CIR ESP. 2009;**85(4)**:222-228

CIRUGÍA ESPAÑOLA

www.elsevier.es/cirugia

Original article

The effects of ileal transposition, gastrojejunal bypass and vertical gastroplasty on the regulation of ingestion in an experimental obesity model associated with diabetes mellitus type 2

*Fàtima Sabench Pereferrer, Mercè Hernàndez Gonzàlez, Santiago Blanco Blasco, Antonio Sánchez Marín, Antonio Morandeira Rivas, and Daniel del Castillo Déjardin**

Departamento de Cirugía, Facultad de Medicina y Ciencias de la Salud, Hospital Universitario de Sant Joan, Universitat Rovira i Virgili, IISPV (Instituto de Investigación Sanitaria Pere Virgili), Reus, Tarragona, Spain

ARTICLE INFORMATION

Article history:
Received July 21, 2008
Accepted October 24, 2008
On-line March 26, 2009

Keywords:
Ileal transposition
Morbid obesity
GLP-1

A B S T R A C T

Aim: The continual advances in our knowledge of the pathogenesis and hormonal disorders of morbid obesity lead to new studies in experimental animals and the development of new technical options. The aim is to asses whether ileal transposition can be a good treatment of morbid obesity associated with diabetes mellitus due to the action of intestinal peptide Glp-1 (enteroglucagon) compared to gastric bypass and vertical gastroplasty (VGB).

Material and methods: Trial enviroment: experimental animals ZDF rats (Zucker Diabetic Fatty rats). Subjects of the study: 3 groups of 10 animals each one divided as: *a)* ileal tranposition; *b)* gastro-jejunal bypass; and *c)* vertical gastroplasty. Parameters to determine: weight loss, levels of glycaemia, enteroglucagon, insulin, and ghrelin in blood, one week before the operation as a baseline control, and 15 days after the surgical procedure.

Results: Gastrojejunal bypass produces the most significant weight loss. There is a significant decrease in intake in all groups. Hyperinsulinaemia and hyperglycaemia tend to decrease after surgery in all groups, but in ileal transposition there is better control of ketosis. After gastrojejunal bypass and ileal transposition, we observed an increase in GLP-1 levels but were only significant in ileal transposition.

Conclusions: Ileal transposition produces a decrease in plasma glucose and better control of diabetes mellitus, which could benefit patients affected by morbid obesity and poor metabolic control. More studies are needed on other models of obesity. A model of exogenous and reversible obesity could be a good option to study the real benefits of the interventions.

*Author for correspondence.
E-mail address: ddelcastillo@grupsagessa.cat (D. del Castillo Déjardin).

Efectos de la transposición ileal, el bypass gastroyeyunal y la gastroplastia vertical en la regulación de la ingesta en un modelo experimental de obesidad relacionada con diabetes mellitus tipo 2

Palabras clave:
Transposición ileal
Obesidad mórbida
GLP-1

R E S U M E N

Introducción: En el marco de la cirugía metabólica, este estudio pretende valorar la transposición ileal como tratamiento quirúrgico de la obesidad mórbida relacionada con la diabetes mellitus no insulinodependiente gracias a la acción del péptido GLP-1, en relación con el *bypass* gástrico y la gastroplastia vertical. También determinar las concentraciones de grelina y su contribución a la pérdida de peso para cada técnica.

Material y métodos: Animales de experimentación del tipo Zucker Diabetic Fatty, ratas obesas y modelo de diabetes tipo 2. Tres grupos de 10 animales cada uno: *a)* transposición ileal; *b) bypass* gastroyeyunal, y *c)* gastroplastia vertical. Parámetros determinados: pérdida de peso, cambios en la ingesta, valores de glucemia, GLP-1, insulina y grelina en sangre de cada uno de los animales una semana antes de la intervención quirúrgica y a los 15 días de la cirugía.

Resultados: La intervención que produce una mayor pérdida de peso es el *bypass* gastroyeyunal. Hay una disminución de la ingesta calórica significativa para los tres tipos de intervención. No se consigue corregir el estado de hiperglucemia intensa en los tres grupos, aunque en el grupo de la transposición se logra frenar el estado de cetosis. El aumento de GLP-1 es sólo significativo en la transposición ileal.

