Nutrición Hospitalaria

Nutr Hosp. 2015;32(2):957-957
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Original/*Otros*

Organic inflammatory response to reduced preoperative fasting time, with a carbohydrate and protein enriched solution; a randomized trial

Guilherme de Andrade Gagheggi Ravanini[1], Pedro Eder Portari Filho[2], Renato Abrantes Luna[3] and Vinicius Almeida de Oliveira[4]

[1]Oncologic Surgeon of the Gaffreé and Guinle University Hospital, Rio de Janeiro. [2]General Surgeon, Professor of the Rio de Janeiro School of Medicine and Surgery, Chief of Surgery Department B of the Federal University of the State of Rio de Janeiro (UNIRIO), Rio de Janeiro. [3]General Surgeon of the Hospital of the State Officials of Rio de Janeiro, Rio de Janeiro. [4]Graduate Student of Medicine, Federal University of the State of Rio de Janeiro —UNIRIO (Brazil).

Abstract

Background: **this study aims to assess the organic inflammatory response of the video laparoscopic cholecystectomy, with abbreviation of the preoperative fasting to 2h using a carbohydrate and protein enriched solution.**

Methods: **this is a randomized, prospective study with patients divided into the following 2 groups: group A, conventional fasting and group B, 2h abbreviated fasting with oral ingestion of a carbohydrate and protein solution. Serum glucose, insulin, interleukin 1, and TNF-α were mesasured before ingestion of the solution, during induction of anesthesia, and 4 h after the end of surgery.**

Results: **thirty-eight patients completed the study without presenting pulmonary complications associated with bronchoaspiration. The postoperative HOMA-IR variance was greater in group A (p = 0.001).**

Conclusion: **the abbreviation of preoperative fasting for 2 h, using carbohydrate and protein enriched solutions, is safe, reduces insulin resistance, and does not increase the risk of bronchoaspiration.**

(*Nutr Hosp*. 2015;32:957-957)

DOI:10.3305/nh.2015.32.2.8944

Key words: *Preoperative fasting. Carbohydrate and protein solution. Organic inflammatory response.*

LA RESPUESTA ORGÁNICA INFLAMATORIA EN LA REDUCCIÓN DEL TIEMPO DE AYUNO PREOPERATORIO CON UNA SOLUCIÓN ENRIQUECIDA CON CARBOHIDRATOS Y PROTEÍNAS; UN ESTUDIO ALEATORIZADO

Resumen

Introducción: **El objetivo de este estudio es la evaluación de la respuesta inflamatoria orgánica a la colecistectomía laparoscópica mediante vídeo con una reducción del tiempo de ayuno preoperatorio a 2h y empleando una solución enriquecida con carbohidratos y proteínas.**

Métodos: **Se trata de un estudio aleatorizado, prospectivo con pacientes divididos en los dos grupos siguientes: grupo A, ayuno convencional y grupo B, ayuno abreviado de 2h con ingesta oral de una solución enriquecida con carbohidratos y proteínas. Antes de la ingesta de la solución, se hicieron mediciones de glucosa sérica, insulina, interleucina 1y TNF-α; también se realizaron mediciones durante la inducción de la anestesia y 4h después de la intervención quirúrgica.**

Resultados: **Treinta y ocho pacientes completaron el estudio sin presentar complicaciones pulmonares relacionadas con el broncoaspirado. La varianza HOMA-IR postoperatoria fue superior en el grupo A (p = 0,001).**

Conclusión: **La reducción del tiempo de ayuno preoperatorio a 2h, empleando soluciones enriquecidas con carbohidratos y proteínas, es segura, reduce la resistencia a la insulina, y no aumenta el riesgo de broncoaspirado.**

(*Nutr Hosp*. 2015;32:957-957)

DOI:10.3305/nh.2015.32.2.8944

Palabras clave: *Ayuno preoperatorio. Solución de carbohidratos y proteínas. Respuesta inflamatoria orgánica.*

Correspondence: Guilherme de Andrade Gagheggi Ravanini.
Oncologic Surgeon of the Gaffreé and Guinle University Hospital.
Rua Antonio Arthur Braga 100, Bl 1 Apt 502,
Barra da Tijuca. Rio de Janeiro, RJ. (Brazil).
Postal Code: 22793-105.
Email: guilhermeravanini@gmail.com

Recibido: 11-III-2015.
1.ª Revisión: 8-IV-2015.
Aceptado: 18-V-2015.

