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# Acute and Chronic Inflammation Affects Plasma Insulin and Glucagon Levels in Piglets

Joanna Zubel-Łojek*, Ewa Ocłoń, Anna Latacz, and Krystyna Pierzchała-Koziec

*Department of Animal Physiology and Endocrinology, University of Agriculture, Al.Mickiewicza 24/28, 30-059 Cracow, Poland*

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The present study was performed to examine the influence of acute and chronic inflammation on the plasma insulin and glucagon levels in piglets. The experiment was carried out on young pigs (10-week-old, n=24). Animals (females) were divided into 4 experimental groups: I – control, II – chronic inflammation (overweight), III – acute inflammation, and IV – overweight with acute inflammation. Piglets in groups II and IV received high-calorie diet in order to develop overweight. In order to induce acute inflammation the animals received a single i.p. injection of streptozotocin. Twenty-four hours after injection, blood was taken from all the piglets. Plasma levels of glucose, IL-6, insulin and glucagon were determined using commercial kits. During induced inflammation the plasma level of IL-6 was significantly increased parallel to an elevated level of glucose. Significant changes in the plasma insulin level were observed in piglets in groups III and IV. In contrast, the plasma level of glucagon was significantly decreased under inflammation. The obtained results clearly indicate that higher activity of the immune system, manifested by an elevated cytokine level, may have an impact on the hormones regulating glucose metabolism.

**Key words:** inflammation, insulin, glucagon, piglets

## INTRODUCTION

Numerous studies have shown that alterations in the function of the immune system are intrinsically linked to metabolic pathways (Pickup and Crook, 1998; Festa et al., 2000; Fernandez-Real and Ricard, 2003). Obesity is associated with a complex systemic inflammatory state that has been implicated in the development of common, medically important complications, including cardiovascular diseases and insulin resistance (Pickup, 2004; Berg and Scherer, 2005). It is recognized that adipose tissue produces multiple peptides, named adipokines, which not only have an impact on the local functions but also affect many metabolic pathways through the bloodstream (Chaldakov et al., 2003). Obesity is considered as a low-degree chronic inflammatory state of the adipose tissue, caused by the immune system activation that generates obesity-

* e-mail: jzubel@ar.krakow.pl

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related metabolic disorders, mainly insulin resistance (Hottamisligil, 2006). The adipose tissue is an endocrine organ that produces adipokines with various biological activities (Fruhbeck and Salvador, 2004; Jensen, 2006). Some of them, such as interleukin 6 (IL-6) and interleukin 18 (IL-18) or tumor necrosis factor (TNF) are of a pro-inflammatory character and modulate metabolism and activity of insulin (Senn et al., 2002). Obesity, associated with glucose intolerance, insulin resistance and hypertension, is an integral feature of the so-called metabolic syndrome (Ridker et al., 2003). The altered production of pro-inflammatory cytokines seems to be directly implicated in obesity-related metabolic disorders. While acute inflammation as part of innate and adaptive immunity is beneficial, excessive or uncontrolled inflammation can promote tissue injury. Indeed, chronic, low level inflammation is thought to be a characteristic feature seen at sites of diabetic complications. On the other hand, hyperglycemia has been found to influence the synthesis of proinflammatory cytokines acutely and chronically (Esposito et al., 2002).

Blood glucose concentration is controlled by hormonal, neural, and hepatic autoregulatory mechanisms. Hormonal mechanisms are exerted by insulin and the adverse hormones, including glucagon, catecholamines, cortisol, and growth hormone. Energy and protein metabolism is mainly controlled by two key pancreatic hormones – insulin and glucagon which are responsible for the energetic status in many body tissues (Levine and Haft, 1970; Knapke et al., 1989). Insulin regulates energy homeostasis by coordinating storage, mobilization, and utilization of free fatty acid and glucose in adipose tissue, liver and muscle (Ruan and Lodish, 2003). Reduced insulin sensitivity or insulin resistance is the suppressed ability of insulin to induce glucose uptake into cells (Xu et al., 2003). Glucagon is a potent stimulator of gluconeogenesis in the liver (Exton et al., 1970). Glucagon is released in response to low blood glucose levels and to events whereby the body needs additional glucose, such as in response to stress situation. Some data indicate that patients with trauma, burn or sepsis normally exhibit increased plasma levels of glucagon to promote gluconeogenesis, increase circulating glucose, and compensate for the energetic demand of the body during these extreme situations (Wolfe et al., 1979). Otherwise, the deregulation of glucagon levels could lead to hyperglycemia and aggravate their negative effects.

In spite of extensive research on the potential involvement of the immune system in the induction of metabolic diseases, there is little information about the effects of proinflammatory cytokines on the release of metabolic hormones. Additionally, in many aspects, the pig is useful as a model for human physiology and pathophysiology, in vivo as well as in isolated preparations of cells or organs (Miller and Ullrey, 1987). Many organs of the pig anatomically and physiologically resemble those in the human body. Thus, the present study was undertaken to examine the influence of acute inflammation, induced by a single streptozotocin injection, and chronic inflammation caused by permanent overweight, on plasma insulin and glucagon levels in piglets.