Conclusiones: En la respuesta metabólica a la cirugía no sólo se modifica una única hormona, sino que se establece un estado de regulación y contrarregulación como traducción de una determinada acción quirúrgica. Los animales obesos, cuyo exceso de peso es de causa exógena, pueden ser un buen modelo para otros estudios en esta dirección.

Introduction

Obesity is a multifactor illness, in which genetic and environmental factors intervene and directly influences a person's quality of life and life expectancy. In morbid obesity (body mass index, ≥40), the excess of weight is accompanied by metabolic, hormonal, and inflammatory disorders that are difficult to resolve, as well as important medical, psychological, social, and economic comorbidities. Indeed, these comorbidities determine a decrease in life expectancy.[1] Certain comorbidities form the so-called metabolic syndrome, including resistance to insulin, type 2 diabetes mellitus, cardiovascular illnesses, dyslipidemias, and high blood pressure.[2-4]

At present, bariatric surgery is a fundamental pillar for the treatment of morbid obesity and its co-morbidities. The mechanisms to control diabetes mellitus, regarding bariatric surgery, include a reduction in food intake and also hormonal mechanisms.[5]

These mechanisms include, amongst others, an increase of the intestinal peptide GLP-1 or enteroglucagon, synthesised in the terminal ileum and that also increases after surgical techniques with a hypo-absorption component (gastric bypass and biliopancreatic derivation).[6] GLP-1, among its actions, inhibits the secretion of pancreatic glucagon and decreases resistance to insulin.[7] Among the different types of obesity surgery, we include, in an experimental manner, the ileum transposition technique.[8] The ileum transposition produces weight loss and an increase of GLP-1. This technique is done by performing a transposition of a segment of terminal ileum at the superior digestive tract in an isoperistaltic manner.[9] Undigested food in the superior digestive tube stimulates the secretion of GLP-1, which, indirectly, improves the glycaemic index.[10-12] Another important point is the role played by the gastric ghrelin hormone as a hunger-regulator and, therefore, of weight loss.[13,14] Ghrelin is an appetite stimulating hormone that produces weight gain in humans and mice, and that is particularly low in obese individuals.[15-17] It is a regulating factor of food intake in the central nervous system (CNS), and studies that relate concentrations of ghrelin and different bariatric surgical techniques have become indispensable given that ghrelin is synthesised, mostly in the gastric fundus.[18,19]

Establishing a connection between the ileum transposition and metabolic control of obesity leads us to consider the necessity of carrying out an experimental project on animals and, specifically, on obese mice and those affected by type 2 diabetes mellitus (Zucker Diabetic Fatty mice) and that would be the equivalent of the metabolic syndrome in humans.[20] The goal of this study is to determine the changes produced after an ileum transposition in the hormonal regulation of food intake by means of the plasma concentrations of ghrelin, GLP-1, glycaemia, weight changes, and the variation of caloric intake. Comparing it with the Roux-en-Y gastrojejunal bypass, as the current standard in the surgical treatment of morbid obesity, and with the vertical gastroplasty allows us to analyse the differences between techniques with a mixed hypo-absorbing/restrictive component and techniques with a purely restrictive character.

Material and methods

Animals: 13 week-old Zucker Diabetic Fatty (ZDF) males. Charles River Laboratories. Model of obesity and type 2 diabetes mellitus.[21]

Experimental groups: three groups of 10 animals each, according to surgical intervention. Group 1: vertical banded gastroplasty (VBG) as a restrictive technique; group 2: Roux-en-Y gastrojejunal bypass as a technique with mixed hypoabsorbing/restrictive components; group 3: ileum transposition.

Procedure: during the second week of stalling before surgery, we performed the first extraction of blood by puncture in the external jugular vein (1.5 mL) to determine the reference parameters: glucose, GLP-1, total ghrelin, and insulin. Daily weighing of animal 10 days before the intervention. Daily control of intake volume (grams of pellets).

The animal is weighed on the day of the intervention and, after 8 hours of fasting, the designated technique is carried out. After the administration, a liquid diet is given during 72 h and then standard pellets and water ad libitum. Daily control of weight and food intake. Glycaemia control after 7 days taking blood from the coccygeal vein in the tail using the Menarini® (mg/dL) glucometer after 8 h of fasting. Fifty days after the intervention, new blood samples are taken from the contralateral external jugular vein.