Introduction

Prolonged preoperative fasting is a common clinical practice despite the lack of scientific evidence supporting its validity[1]. Previous studies reported that it adds metabolic stress to surgical trauma and contributes to worsening of peripheral insulin resistance, being proportional to the severity of the surgical procedure[2,3].

Abbreviating the fasting for 2h is associated with improvement in insulin sensitivity, which, in turn, improves the metabolic response to surgical trauma, reduces hospitalization time and can result in clinical benefits such as the reduction of nausea, vomiting, hunger, thirst, and anxiety, thus accelerating postoperative recovery[2,4-10]. Some studies have also demonstrated the safety and efficacy of this abbreviated fasting when a carbohydrate solution is enriched with amino acids[5,6,7].

The objective of this study is to assess the postoperative organic response to trauma after video laparoscopic cholecystectomy, with abbreviation of preoperative fasting for 2h, offering patients a solution enriched with carbohydrates and proteins.

Patients and Methods

A prospective, randomized, controlled trial was conducted at the general surgery departmant of the Gaffreé and Guinle's University Hospital (HUGG) of the Federal State University of Rio de Janeiro, Brazil (UNIRIO).

The research was approved by the HUGG's Research and Ethics Committee, Rio de Janeiro-RJ, and all patients signed a free and informed consent form (FICF).

All patients were older than 18 years, female and had abdominal symptoms as colic and nauseas. The diagnosis of cholelithiasis was made by abdominal ultrasound, and they had no history of acute cholecystitis and/or cholelithiasi. All surgeries were electively scheduled and patients' operative risk were II or I (ASA — American Society of Anesthesiologists). Patients with comorbidities as diabetes mellitus, renal insufficiency, gastroesophageal reflux disease, acute cholecystitis, use of corticosteroids for more than 6 months, previous gastric surgery, or who had shown any disagreement with the FICF were excluded from this study.

Patients were hospitalized the day before surgery and randomized by computer software into 2 groups. Group A patients were subjected to regular fasting conditions starting at 10 PM of the previous night, and group B patients received 200 mL of carbohydrate and protein solution 2h before the procedure.

The solution for ingestion was Fresubin® Jucy Drink (produced by the Fresenius Kabi laboratory), which contains 33.5% carbohydrates and 4% proteins with a total volume of 200mL (Annex 1).

The anesthetic technique used was the same for both groups: general anesthesia without spinal block. Cefazolin was used during anesthesia induction for prophylaxis. During anesthesia, peripheral vein infusion

Annex 1

Fresubin® Jucy Drink - Fresenius Kabi. Each bottle contains 200ml, nutritional values

Quantity per 100 ml			
Calories (Kcal)	150	Source of protein	Whey protein (100%)
Protein (% / g)	11% / 4 g	Source of CH	Maltodextrin (88%) and sucrose (12%)
CH (% / g)	89% / 33,5g	Source of Lipids	-
Lipid (% / g)	0% / 0g		
Minerals			
Sodium (mg)	6	Zinc (mg)	1,88
Potassium (mg)	7	Copper (mcg)	375
Chlorine (mg)	190	Manganese (mcg)	0,5
Calcium (mg)	50	Iodine (mcg)	37,5
Phosphorus (mg)	11	Fluorine (mcg)	0,25
Magnesium (mg)	1	Chromium (mcg)	12,5
Iron (mg)	2,5	Molybdenum (mcg)	18,8
Selenium (mcg)	12,5		
Vitamins			
A (mcg RE)	150	B6 (mg)	0,43
D (mcg)	2,5	B12 (mg)	0,75
And (mcg TE)	3,75	C (mg)	18,8
K (mcg)	25	Pantothenic acid (mg)	1,5
B1 (mg)	0,3	Biotin	9,4
B2 (mg)	0,4	Folic Ac	62,5
Niacin (mg)	3,75	Osmolality (mOsm / l)	680

was performed with 0.9% saline solution and/or simple Ringer solution. In the immediate postoperative period, there was no prescription of venous hydration unless emergency hydroelectric restitution was necessary. For analgesia, 30 mg/kg of venous dipyrone was used every 6h and no regular antiemetic medication was prescribed.

