Aus der Medizinischen Klinik und Poliklinik II
der Ludwig-Maximilians-Universität München

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The mechanisms of improved glucose metabolism after weight reduction in morbidly obese patients with type 2 diabetes mellitus: effects of a diet simulating the situation after bariatric surgery

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LIST OF ABBREVIATIONS

AIRg	Acute phase of insulin response to hyperglycemia
BMI	Body mass index
DI	Disposition index
DPP-IV	Dipeptidyl peptidase - 4
EGC	Euglycemic clamp
GIP	Glucose - dependent insulinotropic polypeptide
GIR	Glucose infusion rate
GLP-1	Glucagon - like peptide - 1
HDL	High density lipoprotein
HGC	Hyperglycemic clamp
HOMA-IR	The homeostasis model assessment of insulin resistance
ISI	Insulin sensitivity index
LAGB	Laparoscopic adjustable gastric banding
LDL	Low density lipoprotein
Lp(a)	Lipoprotein (a)
LSG	Laparoscopic sleeve gastrectomy
RYGB	Roux en Y gastric bypass
T2DM	Type 2 diabetes mellitus
TG	Triglyceride
VLDL	Very low density lipoprotein
WC	Waist circumference

1. INTRODUCTION

1.1. Obesity

Obesity is characterized by an excessive fat accumulation that may harm health. It is defined by a body mass index (BMI) greater than or equal to 30 kg/m^2. Prevalence of obesity is increasing quickly and becomes a major challenge in the world. WHO estimated about 10 % of men and 14 % of women having obesity (BMI ≥30 kg/m^2) in 2008 [1]. Obesity is the major risk factor of many comorbidities such as type 2 diabetes mellitus (T2DM), dyslipidemia, hypertension, heart diseases, non-alcoholic fatty liver disease, cancers, sleep apnea, bone-joint diseases, dementia, psychosocial dysfunctions [2]. The classification of obesity in adults is determined by BMI that is calculated by weight in kilograms divided by height in meters squared [weight (kg) / height (m^2)] (Table 1).

Table 1: Classification of obesity in adults by BMI (2000, WHO, Geneva)

Classification of obesity	**BMI**	**Risk of comorbidities**
Underweight	< 18.05	Low (but risk of other clinical problems increased)
Normal range	18.05-24.99	Average
Overweight	≥ 25.00	
Overweight	25.00-29.99	Increased
Obese class I	30.00-34.99	Moderate
Obese class II	35.00-39.99	Severe
Obese class III	≥ 40	Very severe

1.2. Type 2 diabetes mellitus

Diabetes mellitus is a metabolic disorder characterized by elevation of plasma glucose which may be the result of a variety of pathologies. The majority (> 90 %) of patients with diabetes suffer from diabetes type 2. In these patients, chronic hyperglycaemia often associated with disturbances of carbohydrate, fat and protein metabolism result from defects of insulin secretion, insulin action or both. It was accounted that more than 371 million people in the

world had diabetes in 2012 and half of this number was not diagnosed. It is estimated that the number of diabetic patients will be over 552 million by 2030 and will increase in every country in the world [3]. The increase in T2DM prevalence is closely related to an increase in obesity. The prevalence of T2DM in obese patients is much higher than that in normal weight patients. The physiological abnormalities of glucose metabolism in T2DM commonly include insulin resistance, β-cell defects and abnormalities of insulin secretion such as first-phase and second-phase secretion [4]. Insulin resistance can be defined as the inability of insulin to fully perform its normal biological functions at circulating concentrations that are well effective in healthy subjects. Obesity leads to insulin resistance that precedes and predicts T2DM. Insulin resistance manifests itself not only in impaired inhibition of hepatic glucose production but also in decreased peripheral uptake of glucose. In healthy subjects hepatic glucose production is suppressed almost completely after a meal by the increase in plasma insulin and glucose concentrations. This complete suppression of hepatic glucose production helps to keep postprandial glucose concentration in the normal range. The impaired suppression of hepatic glucose production resulting from insulin resistance is one factor leading to the increase of postprandial blood glucose in T2DM. Evidence from many studies indicate that in insulin resistance insulin also fails to inhibit very low density lipoprotein (VLDL) production in the liver which contributes to the increase in serum triglyceride (TG) levels [5]. After a meal, approximately one-third of glucose is taken up by skeletal muscle. The impaired effect of insulin on glucose uptake in skeletal muscle and adipose tissues in T2DM subjects has been established in many studies. This further contributes to postprandial hyperglycaemia [6]. There have been many indexes to assess the insulin resistance such as oral glucose tolerance, the homeostasis model assessment of insulin resistance (HOMA-IR), the insulin sensitivity index (ISI) calculated from the fasting plasma insulin and glucose. However, the index of metabolised glucose during an euglycemic clamp (EGC) is considered gold standard to quantify insulin resistance [7].

In experiments using an acute square wave of hyperglycaemia such as in hyperglycaemic clamp (HGC) experiments but also in in-vitro experiments insulin response occurs in two phases. The acute phase of insulin response to hyperglycemia (AIRg) occurs immediately and lasts for approximately ten minutes. The second-phase is followed by the gradual increase of insulin secretion and continues as long as hyperglycemia persists (Figure 1). The AIRg relates to the exocytosis of insulin containing granules located next to the plasma membrane of β cells. The exocytosis is a very complex mechanism in which intracellular calcium concentration plays a major role. The second phase of insulin secretion relates to the synthesis

of new insulin molecules and the movement of insulin storage granules toward the plasma membrane [8]. For a long time, it was unclear whether ß-cell dysfunction is the underlying pathogenesis of T2DM. Recently, it is believed that impaired ß-cell function and insulin resistance occurs in parallel. As the development of T2DM is considered to pass through five stages, the decrease of ß-cell function can be founded at stage 2. The deterioration in ß-cell function happens further, and as a result, postprandial and fasting glucose concentrations reach levels for diabetic diagnosis at the stage 5. The HGC is considered to be the gold standard for assessing AIRg.

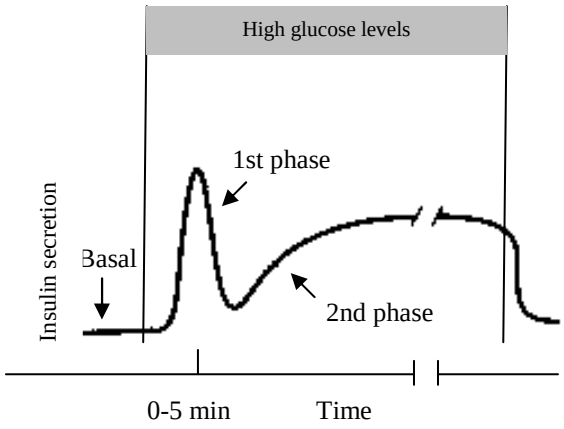


Figure 1: Biphasic insulin secretion in vitro from high glucose perfused pancreas, reproduced from Jones PM et al. [9]**.**

1.3. Obesity and type 2 diabetes mellitus with bariatric surgery

Treatment of T2DM and obesity is challenging and often expensive. Although pharmaceutical treatment of T2DM can improve the prognosis of the affected patients, the long-term treatment of the underlying obesity is often problematic for both patients and care-takers. Over the last 10 years bariatric surgery has been used widely for the treatment of morbidly obese patients because long-term control of obesity is more substantial than with life-style interventions or drug therapy. Bariatric surgery can be classified into three main types, purely restrictive procedures including laparoscopic adjustable gastric banding (LAGB) and laparoscopic sleeve gastrectomy (LSG), malabsorptive techniques such as jejunoileal bypass, and the combined restrictive and malabsorptive techniques presented by Roux-en-Y gastric bypass (RYGB). Besides the effects on weight control that is the principle and classic target of bariatric surgery, most of recent studies have shown that bariatric surgery can induce a

substantial rate of T2DM remission or an improvement in impaired glucose tolerance, not specific for the types of intervention. In a systematic review and meta-analysis on 136 full studies, Buchwald H et al. described complete remission of T2DM in over 75 % of patients after bariatric surgery [10]. Although the remission rate of T2DM is impressive following bariatric surgery, it remains unclear whether this results from caloric restriction and weight loss or other additional factors.

1.4. Caloric restriction, weight loss, and other factors mediating the improvement in type 2 diabetes mellitus after bariatric surgery.

1.4.1. Caloric restriction and weight loss

Caloric restriction starts immediately after surgery. Improvement in glucose metabolism can be detected soon after, well before weight reduction occurs. It is therefore plausible that caloric restriction and not weight loss alone mediates the antidiabetic effect. It is known for a long time that the modification of macro-nutrients and of energy amount can affect glucose metabolism [11]. The weight loss-independent effect of caloric restriction on metabolism was also described in the study of Kelley DE et al. when the author tested the role of caloric restriction in seven obese patients with T2DM [12]. Many study groups have highlighted the role of caloric restriction in the improvement in glucose metabolism after both restrictive and malabsorptive procedures [13, 14].

Obesity is a major risk factor of insulin resistance and T2DM. Risk of T2DM is strongly associated with the duration and the degree of obesity. A large body of evidence shows that there is the strong correlation between obesity and T2DM, and any form of weight loss results in the improvement in glucose metabolism [15, 16]. In an evidence report of NIH conducted in 1998, weight loss was recommended to lower high blood glucose levels in overweight and obese patients with T2DM. A modest weight loss, even as low as 10 % of body weight, could lead to a significant improvement in insulin resistance and T2DM [17, 18]. Even little weight gain, on the other hand, can cause hyperinsulinemia and insulin resistance [19]. LAGB and LSG, the purely restrictive procedures, are thought to improve glucose homeostasis mostly by weight loss and restricted food intake. In a study including 143 obese patients undergoing LAGB, Pontiroli AE et al. showed that there was a significant improvement in glucose metabolism and this improvement was proportional to the degree of weight loss [20]. Hady HR et al. studied the outcomes after LSG on 100 obese patients and described that weight loss after LSG is the main mediator of improved glucose metabolism [21]. Many authors also highlight the correlation between weight loss and the T2DM remission after bariatric surgery

with malabsorptive techniques. The significant correlation between the T2DM remission and excess weight loss was documented in the investigation of Sugerman HJ et al. on a cohort of 1025 obese patients (15 % had T2DM) at 1, 5 and 7 years after gastric bypass [22]. Although weight loss without doubt has an important role in the improvement in T2DM after bariatric surgery, a number of studies indicated that there may be other additional factors.

1.4.2. Additional factors

Many current studies show that the antidiabetic effect is present very early after bariatric surgery before any significant weight loss occurs and that it is not specific for any type of intervention. A strong and significant improvement in glucose metabolism just 3 days after LSG was described by Rizzello et al. in a study of 17 T2DM patients with obesity [23]. In the study of Schauer PR et al. on 240 T2DM patients who underwent RYGB and were treated with medications and/or insulin before surgery, 30 % of patients could stop diabetic treatment immediately after discharge from the hospital before any significant weight loss [24]. In a study by Umeda LM et al. conducted in 10 obese T2DM patients it was shown that there was a significant improvement in HOMA-IR index 7 days after RYGB without significant weight loss [25]. Many trials document that the rate of diabetic remission after bariatric surgery in less obese patients (BMI < 35 kg/m^2) is similar to that in morbidly obese patients [26] and therefore the term “bariatric surgery” is currently being replaced by the term “metabolic surgery” when this form of therapy is used to treat diabetes in non-morbidly obese patients. The non-significant association between the rate of T2DM remission and degree of weight loss after bariatric surgery was also described in many studies [23, 27]. The stronger improvement in glucose metabolism after bariatric surgery than after the marked weight loss from other non-surgical interventions indicated weight loss-independent antidiabetic effects of bariatric surgery. Laferrère B et al. designed a study to compare the effects of weight loss by RYGB versus calorie-restricted diet on glucose metabolism in T2DM patients. This study showed that although weight loss was equivalent in both groups, the improvement on postprandial blood glucose was markedly stronger in the RYGB group [28]. All this indicates that diabetic remission after bariatric surgery may be mediated by additional factors and independent to weight loss.

Many studies indicate that hormonal factors, such as ghrelin and incretin hormones, play an important role in mediating this effect.

Ghrelin: Ghrelin is a gastro-intestinal hormone secreted mainly from gastric fundus. While Ghrelin is secreted in the fasting state and suppressed after a meal, it stimulates appetite and

food intake, inhibits pancreatic insulin secretion, increases hepatic glucogenesis, and leads to hyperglycemia [29]. Nearly total resection of the gastric fundus in LSG leads to low serum ghrelin levels which mediate anorexic and antidiabetic effects. However, the effect of RYGB on ghrelin concentration is heterogeneous, which may relate to the manipulation of the vagal nerve during the operation [30]. Obesity and insulin resistance are usually accompanied by low ghrelin levels, which indicates that a lower ghrelin concentration is not enough to resolve obesity and diabetes [31]. Although low levels of ghrelin may contribute to diabetes remission after bariatric surgery, it is not the main component.

Incretins: In 1964, two independent research groups (Mclntyre N and Elrick H) simultaneously showed that the insulin response of pancreatic ß-cells to oral glucose administration was markedly stronger than that to intravenous glucose administration. This great difference of insulin response was called ‘‘incretin effect’’ [32, 33] (Figure 2).

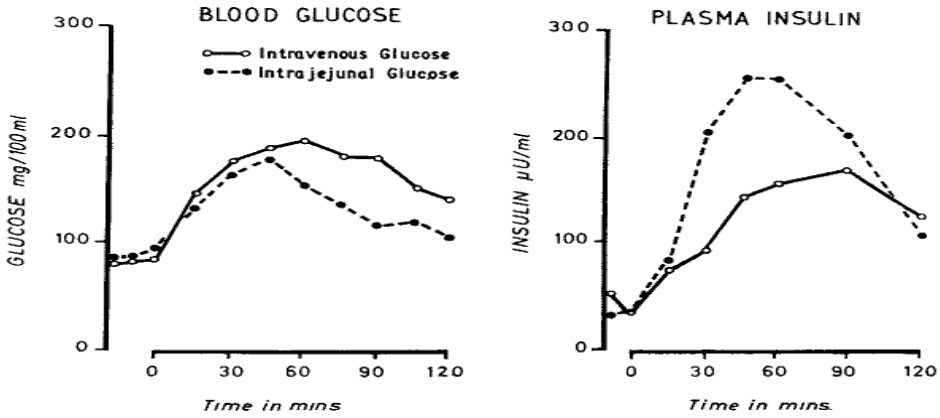


Figure 2: Insulin response to oral and intravenous administration of glucose [32]

Incretin hormones, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are gut peptides that induce a series of physiological responses, especially in glucose metabolism, after a meal. GLP-1, being produced mainly in L cells at the distal small gut and colon, plays the major role in the incretin effect. GLP-1 is formed from proglucagon via processing that requires prohormone convertase-1. Inactive GLP-1(1-37) is processed to bioactive GLP-1(7-36) that is quickly degraded by dipeptidyl peptidase – 4 to GLP-1(9-36) after secreting from the L cells [34]. GLP-1 stimulates the glucose-dependent insulin release via GLP-1 receptor binding, improves both fasting and postprandial blood glucose, retards gastric emptying, inhibits glucagon secretion of α cells,

and induces anorectic effects. GLP-1 also affects the proliferation and the apoptosis of islet ß cells. GIP is released essentially from duodenal K cells, regulates predominately postprandial blood glucose, and has only slight influence on gastric emptying and little anorectic effect (Figure 3). Like GLP-1, bioactive GIP(1-42) is converted to inactive GIP(3-42) by dipeptidyl peptidase - 4 just minutes following the secretion from K cells [35]. Analogues of GLP-1 and GIP as well as dipeptidyl peptidase - 4 inhibitors are used today as a new generation of antidiabetic medications. Under the effect of the incretin hormones that are secreted after the meal, the insulin response after an oral glucose load is greater than that after an isoglycemic intravenous glucose infusion [34].

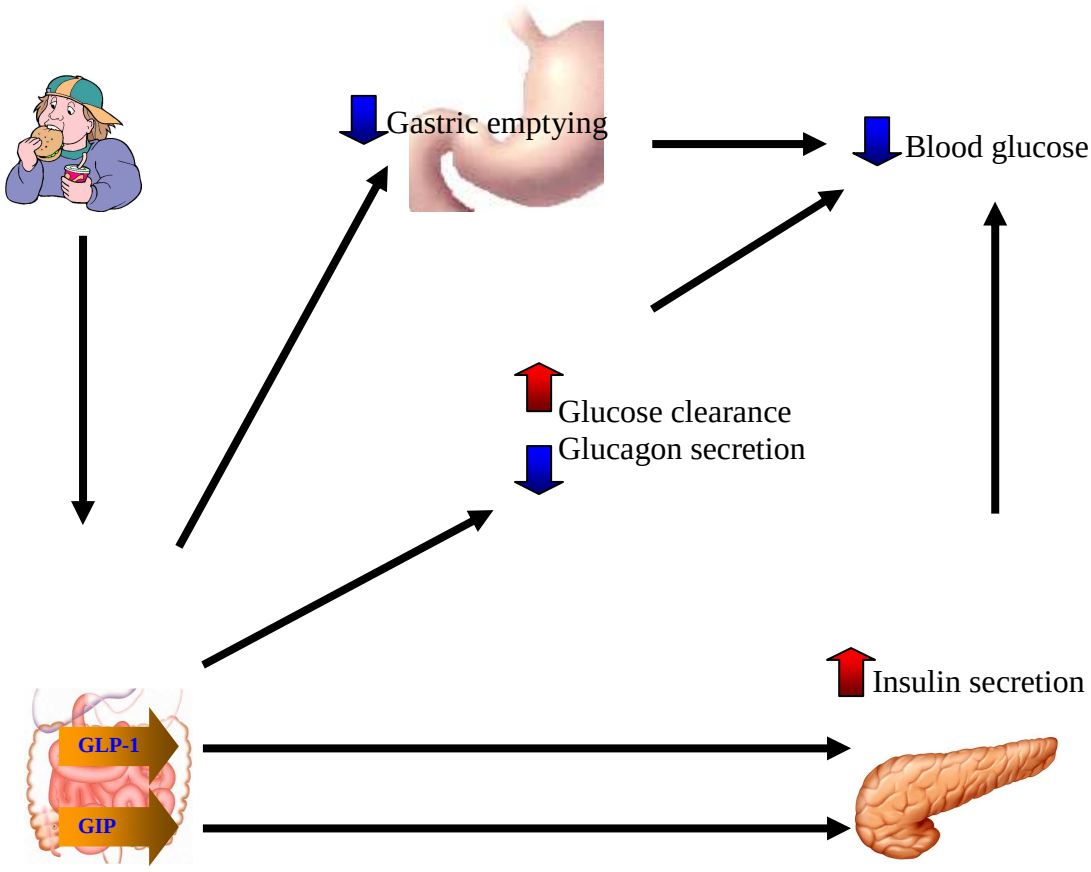


Figure 3: Main effects of incretin hormones on glucose metabolism

In non-diabetic subjects postprandial glucose concentrations are kept at a normal level independent of the amount of glucose in the meal. This is largely related to the incretin effect [36]. Although T2DM patients are characterized by a decreased incretin effect, the degree of

impairment is not constant in various studies, and may relate to duration and severity of diabetes [37]. In individuals with T2DM, postprandial GLP-1 concentrations seem to be decreased, but the effect of exogenous GLP-1 on insulin response persists. Contrary to GLP-1, GIP levels seem to be normal but the effect of GIP on insulin response decreases [38, 39]. An improved IE may play a major role in mediating the antidiabetic effect of bariatric surgery. LSG delays the delivery of ingested foods to the distal portions of the gut and this may lead to an increased stimulation of L-cells and K-cells to release incretin hormones. Two study groups, Valderas et al. and Romero et al., described that postprandial GLP-1 and GIP levels increase significantly after LSG in obese patients [40, 41]. In contrast to LSG, RYGB leads to a status where food bypasses the duodenum. This could theoretically lead to low levels of incretin hormones after surgery. However, the level of incretin hormones and the incretin effect improve significantly after RYGB in many studies. Näslund E et al. studied the response of incretin hormones to the meal in obese patients after jejunoileal bypass. The authors documented that the GLP-1 and GIP response was normalized 9 months after operation. The authors concluded that the improvement in glucose metabolism might result from the increase of incretin hormones [42]. In a study of Laferrère B et al., the authors showed that the greater secretion of incretin hormones and the improved incretin effect early after GBP may be responsible for antidiabetic effects after surgery [28].