The extractions, processing, and interventions have been carried out in the experimental surgery laboratory of the surgery department of the medicine faculty. To determine the GLP-1, an inhibitor of the dipeptidyl peptidase 4 inhibitor was added to avoid its fast degradation. Determination of total ghrelin, insulin and GLP-1 by radioimmunoanalysis (Linco®, special kits for mice).

Stalling: animal facility of the Faculty of Medicine and Health Sciences of the Rovira and Virgili University, following the animal handling and maintenance regulations (DOGC 2.073, 10-7-1995).

Type of pellets: maintenance type AO4 from Panlab®; 3173 kcal/kg. Water: chlorinated ad libitum. Liquid diet: Resource 2.0® (2 kcal/mL).

Anaesthesia and sacrificing: all of the surgical interventions and the blood extractions were performed under general anaesthesia induced by intraperitoneal injections of Zoletil 20® (tiletamin and zolazepam) (20 mg/kg) and atropine (0.01 mg/kg). After the second blood extraction, the animals were sacrificed by and overdose of anaesthesia.

Surgical interventions

VBG: 4 cm middle laparotomy. Dissection of the greater curvature. Ligature of short vessels and gastroepiploic vessels of the antrum region with silk 6/0. The gastric transaction line is defined using "Bulldog" type clamps. Double line of continuous sutures used to close from the fundus to the antrum with polypropylene 5/0. Insertion and fixation of a 15×40 mm Goretex® band around the gastric reservoir.

Roux-en-Y gastrojejunal bypass. Middle laparotomy and transversal section of the stomach with closure of the distal

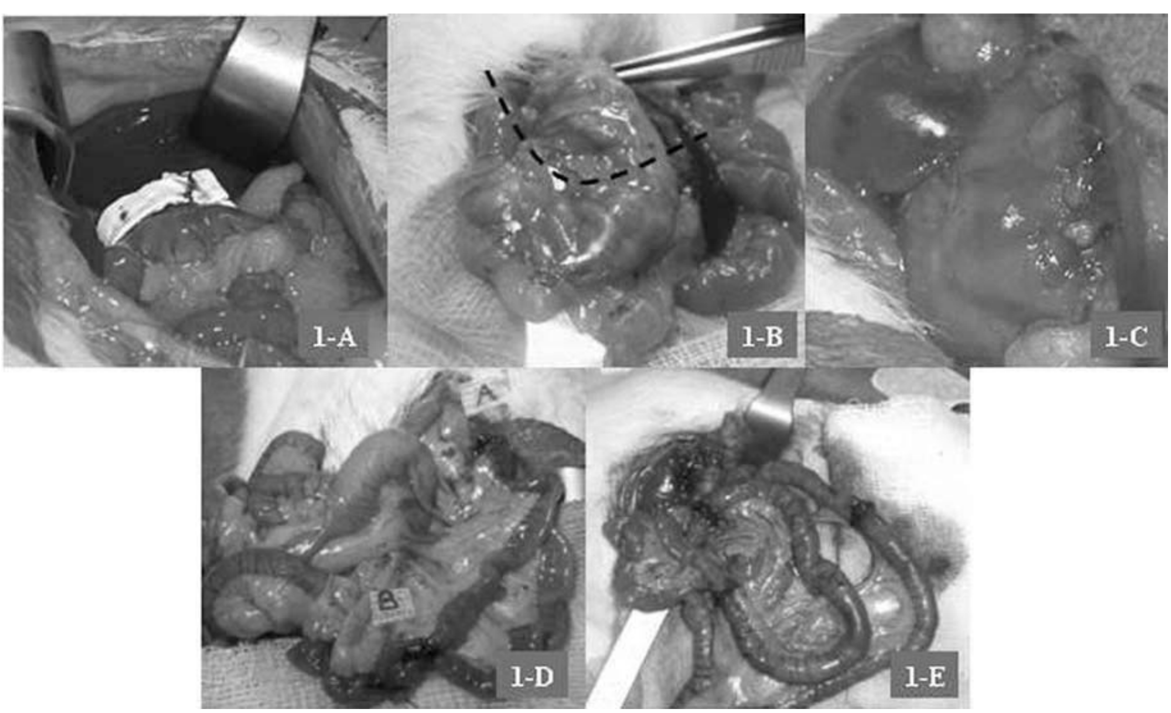

Figure 1 – A: detail of the VBG with a Goretex® band. B: horizontal or transversal gastric transsection line in the Y-en-Roux gastrojejunal bypass. C: detail of the distal or inferior gastric stump. D: gastrojejunal (A) and jejunojejunal (B) anastomosis. E: ileum transposition with detail of the anastomosis.

gastric stump (Figure 1B and C). Jejunal section at 15 cm from the Treitz ligament; this process corresponds with that described by the reviewed studies in existing literature.[20] Gastrojejunal anastomosis and terminolateral jejunojejunal anastomosis with individual sutures of polypropylen 5/0. Ten cm Roux-en-Y loop (Figure 1D).