Clinical parameters such as nausea, vomiting, and pain were assessed at 3 different times: 4, 8, and 24h after surgery. Nausea and vomiting were then assessed as either present or absent, and pain was quantified using the visual analog scale[8].

Serum glucose, insulin, interleukin-1 (IL-1), and tumor necrosis factor-alpha (TNF-α) levels were collected 2h before surgery (immediately before solution ingestion), during anesthesia induction, and 4h after the end of the surgery. In the HUGG's laboratory, the blood samples were separated by centrifugation and frozen to a temperature between -16°C and -20°C.

Glucose levels were measured using an enzymatic method, and insulin levels with electrochemiluminescence at HUGG's laboratory. IL-1 and TNF-α levels were measured at the immunohistochemistry laboratory of the Fundação Oswaldo Cruz (FioCruz) using gel electrophoresis methods by R&D Systems.

Insulin resistance was measured using the *homeostasis model assessment- insulin resistance* (HOMA-IR) proposed by Matthews et al.[9]. [HOMA-IR = insulin (UI/mL) × glycemia (mg/dL) / 405]. IL-1, TNF, and insulin values were expressed in microunits (μU/mL), glycemia in milligrams (mg/dL), and HOMA-IR in absolute values.

Statistical Analysis

The sample number was calculated according to other similar studies[6,10]. To obtain an 85% statistical power (beta error) and a difference of 50% in HOMA-IR, values were calculated using groups of 16 patients. All data were analyzed for homogeneity using the Bartlett test and for normality using the Shapiro test. A statistical α (bilateral) significance level was defined as ≤0.05. For the analysis of categorical data we used chi-square or Fisher tests. The Student's t-test or Mann-Whitney tests were used to analyze continuous data. Data were presented as mean values and standard deviation or as median and interquartile range (IQR). All calculations were conducted on a computer with the R program[11].

Results

Thirty-eight patients were divided into group A (n = 17) and group B (n = 21). No deaths or postoperative complications occurred in either groups. Clinical and demographic characteristics are shown in table I.

No patients presented anesthetic complications, and group B patients did not present any signs of regurgita-

Table I
Groups clinical end demographic analysis, before the 1st solution of carbohydrates and proteins

	Group A	*Group B*	*p*
Sex			
Masc	0	0	
Fem	17	21	
Age (years) average (SD)	41 (14.2)	47.7 (14.5)	0.16
Fasting time (min) average	12h 30min	3h 5min	0.001
Surgery time (min) average	1h 14min	1h 31min	0.03
ASA			1
ASA I	9	12	
ASA II	8	9	
Length of hospital stay	2,05	2,04	0.9
Fasting samples			
IL-1 average (SD)	23.2 (3.8)	25.2 (4.6)	0.172
TNF average (SD)	107.4 (26.5)	122.9 (22.9)	0.061
Glucose average (SD)	79.9 (18.8)	85.4 (13.5)	0.307
Insulin average (IQR)	6.7 (4.8,8.9)	7.3 (5.2,11.4)	0.333
HOMA-IR average (IQR)	1.3 (1,1.8)	1.6 (1.1,2.2)	0.265

IQR – Interquartile range SD – Standard deviation

tion during the induction of anesthesia. There were no significant difference between the groups in terms of nausea, vomiting, and pain (Table II).

Glycemia, insulin, and insulin resistance

Group A presented higher postoperative levels of serum insulin and insulin resistance (HOMA-IR) than group B (Table III). In group A, the postoperative median variance of HOMA-IR was 0.8 (IQR: 0.6,1.5) and in group B it was 0.2 (IQR: -0.2,0.3) (p = 0.001). Insulin variance was also significantly different between the 2 groups. Group A presented an increase of 10.4%, while the postoperative insulin level of group B was lowered by 13.2% (p = 0.01) (Table III).

In relation to glycemia, group B presented in the second sample (anesthetic induction) an 8.8% increase from the value of the first sample, while group A presented a 1.8% reduction. Group A presented a postoperative glycemia increase of 28.8% and group B of 23.6% (p = 0.72).

Interleukin-1 and TNF-α

Inflammatory markers IL-1 and TNF-α did not present differences between groups when we compared the median increase of the first samples with the median of the final samples.