The improvement in glucose metabolism after bariatric surgery has obtained a growing attention in the clinical and scientific community. The underlying mediated mechanisms, however, are still unclear. The role of caloric restriction and weight loss in antidiabetic effects after bariatric surgery is still controversial.

2. AIMS OF STUDY

- To evaluate the effect of caloric restriction and weight loss on glucose metabolism and other clinical outcomes.
- To determine the mechanisms underlying the improved glucose metabolism following caloric restriction and weight loss, focussing on:

  + Incretin effect.

  + Intravenous-glucose stimulated insulin response

  + Insulin resistance.

The results of this study will help to explain the role of caloric restriction and weight loss in the observed improvement of glucose metabolism after bariatric surgery.

3. SUBJECTS AND METHODS

3.1. Approval procedure and informed consent

The study is part of a larger project evaluating “Glucose metabolism after sleeve gastrectomy in obese type 2 diabetic patients”. This study was approved by the Ethics Committee of the Medical Faculty of the Ludwig-Maximilians University, Munich, Germany under project number 120-09. The study was funded by German Research Foundation (DFG) under approved number DFG GZ: BR 151/5-1. Our study is a randomized, single centre, un-controlled, prospective trial. All subjects gave written consent after they had received the information about the study and had carefully read the subjects information.

3.2. Subjects

Twelve obese patients with T2DM participated in a calorie-restricted intervention of 3 months that is equivalent to the diet of patients during the first 3 months after laparoscopic sleeve gastrectomy. Glucose metabolism, potential mechanisms mediating the of improved glucose metabolism, lipid metabolism, and other clinical outcomes are assessed before and after caloric restriction.

3.2.1. Inclusion criteria

- Type 2-diabetes mellitus
- Obesity ≥ grade II (BMI ≥ 35 kg/m²)
- Age 18-65 years
- Signed informed consent

3.2.2. Exclusion criteria

- Type 2-diabetes duration of more than 10 years
- Uncontrolled type 2-diabetes: Fasting glucose > 200 mg/dl, or HbA1c > 10 %
- Patients with intensive insulin therapy
- Therapy with thiazolidindions within the last 3 months
- Type 1-diabetes mellitus
- Alcohol abuse (woman > 70 g/week, man > 140 g/week), nicotine abuse or drug abuse
- Hepatic diseases (except non-alcoholic fatty liver disease)
- Renal insufficiency (glomerular filtration rate < 50 ml/min/1.73)

- Cardiac failure > NYHA I
- Uncontrolled thyroid diseases or other endocrinological diseases
- Pregnancy
- Acute or chronic inflammation
- Malignant diseases
- Anemia (Hemoglobin < 12 mg/dl for woman, < 14 mg/dl for man)
- Using anticoagulation medication under bioactive period, except Aspirin 100mg

3.3. Study procedure and methods

The whole study included a screening visit, 3 visits (visit 1, visit 2, and visit 3), and a calorie-restricted intervention (Figure 4). The oral anti-diabetic medications had to be withdrawn 7 days before every visit. GLP-1 analogues and DPP-IV inhibitor were withdrawn at least 4 weeks before visit 1 and were not taken again during the study. The therapy with long-acting insulin analogues was withdrawn the latest one day before every visit. We regularly contacted the subjects after withdrawing of any anti-diabetic medications to make sure that blood glucose was not over 200 mg/dl.

3.3.1. Screening

Potential candidates were recruited from a number of different sources: announcements in public media, flyers and posters, word of mouth. We also recruited potential candidates from the outpatient metabolic clinic of Medical Department 2, Großhadern. First, we talked with potential candidates by telephone to explain the study, to know the disease history, and to check the inclusion and exclusion criteria. Next, potential candidates came for the official screening visit at the Clinical Research Unit in our hospital, where we described again the study in more detail, handed the ‘‘*patients information documents*’’ over to the subjects, asked about the disease history, and examined the patient clinically after she or he had signed the informed consent form. Blood and urine samples were taken for some parameters. If all inclusion criteria were fulfilled and no exclusion criteria present the subject was included in the study. At the screening visit, the subject also participated in a dietary consultation to ensure full compliance with the calorie-restricted intervention during the study. Plan for visit 1 would be done.

The following criteria were used to diagnose T2DM:

- Fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl), or
- 2-h plasma glucose ≥ 11.1 mmol/l (200 mg/dl), or
- HbA1c ≥ 6.5 %, or
- Treatment with any anti-diabetic medication

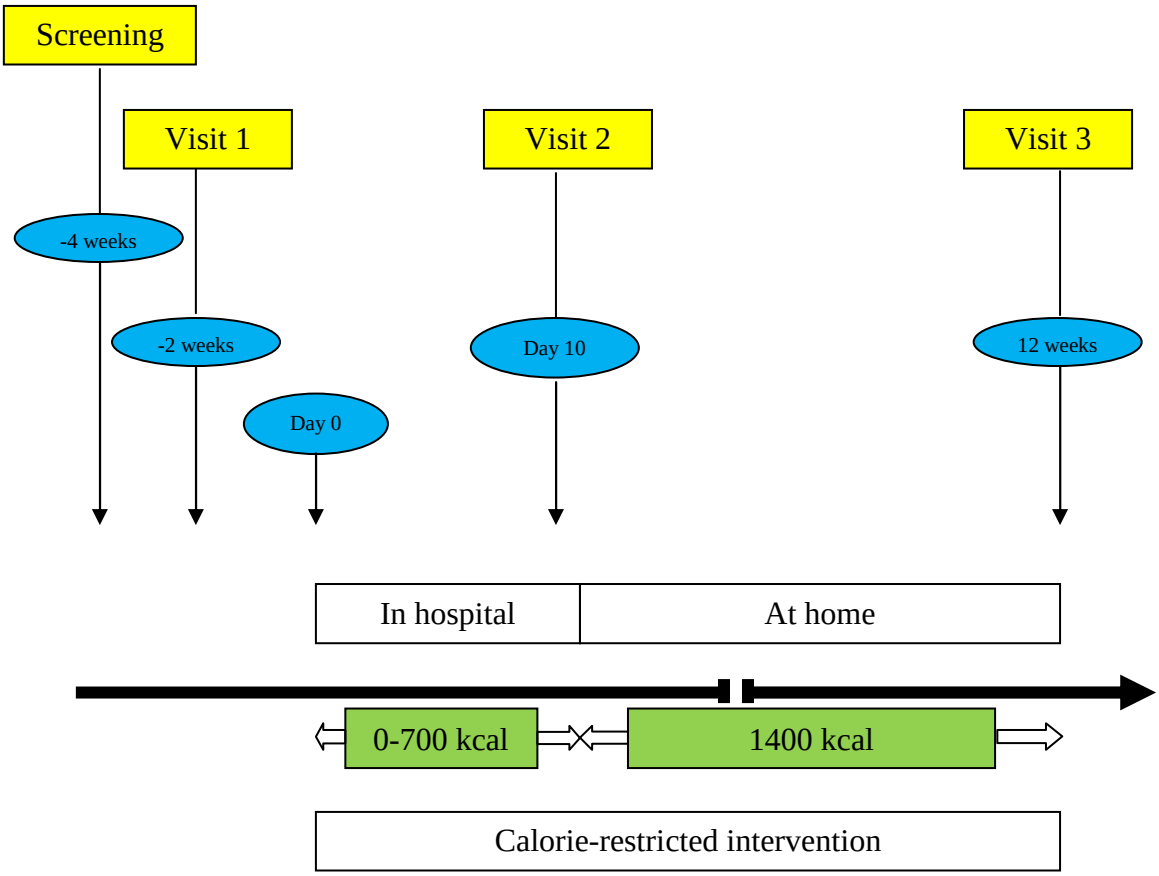


Figure 4: Timeline of study

3.3.2. Visit 1

Visit 1 was scheduled approximately 2 weeks after the screening visit and before caloric restriction started. During the week before visit 1, the subject had to comply with a diet consisting of 18 “Broteinheiten” (corresponding to 216 gr of carbohydrates/day) with approximately 15 % protein, 30 % fat, and 55 % carbohydrates (dietary protocol I) (Figure 7). The physical activity was also kept stable to keep body weight stable (± 2 kg). Visit 1 lasted 3 days (day 1, day 2 and day 3) and supplied the basal data (before caloric restriction).

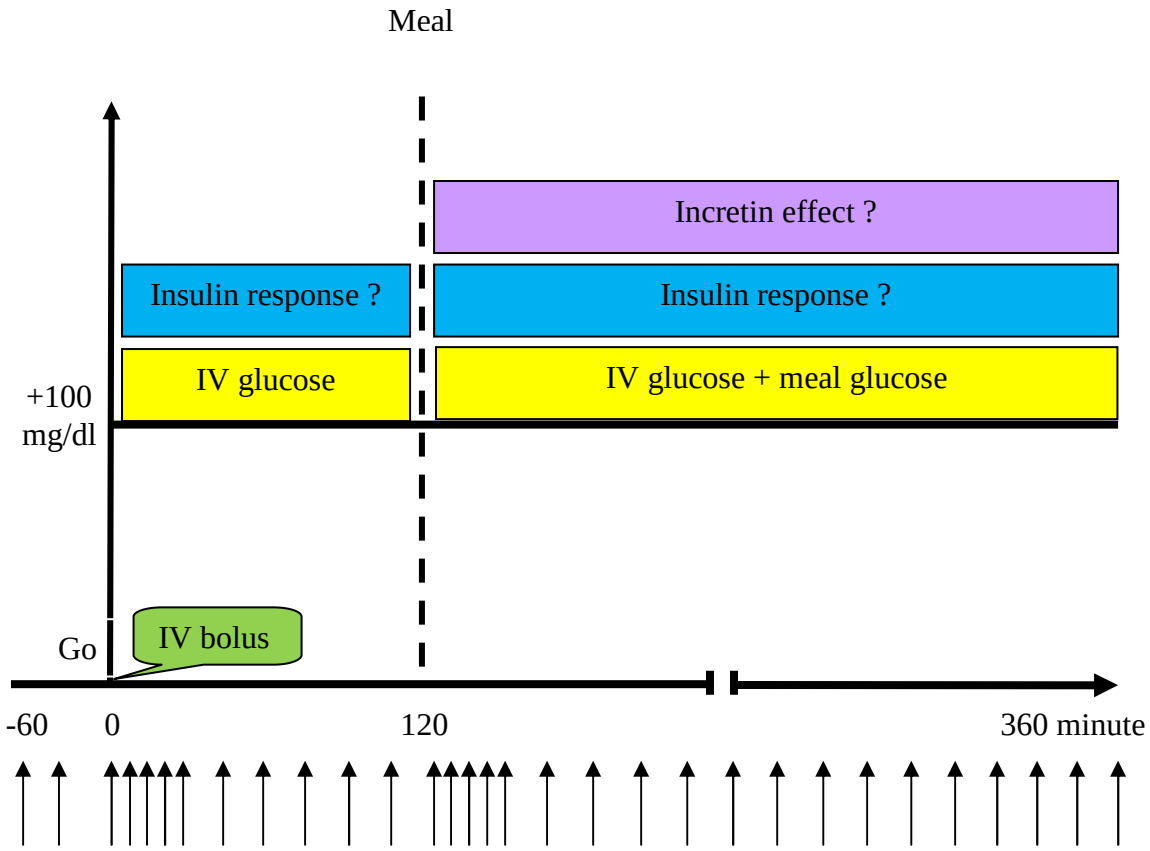
Day 1: hyperglycemic clamp with test meal

In the HGC with test meal, we raised the plasma glucose concentration to 100mg/dl over the fasting concentration and maintained it stable for 6 hours. The glucose supply came from an intravenous glucose infusion for the first 120 minutes and from intravenous glucose infusion plus the oral meal for the last 240 minutes (Figure 5). The subjects were admitted to the clinical research unit in our hospital, between 7:30 and 8:00a.m after an 8 hour fast. Body weight, height, waist circumstance, blood pressure, and pulse were measured. An intravenous catheter was placed in each forearm, one for blood withdrawal and another for glucose infusion. The forearm used for blood-withdrawal was warmed continuously during the clamp with a heating lamp to arterialize the venous blood. One blood sample for lipid profile and four blood samples for the fasting parameters of blood glucose, insulin, and C-peptide at 4 separated time-points were withdrawn after the subject had rested in bed for one hour. Following this (considered as time-point 0 minute), a 20 % glucose bolus (dose see below) was given within 1 minute and followed by a continuous infusion of 20 % glucose solution to raise and maintain the blood glucose concentration 100 mg/dl over fasting blood glucose.

$$\text{Glucose bolus dose (mg)} = \text{Body weight (kg)} \times 100\text{(mg/dl)} \times 1{,}5$$

At 120 minutes, the subject received a 324 kcal-semisolid meal containing 20 % protein, 40 % lipid and 40 % carbohydrate plus 100mg ^{13}C-Sodium Acetate within 5 minutes. To maintain a stable blood glucose at 100 mg/dl over fasting blood glucose during the clamp, the infusion rate of 20 % glucose solution was modified every 5 minutes based on results of arterialized blood glucose. Blood samples for insulin and C-peptide parameters were withdrawn at time-points of 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 125, 130, 135, 140, 145, 150, 160, 170, 180, 195, 210, 225, 240, 255, 270, 285, 300, 315, 330, 345, 360 minutes. The assessment for full consciousness, hunger, nausea, and satiety was done by the visual analogue scales (VAS) at the time-points of 0, 105, 120, 135, 150, 165, 180, 210, 240, 300, and 360 minutes. The estimation of gastric emptying time was performed at the time-points of 120 (before the meal test), 135, 150, 165, 180, 195, 210, 225, 240, 270, 300, 330, and 360 minutes by 13 inflated breathing bags. The measurement of ^{13}C enrichment in breath CO_2 was done by Isotope Ratio Mass Spectrometry (IRMS). The hyperglycaemic clamp can conceptually be divided into 2 stages. The first stage during which glucose supply comes only from the intravenous glucose infusion lasted from 0 to 120 minutes. The main purpose of this first stage was to assess the ß cell response including first-phase and second-phase response to an intravenous glucose infusion [7]. The second phase lasted from 120 to 360 minutes and

started with the test meal. The glucose supply for the body during the second stage comes from intravenous glucose infusion plus the oral meal. The goal of this second phase is to assess the incretin effect. During and at the end of the clamp, urine was collected to determine the urine glucose excretion.



Blood samples for: Blood glucose, insulin, and C-peptide

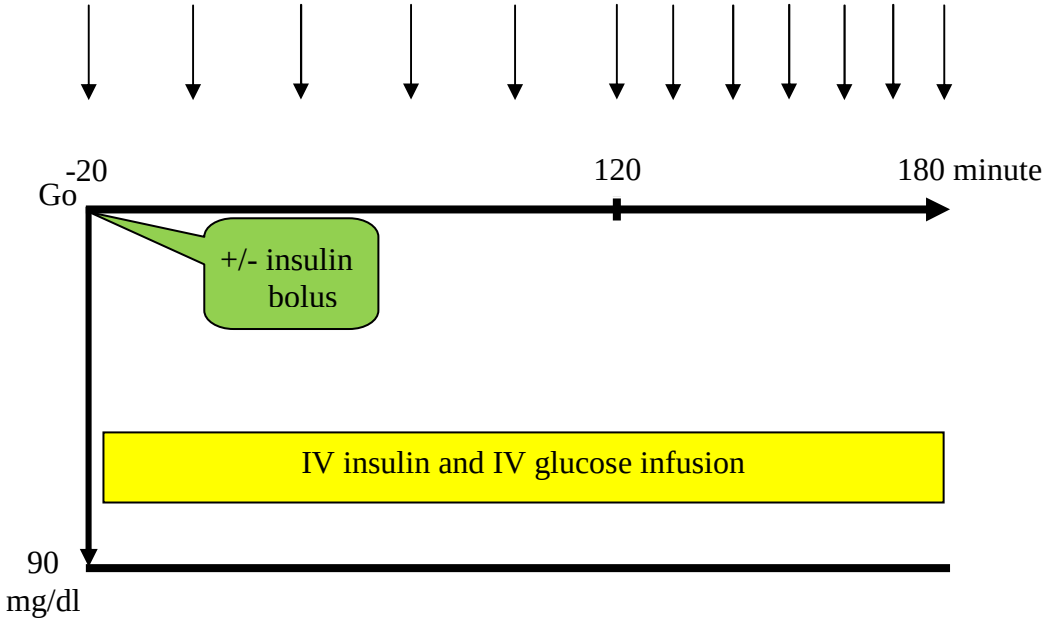
VAS and Gastric emptying time test

Figure 5: Process of hyperglycaemic clamp with the test meal

Day 2: Euglycemic clamp

The EGC was started after an 8 hour fast and lasted for 3.5 hours. One intravenous catheter was again placed in each forearm, one for blood drawing and one for infusing both insulin and 20 % glucose solution. Again the forearm used for blood sampling was warmed as described above to arterialize the blood. After resting in the bed for one hour, the patient received an intravenous insulin infusion with the fixed infusion rate of 1,5 IU/kg/min. The goal of the

insulin infusion was to lower the blood glucose to 90 mg/dl. Insulin infusion with the fixed rate was kept until the end of the clamp. Blood glucose was tested every 5 minutes, and 20 % glucose infusion rate was modified to maintain blood glucose at 90 mg/dl during clamp. Blood samples for insulin and C-peptide assays were regularly withdrawn at time-points of -30, 0, 20, 40, 60, 80, 100, 120, 130, 140, 150, 160, 170, 180 minutes (Figure 6).



