The Role of Nitric Oxide Synthase in Post-Operative Hyperglycaemia

Qader SS
Department of Surgery, Hawler Medical University, Erbil, Iraq
and Department of Clinical Science, Lund University, Malmö, Sweden

Abstract: Post-operative hyperglycaemia is important with regard to outcomes of surgical operations. It affects post-operative morbidity, length of hospital stay, and mortality. Poor peri-operative blood glucose control leads to a higher risk of post-operative complications. Insulin resistance as a cause of post-operative hyperglycaemia has been blamed for some time. Nitric Oxide (NO) is produced by nitric oxide synthase (NOS) isoenzymes. Inducible nitric oxide synthase (iNOS) is not a normal cellular constituent. It is expressed by cytokines and non-cytokines e.g. fasting, trauma, intravenous glucose, and lipid infusion, which are encountered in surgical operations. Review of current published data on postoperative hyperglycaemia was completed. Our studies and others were explored for the possible role of NO in this scenario. Induction and expression of iNOS enzyme in pancreatic islet cells is included in the chaotic postoperative blood glucose control. The high concentrations of iNOS derived NO are toxic to pancreatic β-cells and may inhibit insulin secretion postoperatively. Hence, current peri-operative management is questionable regarding post-operative hyperglycaemia and necessitates development of a new strategy.

Key words: NO, glucotoxicity, lipotoxicity, post-operative hyperglycaemia, pancreatic islets.

Post-operative hyperglycaemia: A real problem
Post-traumatic hyperglycaemia is commonly encountered after surgery and in patients treated in intensive care units (ICU). It carries a higher risk for post-operative complications, prolonged recovery periods, and increased length of stay (LOS) [1,2].

Poor post-operative blood glucose control in diabetic [1] and non-diabetic patients [2] leads to a higher risk of complications. Many studies [3, 4] blame insulin resistance as a cause for post-operative hyperglycaemia. Cytokines [5,6], fasting [7,8], peri-operative feeding [9,10] and immobilization were reported to lead to insulin resistance. Different regimens postulated to overcome the outcome of elective operations [11,12]. Emergency traumatic surgery in conditions e.g. high velocity missile injury and traffic accidents carry an additional risk for post-operative hyperglycaemia because of double trauma.

To decrease post-operative morbidity and mortality, it is essential to explore the molecular mechanism of post-operative hyperglycaemia and its relation to trauma. Pancreatic function during trauma has not been thoroughly studied. It is very important to comprehend post-operative hyperglycaemia and evaluate peri-operative management to improve surgical outcomes.

Review of currently published data on post-operative hyperglycaemia was conducted and the role of nitric oxide in this scenario was investigated.

Nitric oxide and nitric oxide synthase system
Nitric oxide (NO) was described in 1989. NO is the smallest synthetic molecule. It is produced by a family of enzymes known as nitric oxide synthase (NOS) in almost all mammalian cells e.g. vascular endothelium, neurons of the central and enteric nervous system, and cells of the immune system [13,14]. NO is a free radical and an extremely reactive gas [15]. It has a short half life of about 10 seconds. It acts as a signalling molecule, neurotransmitter, and macrophage mediated immunity that can heal or kill. Under conditions of high NO production, a number of enzymes can be inhibited by NO-enzyme interaction [16-18].

According to their expression, activity, and dependence on calcium, NOS isoenzymes are divided into 2 major functional classes:
• Constitutive nitric oxide synthase (cNOS); ncNOS, ecNOS
• Inducible nitric oxide synthase (iNOS).

Nitric oxide and insulin secretion
cNOS and iNOS can be expressed and/or induced by different stimuli in various tissue including pancreatic β-cell [17,19-24]. ncNOS derived NO is recognized as an important signalling molecule in a variety of cellular processes e.g. insulin secretion [19,21,22,24].