Discussion

This study showed that the group that ingested the carbohydrate and protein solution 2h before video laparoscopic cholecystectomy presented less insulin resistance and therefore possibly a reduced organic response to trauma. These patients did not demonstrate signs of regurgitation and/or bronchoaspiration in anesthetic induction, neither increased postoperative morbidity.

The half-life gastric emptying period for water and non-caloric clear liquids is known to be of 10 min. Glucose-enriched liquids initially have a slower emptying process, however after 90 min this difference be-

Table II
Evaluation of nausea, vomiting and pain (VAS) postoperatively, 4h, 8h and 24h after surgery

	Group A	*Group B*	*p*
(4h)			
VAS average (SD)	0.64 (1.05)	0.72 (1.33)	0.838
Nausea	1	1	-
Vomiting	0	1	0.397
(8h)			
EVA average (SD)	0,41 (0.61)	0,54 (1.12)	0.872
Nausea	2	1	0.577
Vomiting	0	1	0.397
(24h)			
EVA average (SD)	0,41 (0.61)	0,31 (0.79)	0.361
Nausea	0	1	0.397
Vomiting	0	0	-

SD – Standard deviation VAS – Visual analog scale

Table III
Average postoperative dosages of insulin and HOMA-IR, and percentage of variation

	Preoperativily	*Postoperativily*	*% Variation*
Group A			
HOMA-IR average (SD)	1.68 (1.74)	2.33 (0.82)	38.7%
Insulin average (SD)	8.26 (7.62)	9.12 (3.59)	10.4%
Grupo B			
HOMA-IR average (SD)	1.74 (0.79)	1.88 (0.70)	7,5%
Insulina average (SD)	8,33 (3.74)	7,23 (3.39)	-13.2%
Variation	*Grupo A*	*Grupo B*	*P*
HOMA-IR	38.7%	7.5%	0.001
Insulin	10.4%	-13.2%	0.01

SD – Standard deviation

comes insignificant. Thus, patients with normal gastric emptying would not present any gastric residue after 2h ingesting these solutions[12].

Prospective and randomized studies showed that reducing the preoperative fasting for 2h, with ingestion of clear carbohydrate liquids, does not increase the risk of gastric content regurgitation and bronchoaspiration during anesthesia[13]. In several trials conducted on preoperative fasting, patients received early discharge without evidence of pulmonary morbidity[14,15]. Meta-analysis of 22 randomized studies, including a Cochrane review, showed that fasting starting at midnight does not reduce the content or the gastric pH, compared with those wiht patients receiving clear liquids 2h before anesthetic induction[13,16].

Some situations and comorbidities as diabetes mellitus, opioid use, mechanical and tumor obstructions, tobacco, alcoholism, functional dyspepsia, first gestational trimester and in labor can prolong gastric emptying[17-19]. In these cases the abbreviated fasting is contraindicated, however this remains a controversial topic within the literature[20,21].

A randomized study of patients submitted to video laparoscopic cholecystectomy, which compared traditional fasting with the use of a carbohydrate solution 2h before surgery, showed that this procedure was safe and not associated with anesthetic complications. Furthermore, patients who received the solution presented a lower occurrence of gastrointestinal complications and 1 day less hospitalization than the control group[22]. Another study using the same group showed that in video laparoscopic cholecystectomy, the abbreviation of fasting with a carbohydrate solution 2h before surgery reduced insulin resistance and the inflammatory response to trauma[10].

Although scarce, reports do suggest that preoperative administration of carbohydrate and milk serum (whey protein) solutions are safe and effective[6,7]. Milk serum whey protein contains a high level of essential amino acids, especially branched-chain amino acids (leucine, isoleucine, and valine)[23,24]. These are rapidly used by skeletal muscle during stress and stimulate protein synthesis. Owing to their high degree of absorption, they are easily digested by the small intestine, in addition to being glutamine precursors and the main source of enterocyte energy. Therefore, the addition of this protein to the carbohydrate solution improves insulin response to trauma in comparison with the ingestion of only carbohydrates[25].

Unlike the vast majority of studies that only assessed formulas with 12.5% carbohydrates[5,10,20,21], our study used a combined solution of 33% carbohydrates and 4% proteins, which was proven to be safe and effective.

Perrone et al. demonstrated that the addition of milk whey protein (14%) to the carbohydrate solution (86%) reduces the inflammatory response of patients submitted to video laparoscopic cholecystectomy. This study demonstrated the safety in using increasingly concentrated protein solutions (86% vs. 12.5%), although in a very small sample size.