Blood samples for: Blood glucose, insulin, and C-peptide

Figure 6: Process of euglycemic clamp

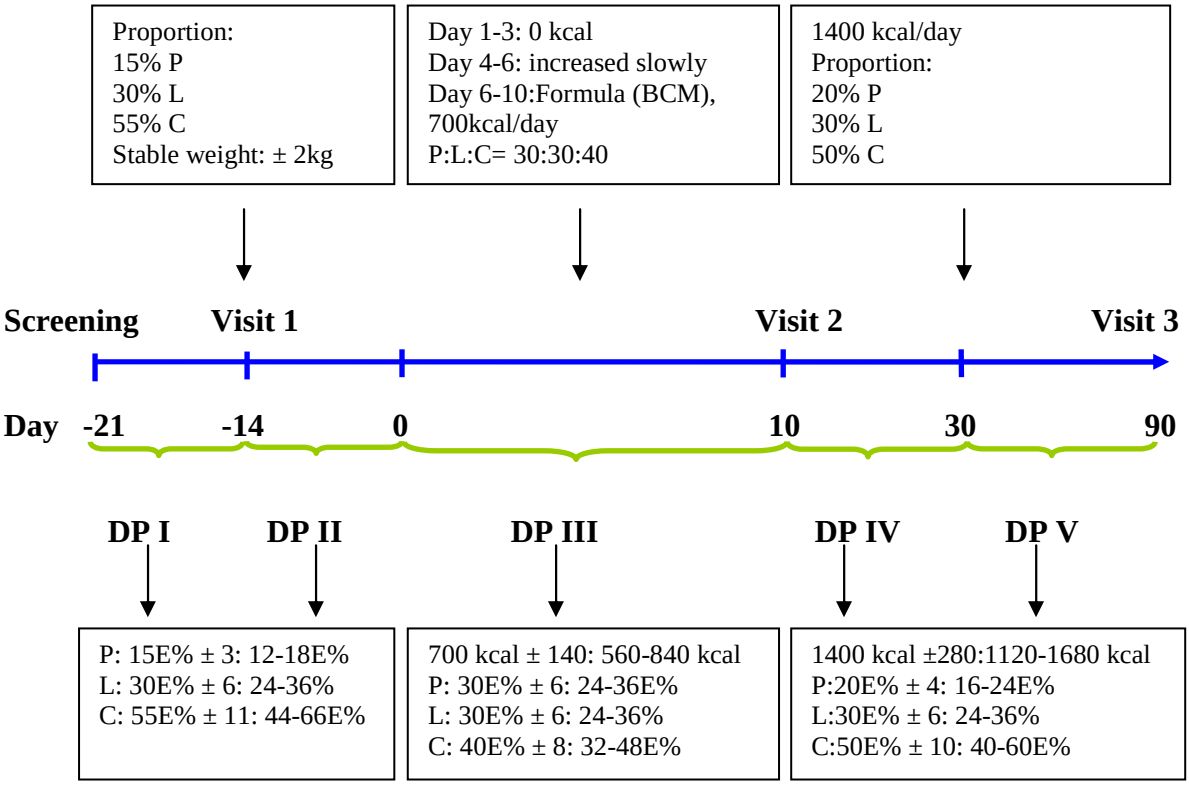
Day 3: Hyperglycemic clamp without the test meal

The HGC without the test meal was performed in the similar manner as HGC with test meal except that the meal was not ingested at 120 minutes. The goal of the HGC without the test meal on the third day of visit 1 was to assess the ß-cell response to the HGC without the meal test and to compare the results with that of the HGC with the test meal.

3.3.3. First phase of caloric restriction

As mentioned above, the caloric restriction started 2 weeks after visit 1 and lasted for 3 months (Figure 4). During the 2 weeks before the caloric restriction, the subject had to fully comply with dietary and activity recommendations to keep a stable weight (± 2kg). The

caloric restriction was divided into 2 phases. The first phase covered the first 10 days, during which the subjects had to stay in the hospital to ensure full compliance with protocol. The subjects performed the same calorie-restricted protocol as that of postoperative patients. The subjects were completely fasting during the first 3 days and were recommended to drink water or unsweetened tea of at least 2.5 liter/day. From day 4 to day 6, the calorie intake was increased slowly like in postoperative patients, using a commercially available product (BCM-basic; www.bcm.de). From day 6 to day 10, the intake was further increased (using the same commercially available formula diet) providing on average 700 kcal/day. The subjects were instructed to continue normal daily activity. Blood glucose, vital parameters, body weight, waist circumference, and VAS were measured every day.



P-Protein; C-Lipid; C-Carbohydrate; DP-Dietary protocol; E-Energy; BCM-basic (formula diet).

Figure 7: Dietary intervention of study

3.3.4. Visit 2

Visit 2 occurred during the last 2 days of the first phase of caloric restriction (Figure 4). Visit 2 included a short HGC without test meal at the first day and an EGC at the second day. The short HGC without test meal lasted for 120 minutes and was performed identical to the first 120 minutes of HGC without test meal at the visit 1. The EGC at the second day was also performed in the same way as that at the visit 1. The goal of visit 2 was to assess the improvement of ß-cell response (first and second phase) to intravenous glucose infusion and the improvement of peripheral insulin sensitivity to exogenous insulin after the first phase of caloric restriction. Lipid profile, the clinical parameters such as body weight, WC, blood pressure, and pulse were also measured at the first day.

3.3.5. Second phase of caloric restriction

After the visit 2, the subject was discharged and started the second phase of caloric restriction. The second phase of the caloric restriction lasted until the end of study (Figure 4). Subjects were allowed to consume 1400 kcal/day and the proportions of protein, lipid, and carbohydrate were 20 %, 30 %, and 50 %, respectively. We regularly instructed them to continue the dietary intervention. Dietary compliance during the second phase was assessed by 2 dietary protocols (dietary protocol IV and dietary protocol V) (Figure 7). Dietary protocol IV estimated the food consumption for the first 30 days, and dietary protocol V estimated the food consumption for the last 50 days. Every dietary protocol included 3 weekdays and 1 weekend-day. Based on these protocols, we calculated the amount of energy consumed and the proportion of the food components. The mean values of these two protocols were used to determine the compliance with dietary intervention (Figure 7).

3.3.6. Visit 3

Visit 3 occurred 3 months after starting caloric restriction. It included 2 days. The study procedures of these 2 days included the HGC with the meal on the first day and the EGC on the second day was performed identical to the first 2 days of visit 1. The lipid profile and other clinical parameters such as body weight, WC, blood pressure, and WC were measured on the first day.

3.4. Sample preparation and laboratory analysis

During the clamp, blood glucose was measured by the glucose oxidase method using 2 standardized Bayer´s Contour® blood glucose analysers. The blood glucose was measured two times, and the mean value of these 2 separated values was used.

The blood samples for insulin and C-peptide were collected in Monovette EDTA blood collection tubes (Sarstedt). After collecting, the blood was centrifuged immediately, and serum samples were stored at -25^{o}C. Insulin and C-peptide were measured by specific and sensitive sandwich immune-luminescence assay (ILMA).

3.5. Calculation

- Acute insulin response to intravenous-glucose induced hyperglycemia (AIRg) was estimated to be the mean increment of insulin during the first 10 minutes of HGC with the test meal [7].
- The basal insulin concentration was obtained as the mean of the four samples drawn at -20, -10, -5, and 0 minute.
- The plasma insulin concentrations of the first 10 minutes and from 60 to 120 minutes were obtained as the mean of all samples in each time period.
- First-phase insulin response: (μU/ml) = $MIC_{0\text{-}10\ min} - MIC_{basal}$
- The incretin effect (%) was calculated as

$$\text{Incretin effect (\%)} = \frac{MIC_{(125\text{-}360\ min)} - MIC_{(90\text{-}120\ min)}}{MIC_{(125\text{-}360\ min)}} \times 100 \quad [33]$$

- Where $MIC_{(90\text{-}120\ min)}$ and $MIC_{(125\text{-}360\ min)}$ are the means of insulin concentration from 90 to 120 min and from 125 to 360 min for HGC with test meal, respectively.
- Glucose infusion rate (GIR) was calculated after at last 60 minutes of HEC and was expressed as mg/kg/min.
- The insulin sensitivity index (ISI = GIR/insulin concentration) was expressed as $mg.kg^{-1}.min^{-1}/\mu U.ml^{-1}$.
- The insulin sensitivity to exogenous insulin was estimated by GIR and ISI.
- The deposition index (DI=AIRg X ISI) was used to estimate the appropriateness of acute ß-cell response relative to prevailing insulin sensitivity [43].
- Insulin resistance index (HOMA-IR) was calculated as:

$$\text{- fasting plasma insulin (}\mu\text{U/mL) x fasting plasma glucose (mg/dl)}$$

3.6. Data analysis

Statistical analysis is conducted using IBM SPSS statistics 20 and GraphPad Prism 5. Data are presented as the mean ± SEM. Paired t-tests are used to compare data between visit 1 and visit 2, visit 1 and visit 3. Spearman´s correlation was used to estimate the correlation between degree of body weight loss and degree of improved glucose metabolism. Statistical significance was set at a p-value less than 0.05.

4. RESULTS

Twelve obese and diabetic subjects (4 men and 8 women, mean age 50.2 ± 2.2 years) were evaluated in the study. All subjects completed all phases of the study and participated in all visits. Serious adverse events did not happen during the study.

4.1. Compliance of subjects with caloric restricted intervention

Caloric intake and macronutritient intake is shown in Table 2 and Figure 8. The average energy intake during the first and the second phase of caloric restriction was 678.8 ± 47.8 and 1407.3 ± 52.7 kcal/day, respectively. Both values are within the range of the protocol (560-840kcal/day during the first phase and 1120-1680 kcal/day during the second phase).

Table 2: Energy intake and proportion of food components (N=12)

Parameters	**Before study**	**1st phase of CR**	**2nd phase of CR**
Energy intake (kcal/day)	2630 ± 141.9	678.8 ± 47.8***	1407.3 ± 52.7***
Protein (%)	19.4 ± 0.7	30.4 ± 1.5***	21.9 ± 0.6**
Lipid (%)	36.7 ± 1.6	31.9 ± 1.1	29.8 ± 1.3**
Carbohydrate (%)	43.3 ± 1.4	37.9 ± 1.0*	48.1 ± 1.8*

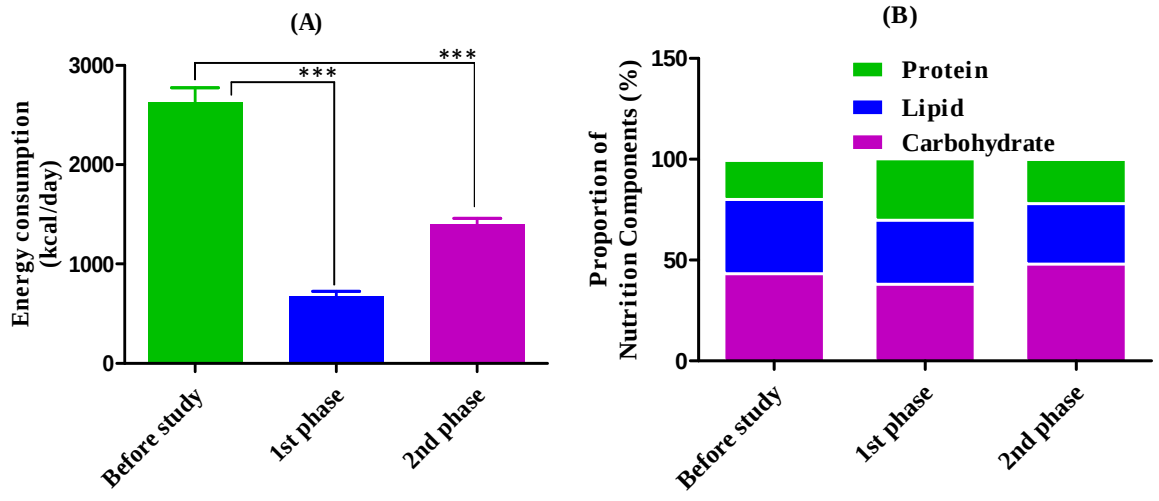


Figure 8: Compliance of subjects with caloric restricted intervention described by energy intake (A) and proportion of food components (B)

During the first phase, three subjects did not fully comply with caloric restriction. Of these three subjects, one consumed 164 kcal/day more than the accepted upper limit and two consumed less than the accepted lower limit, (one 144 kcal/day and one 191 kcal/day less than the lower limit value). During the second phase, eleven subjects complied fully with caloric restriction with one subject consumed 91.5 kcal/day less than the accepted lower limit.

4.2. Weight loss and clinical parameters

Changes in body weight and clinical parameters are shown in Table 3 and Figure 9. Before the caloric restriction, mean BMI was 46.0 ± 2.1 kg/m^2. Nine subjects had grade III obesity (BMI ≥ 40kg/m^2), and three subjects had grade II obesity (35 kg/m^2 ≤ BMI < 40 kg/m^2). All subjects had central obesity (evaluated by waist circumference ≥ 94cm for men and ≥ 80cm for women).

Table 3: Effect of caloric restriction on body weight and clinical parameters (N=12)

Parameters	**Visit 1**	**Visit 2**	**Visit 3**
Weight (kg)	133.5 ± 6.6	127.0 ± 6.3$^{(***)}$	120.5 ± 5.7***
BMI (kg/m^2)	46.0 ± 2.1	43.8 ± 2.0$^{(***)}$	41.7 ± 2.1***
Excess weight loss (%)		11.4 ± 1.0	22.3 ± 3.8
Waist circumference (cm)	136.7 ± 5.3	131.5 ± 5.3	126.2 ± 4.6***
Sys-Blood pressure (mmHg)	132.9 ± 4.1	129.7 ± 3.5	126.9 ± 2.7
Dia-Blood pressure (mmHg)	75.2 ± 2.4	69.2 ± 3.0	69.8 ± 2.9

Weight loss was observed in all subjects after the first phase and the second phase of caloric restriction. Mean body weight decreased from the initial value of 133.5 ± 6.6 kg to 127.0 ± 6.3 kg ($P < .001$) at visit 2 and to 120.5 ± 5.7 kg ($P < .001$) at visit 3. The mean percentage of excess weight loss was 11.4 ± 1.0 % at visit 2 and 22.3 ± 3.8 % at visit 3. The number of subjects who lost > 5 % of their initial body weight was seven and nine at visit 2 and visit 3, respectively. This decrease in weight was associated with a decrease in central obesity (estimated by waist circumference) from 136.7 ± 5.3 cm at visit 1 to 131.5 ± 5.3 cm ($P < .001$) at visit 2 and to 126.9 ± 2.7 cm ($P < .001$) at visit 3. There was an improvement in blood pressure at visit 2 and visit 3, although these changes were not significant.

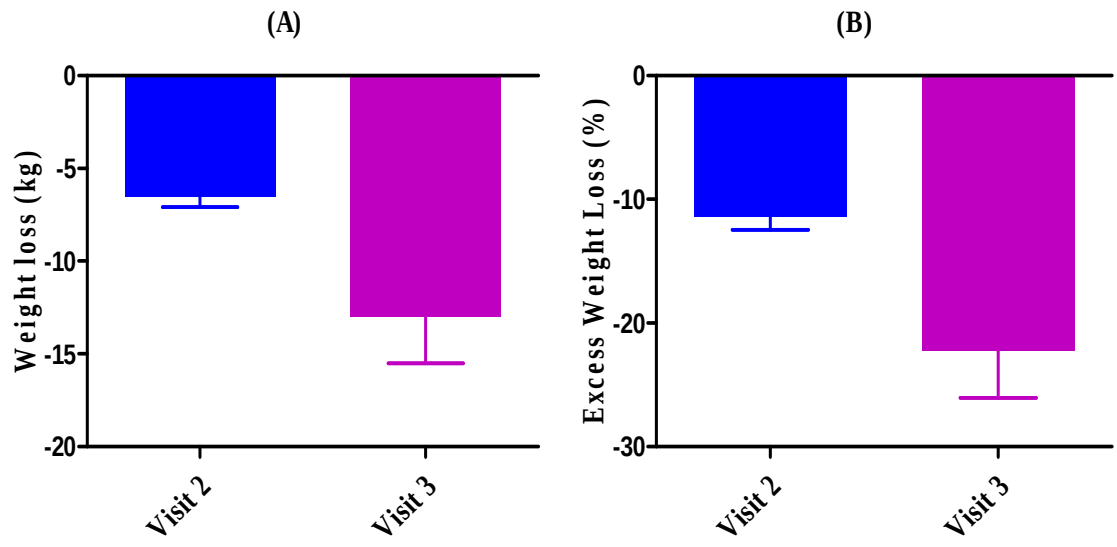


Figure 9: Change of body weight after caloric restriction shown by weight loss (A) and excess weight loss % (B)

4.3.Improvement in glucose metabolism

Before the caloric restriction, the subjects had a mean duration of diabetes of 5.2 ± 0.7 years (from 1.4 to 10 years). Most of them (9 subjects) had good glycemic control with HbA1c ≤ 7 %. Eight subjects took oral antidiabetic medications, two subjects received both oral medications and insulin, and two subjects performed life style modification. There was a significant improvement in main parameters of glucose metabolism after the caloric restriction. The decrease of fasting blood glucose was found in 11/12 subjects at visit 2 and in all subjects at visit 3. The mean of fasting blood glucose dropped by 18.4 ± 8.8 mg/dl ($P < .01$) at visit 2 and 23.1 ± 3.5 mg/dl ($P < .001$) at visit 3. The decrease of HbA1c was observed in 9/12 subjects, and the mean of HbA1c fell from the basal value of 6.7 ± 0.3 % to 6.2 ± 0.1 % ($P = .06$) at visit 3 (Table 4 and Figure 10).