Our laboratory [23,27,29-30] and others [25,31-33] presented biochemical and immuno-cytological evidence for occurrence of ncNOS in mouse and rat pancreatic β-cells. When cNOS is activated, it produces a pulsatile low amount of NO for a short period of time [29,31,34]. Although the effect of ncNOS derived NO on insulin secretion is highly controversial, the results from rat and mouse pancreatic islets suggests that it acts as a negative modulator for glucose-stimulated insulin secretion (GSIS) [27,29].

iNOS is not a normal cellular constituent and can only be expressed in pathophysiological conditions in a response to inflammatory cytokines e.g. IL-1β, TNF-α, and lipopolysaccharide. Under such conditions, pancreatic β-cells produce huge amounts of NO in a more sustained manner [27,34-36] through induction of iNOS, comparing to the cNOS isoforms [15,37-38]. Non-cytokine induction of iNOS in pancreatic islets has also been reported. One hour in vitro incubation of healthy rat and mouse islets with high glucose concentrations [10-20 mmol/L) induced iNOS and ncNOS [27,30,34]. However, the activation of ncNOS was rapid, within minutes. It is at least in part, associated with the glucose-stimulated influx of extracellular Ca2+ into β-cells [27,33]. Glucose activation
of iNOS was slower and detectable after approximately 60 minutes [27]. The mechanism behind glucose-stimulated iNOS expression and activity is poorly understood. It has been suggested that glucose metabolism generates NADPH through the pentose shunt, which is an important stimulus in IL-1β induction of iNOS [39,40]. NADPH is an obligatory substrate for iNOS synthesis of NO [20-21]. This is of great interest since high amounts of iNOS derived NO is detrimental to β-cells [38-39]. Moreover, we showed that 24 hour intravenous (IV) glucose administration induced marked expression and activity of islets iNOS [41].

**Figure 1** Simple scheme illustrating the possible mechanisms for the toxic effects of NOS-derived NO on β-cell function

The inhibitory effect of increased NO production on insulin secretion, due to either enhanced activity of cNOS (physiological) or induction of iNOS (inflammatory condition) [29,38,42]. NO is widely accepted as a mediator of β-cell dysfunction and apoptosis [29,30,34,43-45]. A clear role of iNOS in the pathogenesis of type 1 diabetes mellitus has been reported [38-39].

In addition, the extremely low level of NO metabolizing enzymes, e.g. catalase and glutathione peroxidase, makes pancreatic β-cells extremely susceptible to high levels of intracellular NO (46). High concentration of NO may interact with vital sites in the β-cell such as Kreb’s cycle enzyme aconitase [47], ion channels [48], or other enzymes of importance for β-cell function [30,34,43,48] (Figure 1). Indeed, several studies demonstrated that inhibition of NOS isoenzymes activity by specific inhibitors was accompanied by enhanced GSIS, both in vitro and in vivo [27,29,30,34].

Initial inhibition of insulin release is exerted by nCNOs-derived NO, when the islets are exposed to high glucose concentration [26-27].

**Glucotoxicity**

Chronic hyperglycaemia is detrimental to pancreatic β-cells. It could be implicated in the pathogenesis of type 2 diabetes mellitus (DM) in a process called glucotoxicity [24,26,30,34]. It is markedly suppressed in islets isolated from fasting mice and rats. Besides, GSIS was markedly impaired in islets isolated from fasting mice, and associated with a decreased production of CO and HO-2 expression. Hence two potent inhibitors of iNOS NO production; CO and cyclic AMP, were markedly suppressed by IV glucose administration, or hyperlipidemia by IV intralipid infusion, caused marked induction and expression of iNOS in rat β-cells [41]. This is consistent with previous reports, that plasma insulin response was much greater following glucose ingestion than IV glucose administration despite an equivalent increase in plasma glucose concentration. This is explained by the release of incretin hormones from endocrine cells in the gastrointestinal tract e.g. glucagon-like peptide-1 (GLP-1) [52-53]. The expression of iNOS after IV infusion of glucose could be explained by suppression of release of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) whose secretion is dependant upon ingestion of carbohydrates (for GLP-1) or FFA (for GIP).

The relative importance of glucotoxicity versus lipotoxicity in inducing β-cell dysfunction and apoptosis remains controversial. Although it has been reported that lipotoxicity alone will not affect β-cell function without signs of glucotoxicity [54], we showed that glucose or intralipid infusion for 24h induced marked expression and activity of iNOS [41]. This is in line with results of cultured cell lines exposed to high glucose or FFA for 24-48 hours [55-56]. In this context, it is conceivable that iNOS derived NO might be a contributing factor in this process [55].

Furthermore, IV infusion of nutrients is commonly prescribed as an important treatment model both pre- and post-operatively [6,57], in burn patients and in some patients as a life long treatment when they can not take oral food. If IV nutrients induce NO-production in human β-cells, this may explains post-operative hyperglycaemia to some degree.