Our study used larger groups to guarantee the reliability of results. This study confirmed the same benefits but with a population of almost twice the size. Another important factor was that our study used a higher carbohydrate concentration than that used in other studies, and also, there were no signs of regurgitation and/or bronchoaspiration.

Insulin resistance is a transitory event occurring in the first days after surgery and lasting for up to 3 weeks. This metabolic state is similar to the physiopathology of type II diabetes mellitus, in which glucose cell capture is reduced because of the low activity of the GLUT-4 membrane transporter. In addition to the postoperative release of inflammatory cytokines and regulatory hormones such as glucagon, this leads to a state of hyperglycemia and can increase the risk of complications and morbidity[26].

During the surgical procedure, inflammatory mediators such as IL-1, IL-6, and TNF-α are released, leading to insulin resistance and hyperglycemia[27,28]. The organic response to surgical trauma is essentially dependent to the level of surgery and is measured by a variety of mechanisms such as insulin resistance[26]. In this study we observed reduced insulin resistance in the group with an abbreviated fasting period – probably meaning a reduced organic metabolic response to trauma.

Besides their important role as inflammatory mediators, IL-1 and TNFα have very short half-lives, 6 and 20 min, respectively, being involved in the initial activation process of inflammation. The greatest effect of these cytokines is seen at the cell lesion site, in an autocrine or paracrine manner without major variation in plasma[29,30]. Perhaps for this reason, we could not find a significant difference between IL-1 and TNF-α levels in the assessed groups. Studies on inflammatory cytokines that compared conventional video cholecystectomy and laparoscopy also failed to measure IL-1 and TNF-α as inflammation variables; however, they obtained significantly different values when they comparing IL-6 levels[28,29,31].

Another important shortcoming is the lack of a group receiving only one solution of 33% carbohydrates. This would have made it possible to analyze whether the postoperative inflammatory pattern improvement was due to the carbohydrate solution or to the synergy of these carbohydrates with protein.

Conclusion

The preoperative abbreviation of fasting for 2h with ingestion of a liquid formula of 33% carbohydrates and 4% proteins reduces insulin resistance and consequently the organic response to trauma by patients submitted to elective video laparoscopic cholecystectomy, and does not increase the incidence of perioperative pulmonary complications.