Table 4: Effect of caloric restriction on diabetic improvement (N=12)

Parameters	Visit 1	Visit 2	Visit 3
Fasting glucose (mg/dl)	133.1 ± 6.4	114.6 ± 6.7$^{(**)}$	110 ± 4.7***
HbA1c (%)	6.7 ± 0.3		6.2 ± 0.1
Diabetic medication **Decrease** **Withdrawing** **Keeping same dose**	10		10 5/10 0/10 5/10

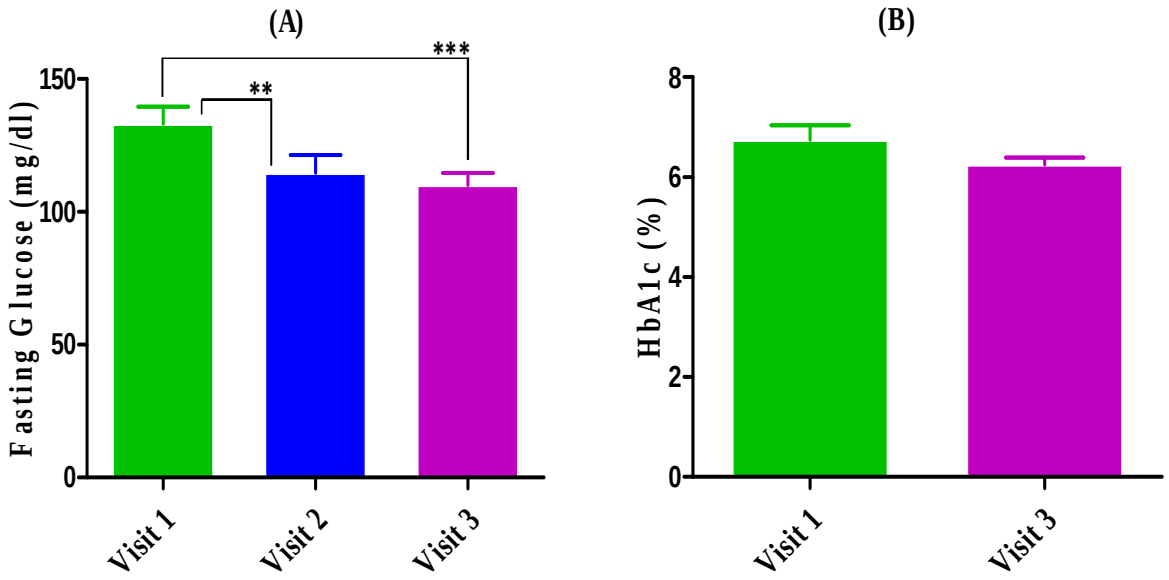


Figure 10: The improvement in fasting blood glucose (A) and HbA1c (B) after caloric restriction (N=12)

The improvement in diabetes (simultaneous decrease of HbA1c and fasting blood glucose without the increase of diabetic medications) was observed in nine subjects. Among these

nine subjects, five decreased diabetic medications, four kept the same doses. Among the three remaining subjects, one improved in fasting glucose but without change of HbA1c and medication dose, while two had an improvement in fasting glucose and an increase in HbA1c. Of these latter two subjects, one had a duration of diabetes of 10 years, and one had very good glycemic control before the study (HbA1c = 5.6 % and fasting glucose = 104 mg/dl). Both had a rise of HbA1c (5.8 % at visit 1 compared to 6.0 % at visit 3, 5.6 % at visit 1 compared to 6.0 % at visit 3) and a decrease of fasting blood glucose (120 mg/dl at visit 1 compared to 109 mg/dl at visit 3, 104 mg/dl at visit 1 compared to 97 mg/dl at visit 3).

4.4. Mechanisms of improved glucose metabolism

The mean values of glucose concentrations during the hyperglycemic and euglycemic clamps are shown in Figure 11. Clamped levels of blood glucose showed a very small fluctuation (during the clamp). The mean increments above fasting blood glucose of clamped blood glucose during hyperglycemic lamps were 99.5 ± 0.7 mg/dl at visit 1 and 99.3 ± 0.4 mg/dl at visit 3 (Figure 11A). The means of clamped blood glucose (120-180 min) during EGC at visit 1, visit 2, and visit 3 were 89.8 ± 0.5, 89.2 ± 1.6, and 90.9 ± 0.9 mg/dl, respectively (Figure 11B).

4.4.1. Incretin effect

Incretin effect was observed after the test meal during the HGC, and it was not present during the HGC without the test meal at day 3 of visit 1. The incretin effect, which was estimated by the increment of values (glucose infusion rate, insulin concentration, and C-peptide concentration) between 90 – 120 min and 125 – 360 min, was larger at visit 3 in comparison with that at visit 1 (Figure 12A, 12B, 12C). A two-fold increase of insulin concentration after the test meal compared to that before the test meal was found at visit 1. Meanwhile, a four-fold increase was observed at visit 3. This difference was also present for the glucose infusion rate and C-peptide concentration (Figure 13, Table 5). Percent incretin effect for insulin increased from the initial value of 51.2 ± 5.0 % to 70.6 ± 4.7 % ($P < .05$) at visit 3. The improvement in the percent incretin effect was also observed when it was estimated by glucose infusion rate and C-peptide concentration (Figure 14).

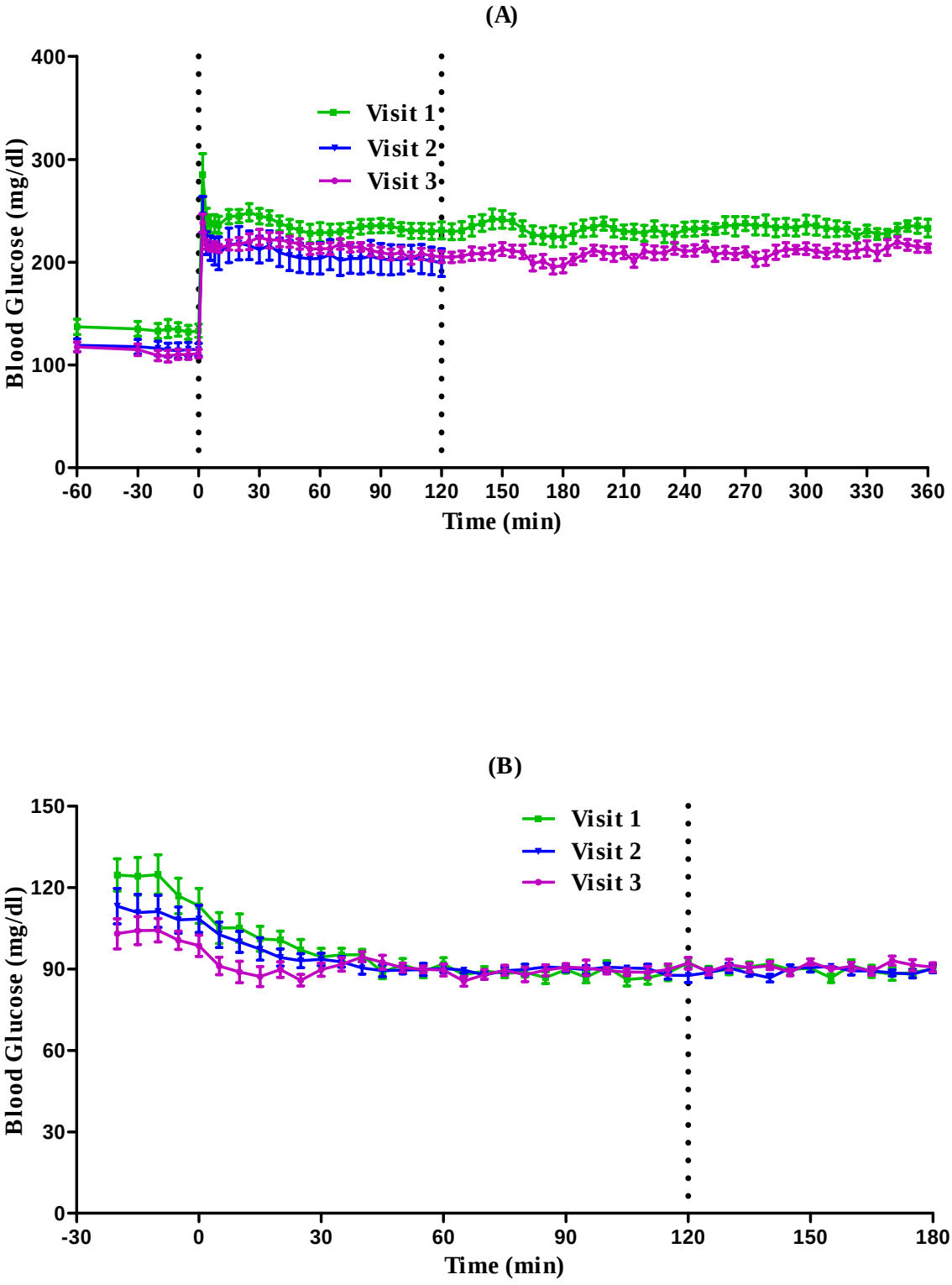


Figure 11: Blood glucose during hyperglycemic clamp (A) and euglycemic clamp (B)

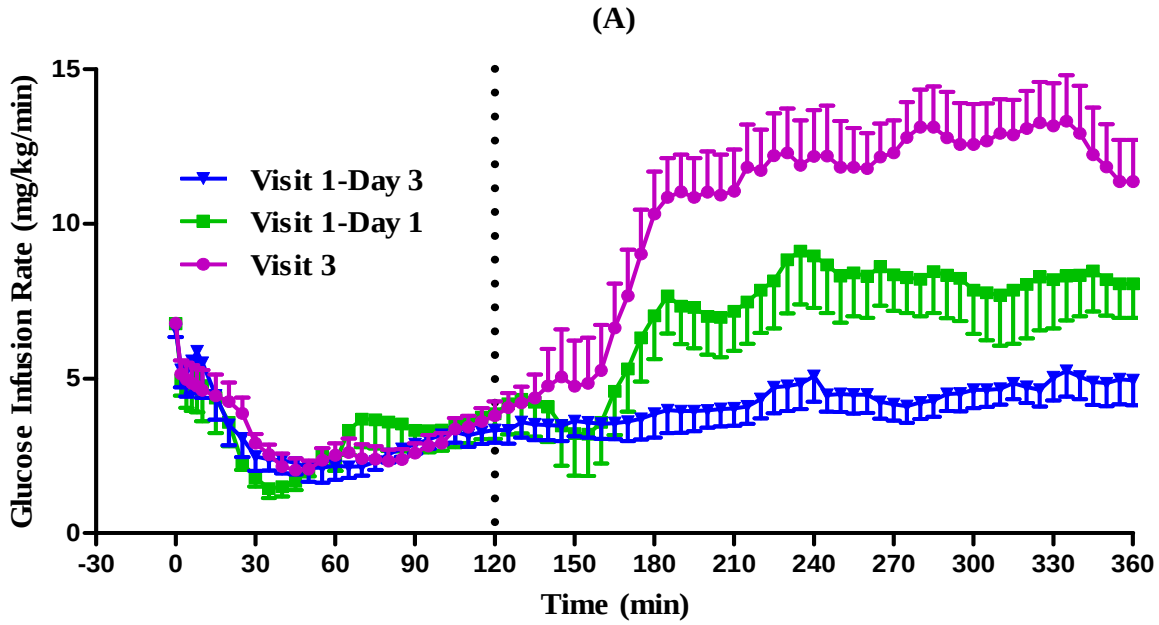


Figure 12A: Changes in the incretin effect described by glucose infusion rate during the HGC with the test meal. For comparisondata from visit 1 day 3 (HGC without the test meal) are also shown (N=12).

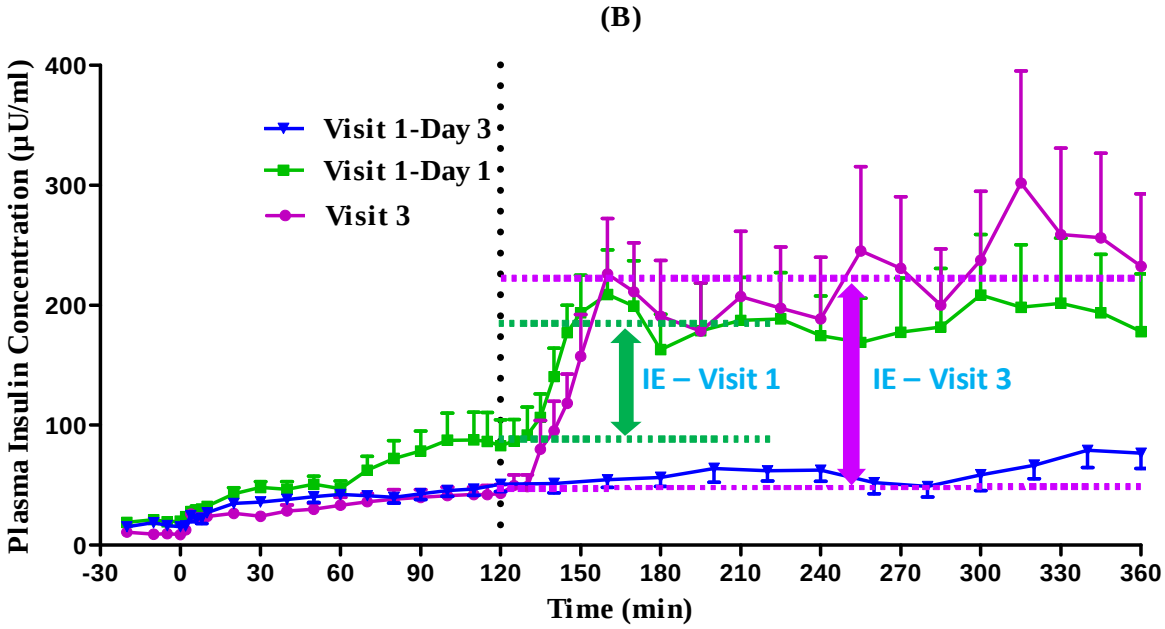


Figure 12B: Changes in the incretin effect described by plasma insulin concentration during the HGC with the test meal. For comparisondata from visit 1 day 3 (HGC without the test meal) are also shown (N=10).

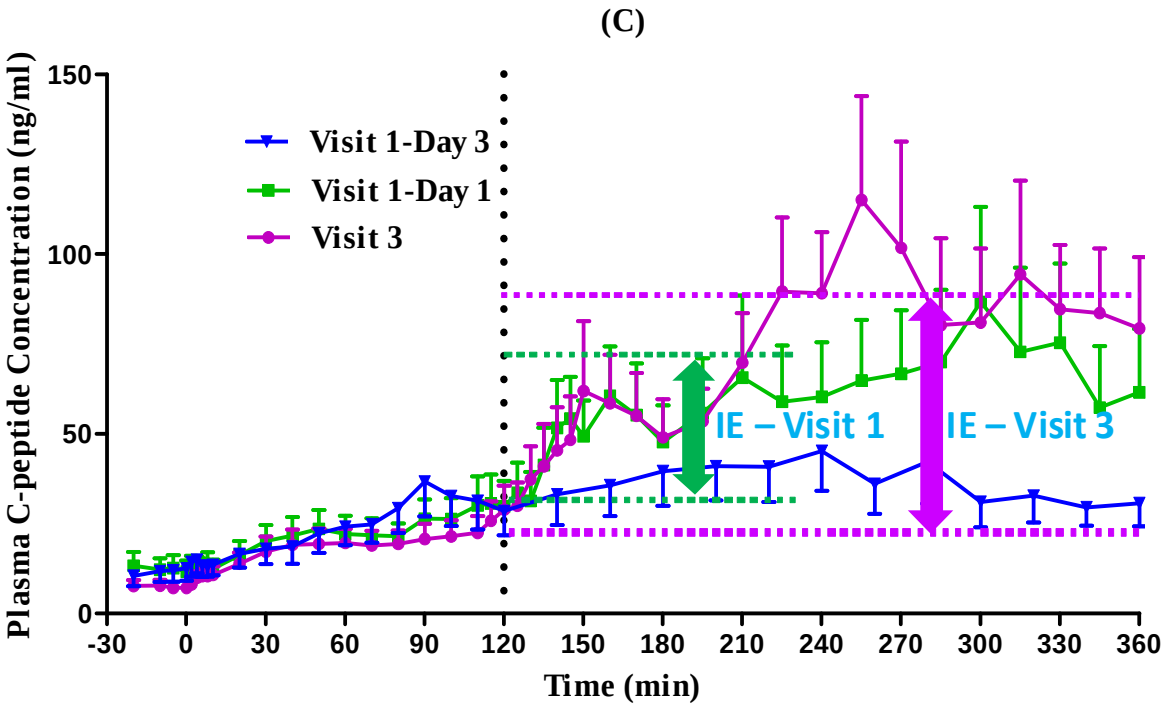


Figure 12C: Changes in the incretin effect described by plasma C-peptide concentration during the HGC with the test meal. For comparisondata fromvisit 1 day 3 (HGC without the test meal) are also shown (N=10).