## MATERIALS AND METHODS

All animal procedures were approved by the First Local Animal Ethics Commission in Cracow, Poland (No. 14/2009).

The experiment was performed on 10-week-old piglets (n=24) of the Polish Landrance strain. The animals (females) were kept under standard conditions and divided into 4 experimental groups: I – control (C), II – chronic inflammation – overweight (O), III – acute inflammation (STZ) and IV – overweight with acute inflammation (O+STZ). Piglets in groups I and III were fed a commercial diet, whereas those in groups II and IV received high-fat feed so as to develop overweight. In order to develop acute inflammation, the animals received a single i.p. injection of streptozotocin (STZ, 100 mg/kg b.w.). The piglets were weighed prior to injection, and STZ was freshly dissolved in dilution buffer (0.1 M sodium citrate, pH 4.5, with HCl, stored at 4°C). Twenty-four hours after injection, blood was taken to heparinized tubes, centrifuged for 20 min with 3000xg at 4ºC, and stored at -20ºC until further analysis. The plasma glucose level was measured by commercial kits (Pointe Scientific INC, USA), using an enzymatic method. Plasma levels of insulin and glucagon were determined using commercial radioimmunological kits from Cis bio International and Dia Source, respectively. IL-6 was measured by

a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay kit from Diaclone. The results were expressed as mean±SE and their statistical comparison was made by analysis of variance followed by Duncan's test [ANOVA].

## RESULTS

All the pigs gained weight throughout the study, both those fed the control feed and those fed the experimental diet, although weight gain was significantly higher in the groups fed the high-fat diet (10.7±0.7 kg) compared with the group fed the control feed (4.9±0.3 kg), which confirmed the induction of overweight ($P<0.05$). Body weight gain was determined as the difference between final and initial weights.

The results showed (Fig. 1.) significantly increased blood glucose levels (from 19% to 33%, $P<0.05$) in all the experimental groups compared with the control animals (4.3±0.3 mmol/l). The obtained data confirmed the effectiveness of inflammation induction (Fig. 2.) using the above methods. The plasma level of IL-6 in overweight animals was 3.08±0.23 pg/ml, in STZ-treated animals – 4.76±0.30 pg/ml, and in overweight pigs with acute inflammation – 4.62±0.26 pg/ml, compared with the control group (2.45±0.15 pg/ml, $P<0.01$). As shown in Fig. 3, a significant difference in the level of insulin was observed in the blood of piglets in experimental groups III and IV. During acute inflammation the plasma level of insulin significantly increased, by 71%, whereas during acute and chronic inflammation it decreased by 21% compared with the control level – 24.2±0.18 μIU/ml. The obtained data showed that in control animals the total plasma level of glucagon was 60.5±4.8 pg/ml (Fig. 4.), and it was significantly decreased during inflammation (both acute and chronic states, a decrease of 27% and 30%, respectively, $P<0.05$).

## DISCUSSION

Obesity is associated with a complex systemic inflammatory state that has been implicated in the development of common, medically important complications (Pickup, 2004; Wellen and Hotamisligil, 2005). Several studies have reported

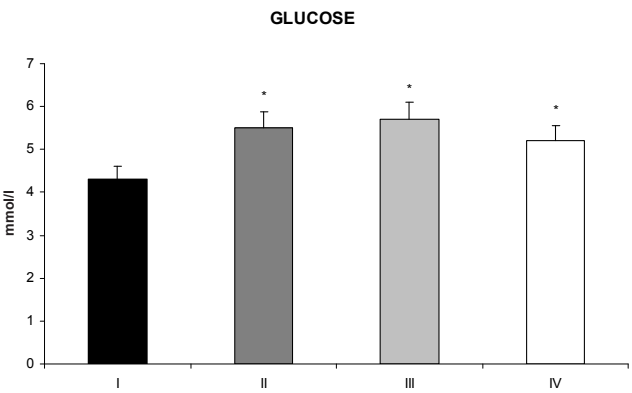


**Fig. 1.** The plasma glucose level in piglets (mmol/l, X±SE, *P<0.05-0.01 – compared with the control values). I – control, II – chronic inflammation, III – acute inflammation, IV – chronic and acute inflammation.

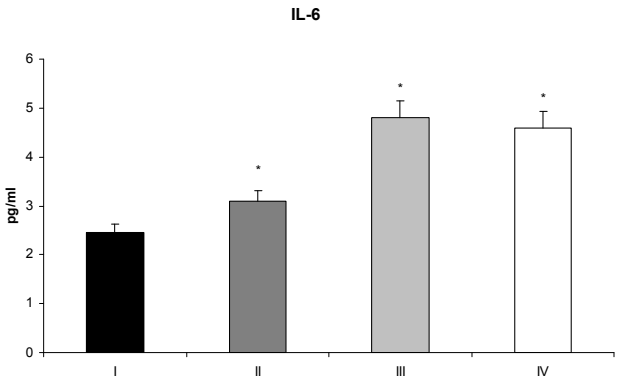


**Fig. 2.** Changes in the plasma IL-6 level (pg/ml, X±SE, * P<0.05-0.01 – compared with the control values). I – control, II – chronic inflammation, III – acute inflammation, IV – chronic and acute inflammation.