References

1. Cestonaro T, Schieferdecker ME, Thieme RD, et al (2014) The reality of the surgical fasting time in the Era of the ERAS protocol. *Nutr Hosp* 29(2): 437-443
2. Thorell A, Nygren J, Ljungqvist O (1999) Insulin resistance: a marker of surgical stress. *Curr Opin Clin Nutr Metab Care* 2(1):69-78.
3. Soop M, Nygren J, Thorell A,et al (2007) Stress-induced insulin resistance: recent developments. *Curr Opin Clin Nutr Metab Care* 10(2):181-6.
4. Ljungqvist O (2011) ERAS - Enhanced Recovery After Surgery. *J Visc Surg* 148(3):e157-9.
5. Henriksen MG, Hessov I, Dela F, et al (2003) Effects of preoperative oral carbohydrates and peptides on postoperative endocrine response, mobilization, nutrition and muscle function in abdominal surgery. *Acta Anaesthesiol Scand* 47:191-199.
6. Perrone F, da-Silva-Filho AC, Adorno IF, et al (2011) Effects of preoperative feeding with a whey protein plus carbohydrate drink on the acute phase response and insulin resistance. A randomized trial. *Nut* 10:66-72.
7. Aguilar-Nascimento JE, Caporossi C, Metelo JS, et al (2014) Safe intake of an oral supplement containing carbohydrates and whey protein shotly before sedation to gastroscopy; A double blind, radomized trial. *Nutr Hosp* 29(3) 681-6.
8. Miller MD, Ferris DG (1993) Measurement of subjective phenomena in primary care research: the visual analogue scale. *Fam Pract Res J* 13:15-24.
9. Matthews DR, Hosker JP, Rudenski AS, et al (1985). "Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man." *Diabetologia* 28 (7): 412-9.
10. Faria MS, Aguilar-Nascimento JE, Pimenta OS, et al (2009) Preoperative fasting of 2 hours minimizes insulin resistance and organic response to trauma after video-cholecystectomy: a randomized, controlled, clinical trial. *World J Surg* 33(6):1158-64.
11. R CORE TEAM (2001, Janeiro). What's R? R News: The Newsletter of the R Project [Online], vol. 1, no. 1, pp. 2-3. URL: http://cran.r- project.org/doc/Rnews/Rnews_2001-1.pdf
12. Nygren J, Thorell A, Jacobsson H, et al (1995) Preoperative gastric emptying: the effects of anxiety and carbohydrate administration. *Ann Surg* 222: 728-34.
13. Brady M, Kinn S, Stuart P (2003) Preoperative fasting for adults, to prevent perioperative complications. Cochrane Database Syst Rev 4:CD004423
14. Gustafsson UO, Scott MJ, Schwenk W, et al (2012) Guidelines for perioperative care in elective colonic surgery: Enhanced Recovery After Surgery (ERAS) society recommendations. *Clin Nutr* 31(6):783-800.
15. Nygren J, Thorell A, Ljungqvist O (2007) Are there any benefits from minimizing fasting and optimization of nutrition and fluid management for patients undergoing day surgery? *Curr Opin Anaesthesiol* 20(6):540-4.
16. Petring OU, Blake DW (1993) Gastric emptying in adults: an overview related to anaesthesia. *Anaesth Intensive Care* 21:774-81.
17. Horowitz M, O'Donovan D, Jones KL, et al (2002) Gastric emptying in diabetes: clinical significance and treatment. *Diabet Med* 19: 177-94.
18. Whitehead EM, Smith M, Dean Y, et al (1993) An evaluation of gastric emptying times in pregnancy and the puerperium. *Anaesthesia* 48: 53-7.
19. Harter RL, Kelly WB, Kramer MG, et al (1998) A comparison of the volume and pH of gastric contents of obese and lean surgical patients. *Anesth Analg* 86(1):147e52.
20. Maltby JR, Pytka S, Watson NC, et al (2004) Drinking 300 mL of clear fluid two hours before surgery has no effect on gastric fluid volume and pH in fasting and non-fasting obese patients. *Can J Anaesth* 51(2):111e5.
21. Gustafsson UO, Nygren J, Thorell A, et al (2008) Pre-operative carbohydrate loading may be used in type 2 diabetes patients. *Acta Anaesthesiol Scand* 52(7):946e51.
22. Aguilar-Nascimento JE, Bicudo-Salomao A, Caporossi C, et al (2008) Enhancing surgical recovery in Central-West Brazil: The ACERTO protocol results. e-SPEN, *Eur J Clin Nutr* 3(2):e78-e83.
23. Haraguchi FK, Abreu WC, Paula H (2006) Proteínas do soro do leite: composição, propriedades nutricionais, aplicações no esporte e benefícios para a saúde humana. *Rev Nutr* 19:479-488.
24. Sax HC, Talamini MA, Fischer JE (1986) Clinical use of branched-chain amino acids in liver disease, sepsis, trauma, and burns. *Arch Surg* 121:358-366.
25. van Loon LJ, Saris WH, Verhagen H, et al (2000) Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. *Am J Clin Nutr* 721:96-105.
26. Hill AG, Hill GL (1998) Metabolic response to severe injury. *Br J Surg* 85:884-890.
27. Nawabi MD, Block KP, Chakrabarti MC, et al (1990) Administration of endotoxin, tumor necrosis factor, or interleukin 1 to rats activates skeletal muscle branched-chain alpha-keto acid dehydrogenase. *J Clin Invest* 85:256-263.
28. Aguilar-Nascimento JE, Marra JG, Slhessarenko N, et al (2007) Efficacy of National Nosocomial Infection Surveillance score, acute-phase proteins, and interleukin-6 for predicting postoperative infections following major gastrointestinal surgery. *Sao Paulo Med J* 125:34-41.
29. Lin E, Lowry SF (1999) Inflammatory cytokines in major surgery: a functional perspective. *Intensive Care Med* 25(3):255-7.
30. Bellón JM, Manzano L, Bernardos L, et al (1997) Cytokine levels after open and laparoscopic cholecystectomy. *Eur Surg Res* 29(1):27-34
31. Karayiannakis AJ, Asimakopoulos B, Efthimiadou A, et al (2005) Serum leptin levels and their response during laparoscopic and open cholecystectomy. *Eur Cytokine Netw*. 16(1):91-6.