Table 5: Effect of caloric restricted intervention on the incretin effect (N=10)

Parameters	Visit 1	Visit 3
Increase after test meal (fold)		
GIR	2.2 ± 0.3	3.5 ± 0.5
Insulin	2.2 ± 0.2	4.4 ± 0.9
C-pepetide	2.0 ± 0.2	3.1 ± 0.5
Incretin effect (%)		
GIR	44.4 ± 6.8	67.0 ± 3.7**
Insulin	51.2 ± 5,0	70.6 ± 4.7$^{(*)}$
C-peptide	47.5 ± 4.1	63.1 ± 4.2$^{(*)}$

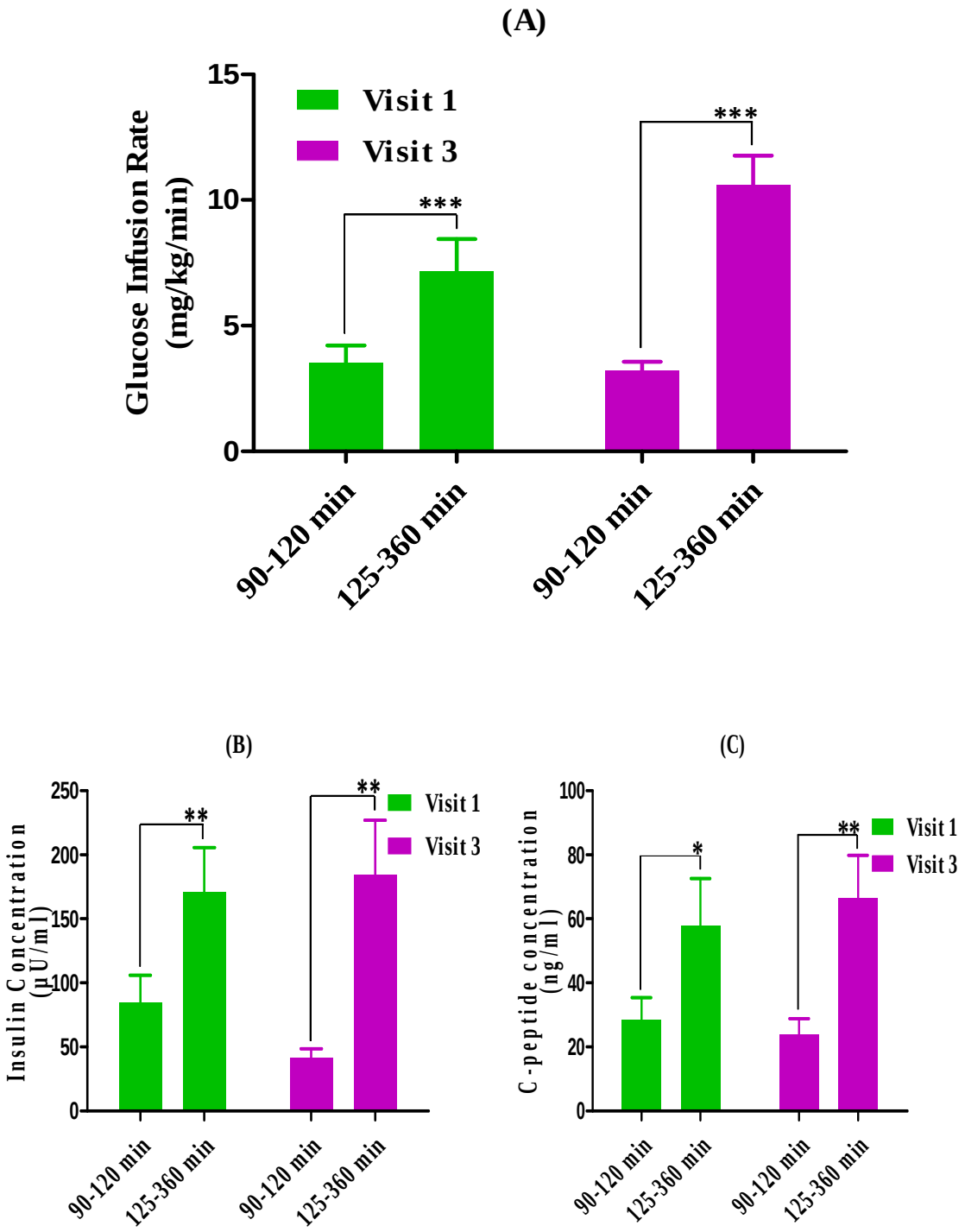


Figure 13: The difference of glucose infusion rate (A, N=12), insulin concentration (B, N=10), and C-peptide concentration (C, N=10) before and after the test meal at visit 1 and visit 3. The glucose infusion rate between 90-120min and 125-360min increased 2.2-fold at visit 1, while it increased 3.5-fold at visit 3 (A). A similar effect was seen when insulin (B) and C-peptide (C) data were analyzed.

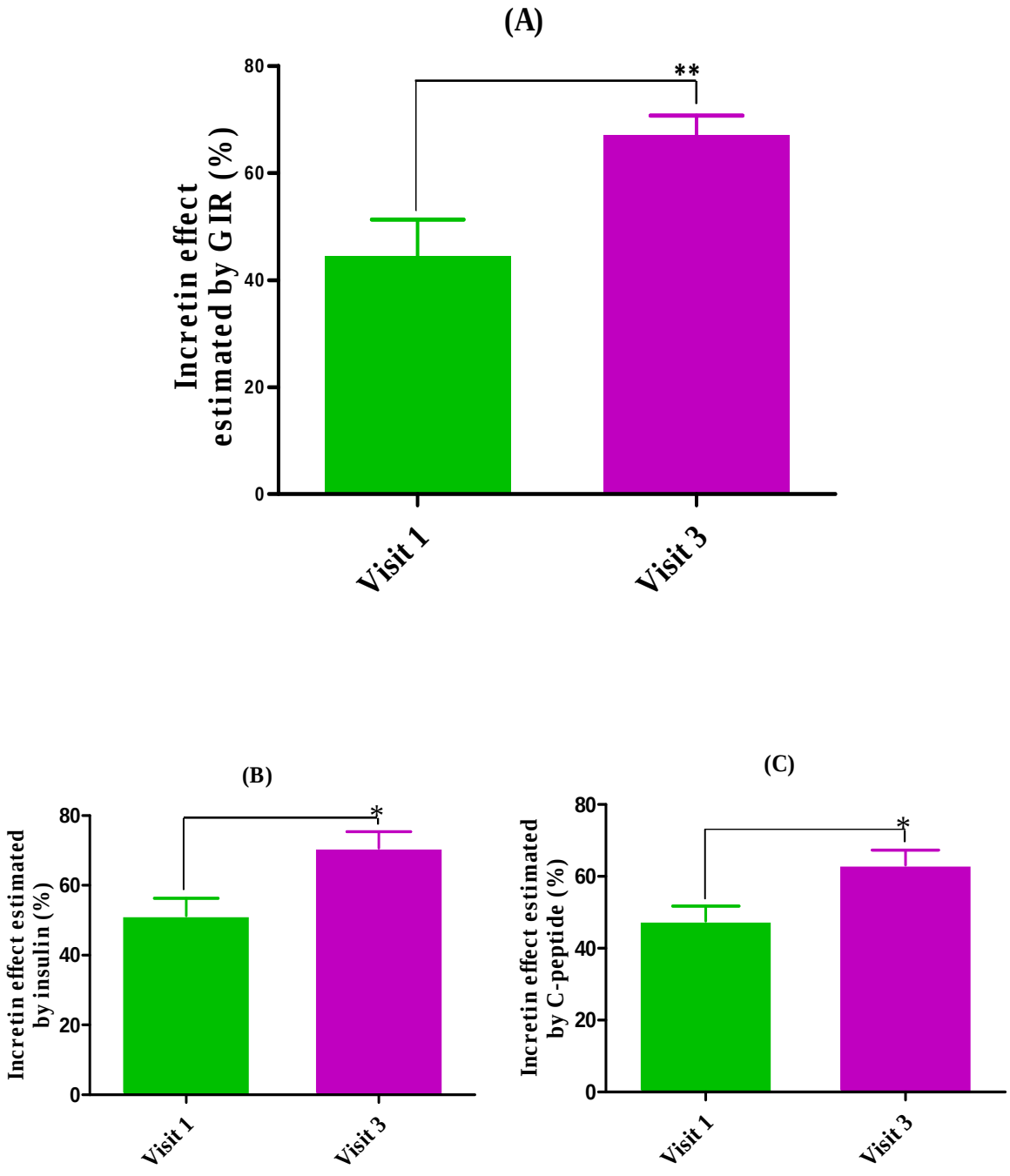


Figure 14: The increase in the incretin effect before and after caloric restriction estimated by glucose infusion rate (A, N=12), insulin concentration (B, N=10), and C-peptide concentration (C, N=10)

4.4.2. Insulin sensitivity

Insulin sensitivity was estimated by several variables. As assessed by the EGC, insulin sensitivity improved significantly at visit 3. An increased glucose infusion rate during the last 60 min of EGC was present in 11/12 subjects at visit 3, and the mean of glucose infusion rate increased from 3.7 ± 0.5 at visit 1 to 5.5 ± 0.6 mg/kg/min at visit 3 ($P < .01$). Similarly, an increase of the insulin sensitivity index (ISI) was observed in all subjects, and the mean of insulin sensitivity index increased from 22.9 ± 3.8 at visit 1 to 45.5 ± 9.8 at visit 3 ($P < 0.05$) (Table 6, Figure 15). As estimated by HOMA-IR, insulin sensitivity also improved significantly at visit 3 (6.7 ± 0.9 at visit 1 and 2.7 ± 0.8 at visit 3, $P < .001$), and HOMA-IR decreased in all subjects at visit 3. On the other hand, fasting concentration of insulin decreased significantly at visit 3 (compared to visit 1). Although caloric restriction during the first phase (between visit 1 and 2) resulted in a small improvement in GIR, ISI, HOMA-IR, and fasting insulin concentration, these changes were not significant.

Table 6: The effect of caloric restriction on insulin sensitivity (N=12)

Parameters	**Visit 1**	**Visit 2**	**Visit 3**
Fasting insulin (µU/ml)	20.0 ± 2.5	15.9 ± 2.7	9.3 ± 2.5*
Fasting C-peptide (ng/ml)	12.4 ± 3.4	12.2 ± 3.0	7.4 ± 1.5
GIR (mg/kg/min)	3.7 ± 0.5	3.9 ± 0.3	5.5 ± 0.6**
ISI	22.9 ± 3.8	27.4 ± 5.0	45.5 ± 9.8$^{(*)}$
HOMA-IR	6.7 ± 0.9	4.6 ± 1.0	2.7 ± 0.8***

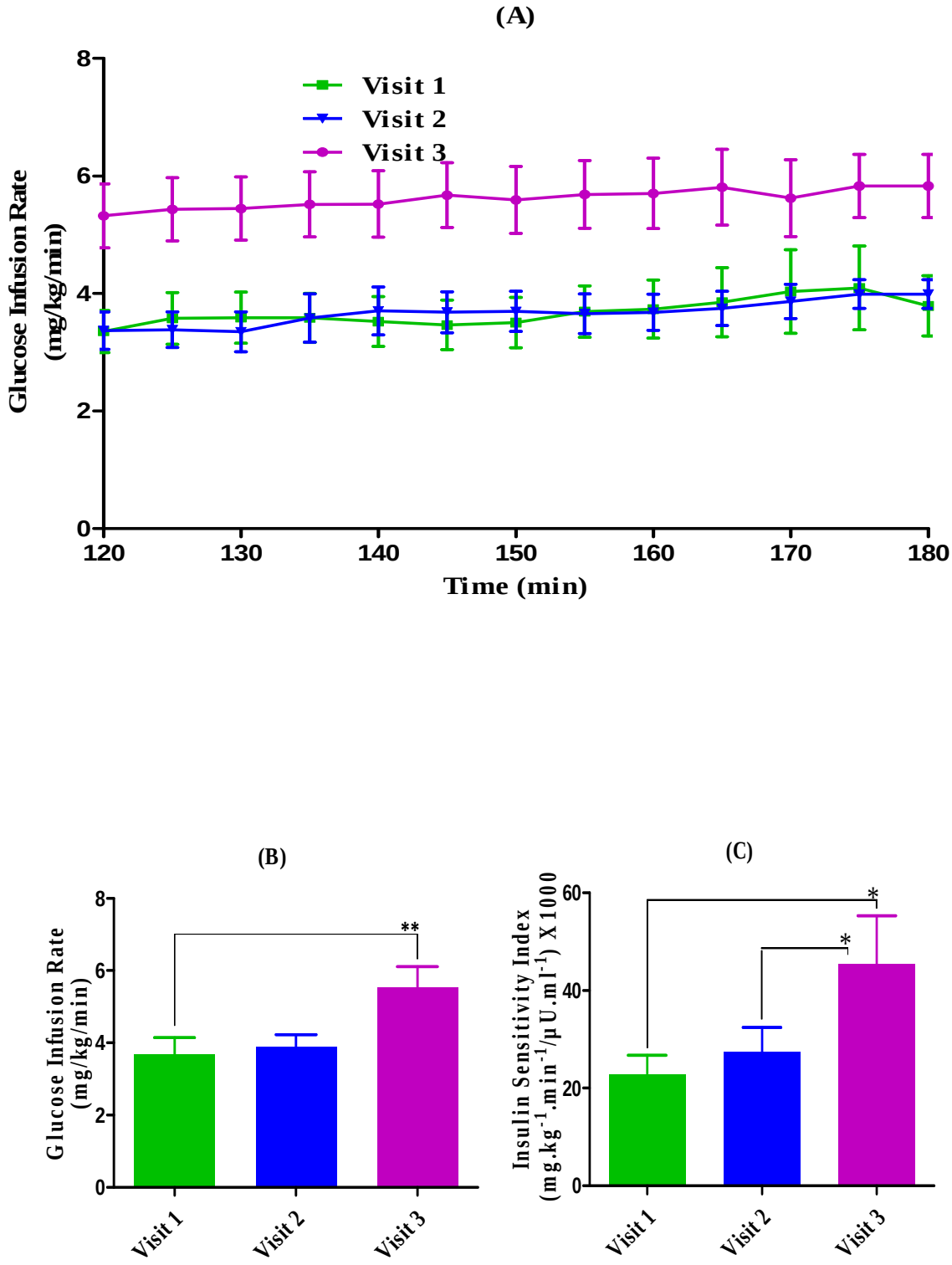


Figure 15: The improvement in insulin sensitivity after caloric restriction shown by GIR (A,B, N=12) and ISI (C, N=10) during the last 60 minutes of EGC.

4.4.3. ß-cell function

ß-cell function was determined by acute insulin response to hyperglycemia (AIRg) and deposition index (DI). AIRg was observed for both insulin and C-peptide concentrations during the first 10 min of the HGC (Figure 16A and 16B). Caloric restriction induced an increase of AIRg for insulin and C-peptide at visit 2 and visit 3. However, the increase reached statistical significance only for insulin at visit 2 (Table 7, Figure 16C and 16D). Deposition index (a useful measure of ß-cell compensation) increased from 244.2 ± 131.1 at visit 1 to 516.0 ± 237.5 ($P > 0.05$) at visit 2 and to 542.1 ± 201.0 ($P < .05$) at visit 3. The increase of deposition index (estimated as AIRg X ISI) resulted from the combined increase of acute insulin response and insulin sensitivity index. Acute insulin response increased from 8.4 ± 3.5 µU/ml at visit 1 to 14.5 ± 5.1 µU/ml at visit 2 and to 12.3 ± 2.8 µU/ml at visit 3 (Table 7). Meanwhile, insulin sensitivity index also increased from 22.9 ± 3.8 at visit 1 to 27.4 ± 5.0 at visit 2 and to 45.5 ± 9.8 at visit 3 (Table 6).

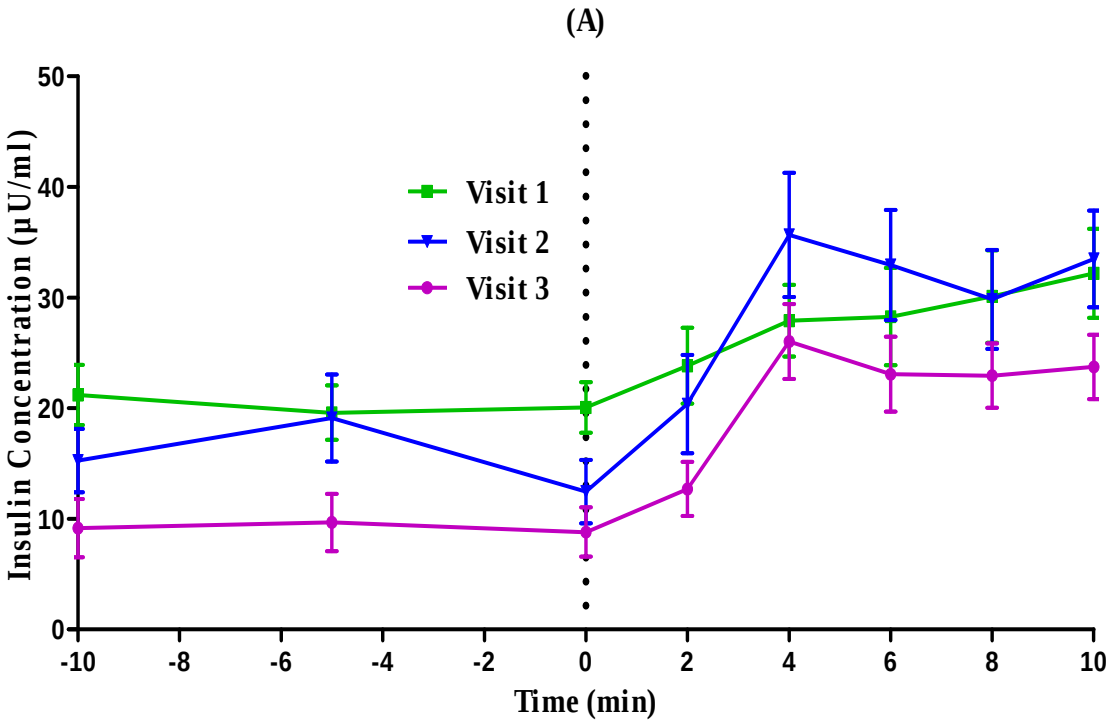


Figure 16A: The changes of acute response of ß cells described by insulin concentration (N=10).