**Lipotoxicity**

Long term Total Parenteral Nutrition (TPN) in rats for 10 days resulted in increased iNOS and decreased ncNOS activity in pancreatic islets [30,34]. Infusion of lipids for 24 hours induced suppression of insulin secretion [41]. This is in agreement with previous studies [30,34,58]. Although the induction of iNOS could not account entirely for alteration in β-cell survival, it might negatively modulate the secretory function of β-cells. In addition, long term exposure of β-cells to FFA resulted in a marked production of reactive oxygen species e.g. superoxide anion (O-2-) [59]. Combination of NO and O-2- resulted in the formation of peroxynitrite, which is a powerful oxidant and cytotoxic molecule. The increase in NO, O-2- and peroxinitrite concentrations were positively correlated with mitochondrial and DNA damage in β-cells [44]. It has been reported that an increased plasma FFA obtained by IV infusion of lipids resulted in decreased plasma levels of glucagon in humans [60]. The suppression of insulin secretion during TPN could partly be due to the absence of incretin hormone which may be normalized by injection of GLP-1 [61].

**Fasting and pancreatic function**

Cyclic AMP is a potent inhibitor of islet’s NOS activity [24,26,30,34]. It is markedly suppressed in islets isolated from fasting mice and rats. Besides, GSIS was markedly impaired in islets isolated from fasting mice, and associated with a decreased production of CO and HO-2 expression. Hence two potent inhibitors of iNOS NO production; CO and cyclic AMP, were markedly suppressed
in islets isolated from fasting mice and rats. This may explain the increased iNOS activity in β-cells and suppression of insulin secretion in these animals.

Taken together, decreased CO production and increased iNOS-derived NO production is associated with a diabetic condition in islets β-cells [62].

Preoperative fasting or IV glucose infusion for 24 hours induced strong expression and activity of iNOS in rat pancreatic islets post-operatively (data not published), which was stronger than those seen in rats that received preoperative oral glucose or were freely fed. This is of significant clinical importance if the same thing happens in human pancreatic islets. Since, preoperative fasting and/or post-operative IV glucose infusions are applied in surgical patients, especially in abdominal operations. Hence both fasting and IV glucose administration play a role, at least partly, in suppression of insulin secretion and induction of post-operative hyperglycaemia. Although post-operative insulin resistance is still blamed, fasting and IV glucose may act as contributors to insulin suppression by inducing post-operative hyperglycaemia, which needs further study in humans.

Trauma and β-cell function

Trauma-induced iNOS expression and activity has been noted in rat pancreatic islets [63]. During trauma, the body responds with a series of reactions e.g. a change in metabolism, to a catabolic state, and an expression of insulin resistance [64]. The consequence of post-operative insulin resistance is that patients in the post-operative period are in a metabolic state similar to T2DM [13]. Insulin resistance persists for about 2-3 weeks after uncomplicated elective upper abdominal surgery [65]. It negatively affects the post-operative recovery, convalescent period, and LOS.

Surprisingly, in spite of insulin resistance and its role in post-operative hyperglycaemia, iNOS isoenzyme may be involved very early in the impairment of the insulin secretion. Hence, β-cells seem to be unable to respond adequately to a glucose challenge. It seems reasonable to assume that an improvement in the insulin secretory capacity of the pancreas may positively affect the post-operative glycemic state and ultimately the outcome of surgery.

The present findings may stir more debate in the explanation of post-operative hyperglycaemia.

Conclusions

1. Trauma, fasting, hyperglycaemia, hyperlipidemia and route of nutrient administration possibly are other factors contribute to post-operative hyperglycaemia.

2. It is recommended to investigate the molecular mechanism behind the pathophysiology of post-traumatic hyperglycaemia in human beings. The role of nitric oxide in this scenario should be appreciated.

3. New strategy should be developed regarding peri-operative management and postoperative hyperglycaemia.

4. A possible pharmacological target is to suppress iNOS activity in pancreatic islets with agents stimulating cyclic AMP/PKA pathway e.g. PACAP. This may be a hope to restore adequate insulin secretion post-operatively.