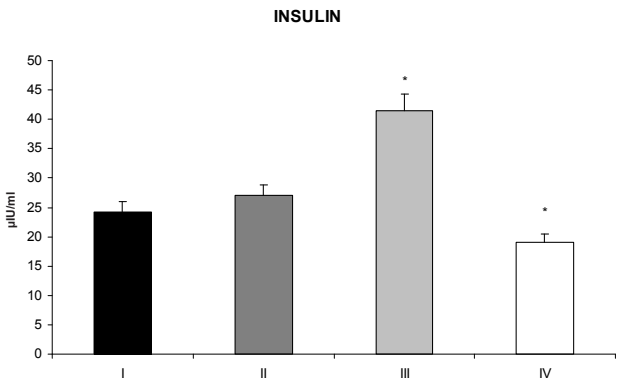


**Fig. 3.** Changes in the plasma insulin level (μIU/ml, X±SE, * P<0.05-0.01 – compared with the control values). I – control, II – chronic inflammation, III – acute inflammation, IV – chronic and acute inflammation.

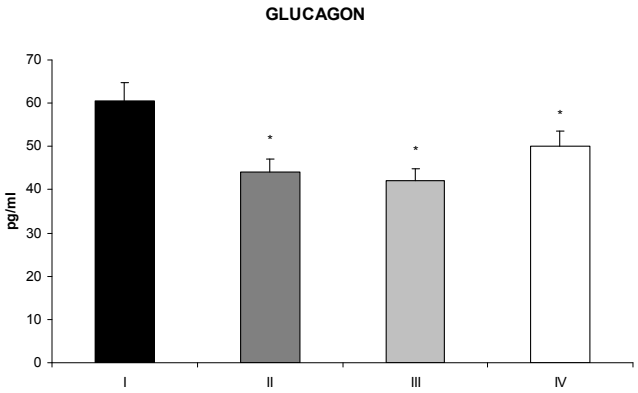


**Fig. 4.** Changes in the plasma glucagon level (pg/ml, X±SE, * P<0.05-0.01 – compared with the control values). I – control, II – chronic inflammation, III – acute inflammation, IV – chronic and acute inflammation.

that obesity induced by feeding with a high-fat diet for 10 weeks can be considered as an adequate model of human obesity (Lin et al., 2000; Gaiva et al., 2001). It has been demonstrated that the long-term supplementation of a diet containing 40-60% fat promotes metabolic alterations, proinflammatory cytokine production and, consequently, obesity, insulin resistance, and hypertension (Flanagan et al., 2008). Interestingly, in our study we found that after only three weeks of feeding with high-fat diet, increased body weight gain of approximately 23% (P<0.05) was observed that caused overweight in piglets. Additionally, the plasma level of the pro-inflammatory factor – IL-6 confirmed the development of inflammation in the experimental animals. Numerous studies have shown that compared with healthy lean individuals, obese individuals have higher levels of pro-inflammatory cytokines and lower levers of anti-inflammatory cytokines, which defines obesity as a chronic inflammatory disease (Dixit, 2008; Nathan, 2008). IL-6 is a circulating protein produced by multiple organs and tissues, including adipose tissue, liver, and leukocytes. This cytokine, released in the circulation, acts as a hormone to regulate the acute phase reaction and influences the major endocrine axes and metabolism (Reichlin, 1993). In the present study it was found that after a relatively short time, the high-fat nutrition also increased the plasma glucose level, compared with the animals fed the standard diet. Similar results were observed in the animals treated with streptozotocin (acute inflammation). In the present data STZ, administered once, caused a twofold increase in the IL-6 level compared with the control animals. It is known that proinflammatory cytokines contribute to elevated glucose synthesis by stimulating gluconeogenesis and glycogenolysis and by increasing indirectly the release of glucagon (Mizock, 2001). However, unexpectedly, we observed a decrease in the plasma glucagon level in both chronic and acute inflammatory states. The observed reduction in the level of glucagon in plasma of animals with inflammation could be explained by its rapid transfer to the liver where it can stimulate the process of gluconeogenesis, thereby increasing glucose production. In the present experiment the high-fat diet did not influence the plasma insulin level, whereas its combination with streptozotocin caused a decrease in this parameter. The plasma insulin level was increased only in the group treated with streptozotocin. The highest level of IL-6 and insulin in this group suggests strong association of this cytokine with insulin resistance. These observations raise the question whether inflammation is really important in the development of type 2 diabetes. The obtained results clearly indicate that higher activity of the immune system, manifested by an elevated cytokine level, may have an impact on the hormones regulating glucose metabolism. The obtained results may confirm the suggestion that glucagon might be an important link between immune and metabolic parameters in piglets with metabolic disorders.

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