in the data both before and after BPD. The diurnal variation as well as the pulsatility of leptin secretion were entrained to meal patterns, in agreement with previous data[29].

Based on Granger statistical modelling, lowering of daily FFA levels was linked to decreased circulating leptin concentrations which, in turn, were related to the lowering of daily insulin excursions. A positive correlation between 24-h circulating levels of leptin and FFAs, and a negative correlation between 24-h concentrations of insulin and FFAs, were found after BPD, when insulin sensitivity was restored.

The presence of a significant inverse correlation between insulin and FFA levels only after BPD suggests that the normalization of insulin sensitivity restores regulation of lipolysis. In contrast, the insulin resistance present before BPD might be accounted for by increased lipolysis and a raised FFA level.

BPD is mainly a malabsorptive procedure causing steatorrhoea. It has been demonstrated previously[30] that it also causes a marked reduction in circulating lipid levels and fat deposition. The reduction in plasma FFA levels is therefore probably attributable to the reduced fat absorption.

In contrast to both FFA and leptin levels, circulating insulin levels were lowest during the night, particularly after BPD. Based on the Granger causality test, leptin influenced the insulin pattern whereas the opposite did not hold, suggesting that leptin might control insulin secretion. Leptin consistently reduces insulin secretion in isolated pancreatic β-cells[31] and in perfused pancreas[32] of *ob*/*ob* mice. The acute increase in plasma leptin concentration in rats suppresses glucose-stimulated insulin secretion in a dose-dependent manner[33]. Chronic hyperleptinaemia is proposed to uncouple the action of leptin on its receptor in the hypothalamus, thereby attenuating signal transduction pathways that exert resistance to this hormone on satiety and energy expenditure and metabolism[34]. Therefore, high leptin levels associated with leptin resistance, as observed before BPD, may not contribute to the modulation of insulin secretion, whereas the action of leptin on insulin secretion control is restored after BPD.

As shown elsewhere[35] following major weight reduction, a significantly lower relative amplitude of leptin concentration was seen after BPD.

The present study has limitations. Only women were included so the data may not be applicable to men, who generally have lower leptin levels than women. Patients with impaired glucose tolerance were not included, so the clinical application is somewhat limited. The type of surgery may also have played a specific role in the results, and data from other types of surgery might help in the interpretation of the present findings.

Lowering circulating levels of FFA by BPD may induce a reduction in leptin secretion which, in turn, can decrease insulin secretion. Modification of other bariatric operations, such as gastric bypass, by increasing the length of the biliopancreatic limb to promote lipid malabsorption, or pharmacological treatments aimed at reducing circulating FFA levels, might allow similar results in terms of reducing insulin resistance.

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