References

1. Zarr K, Furnary A, Grunkemeier G, Bookin S, Kanhere V, Starr A. Glucose control lowers the risk of wound infection in diabetics after open heart operation. Ann Thorac Surg 1997; 63:356-361.

2. Thorell A, Nygren J, Ljungqvist O. Insulin resistance–a marker of surgical stress. Curr Opin Nutr Met Care 1999; 2:69-79.

3. Ljungqvist O, Nygren J, Thorell A. Modulation of post-operative insulin resistance by pre-operative carbohydrate loading. Proc Nutr Soc 2002; 61(3):329-336.

4. Hrebicek S, Rypka M, Chmela Z, Vesely J, Kantorova M, Golch V. Tumor necrosis factor alpha in various tissues of insulin-resistant obese Koletsky rats: relations to insulin receptor characteristics. Physiol Res 1999; 48:83-86.

5. Qi C, Pekala PH. Tumor necrosis factor-alpha-induced insulin resistance in adipocytes. Proc Soc Exp Biol Med. 2000 Feb; 223(2):128-135.

6. Ljungqvist O, Soreide E. Preoperative fasting. Br J Surg 2003; 90(4):400-406.

7. Nygren J, Thorell A, Soop M, Efendic S, Brismar K, Karpe F, Nair KS, Ljungqvist O. Perioperative insulin and glucose infusion maintains normal insulin sensitivity after surgery. Am J Physiol 1998; 275:E140-E148.

8. Nygren J, Thorell A, Brismar K, Karpe F, Ljungqvist O. Short time hypocaloric nutrition but not bed rest decrease insulin sensitivity and IGF-1 bioavailability in healthy subjects: the importance of glucagon. Nutrition 1997; 13:945-951.

9. Ljungqvist O, Thorell A, Gentian M, Highmark T, Efendic S. Glucose infusion instead of preoperative fasting reduces postoperative insulin resistance. J Am Coll Surg 1994; 178:329-336.

10. Nygren J, Thorell A, Jacobsson H, Schnell PO, Ljungqvist O. Preoperative gastric emptying; the effects of anxiety and carbohydrate administration. Ann Surg 1995; 222:728-734.

11. Nygren J, Soop M, Thorell A, Efendic S, Nair KS, Ljungqvist O. Preoperative oral carbohydrate administration reduces postoperative insulin resistance. Clin Nutr 1998; 17:65-71.

12. Soop M, Nygren J, Moreno’s P, Thorell A, Ljungqvist O. Preoperative oral carbohydrate treatment attenuates endogenous glucose release 3 days after surgery. Clinical Nutrition 2001; 2004(23):733-741.

13. Ljungqvist O, Nygren J, Thorell A. Insulin resistance and elective surgery. Surgery 2000; 128(5):757-760.

14. Moncada S. The L-arginine: nitric oxide pathway. Acta Physiol Scand. 1992; 145:201-227.

15. Nathan CF and Xie Q-w. Nitric oxide synthase: Roles, tolls and controls. Cell 1994; 78:915-918.

16. Nathan C. Nitric oxide as a secretory product of mammalian cells. FASEB J 1992; 6:3051-3064.

17. Feldman PI, GQ, Stuehr DJ. The surprising life of nitric oxide. Chem Eng News. 1993; 71:26-39.

18. Dragier JC, H. JJ. Murine cytotoxic activated macrophages inhibit acoititis in tumor cells. Inhibition involves the iron-sulfur prosthetic group and is reversible. J Clin Invest. 1986; 78:790-797.

19. Stuehr DJ, N C. Nitric oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. J Exp Med 1989; 169:1543-1555.

20. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. Biochem J 2001; 357:593-615.

21. Knowles RG, Moncada S. Nitric oxide synthases in mammals. Biochem J 1994 Mar 1; 298 (Pt 2):249-58.

22. Henningsson R, Salehi A, Lindqvist I. Role of nitric oxide synthase isoforms in glucose-stimulated insulin release. Am J Physiol Cell Physiol 2002; 283(1):C296-304.

23. Alm P, Ekstrom P, Henningsson R, Lindqvist I. Morphological evidence for the existence of nitric oxide and carbon monoxide pathways in the rat islets of Langerhans: an immunocytochemical and confocal microscopic study. Diabetologia 1999; 42(978-986).
24. Henningsson R, Alm P, Lundquist I. Evaluation of islet heme oxygenase-CO and nitric oxide synthase-NO pathways during acute endotoxemia. Am J Physiol Cell Physiol 2001; 280:C1242-254.