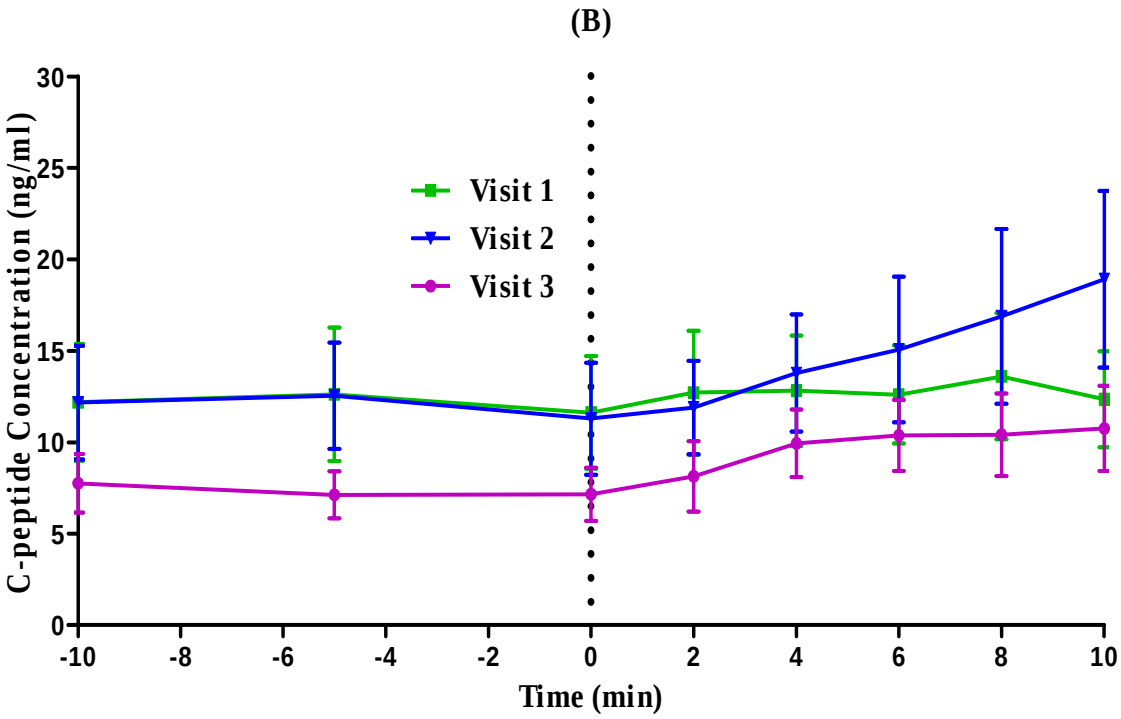


Figure 16B: The changes of acute response of ß cells described by C-peptide concentration (N=10).

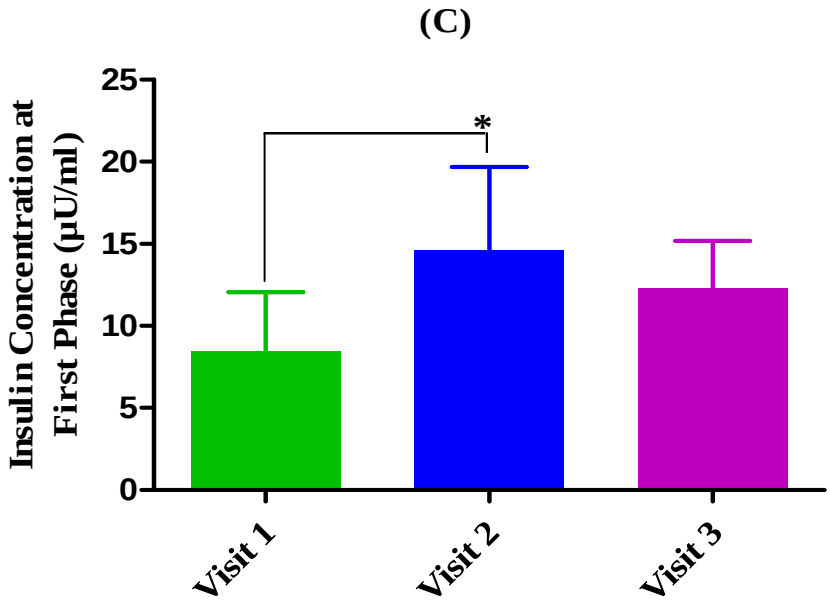


Figure 16C: Insulin concentration during AIRg increases at visit 2 and visit 3 in comparison with that at visit 1 (N=10)

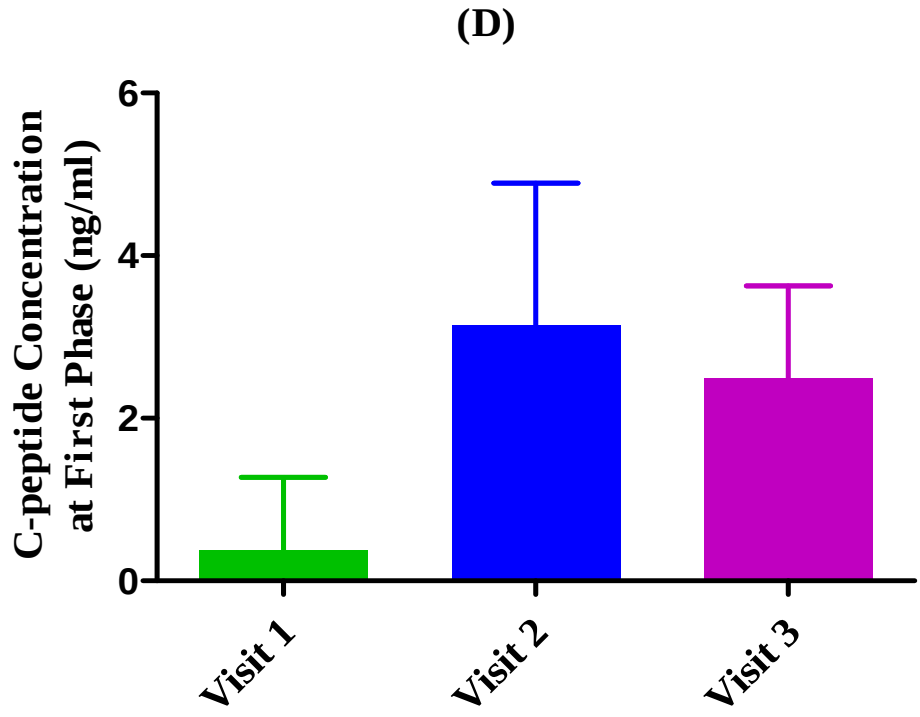


Figure 16D: C-peptide concentration during AIRg increases at visit 2 and visit 3 in comparison with that at visit 1 (N=10)

Table 7: Effect of caloric restriction on ß cell function (N=10)

Parameters	**Visit 1**	**Visit 2**	**Visit 3**
AIRg			
Insulin (µU/ml)	8.4 ± 3.5	14.5 ± 5.1(*)	12.3 ± 2.8
C-peptide (ng/ml)	0.4 ± 0.9	3.1 ± 1.7	2.5 ± 1.1
Disposition index	244.2 ± 131.1	516.0 ± 237.5	542.1 ± 201.0(*)

4.5. Clinical improvement in diabetes and result of mechanistic studies (on individual basis)

Although the clinical improvement in diabetes was less pronounced in 2/12 subjects (subject one and subject two in Table 8), mechanistic studies (incretin effect, insulin sensitivity, and ß-cell function) show an improvement in both subjects. Among the remaining ten subjects having clear clinical improvement, six had an improvement in most mechanisms, four had the improvement in the incretin effect and the insulin sensitivity (Table 7).

Table 8: Clinical improvement and improved mechanisms at visit 3: GIR – Glucose infusion rate; ISI – Insulin sensitivity index; AIR-Insulin – Acute insulin response estimated for insulin; AIR-C-peptide – Acute insulin response estimated for C-peptide; DI – Disposition index (subjects 10-12 not all data available). (+): improvement; (-): no improvement; (+/-): decreased fasting blood glucose and increased HbA1c.

Subject	Diabetic improvement	Improved mechanisms								
		Incretin effect (%)			Insulin sensitivity			ß-cell function		
		GIR	Insulin	C-peptide	GIR	ISI	HOMA	AIR-Insulin	AIR-C-peptide	DI
1	-/+	+	+	+	+	+	+	+	+	+
2	-/+	+	+	+	+	+	+	-	+	-
3	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	-	+	+	-	-	-
5	+	-	-	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	-	+	+
8	+	-	+	-	+	+	+	+	+	+
9	+	+	-	+	+	+	+	+	+	+
10	+	+			+					
11	+	+			+					
12	+	+			+					

4.6. Lipid profile

Changes of lipid parameters are shown in Table 9 and Figure 17. Caloric restriction induced significant decreases in total-cholesterol and Lp(a) at visit 2 and visit 3. Although triglycerides and VLDL-cholesterol tended to decrease at visit 2 and visit 3, the changes in these parameters were not significant. LDL-cholesterol decreased significantly only at visit 3, while, VLDL-triglycerides decreased significantly only at visit 2. Interestingly, HDL-cholesterol decreased significantly at visit 2.

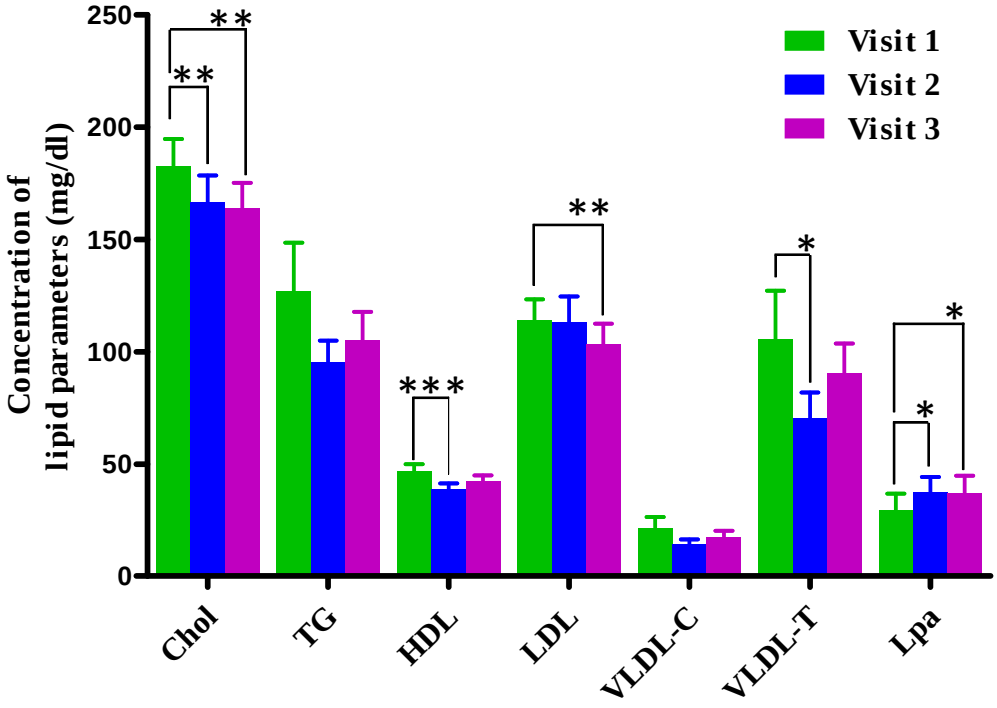


Figure 17: The effect of caloric restriction on lipid parameters; Chol – total cholesterol; TG – triglycerides; HDL – HDL-cholesterol; LDL – LDL-cholesterol; VLDL-C – VLDL-cholesterol; VLDL-T – VLDL-triglyceride; Lp(a) – Lipoprotein (a)

Table 9: Effect of caloric restricted intervention on lipid metabolism (N=12)

Lipid parameters	**Visit 1**	**Visit 2**	**Visit 3**
Cholesterol (mg/dl)	182.8 ± 11.9	166.4 ± 12.1$^{(**)}$	163.8 ± 11.3**
Triglycerides (mg/dl)	127.1 ± 21.5	95.3 ± 9.5	105.2 ± 12.6
HDL-chol (mg/dl)	47.0 ± 3.0	38.8 ± 2.5$^{(***)}$	42.6 ± 2.2
LDL-chol (mg/dl)	114.2 ± 9.2	113.2 ± 11.4	103.4 ± 9.0**
VLDL-cholesterol (mg/dl)	21.7 ± 4.8	14.3 ± 2.0	17.6 ± 2.6
VLDL-triglyceride (mg/dl)	105.5 ± 21.7	70.3 ± 11.6$^{(*)}$	90.3 ± 13.4
Lp(a) (mg/dl)	27.7 ± 7.6	37.4 ± 6.7$^{(*)}$	37.2 ± 7.5*

5. DISCUSSION

5.1. Caloric intake and weight loss

The protocol of our study was such that we tried to imitate nutritional intake following bariatric surgery. During the first phase of the study (day 0 until day 10 of caloric restriction) average caloric intake was 678.8 ± 47.8 kcal/day and while it was 1400.8 ± 52.0 kcal/day during the second phase of caloric restriction (day 11 until 3 months). The recommended caloric intake following bariatric surgery is about 500 kcal/day for the first 2 weeks after surgery [44]. Golpaie A et al. evaluated caloric intake in 30 severely obese subjects treated with laparoscopic restrictive bariatric surgery and reported a mean caloric intake of 621.7 ± 301.0 kcal/day during the first 6 weeks following surgery [45]. Similarly, Trostler N et al. reported a caloric intake of 694 ± 105 kcal/day in men and 579 ± 111 kcal/day in women during the first 30 days after gastric bypass Roux-en-Y for morbidly obese patients [46]. Thus, the caloric intake immediately after bariatric surgery is very homogenous in previously published studies and is very similar to the one observed during the the first phase of caloric restriction in our study. This also indicates that our subjects were very compliant with the protocol during this study period. The fact that subjects were admitted to the hospital was an important factor in achieving this compliance. While caloric intake shows little variation immediately after surgery, it is more variable 3 months later. The caloric intake 3 months after bariatric surgery was 535 ± 158 kcal/day and 529.4 ± 300.2 kcal/day in studies of Trotsler N et al. and Dias MC et al., respectively [46, 47]. Bobbioni-Harsch E et al. showed a caloric intake of 966 ± 42 kcal/day at 3 months after gastric bypass in a cohort of fifty obese women [48]. Another study reported a caloric intake of 899 ± 41 kcal/day and 871 ± 47 kcal/day at 4 months after gastric bypass and sleeve gastrectomy, respectively [49]. Although caloric intake during the second phase of caloric restriction in our study is higher than in the above mentioned studies, it is still significantly lower compared to the period before the study.

The weight loss observed in our study is due to caloric restriction. There is abundant evidence that bariatric surgery induces weight loss short term after surgery. A decrease in BMI at visit 2 in our study is line with that in postoperative patients in some previously published studies. Rizeello M et al. described a decrease from the initial value of 44.76 ± 7.2 kg/m^2 to 43.3 ± 7.7 kg/m^2 at 15 days after sleeve gastrectomy [23]. Umeda LM et al. studied 10 obese patients with type 2 diabetes treated with gastric bypass and showed a decrease from 39.7 ± 1.9 kg/m^2 before surgery to 37.0 ± 2.1 kg/m^2 at 7 days after surgery [25]. The effect on BMI 3 months after surgery is much more variable ranging from 4 to 10 kg/m^2 [25, 46-48, 50-53]. The BMI

in our study dropped by 5.7 kg/m^2 at visit 3 (3 months). This decrease is less than the one described in some but not all previously published studies. While Trostsler N et al. and Harsch E et al. [46, 48] describe a decrease of more than 8 kg/m², Ballantyne GH et al. and Dias MC et al. [47, 50] describe a decrease similar to the one observed in our study. These differences may be the consequence of different caloric intakes in the different studies.

5.2. Improvement in glucose metabolism

Bariatric surgery leads not only to weight loss but also to an improvement in glucose metabolism [50, 52, 54]. The role of caloric restriction and weight loss in improved glucose metabolism after bariatric surgery is still controversial. Although the role of caloric restriction in the improvement in glucose metabolism is established in the literature, many studies show that the improvement in glucose metabolism occurs after bariatric surgery before any significant weight loss is observed. The data from these studies support a weight-loss independent mechanism of improved glucose metabolism after bariatric surgery [23-25, 27, 28, 55]. Our study shows that the improvement in glucose metabolism was present in most of the subjects despite the fact that the weight loss was less or equivalent to that observed after bariatric surgery. The improvement in glucose metabolism at visit 2 in our study was assessed by the significant decrease of fasting blood glucose. Because visit 2 occurred on the 10th day of first phase of caloric restriction, changes in HbA1c and diabetic medications were not expected. The diabetic improvement at visit 3 was evident by a decrease in fasting glucose, medication doses, and HbA1c. Two subjects had an increase in HbA1c (despite a decrease in fasting blood glucose) at visit 3. One subject had aduration of diabetes of 10 years. It has been described extensively in the past that duration of diabetes and glycemic response to treatment is correlated to each other. Increasing duration of diabetes is associated with decreasing ß-cell function [56, 57] and insulin resistance may increase with duration of diabetes [58]. Duration of diabetes is also a major predictor of whether or not diabetes remission will occur after bariatric surgery in patients with type 2 diabetes, and it seems that ten-year duration is a cut point in this respect [54, 59, 60]. The other subject with an increase in HbA1c had a baseline value of 5.6 % at visit 1 which increased to 6.0 % at visit 3. However, fasting blood glucose dropped by 7 mg/dl, and the dose of metformin decreased from 1.5 g/day at visit 1 to 1 g/day at visit 3. In our study, the improvement in glucose metabolism occurred in the context of a significant weight loss. However, the degree of improvement of fasting glucose and of HbA1c was not associated with the degree of weight loss. This dissociation could be explained by a small variation in weight loss and by the small study group. Some studies report a weight loss

independent effect of caloric restriction on the improvement in glucose metabolism [12]. Our study design does not allow to seperate effect of caloric restriction from the effect of weight loss in the improvement in glucose metabolism. After bariatric surgery, weight loss commonly occurs in the context of caloric restriction. The results in our study confirm the role of caloric restriction and weight loss in the improvement in glucose metabolism, although there may be additional factors mediating the improved glucose metabolism after bariatric surgery.

5.3. Mechanisms of the improved glucose metabolism

5.3.1. Incretin effect

An improved incretin effect (increase in incretin levels and effect) is considered to be an important mediator for the improved glucose metabolim following bariatric surgery [41, 61]. Because caloric restriction and weight loss occur very early after bariatric surgery, it is still controversial whether the improved incretin effect results from caloric restriction and weight loss or from other additional factors [62, 63].

Our study assessed the effect of caloric restriction and weight loss on the incretin effect. The results from our study highlight two important aspects of the incretin effect.