Ileum transposition: middle laparotomy and localisation of the 10 cm long ileal segment at 10 cm from the terminal ileum. Ligature of vessels of the adjacent epiploon. Section of the segment and jejunal transposition at 2 distal cm from the Treitz ligament in the peristaltic direction. Terminoterminal anastomosis (polypropylene 5/0) (Figure 1E).

Statistical analysis

Sample calculations: formulas were used corresponding to the comparison of averages and the analysis of the variance for each one of the variables (Sample Power 2.0); weight was the determinant variable.

To compare averages in the same group and between different groups, we use the *t* of Student-Fisher test (for paired data and non-paired data respectively). General analyses were done with the SPSS-PC 13.1 statistics program.

Results

Changes in weight and food intake: in the VBG group, a significant weight decrease was observed in the first week after the intervention, although the animals recuperated the initial weight at the end of the second week. In the gastrojejunal bypass and the ileum transposition groups, however, a significant decrease was observed in the average weight during the first week and that remained stable during the second week ($P<.001$). Regarding intake, a significant decrease was observed after the first week of the intervention in the VBG group that was recuperated during the second week but did not reach the initial values. Even so, the general decrease in intake at the end of the second week is statistically significant. In the gastrojejunal and ileum transposition groups, there is a significant decrease in intake after the first week after the intervention that is maintained during the second week, also in a significant manner ($P<.001$). These changes are shown in Table 1. The weight and intake variations throughout the study are reflected in Figure 2 and Figure 3, respectively.

Biochemical changes: in the gastroplasty group there is a significant increase in the concentrations of ghrelin after the intervention. There is a tendency to decrease in the other 2 groups regarding the serum concentrations of ghrelin, but without significant statistical changes. Regarding the GLP-1 values, in the gastroplasty group a significant decrease

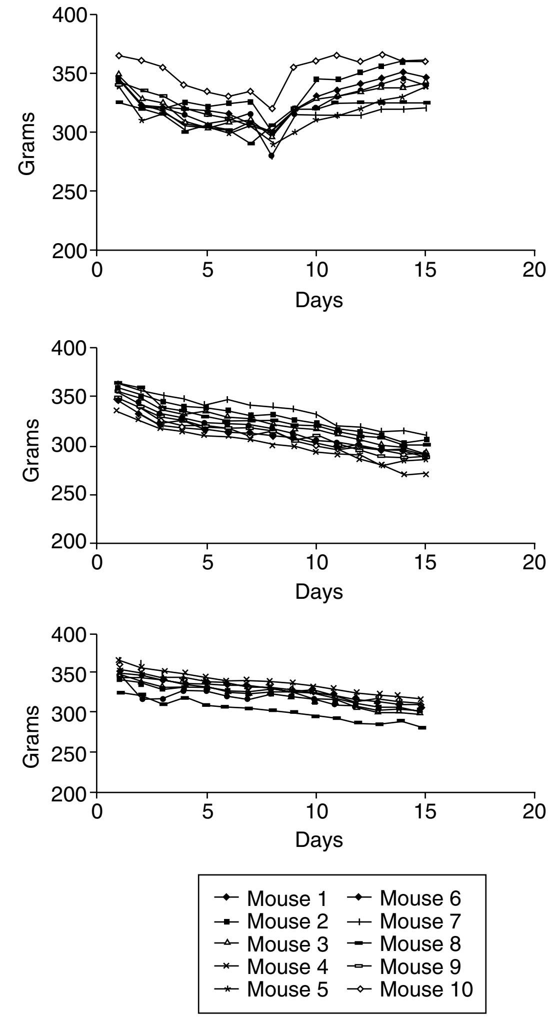

Figure 2 – Evolution of weight after surgery in the 3 experimental groups.