25. Lajoix AD, Reggio H, Chardes T, Peraldi-Roux S, Tribillic F, Roye M, Dietz S, Broca C, Manteghetti M, Ribes G, Wollheim CB, Gross R. A neuronal isoform of nitric oxide synthase expressed in pancreatic beta-cells controls insulin secretion. Diabetes 2001; 50:1311-1323.

26. Jimenez-Feltstrom J, Lundqftlst I, Salehi A. Glucose stimulates the expression and activities of nitric oxide synthases in incubated rat islets: an effect counteracted by GLP-1 through the cyclic AMP/PKA pathway. Cell Tissue Res 2005; 319:221-230.

27. Henningsson R, Salehi A, Lundquist I. Role of nitric oxide synthase isoforms in glucose-stimulated insulin release. Am J Physiol Cell Physiol 2002; 283:C296-C304.

28. Salehi A, Carberg M, Henningsson R, Lundquist I. Cell constitutive nitric oxide synthase: biochemical determination and regulatory function. Am J Physiol 1996; 270:C1634-1641.

29. Salehi A, Ekelund M, Lundquist I. Total parenteral nutrition-stimulated activity of nitric oxide synthase in rat pancreatic islets is suppressed by glucagon-like peptide-1. Horm Metab Res 2003; 35(1):48-54.

30. Schmidt HH, Warner TD, Ishii K, Sheng H, Murad F. Insulin secretion from pancreatic B cells caused by l-arginine-derived nitrogen oxides. Science 1992; 255:721-723.

31. Gross R, Roje M, Manteghetti M, Broca C, Hillaire-Boys D, Masiello P, Ribes G. Mechanisms involved in the effect of nitric oxide synthase inhibition on L-arginine-induced insulin secretion. Br J Pharmacol 1997; 120:495-501.

32. Smukler SR, Tang L, Wheeler MB, Salapatek AM. Exogenous nitric oxide and endogenous glucose-stimulated beta-cell nitric oxide augment insulin release. Diabetes 2001; 50:1345-1350.

33. Salehi A, Ekelund M, Henningsson R, Lundquist I. Total parenteral nutrition modulates hormone release by stimulating expression and activity of inducible nitric oxide synthase in rat pancreatic islets. Endocrine 2001a; 16(2):97-104.

34. Flodstrom M and Eizirik DL. Interferon-gamma-induced nitric oxide synthase expression in insulin-producing cells. Diabetes 2002; 51:3450-3460.

35. McDaniel ML, Corbett JA, Kwon G, Hill JR. A role for nitric oxide and other inflammatory mediators in cytokine-induced pancreatic beta-cell dysfunction and destruction. Adv Exp Med Biol 1997; 426:313-319.

36. Nathan CF. Perspective series: nitric oxide and nitric oxide synthases. Inducible nitric oxide synthase: what difference does it make? Journal of Clinical investigation 1997; 100:2417-2423.

37. Corbett JA, McDaniel ML. Does nitric oxide mediate autoimmune destruction of beta-cells? Possible therapeutic interventions in IDDM. Diabetes 1992; 41:897-903.

38. Mandrup-Poulsen T. The role of interleukin-1 in the pathogenesis of IDDM. Diabetologia 1996; 39:1005-1029.

39. Guo L, Zhang Z, Green K, Stanton RC. Suppression of interleukin-1 beta induced nitric oxide production in RINm5F cells by inhibition of glucose-6-phosphate dehydrogenase. Biochemistry 2002; 41:14726-14733.

40. Ekelund M, Qader SS, Jimenez-Feltstrom J, Salehi A. Selective induction of inducible nitric oxide synthase in pancreatic islet of rat after an intravenous glucose or intralipid challenge. Nutrition. 2006 Jun; 22(6):652-660.

41. Delaney CA, Eizirik DL. Intracellular targets for nitric oxide toxicity to pancreatic beta-cells. Braz J Med Biol Res 1996; 29(5):569-579.

42. Salehi A, Fan BG, Ekelund M, Nordin G, Lundquist I. TPN- evoked dysfunction of islet lysosomal activity mediates impairment of glucose-stimulated insulin release. Am J Physiol Endocrinol Metab 2001b; 281(1):E171-179.