The first is that the incretin effect is retained in morbidly obese patients with type 2 diabetes. This is evident by comparing the results of the HGC with test meal at day 1 of visit 1 with those of the HGC without test meal at day 3 of visit 1. The significant increase of plasma insulin concentration, C-peptide concentration, and glucose infusion rate after the test meal indicates an increase in glucose-stimulated insulin secretion after the test meal. Although GLP-1 and GIP were not quantified in our study, the increase in glucose-stimulated insulin secretion after the test meal most likely corresponds to the effect of incretin hormones on beta cells. Thus the incretin effect is retained in the subjects in our study. Many studies show that the incretin effect in individuals with T2DM is impaired compared to controls [64]. However, there is evidence that in individuals with well-controlled diabetes, the effects of endogenous GLP-1 on insulin secretion is comparable to that in nondiabetic individuals [65]. Most of the patients included in the current study had well-controlled diabetes with an HbA1c < 7 %. Obesity also induces a decreased GLP-1 secretion which is related to the impaired incretin effect [66]

The second observation is the significant improvement in the incretin effect after caloric restriction and weight loss. The data from our study support a significant improvement in

glucose-stimulated insulin secretion after the test meal at visit 3. This improvement may result from an improved secretion of endogenous incretin hormones or/and an improvement in the sensitivity of insulin secretion to endogenous incretin hormones. Because incretin hormones were not quantified, these two features cannot be separated in our study. The effects of incretin hormones on glucose metabolism have been shown by the administration of GLP-1 receptor agonists and DPP-4 inhibitors both also used for treatment of diabetes. Studies evaluating the effect of dietary interventions on the incretin effect in individuals with type 2 diabetes are scarce. Laferrere B et al. showed an unchanged GLP-1 and GIP secretion after an hypocaloric diet, while there was an increased GLP-1 and GIP secretion after gastric bypass surgery in patients with type 2 diabetes despite similar weight loss [28]. Similar results were also described by Valderas JP et al. in obese patients without diabetes treated with sleeve gastrectomy or with medication for obesity. Although patients achieved similar weight loss 2 months after the treatment for obesity, an increase in GLP-1 secretion was present only after sleeve gastrectomy, not after medical treatment [40]. The differences between our study and the above two studies may be due to the absence of diabetes (in study of Valderas JP et al.), a smaller weight loss (9.8 kg in study of Laferrere B et al), and shorter duration dietary intervention (two months in study of Valderas and one month in study of Laferrere).

Thus, our study confirms that caloric restriction and weight loss leads to a significant improvement in the incretin effect that may be one of the major mediators of the improved glucose metabolism.

5.3.2. Insulin sensitivity

Bariatric surgery induces an improvement in insulin sensitivity that contributes to the improved glucose metabolism after surgery. Interestingly, many previous studies show that the improvement in insulin sensitivity occurs very early after both malabsorptive and restrictive procedures before any significant weight loss occurs. In the studies of Rizzello M et al., Peterli R et al., and Wickremesekera K et al., insulin sensitivity (estimated by HOMA-IR and EGC) improved already one week after sleeve gastrectomy and gastric bypass before weight had significantly dropped. The authors of these studies concluded that a hormonal mechanism may be involved in these changes [23, 63, 67]. Hady HR et al. studied the improvement in insulin sensitivity on day 7 after restrictive bariatric surgery and showed that the insulin sensitivity improved significantly before significant weight loss occurred. Our study shows a significant improvement in insulin sensitivity in the context of a significant weight loss following caloric restriction. The improvement in insulin sensitivity was

estimated by changes in fasting insulin concentration, HOMA-IR, glucose infusion rate, and insulin sensitivity index during the EGC performed before and after caloric restriction. These variables tended to change (decrease in fasting insulin concentration and HOMA-IR, increase in glucose infusion rate and insulin sensitivity index during the EGC) at visit 2 and improved significantly at visit 3. The correlation between weight loss and insulin sensitivity has been established in the literature [68-70]. Many possible mechanisms for the improvement in insulin sensitivity after weight loss have been discussed in previous studies. Schenk S et al. showed that weight loss leads to decreased systemic fatty acid mobilization and uptake resulting in improvement in insulin sensitivity [71]. The improvement in hepatic insulin sensitivity after weight loss is related to a decrease in liver fat [72, 73]. Weight loss may also decrease subclinical inflammation which plays an important role in inducing and maintaining insulin resistance [74].

The degree of the improvement in insulin sensitivity seems to be related to the degree of weight loss. Borges RL et al. studied female patients with abdominal obesity and showed that a weight loss of more than 5 % was associated with improved insulin sensitivity [75]. The result in the study by Borges would fit to the observations from our study, where no significant improvement in insulin sensitivity was observed at visit 2 with a weight loss of 4.9 ± 0.3 %, while there was a significant improvement at visit 3 with a weight loss of 9.5 ± 1.3 %. Interestingly, although the decrease in body weight between visit 1 and visit 2 (6.5 kg, after 10 days of caloric restriction) was similar to that between visit 2 and visit 3 (also 6.5 kg, after 10 weeks), the difference in insulin sensitivity was significant only between visit 1 and visit 2, but not between visit 2 and visit 3. This implicates that acute or chronic weight loss may be related to the improvement in the insulin sensitivity. Very few studies have addressed this issue previously. Kirk E et al. studied the effect of acute caloric restriction on the insulin sensitivity and described that glucose infusion rate during EGC changed at 11 weeks with weight loss of 7.5 ± 0.4 %, but not at 48h with weight loss of 2.0 ± 0.2 % [73]. However, this difference may result from the differences in lost weight rather than differences in time. The role of weight loss on the improvement in the insulin sensitivity early after bariatric surgery has not been evaluated, while a number of studies have evaluated the longer term effect of weight loss following bariatric surgery on this parameter. In the studies of Valdera JP et al. and Nosso G et al, the improved insulin sensitivity was observed when significant weight loss had occurred 2 months and 3 months after sleeve gastrectomy, respectively. These authors also mention the role of weight loss in the improved insulin sensitivity [40, 52]. In the study by Hady et al. HOMA-IR had improved on the 7th day after surgery before any significant

weight loss had occurred, but both improvement in insulin sensitivity and weight loss continued at 1 and 3 months after surgery [21].

The results from our study confirm that caloric restriction and weight loss lead to an improvement in insulin sensitivity. The degree of improvement may be related to the degree of weight loss but also to the duration of weight loss.

5.3.3. ß-cell function

An absence or decrease in the acute phase (first phase) of insulin secretion is commonly observed in patients with type 2 diabetes mellitus. In our study, the AIRg was 8.4 ± 3.5 µU/ml for insulin and 0.4 ± 0.9 ng/ml for C-peptide at visit 1. AIRg in our study was blunter than that in some previous studies which investigated the AIRg by HGC in normal individuals. Caumo A et al. used HGC to investigate the AIRg in 7 normal subjects and reported that AIRg was approximately 200 pmol/l (28.8 µU/ml) for insulin and 0.5 mmol/l (1.5 ng/ml) for C-peptide [76]. Many studies show that bariatric surgery leads to an improvement in the acute phase insulin secretion which may mediate the improvement in glucose metabolism after surgery. However, the role of caloric restriction and weight loss for the improvement in AIRg after bariatric surgery is still unclear. AIRg restoration was present one month after BPD in type 2 diabetic patients and was associated with normalized fasting blood glucose in the study of Briatore L e al. [77]. Salinari S et al investigated the acute insulin secretion one month after malabsorptive surgery in nine morbidly obese patients with type 2 diabetes and reported that the full normalization of the acute insulin secretion may be related to changes in intestinal factors [78]. The results from our study show an improvement in the acute insulin secretion at visit 2 and visit 3, although a significant improvement was observed only at visit 2. We understand that there are some limitations of using peripheral insulin and C-peptide concentrations to estimate the acute insulin secretion, which include the primary hepatic degradation of insulin and the peripheral clearance of insulin and C-peptide. When only peripheral insulin and C-peptide are used, the first phase insulin secretion may be delayed and blunted [76]. However, the above limitations may be negligible since the acute insulin secretion was estimated before and after caloric restriction (the same limitations are present at both time points). Because the improvement in insulin sensitivity was not significant and the incretin effect was not measured at visit 2, the improvement in the acute insulin secretion was the only mechanism contributing to the improved glucose metabolism at that time point. Insulin is an essential hormone in glucose metabolism. It stimulates glucose uptake and inhibits endogenous glucose production. Thus, the improvement in first phase insulin secretion may lead to an improvement in all insulin-related processes, particularly in

postprandial glucose homeostasis [79]. Mitrakou A al. studied the relation between 30 min insulin levels and 2-hour blood glucose levels after an oral glucose test and showed that the improved acute insulin secretion seems to ensure a better postprandial blood glucose [80]. The correlation between the impairment of the acute insulin secretion and the impaired fasting glucose was also described in previous studies. Kanat M et al. studied ß-cell function in patients with impaired fasting glucose and describes an impaired first phase secretion of insulin [81]. Ozaki K et al. studied 8923 subjects and shows that there is a decrease in the acute insulin secretion in patients with fasting plasma glucose > 110 mg/dl [82].

In type 2 diabetes mellitus, it is likely that insulin resistance precedes the impaired ß-cell function. Insulin resistance modifies ß-cell function in order to maintain a normal glucose homeostasis [83]. The relationship between the insulin sensitivity and ß-cell function is that as insulin sensitivity decreases, insulin secretion This relationship can be described and estimated by calculating the product of the acute insulin secretion and insulin sensitivity index (deposition index: ID=AIRg X ISI). This product should be constant as long as the ß-cell has enough capacity to compensate for an increase in insulin resistance. A decrease in this product indicates that ß cells are unable to fully compensate for insulin resistance. A decrease in this deposition index is commonly present in patients with impaired glucose tolerance, impaired fasting blood glucose, and type 2 diabetes [84]. The marked improvement (a two-fold increase at visit 2 and visit 3) in the deposition index, which results from the combined improvement in AIRg and ISI, confirms the effect of caloric restriction and weight loss on ß-cell function.

5.4. Improvement in lipid metabolism

Dyslipidemia is associated with an increased risk for cardiovascular disease. Although dyslipidemia was not pronounced before caloric restriction in our subjects, others report a common dyslipidemia in patients with T2DM and/or obesity [85, 86]. Despite the fact that dyslipidemia was only mild in our subjects triglycerides and non-HDL cholesterol decreased after caloric restriction in our study. The role of caloric restriction and weight loss on lipid metabolism has been described in previous studies. Bouwman FG et al. studied the effect of a very low caloric diet on lipid metabolism in overweight/obese subjects and reported a significant decrease in total cholesterol and LDL-cholesterol [87]. A significant decrease in triglycerides and total cholesterol was also observed after a weight loss of 11kg in a study of Jourdan M et al. [88]. Similarly, a decrease in triglycerides and non-HDL cholesterol was also observed after bariatric surgery. Hady et al. described a significant decrease in total cholesterol, triglycerides, and LDL-cholesterol in 100 obese patients 3 months after sleeve

gastrectomy [89]. In general, weight loss results in a decrease in triglycerides, VLDL-triglyceride, and non-HDL cholesterol [90]. The decrease in triglycerides after weight loss may result from a decrease in the hepatic VLDL secretion, which may be the consequence of a decreased substrate flux (fatty acids) for VLDL production [91]. Non-HDL-cholesterol is a calculated parameter which encompasses LDL-cholesterol and remnant-cholesterol [92]. An improvement in this parameter reflects either an increase in HDL-cholesterol or a decrease in LDL and/or remnant cholesterol. In our study the improvement was mostly related to a decrease in remnant cholesterol. With respect to lipid metabolism, two unexpected changes were observed in our study: a decrease in HDL-cholesterol and an increase in Lp(a) were observed after caloric restriction and weight loss. Although most of the previous studies reported an increase in HDL-cholesterol after weight loss, a decrease was also observed in study of Thompson PD et al. [93]. The mechanism behind this observation is unclear but factors such as a dramatically decreased cholesterol intake during caloric restriction may be involved. Although elevated concentrations of Lp(a) are an established risk factor for cardiovascular diseases, very little is known about the metabolism of these particles. In contrast to other lipid parameters, Lp(a) levels change very little with lipid lowering agents. The effect of weight loss after bariatric surgery or other medical interventions on Lp(a) levels was variable in previous studies. Some studies show a significant decrease [94, 95], while others describe a significant increase or no change [96, 97]. The importance of this finding is currently unclear.

6. LIMITATIONS OF STUDY

The current study has several limitations, some of which will be overcome by future analyses. We did not measure the incretin effect at visit 2 and therefore cannot say whether the incretin effect has improved already at visit 2 (like beta-cell function) or only at visit 3 (like insulin sensitivity). Furthermore, we so far have not measured incretin levels, which would be important to decide which of the involved hormones is primarily affected. Finally, we have not yet compared the results of the dietary intervention directly to the results of the surgical procedure. Measuring of incretin levels and direct comparison will be performed once the whole project is finished.

7. CONCLUSION

In this study we used euglycemic and hyperglycemic clamps to evaluate the mechanisms of improved glucose metabolism in obese type 2 diabetic patients after caloric restriction and weight loss. The results of the current study show that:

- Caloric restriction and weight loss lead to an improvement in glucose and lipid metabolism.
- Improved glucose metabolism after caloric restriction and weight loss may be mediated by an improvement in the incretin effects, insulin sensitivity, and ß-cell function.
- Improvement in glucose metabolism after bariatric surgery may be at least partly explained by caloric restriction and weight loss, not specific for the type of intervention.
- Properly controlled studies with larger cohorts are necessary to elucidate the beneficial effects of bariatric surgery.

8. SUMMARY

Objective

The prevalence of obesity and type 2 diabetes mellitus is increasing quickly and is a major challenge to health care systems in the world. Bariatric surgery, which has been used widely for the treatment of morbidly obese patients, results not only in weight loss but also in a dramatic improvement in glucose metabolism. It is however unclear whether caloric restriction, weight loss or other surgery related factors mediate the improved glucose metabolism after bariatric surgery. Caloric restriction and weight loss occurs after bariatric surgery. However, there is evidence that the improvement in glucose metabolism is present immediately after surgery before any significant weight loss occurs. This evidence, therefore, supports the role of other weight loss-independent mechanisms in mediating the antidiabetic effect of bariatric surgery.

To better understand the beneficial effects of bariatric surgery we designed a study in which morbidly obese subjects with type 2 diabetes follow a dietary protocol identical to one that patients receiving bariatric surgery have to follow. Our aim was to evaluate the role of caloric restriction and weight loss on glucose and lipid metabolism and analyse the mechanisms behind the improved glucose metabolism.

Subjects and methods

Morbidly obese subjects with type 2 diabetes followed the same dietary protocol as patients receiving bariatric surgery. The dietary protocol includes two phases. The first phase lasts 10 days and subjects have to stay in hospital to comply fully with the protocol. The second phase lasts about 10 weeks and subjects are seen on an out-patient basis. Glucose and lipid metabolism was assessed before beginning caloric restriction (visit 1), and again after 2 weeks (visit 2, immediately after the first phase of caloric restriction) and 12 weeks (visit 3, immediately after the second phase of caloric restriction). The improvement in glucose metabolism was evaluated by changes in fasting blood glucose, HbA1c, and dose of antidiabetic medications. The improvement in lipid metabolism was evaluated by changes in lipid parameters. Potential mechanisms mediating the improved glucose metabolism, which were evaluated in the current study, include changes of the incretin effect, of insulin sensitivity and of ß-cell function. Incretin effect was estimated by the increment of values (glucose infusion rate, insulin concentration, and C-peptide concentration) between 90 – 120 min (before test meal) and 125 – 360 min (after test meal) during the 6-hour hyperglycemic clamp with a test meal. Changes in insulin sensitivity were evaluated by changes in fasting plasma insulin, fasting plasma C-peptide, HOMA-IR, glucose infusion rate during last 60

minutes of a 3-hour euglycemic clamp, and the insulin sensitivity index. The changes in acute insulin secretion to hyperglycemia (AIRg) and disposition index were used to evaluate changes in ß-cell function.

Results

Twelve subjects were included in the study (8 women, 4 men; mean age 50.2 ± 2.2 years; mean duration of diabetes 5.2 ± 0.7 years). Most of the subjects had good glycemic control with mean HbA1c of 6.7 ± 0.3 % before beginning of caloric restriction. The average energy intake during the first phase and the second phase of caloric restriction was 678.8 ± 47.8 kcal/day and 1407.3 ± 52.7 kcal/day, respectively. BMI decreased significantly from 46.0 ± 2.1 kg/m2 to 43.8 ± 2.0 kg/m2 and 41.7 ± 2.1 kg/m2 at visit 2 and visit 3, $p < .001$ for both.

There was a significant improvement in glucose metabolism after the first phase and the second phase of caloric restriction. This improved glucose metabolism is evident from a significant decrease in fasting blood glucose at visit 2 (decreased from 133.1 ± 6.4 mg/dl to 114.6 ± 6.7 mg/dl, $p < .01$), and from the combined decrease in fasting blood glucose, HbA1c, and dose of antidiabetic medications at visit 3 (fasting blood glucose deceased from 133.1 ± 6.4 mg/dl to 110 ± 4.7 mg/dl, $p < .001$; HbA1c decreased from 6.7 ± 0.3 % to 6.2 ± 0.1 %, $p = 0.06$; dose of antidiabetic medications decreased in 5/10 subjects). A significant improvement in lipid metabolism was also present at visit 2 and visit 3 (total cholesterol decreased significantly from 182.8 ± 11.9 mg/dl to 166.4 ± 12.1 mg/dl and 163.8 ± 11.3 mg/dl; triglyceride decreased from 127.1 ± 21.5 mg/dl to 95.3 ± 9.5 mg/dl and 105.2 ± 12.6 mg/dl; LDL-cholesterol decreased significantly from 114.2 ± 9.2mg/dl to 113.2 ± 11.4 mg/dl and 103.4 ± 9.0 mg/dl; VLDL-cholesterol decreased from 21.7 ± 4.8 mg/dl to 14.3 ± 2.0 mg/dl and 17.6 ± 2.6 mg/dl; VLDL-triglyceride decreased from 105.5 ± 21.7 mg/dl to 70.3 ± 11.6 mg/dl and 90.3 ± 13.4 mg/dl).