Table 1 – Variation of weight and food intake before and after surgery

	Weight, g	Food intake, kcal/d
Gastroplasty		
Before the surgery	344 (10)	128 (11)
1 Week after surgery	298 (10)[a]	66 (6)[a]
2 Weeks after surgery	341 (14)	107 (16)[a]
Gastrojejunal bypass		
Before the surgery	352 (9)	132 (10)
1 Week after surgery	318 (11)	80 (15)[a]
2 Weeks after surgery	291 (11)a	85 (17)[a]
Ileum transposition		
Before the surgery	351 (12)	131 (11)
1 Week after surgery	328 (11)	67 (6)[a]
2 Weeks after surgery	304 (15)[a]	76 (13)[a]

The values indicate average (standard deviation).
[a]$P<.001$.

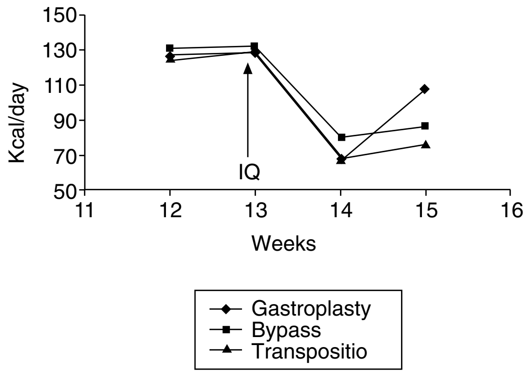

Figure 3 – Average variation of caloric intake in the 3 experimental groups before and after surgery.

Table 2 – Hormonal differences before and after surgery

	Insulin, mU/L	Ghrelin, pg/mL	GLP-1, pmol/L
Gastroplasty			
Before the surgery	1.8 (0.3)	1632 (372)	15.2 (5.5)
Post-surgery	1.9 (0.6)	2401 (343)[a]	8.1 (3)[a]
Gastrojejunal bypass			
Before the surgery	1.7 (0.3)	1503 (189)	14.4 (5)
Post-surgery	2.1 (0.3)[a]	1399 (191)	16.3 (2.5)
Ileum transposition			
Before the surgery	1.7 (0.4)	1726 (236)	14.1 (3.6)
Post-surgery	1.9 (0.3)	594 (89)	18.5 (2.7)[a]

The values indicate average (standard deviation).
[a]P<.001.

was observed after the intervention. However, in the gastrojejunal bypass group there is a tendency to increase GLP-1 concentrations, although not significantly. In the ileum transposition group a statistically significant increase in GLP-1 is produced. Concerning the insulin concentrations, there is an increase in all three animal groups, although it is only significant in the gastrojejunal bypass group (Table 2). The levels of glucose in blood undergo significant changes after the first week, without reaching normal values, although later they are recuperated during the second week, and they maintain an intense state of hyperglycaemia (groups 1 and 2). In the ileum transposition group, these changes are maintained during the second week after the intervention (Table 3).

Post-surgical complications: they are principally found during the second week after the intervention. The following stand out: three eventrations in group 1 (VBG); 2 animals presented cases of persistent diarrhoea during the second week after gastrojejunal bypass.

Table 3 – Changes in the glycaemic values before and after surgery

	Glycaemia, mg/dL		
	Before the surgery	Days after 7 surgery	14 Days after surgery
Gastroplasty	510 (74)	258 (84)[a]	525 (98)
Gastrojejunal bypass	498 (86)	281 (91)[a]	471 (68)
Ileum transposition	512 (69)	317 (72)[a]	321 (98)[a]

The values indicate average (standard deviation).
[a]P<.001.

Mortality occurred during the first 72 h of the postoperative period. Gastrojejunal bypass: 3 (23%) cases. Ileum transposition: 2 (16%) cases. The gastroplasty group did not register any deaths.

Discussion

The procedure that achieves the greatest weight loss is the gastrojejunal Roux-en-Y bypass, although the weight lost in the ileum transpositions is equally comparable. The gastroplasty produces a significant initial weight loss that is later recuperated, although not corresponding to the values that the animal should have for its age. Keeping in mind the fact that the mice gained weight in a manner inherent to their physiology throughout the weeks studies, the final weight of this group of animals is found in the value of their ideal weight according to anthropometric parameters of the control ZDF mice provided by the Charles Rives® Laboratories (week 13, ideal weight of 320 g, and week 15, ideal weight of 344 g).