43. Eizirik DL, Delaney CA, Green MH, Cunningham JM, Thorpe JR, Pipeleers DG, Hellerstrom C, Green IC. Nitric oxide donors decrease the function and survival of human pancreatic islets. Mol Cell Endocrinol 1996; 118(1-2):71-83.

44. de-Mello MA, Flodstrom M, Eizirik DL. Ebselen and cytokine-induced nitric oxide synthase expression in insulin-producing cells. Biochem Pharmacol1996; 52(11):1703-1709.

45. Ammon HP, Mark M. Thiols and pancreatic beta-cell function: a review. Cell Biochem Funct 1985; 3:157-171.

46. Welsh N, Sandler S. Interleukin-1 beta induces nitric oxide production and inhibits the activity of aconitase without decreasing glucose oxidation rates in isolated mouse pancreatic islets. Biochem Biophys Res Commun 1992; 182(1):333-340.

47. Tsumura Y, Ishida H, Shinomura T, Nishimura M, Seino Y. Endogenous nitric oxide inhibits glucose-induced insulin secretion by suppression of phoshofructokinase activity in pancreatic islets. Biochem Biophys Res Commun 1998; 252(1):34-38.

48. Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H. Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. Diabetes 2003; 52:581-587.

49. Rossetti L, Giacca A, DeFronzo RA. Glucose toxicity. Diabetes Care 1990; 13:610-630.

50. Hansotia T, Drucker DJ. GIP and GLP-1 as incretin hormones: lessons from single and double incretin receptor knockout mice. Regl Pept 2005; 128(2):125-134.

51. Holst JJ, Orskov C. The incretin approach for diabetes treatment: modulation of islet hormone release by GLP-1 agonism. Diabetes 2004; 53:S197-204.

52. Robertson RP, Harmon J, Tran PO, Poiltout V. Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. Diabetes 2004; 53:S119-124.

53. Kajimoto Y, Kaneto H. Role of oxidative stress in pancreatic beta-cell dysfunction. Ann N Y Acad Sci 2004; 1011:168-176.

54. Harmon JS, Gleason CE, Tanaka Y, Poiltout V, Robertson RP. Antecedent hyperglycaemia, not hyperlipidemia, is associated with increased islet triacylglycerol content and decreased insulin gene mRNA level in Zucker diabetic fatty rats. Diabetes 2001; 50(11):2481-2486.

55. Knape CM, Owens JP, Mirtallo JM. Management of glucose abnormalities in patients receiving total parenteral nutrition. Clin Pharm 1989; 8(2):136-144.

56. Lupi R, Dotta F, Marselli L, Del Guerra S, Masini M et al. Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. Diabetes 2002; 51(5):1437-1442.

57. Koshkin V, Wang X, Scherer PE, Chan CB, Wheeler MB. Mitochondrial functional state in clonal pancreatic beta-cells exposed to free fatty acids. J Biol Chem.2003 May 30; 278(22):19709-19715.

58. Gerich JE, Langlois M, Schneider V, Karam JH, Noacco C. Effects of alternations of plasma free fatty acid levels on pancreatic glucagon secretion in man. J Clin Invest 1974; 53(5):1284-1289.

59. Nauck MA, Walberg J, Vethace A et al. Blood glucose control in healthy subject and patients receiving intravenous glucose infusion or total parenteral nutrition using glucagon-like peptide 1. Regul Pept 2004; 118:89-97.

60. Grey NJ, Goldring S, Kipnis DM. The effect of fasting, diet, and actinomycin D on insulin secretion in the rat. J Clin Invest 1970; 49:881-889.

61. Qader SS, Ekelund M, Andersson R, Obermuller S, Salehi A. Acute pancreatitis, expression of inducible nitric oxide synthase and defective insulin secretion. Cell Tissue Res 2003; 313:271-279.

62. Holmes E. The effect of toxemia on metabolism. Physiol Rev 1939; 4:439-471.

63. Thorell A, Hågmark T, Gutiark M, Efendic S, Ljungqvist O. Insulin resistance after abdominal surgery. Br J Surg 1994; 81:59-63.

64. Abramson SB, CA, Clancy RM, Attur M. The role of nitric oxide in tissue destruction. Best Pract Res Clin Rheumatol 2000; 15:831-45.