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Is hyperoxaluria in a porcine model of Roux-en-Y gastric bypass (RYGB) associated with exocrine pancreatic insufficiency?

Czy mocznica szczawianowa obserwowana na świńskim modelu ominięcia żołądka Roux-en-Y (RYGB) związana jest z niewydolnością zewnątrzwydzielniczą trzustki?

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Key words

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Słowa kluczowe

mocznica szczawianowa, świnie, zabieg ominięcia żołądka Roux-en-Y, zewnątrzwydzielnicza niewydolność trzustki

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S u m m a r y

Introduction. Exocrine pancreatic insufficiency (EPI) is always provided by efficient Roux-en-Y gastric bypass (RYGB) surgery. In the same time, one of the complications of EPI as well as bariatric surgery is oxalate nephropathy.

Aim. The aim of the study was to develop a porcine model of enteric hyperoxaluria after RYGB to investigate its adverse effects in humans.

Material and methods. A total of 11 pigs (n = 7 Roux-en-Y gastric bypass surgery and n = 4 intact – controls) were used in the study. Pigs were fed either regular pig feed (RF), a low calcium pig feed or human type food. RF was enriched with 0.5 or 1% potassium oxalate (KOx).

Results. Following surgery, pigs displayed complete growth arrest for up to 6 weeks, after which they began to grow slightly. Growth of intact control pigs was normal. During the 51 days following surgery (P1 period), daily urinary oxalate excretion levels did not differ between bariatric and control pigs and was approximately 20 ± 10.6 mg/24 h at day 51. Inclusion of 0.5% (P2 period) and 1% (P3 period) KOx to the regular pig feed, increased urinary oxalate excretion both in bariatric and intact control pigs (43.7 ± 32.1 mg/24 h vs. 23.2 ± 18.4 mg/24 h and 51.4 ± 20.5 mg/24 h vs. 48.7 ± 39.2 mg/24 h, respectively) but significant differences compared to P1 period were found only after the 1% inclusion level ($P < 0.05$). Oxalate excretion in RYGB pigs remained at relatively higher levels after KOx removal from the diet. Pig fed low calcium diet showed higher levels of urinary oxalate

excretion as compared to pigs fed regular diet. Surprisingly total calcium depletion from urine was found in pigs after RYGB.

Conclusions. Dietary oxalate governs the development of enteric hyperoxaluria in bariatric pigs, which are more sensitive to it than intact control pigs. The pig model described in the present study could be a sensitive tool used to highlight the mechanisms of enteric hyperoxaluria following RYGB in humans. The interaction between exocrine pancreatic insufficiency and bariatric surgery should be also more profoundly explored.

S t r e s z c z e n i e

Wstęp. Niewydolność zewnątrzwydzielnicza trzustki (NZT) z reguły występuje po udanym zabiegu chirurgicznego ominięcia żołądka Roux-en-Y. Jednocześnie jednym z problemów występujących zarówno w przypadku NZT, jak i zabiegów bariatrycznych jest nefropatia szczawianowa.

Cel pracy. Celem badań było tworzenie świńskiego modelu pokarmowej mocznicy szczawianowej po zabiegu ominięcia żołądka, aby określić jego negatywne skutki u ludzi.

Materiał i metody. Badania przeprowadzono na 11 świniach (n = 7 z zabiegiem ominięcia żołądka (Roux-en-Y) i n = 4 nieoperowanych – kontrolne). Świnie były żywione standardową karmą dla świń (SK), karmą zawierającą niską zawartość wapnia lub karmą typu ludzkiego. SK była wzbogacona 0,5 lub 1% szczawianu potasowego (KOx).