The improvement in glucose metabolism after caloric restriction in our study is accompanied by an improved incretin effect, enhanced insulin sensitivity, and better ß-cell function. Percent incretin effect increased significantly from visit 1 to visit 3 (not evaluated at visit 2) (from 51.2 ± 5.0 % to 70.6 ± 4.7 %, $P < .05$, estimated by insulin; from 47.5 ± 4.1 % to 63.1 ± 4.2 %, $P < .05$, estimated by C-peptide; from 44.4 ± 6.8 % to 67.0 ± 3.7 %, $P < .01$, estimated by glucose infusion rate). Enhanced insulin sensitivity was present only at visit 3, but not at visit 2, and evident from the significant improvement in fasting plasma insulin (decreased from 20.0 ± 2.5 µU/ml to 9.3 ± 2.5 µU/ml, $p < .05$), HOMA-IR (decreased from 6.7 ± 0.9 to 2.7 ± 0.8, $p < .001$), glucose infusion rate during last 60 min of 3-hour euglycemic clamp (increased from 3.7 ± 0.5 mg/kg/min to 5.5 ± 0.6 mg/kg/min, $p < 0.01$), and insulin sensitivity index

(increased from 22.9 ± 3.8 to 45.5 ± 9.8, $p < .05$). Better ß-cell function was evident from a significant improvement in acute insulin secretion to hyperglycemia of ß cells at visit 2 (mean increment of insulin concentration between the fasting and the first ten minutes after glucose bolus during hyperglycemic clamp increased from 8.4 ± 3.5 µU/ml at visit 1 to 14.5 ± 5.1 µU/ml at visit 2, $p < .05$) and a significant increase in disposition index at visit 3 (increased from 244.2 ± 131.1 at visit 1 to 542.1 ± 201.0 at visit 3, $p < .05$). The improvement in acute insulin secretion of ß cells was also present at visit 3 and the increase in disposition index was also present at visit 2. However, these changes were not significant.

Conclusion

This is a study evaluating the role of caloric restriction and weight loss on glucose and lipid metabolism and determining potential mechanisms mediating the improved glucose metabolism in obese type 2 diabetic patients. The results from our study show that caloric restriction and weight loss lead to a significant improvement in glucose and lipid metabolism. This improved glucose metabolism is mediated by an improved incretin effect, enhanced insulin sensitivity, and better ß-cell function. However, these improvements occur at different time points. While better beta-cell function is detectable very early after initiation of caloric restriction, insulin sensitivity only improves after several weeks and months. Due to a dietary protocol which is comparable to that of patients undergoing bariatric surgery, our subjects had the same average energy intake and weight loss after the first phase and second phase of caloric restriction. Thus, our study supports the concept that the improvement in glucose metabolism after bariatric surgery is at least partially (if not fully) explained by the caloric restriction and weight loss. Further studies directly comparing patients undergoing bariatric surgery with those undergoing a dietary intervention are necessary to further understand the beneficial effects of caloric restriction and weight reduction.

ZUSAMENFASSUNG

Zielsetzung

Die Prävalenz von Adipositas und Typ-2 Diabetes mellitus nimmt rasant zu und stellt eine große Herausforderung für Gesundheitssysteme weltweit dar. Bariatrische Chirurgie, die in großem Umfang zur Behandlung von krankhaft adipösen Patienten verwendet wird, führt nicht nur zu Gewichtsverlust, sondern auch zu einer dramatischen Verbesserung des Glukosemetabolismus. Trotzdem ist unklar, ob die kalorische Restriktion, der Gewichtsverlust oder andere, mit der Operation einhergehende Faktoren einen verbesserten Glukosestoffwechsel nach bariatrischer Chirurgie bedingen. Sowohl kalorische Restriktion als auch Gewichtsverlust treten nach einer bariatrischen Operation auf. Es gibt jedoch Belege dafür, dass eine Verbesserung des Glukosemetabolismus unmittelbar nach der chirurgischen Intervention und noch vor einem signifikanten Gewichtsverlust eintritt. Diese Erkenntnis unterstützt ihrerseits die Bedeutung von anderen, vom Gewichtsverlust unabhängig vermittelten Mechanismen, die für den antidiabetischen Effekt nach bariatrischen Operationen verantwortlich sein könnten.

Um die positiven Auswirkungen der bariatrischen Chirurgie auf den Stoffwechsel besser verstehen zu können, führten wir eine Studie durch, in der stark adipöse Typ-2-Diabetiker ein Ernährungsverhalten annehmen, das dem von bariatrisch operierten Patienten entspricht. Dies erlaubte uns die Rolle von kalorischer Restriktion und Gewichtsverlust bezüglich der Verbesserung des Glukose- und Lipidmetabolismus und die dahinter steckenden Mechanismen zu beurteilen.

Probandenkollektiv und Methodik

Stark adipöse Typ-2 Diabetiker ernährten sich analog zu Patienten, die sich einer bariatrischen Operation unterzogen. Der Ernährungsplan umfasste zwei Phasen. Die erste, zehntägige Phase mussten die Probanden in der Klinik verbringen um eine gute Compliance bezüglich der Ernährungsvorgaben zu erreichen. Die zweite Phase dauerte zehn Wochen und fand bei den Probanden zu Hause statt. Daten zu Glukose- und Fettstoffwechsel wurden vor dem Beginn der kalorischen Restriktion (Visit 1), nach zwei Wochen (Visit 2, unmittelbar nach der ersten Phase der kalorischen Restriktion) und zwölf Wochen (Visit 3, unmittelbar nach der zweiten Phase der kalorischen Restriktion) erhoben. Die Verbesserung des Glukosemetabolismus wurde in Form von einer veränderten Nüchternblutglukose, des HbA1c-Werts und der Dosis der antidiabetischen Medikamente erfasst. Die Verbesserung des Fettstoffwechsels wurde als Änderung der Lipidparameter erfasst. Die in unserer Studie untersuchten Mechanismen, die zu einer Verbesserung des Glukosestoffwechsels geführt haben, umfassen den Inkretineffekt,

die Insulinsensitivität und die ß-Zellfunktion. Der Inkretineffekt wurde als Verbesserung von Messwerten (Glukoseinfusionsrate, Insulinkonzentration, C-Peptid-Konzentration) zwischen 90-120 Minuten (vor der Testmahlzeit) und 125-360 Minuten (nach der Testmahlzeit) während des sechsstündigen hyperglykämischen Clamp mit Testmahlzeit festgelegt. Die Verbesserung der Insulinsensitivität wurde in Form von Veränderungen des Nüchternplasmainsulins, des Nüchternplasma-C-Peptids, des HOMA-IR, der Glukoseinfusionsrate während der letzten 60 Minuten des dreistündigen euglykämischen Clamps und des Insulinsensitvitätsindex erfasst. Die Veränderungen bezüglich der akute Insulin response auf Hyperglykämie (AIRg) und der Disposition Index wurden zur Beurteilung der ß-Zell-Funktion herangezogen.

Ergebnisse

Zwölf Patienten wurden in die Studie eingeschlossen (8 Frauen, 4 Männer; mittleres Alter 50,2 ± 2,2 Jahre; mittlere Diabetesdauer 5,2 ± 0,7 Jahre). Die meisten Probanden hatten einen gut eingestellten Blutzucker mit einem mittleren HbA1c von 6,7 ± 0,3 % vor dem Beginn der kalorischen Restriktion. Die mittlere Energieaufnahme während der ersten Phase und während der zweiten Phase der kalorischen Restriktion war 678,8 ± 47,8 kcal/Tag bzw. 1407,3 ± 52,7 kcal/ Tag. Der BMI nahm signifikant von 46,0 ± 2,1 kg/m2 auf 43,8 ± 2.0 kg/m2 (Visit 2, p <,001) und 41,7 ± 2,1 kg/m2 (Visit 3, p <,001) ab.

Eine signifikante Verbesserung des Glukosemetabolismus war sowohl nach der ersten Phase als auch nach der zweiten Phase der kalorischen Restriktion feststellbar. Dieser verbesserte Glukosestoffwechsel lässt sich aus der signifikanten Senkung des Nüchternblutzuckers bei Visit 2 (Rückgang von 133,1 ± 6,4 mg/dl auf 114,6 ± 6,7 mg/dl, p <,01), und aus der kombinierten Senkung des Nüchternblutzuckers, des HbA1c-Werts, und der Dosis von antidiabetisch wirksamen Medikamenten bei Visite 3 (Rückgang des Nüchternblutzuckers von 133,1 ± 6,4 mg/dl auf 110 ± 4,7 mg/dl, p <,001; Senkung des HbA1c von 6,7 ± 0,3 % auf 6,2 ± 0,1 %, p = 0,06; Dosisreduktion von Antidiabetika in 5/10 Probanden) ableiten. Auch eine signifikante Verbesserung des Fettstoffwechsels war bei Visit 2 und 3 evident (Gesamtcholesterin sank signifikant von 182,8 ± 11,9 mg/dl auf 166,4 ± 12,1 mg/dl bzw. 163,8 ± 11,3 mg/dl , Triglyceride sanken von 127,1 ± 21,5 mg/dl auf 95,3 ± 9,5 mg/dl bzw. 105,2 ± 12,6 mg/dl; LDL-Cholesterin sank signifikant von 114,2 ± 9,2 mg/dl auf 113,2 ± 11,4 mg/dl bzw. 103,4 ± 9,0 mg/dl; VLDL-Cholesterin sank von 21,7 ± 4,8 mg/dl auf 14,3 ± 2,0 mg/dl bzw. 17,6 ± 2,6 mg/dl; VLDL-Triglyceride sanken von 105,5 ± 21,7 mg/dl auf 70,3 ± 11,6 mg/dl bzw. 90,3 ± 13,4 mg/dl).

Die Verbesserung des Glukosestoffwechsels nach kalorischer Restriktion wird in unserer Studie vom einem verbesserten Inkretineffekt, einer gesteigerten Insulinsensitivität und einer besseren ß-Zell-Funktion begleitet. Die prozentuale Verbesserung des Inkretineffekts von Visit 1 bis Visit 3 (nicht eruiert bei Visit 2) war im signifikanten Bereich (von 51,2 ± 5,0 % auf 70,6 ± 4,7 %, p <0,05, anhand von Insulindaten ermittelt; von 47,5 ± 4,1 % auf 63,1 ± 4,2 %, P <0,05, anhand von C-Peptid-Daten ermittelt; von 44,4 ± 6,8 % auf 67,0 ± 3,7 %, p <0,01, anhand der Glukoseinfusionsrate ermittelt). Eine verbesserte Insulinsensitivität war nur bei Visit 3 feststellbar, nicht jedoch bei Visit 2, und aus der signifikanten Verbesserung des Nüchternplasmainsulin (Verringerung von 20,0 ± 2.5 μU/ml auf 9,3 ± 2.5 μU/ml, p <.05), des HOMA -IR (Verringerung von 6,7 ± 0,9 auf 2,7 ± 0,8, p <.001), der Glukoseinfusionsrate während der letzten 60 Minuten des dreistündigen euglykämischen Clamps (Anstieg von 3,7 ± 0.5 mg/kg/min auf 5,5 ± 0.6 mg/kg / min, p <0,01), und des Insulinsensitivitätsindex (Erhöhung von 22,9 ± 3,8 auf 45,5 ± 9,8, p <.05) ableitbar. Eine bessere ß-Zellfunktion war aus einer signifikanten Verbesserung der akute Insulin response auf Hyperglykämie bei Visit 2 ersichtlich (Anstieg der mittleren Differenz der Insulinkonzentrationen im nüchternen Zustand und während der ersten zehn Minuten nach dem Glukosebolus während des hyperglykämischen Clamps von 8,4 ± 3.5 μU / ml bei Visit 1 auf 14,5 ± 5.1 μU/ml bei Visit 2, p <.05). In diesem Zusammenhang stieg auch der Disposition Index bei Visit 3 an (Anstieg von 244,2 ± 131,1 bei Visite 1 auf 542,1 ± 201,0 bei Visite 3, p <. 05). Die akute Insulinsekretion von ß-Zellen verbesserte sich auch bei Visit 3 und der Disposition Index erhöhte sich auch bei Visit 2. Allerdings waren diese Veränderungen nicht signifikant.

Fazit

Diese Studie beurteilte die Rolle der kalorischen Restriktion und des Gewichtsverlusts bezüglich des Glukose- und Lipidmetabolismus und ermittelte Mechanismen, die hinter einem verbesserten Glukosemetabolismus stehen. Die Ergebnisse unserer Studie zeigen, dass kalorische Restriktion und Gewichtsverlust zu einer signifikanten Verbesserung des Glukose- und Lipidmetabolismus führen. Dieser verbesserte Glukosemetabolismus wird durch einen verbesserten Inkretineffekt, eine gesteigerte Insulinsensitivität und eine bessere ß-Zell-Funktion vermittelt. Allerdings treten diese Verbesserungen zu unterschiedlichen Zeitpunkten auf. Während sich die beta-Zellfunktion sehr schnell nach Beginn einer Kalorienrestriktion verbessert, kommt es erst im Verlauf von Wochen bis Monaten zu einer Verbesserung der Insulinempfindlichkeit. Aufgrund einer Ernährung, die der vergleichbar ist von Patienten, die sich einer bariatrischen Operation unterzogen, hatten unsere Probanden auch die gleiche durchschnittliche Energieaufnahme und einen ähnlichen Gewichtsverlust wie operierte

Patienten. Folglich unterstützen unsere Ergebnisse das Konzept, dass die Verbesserung des Glukosemetabolismus nach bariatrischen Operationen zumindest teilweise (wenn nicht ganz) durch kalorische Restriktion und Gewichtsreduktion erklärt werden kann. Weitere Studien, in welchen der Effekt einer bariatrischen Operation direkt mit diätetischen Maßnahmen verglichen wird, sind notwendig, um die positiven Effekte einer Kalorienrestriktion bzw. Gewichtsreduktion besser zu verstehen.

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Scientific research

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APPENDICES

Protocol of hyperglycemic clamp with test meal (Day 1 of visit 1 and visit 3)

time min	actual time (h:min)	blood glucose / in duplicate (mg/dl)	blood sample EDTA (1,5 ml)	Breath sample 13C	VAS	Circulation RR (mmHg)	Circulation Puls (/min)	notes	Glucose infusion rate D 20% (ml/h)
			√	√	√				
-60		/						blood glucose by finger stick	
-30		/						take blood for adipokines	
-20		/	1					2x9ml EDTA+ 2x9 ml serum	
-15									
-10		/	2						
-5		/	3						
0		/	4		1				
2		/	5						
4		/	6						
6		/	7						
8		/	8						
10		/	9						
15		/							
20		/	10						
25		/							
30		/	11						
35		/							
40		/	12						
45		/							
50		/	13						
55		/							
60		/	14						
65		/							
70		/	15						
75		/							
80		/	16						
85		/							
90		/	17						
95		/							
100		/	18						
105		/			2				

110		/	19						
115		/	20						
120		/	21	1	3				
125		/	22						
130		/	23						
135		/	24	2	4				
140		/	25						
145		/	26						
150		/	27	3	5				
155		/							
160		/	28						
165		/		4	6				
170		/	29						
175		/							
180		/	30	5	7				
185		/							
190		/							
195		/	31	6					
200		/							
205		/							
210		/	32	7	8				
215		/							
220		/							
225		/	33	8					
230		/							
235		/							
240		/	34	9	9				
245		/							
250		/							
255		/	35						
260		/							
265		/							
270		/	36	10					
275		/							
280		/							
285		/	37						
290		/							
295		/							

300		/	38	11	10				
305		/							
310		/							
315		/	39						
320		/							
325		/							
330		/	40	12					
335		/							
340		/							
345		/	41						
350		/							
355		/							
360		/	42	13	11				
380									
400									
420									

Protocol of euglycemic clamp (day 2 of visit 1, visit 2, and visit 3)

time min	actual time (h:min)	blood glucose / in duplicate (mg/dl)	blood sample EDTA (1,5 ml)	Glucose infusion rate D 20% (ml/h)	Circulation RR (mmHg)	Circulation Puls (/min)	notes .
-60		/					blood glucose by finger stick
Insertion i.v.-cannula or check if already inserted cannulas are patent							
-50							Take fat tissue biopsie: see page 10
-40							
-30		/	1				
Start insulin infusion at 1,5 IU/kg BW, Bolus:							
-20		/		ml/h			Insulin Bolus: IU
-15		/		ml/h			Insulin infusion rate: ml/h
-10		/		ml/h			Start glucose (D20%) infusion when blood glucose is <100 mg/dl
-5		/		ml/h			
0		/	2	ml/h			
5		/		ml/h			
10		/		ml/h			
15		/		ml/h			

20		/	**3**	ml/h			
25		/		ml/h			
30		/		ml/h			
35		/		ml/h			
40		/	**4**	ml/h			
45		/		ml/h			
50		/		ml/h			
55		/		ml/h			
60		/	**5**	ml/h			
65		/		ml/h			
70		/		ml/h			
75		/		ml/h			
80		/	**6**	ml/h			
85		/		ml/h			
90		/		ml/h			
95		/		ml/h			
100		/	**7**	ml/h			
105		/		ml/h			
110		/		ml/h			
115		/		ml/h			
120		/	**8**	ml/h			
125		/		ml/h			
130		/	**9**	ml/h			
135		/		ml/h			
140		/	**10**	ml/h			
145		/		ml/h			
150		/	**11**	ml/h			
155		/		ml/h			
160		/	**12**	ml/h			
165		/		ml/h			
170		/	**13**	ml/h			
175		/		ml/h			
180		/	**14**	ml/h			
Stop insulin infusion, continue glucose infusion, diabetes lunch							
200							
220							
240							

Protocol of hyperglycemic clamp without test meal (day 3 of visit 1)

time min	actual time (h:min)	blood glucose / in duplicate (mg/dl)	blood sample EDTA (1,5 ml)		Circulation RR (mmHg)	Circulation Puls (/min)	notes	Glucose infusion rate D 20% (ml/h)
			√					
-60		/					blood glucose by finger stick	
-30		/						
-20		/	1					
-15		/						
-10		/	2					
-5		/	3					
0		/	4					
2		/	5					
4		/	6					
6		/	7					
8		/	8					
10		/	9					
15		/						
20		/	10					
25		/						
30		/	11					
35		/						
40		/	12					
45		/						
50		/	13					
55		/						
60		/	14					
65		/						
70		/	15					
75		/						
80		/	16					
85		/						
90		/	17					
95		/						

100		/	18				
105		/					
110		/	19				
115		/					
120		/	20				
125		/					
130		/					
135		/					
140		/	21				
145		/					
150		/					
155		/					
160		/	22				
165		/					
170		/					
175		/					
180		/	23				
185		/					
190		/					
195		/					
200		/	24				
205		/					
210		/					
215		/					
220		/	25				
225		/					
230		/					
235		/					
240		/	26				
245		/					
250		/					
255		/					
260		/	27				
265		/					
270		/					
275		/					
280		/	28				
285		/					

290		/					
295		/					
300		/	**29**				
305		/					
310		/					
315		/					
320		/	**30**				
325		/					
330		/					
335		/					
340		/	**31**				
345		/					
350		/					
355		/					
360		/	**32**				
380							
400							
420							