There is a statistically significant general decrease in caloric intake for the 3 groups. This type of animal has an intake behaviour that is substantially different than other types of animals. The baseline caloric intake is approximately 50% higher than that observed in Sprague-Dawley or Zucker mice. Even before a predetermined genetic situation, the effects of the surgery and its metabolic improvements lead to the attribution of said effects.[22] Other studies with non-obese mice, but with type 2 diabetes behaviour and to those that have undergone an ileum transposition, show no effects on intake or weight but they do show effects on the GLP-1.[23] This would demonstrate the effectiveness and the possibility to indicate the intervention in situations of obesity related to type 2 diabetes mellitus.

The significant increase in ghrelin concentrations after VBG, as well as in other restrictive techniques,[24] indicates a tendency towards the normalisation of values and that may be caused by a residual stimulation of the fundus and/ or by a possible extra-gastric production of ghrelin that tries to compensate a situation of weight loss and lowered food intake. However, the serum concentrations of ghrelin do not significantly vary in the other groups. This low variation in the bypass group would support the hypothesis that the direct stimulation by food in the fundus is the cause of the ghrelin increase[25]; in this case, a good portion of the fundus is excluded (Figures 1B and 3C) and, in spite of a weight decrease greater than in the gastroplasty group, the values of ghrelin have not changed. Weight loss alone is not capable of compensating the low concentrations of ghrelin that are found in situations of obesity. In the ileum transposition group, the levels of ghrelin also have not changed significantly, as the stomach was not affected in this case.

In the gastroplasty group, the significant decrease of the GLP-1 levels after surgery could explain this as a possible compensation to a decreased intake and weight caused by its known anorexigenic effect.[26] The intense hyperglycaemic state is not corrected in the gastroplasty and gastrojejunal bypass groups. However, in the ileum transposition group, the level of glucose is decreased in a significant and sustained manner, although without reaching a normoglycaemic state.[27] This could make us think that the genetic contribution could make it impossible to reach normal levels of serum glucose.

The increase in insulin concentrations, contrary to what expected, does not reflect a worsening of the resistance to insulin, but a metabolic improvement, as it deals with animals with very low insulin reserves due to elevated and persistent glycaemia levels.[28] This increase could indicate a possible recuperation in the production of insulin. Other experimental studies contrast these results with different obesity models, where the results are different according to their genetic contributions.[22] In clinical practice, this genetic contribution could be evaluated in some way, in a preoperative manner, as another variable to keep in mind, as this study points out.[29]

Regarding post-surgical complications, the deaths (3 cases) were in the bypass group and always within the first 72 h, without evident macroscopic findings in the necropsy. Possibly this could be due to a serious metabolic failure from intense hyperglycaemia before an intervention that lasts 50% more than the others (100 min for the bypass compared with 60 min for the transposition and 45 min for the gastroplasty). The diarrhoea cases observed (2 cases) also belong to the gastrojejunal bypass group; this event was rather predictable in interventions with a hypo-absorbing component.

Conclusions

The intervention that produces the greatest weight loss is the Roux-en-Y gastrojejunal bypass. A statistically significant general decrease in caloric intake is produced in the 3 intervention types. The intense hyperglycaemic state cannot be corrected in the 3 surgical groups, although in the transposition group, an increase to degrees of cetosis is stopped. There is a tendency to increase levels of serum insulin in the three groups that could imply a good metabolic response in a group of animals with very poor pancreatic reserves of insulin. The changes observed in the serum ghrelin may point to a joint regulation from the weight loss, decreased food intake and anatomical changes that each surgical intervention implies. The increase of GLP-1 is found in the techniques where we have carried out a change in the anatomical intestinal position, which justifies the hyper-stimulation with undigested food as the principal production mechanism. More studies are needed in this direction which evaluate different models of animal obesity (not genetic) and objectify the real hormonal changes of the principal peptides that regulate food intake.

Financing

Study financed by the Carlos III Health Institute. Health Investigation Fund.

Awards

First place award for an oral presentation in the 10th Conference of the Spanish Society of obesity surgery (*Sociedad Española de Cirugía de la Obesidad*). Barcelona, April 23-25, 2008.

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