Wyniki. Po zabiegu chirurgicznym świnie wykazały całkowite zahamowanie wzrostu przez okres 6 tygodni, po czym zaczęły minimalnie rosnąć. Świnie nieoperowane rosły normalnie w tym czasie. Podczas 51 dni po operacji (okres P1) dzienne wydalanie szczawianów w moczu nie różniło się pomiędzy zwierzętami bariatrycznymi a kontrolnymi i wynosiło 20 ± 10,6 mg/dobę w 51. dniu. Dodanie 0,5% (okres P2) i 1% (okres P3) szczawianu potasu do standardowej paszy dla świń spowodowało wzrost wydalania szczawianów w moczu zarówno u świń bariatrycznych, jak i kontrolnych (odpowiednio: 43,7 ± 32,1 mg/24 dobę vs. 23,2 ± 18,4 mg/24 dobę i 51,4 ± 20,5 mg/24 dobę vs. 48,7 ± 39,2 mg/24 dobę), lecz istotne różnice zaobserwowano tylko po zastosowaniu 1% dodatku do paszy ($P < 0,5$). Wydalanie szczawianów w moczu utrzymało się na względnie wysokim poziomie u świń RYGB po zaprzestaniu stosowania dodatku szczawianu potasu. Świnie żywione paszą z niską zawartością wapnia wykazywały wyższe poziomy szczawianów w moczu w porównaniu ze zwierzętami żywionymi paszą standardową. Ciekawym odkryciem było stwierdzenie braku jonów wapniowych w moczu świń po zabiegu RYGB.

Wnioski. Szczawiany w diecie odpowiedzialne są za rozwój pokarmowej mocznicy szczawianowej u bariatrycznych świń, które są bardziej wrażliwe niż nieoperowane świnie kontrolne. Opisany w badaniach świński model może być czułym narzędziem do badania mechanizmów pokarmowej mocznicy szczawianowej po zabiegu RYGB u ludzi. Interakcje pomiędzy niewydolnością zewnątrzwydzielniczą trzustki a zabiegami bariatrycznymi powinny być zbadane bardziej szczegółowo.

INTRODUCTION

Roux-en-Y gastric bypass (RYGB) is considered as an effective, acceptable and safe treatment of human obesity (1-3). Sufficient RYGB is mainly dependent on self-digestion on pancreatic enzymes before their contact with digest, which can be compared to the condition of both physiological (neonates, elderly) and pathological exocrine pancreatic insufficiency (EPI). After RYGB patients experience several benefits, including significant weight reduction, reversal of insulin resistance, improvement in hypertension and other cardiovascular risks, and decreased mortality. However, studies have shown that patients undergoing this surgical procedure are at risk for developing hyperoxaluria, nephrolithiasis and oxalate nephropathy (2, 4, 5), among other systemic disorders.

Renal failure due to oxalate nephropathy has been reported by Sinha et al. (4) and Navarro-Díaz et al. (6), who observed that hyperoxaluria may be prevalent in non-stone formers who undergo RYGB surgery as a treatment for weight loss. This has also been observed in other studies, e.g. by Matlaga et al. in which 7.65% of patients were diagnosed with urolithiasis following RYGB surgery, compared to the 4.63% of obese patients in the control group. Moreover, patients after RYGB surgery were more pronounced to undergo shock wave lithotripsy (81 (1.75%) vs. 19 (0.41%)) and ureteroscopy (98 (2.11%) vs. 27 (0.58%)) (7). Furthermore, oxalate nephropathy following RYGB may lead to the development of end stage renal disease (ESRD), thus patients would require dialysis or even renal transplant (8). Importantly, obesity is an independent risk factor for the development of end-stage renal disease (ESRD). Additionally, morbid obesity has been shown to be associated with nephrotic syndrome and it has been reported that proteinuria and segmental glomerulosclerosis can be present in obese patients even in the absence of diabetes (9). On the other hand, both obesity and RYGB surgery are separately associated with an increased risk of kidney deposits (10). Thus, hyperoxaluria together with a high number of renal deposits appears to develop at least 6 months following RYGB surgery (6, 11).

The etiology of post-RYGB hyperoxaluria is not completely understood, but is believed to arise from

enteric malabsorption due to reduced small intestine surface area available for nutrient absorption as well as a shortened gastrointestinal transit time. Moreover, limited contact of food with pancreatic enzymes reduces digestion and a malabsorptive state is established, leading to derangements in calcium and oxalate metabolism. Calcium metabolism is altered by its reduced absorption in the bypassed segments of the small intestine. Under normal physiological conditions, free fatty acids in the intestinal lumen saponify calcium, thus preventing the formation of calcium salts (i.e. containing oxalate), instead forming an insoluble product, which is excreted in the faeces (12). However, some fatty acids are believed to promote paracellular intestinal transport and facilitate oxalate transport into the bloodstream (13). What is interesting, EPI condition, which can arise in patients after RYGB surgery (14), can evoke oxalate nephropathy (15). Oxalate nephropathy is an uncommon complication of chronic pancreatitis. The pathophysiological mechanisms, risk factors, and the clinical course of oxalate nephropathy in chronic pancreatitis are poorly known.

To understand the mechanisms of hyperoxaluria development, following bariatric surgery, several animal models have been developed (16, 17). Rats are commonly used for the study of nephrolithiasis (18-21). Despite similarities between human and rodent genomes, there are significant dietary, anatomic, metabolic, and physiological differences (3, 22). In contrast, pigs seem to be a more appropriate model for the study of enteric hyperoxaluria in humans, since pigs and humans are both omnivores and have similarities in the structure and function of the porcine and the human kidneys (23, 24). According to USDA (25) the gastrointestinal tract (GIT) of a 30-40 kg pig has a similar length to that of an adult human and the nutritional requirements of the pig are very similar to humans, however quantitatively there are species differences. Hyperoxaluria in pigs is usually induced by oral administration of hydroxy-L-proline (HLP). The addition of 5% HLP to the diet results in hyperoxaluria, calcium oxalate crystalluria, and nephrolithiasis (26, 27).

The prevalence of hyperoxaluria after RYGB surgery appears to be high, but the incidence of hyperoxaluria-related complications remains unknown. It is of great interest to use an animal model for studying the development of hyperoxaluria to mimic the situation after RYGB in humans.

AIM

The aim of the study was to explore the sensitivity and applicability of the surgical pig model of human Roux-en-Y gastric bypass to study the dependency of enteric hyperoxaluria on dietary oxalate and calcium levels.

MATERIAL AND METHODS

Animals

The study made use of 11 growing pigs, 5 to 7 weeks of age and was performed in Poland, Lublin, at the University of Life Sciences and in Sweden, Lund, at Lund University. Pigs were randomly chosen from 2 farms. Six crossbred pigs ((Yorkshire × Swedish Landrace) × Hampshire) were selected from the university herd at Odarslöv, Swedish Agricultural University, and 5 Polish crossbreeds (Polish Landrace x Pietrain) were obtained from a private pig farmer.

Roux-en-Y gastric bypass procedure

At the time of surgery, pigs between 6 and 8 weeks of age. Seven pigs were fasted for 12 hours and premedicated with azaperone (Stresnil; Janssen Pharmaceutica NV, Belgium; 4 mg/kg intramuscularly). Pigs were anaesthetized using a 0.5 to 1.5% air mixture of isoflurane (Forene, isoflurane 100, Abbot Scandinavia AB) and carrier oxygen at approximately 0.5 l/min. An incision was made posterior to the sternum along the linea alba, and the stomach was visualized. From the upper part of the stomach, a small pouch of about 20 mL of volume was created. The lower part of the stomach was closed. For the duodenal loop, the intestine was cut about 30 cm caudally from the pylorus. The jejunum was connected to the stomach pouch, allowing for the passage of feed. About 10 cm caudally from the gastrojejunostomy, the ending of the duodenal loop was connected (end to side). The peritoneum, abdominal muscles, and skin were stitched separately. A silicon catheter was implanted into the right jugular vein (28). The studies were reviewed and approved by the Malmö/Lund Ethics Review Committee on Animal Experiments, Lund's city court, and the Ethics Committee of the University of Life Sciences in Lublin.

The general study design is shown in figure 1. The pigs were individually housed in metabolic cages, in which they are able to move freely. Each cage had a perforated floor allowing for the collection of urine samples, free of feed and faeces contamination and was equipped with a drinking nipple and a heating lamp. All pigs were acclimated to the metabolic cages

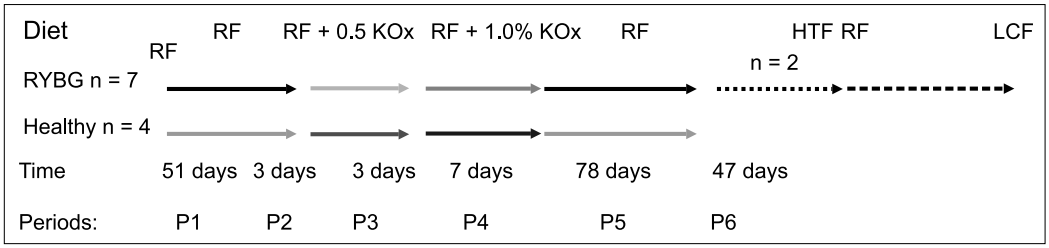

Fig. 1. Study design.
RF – regular feed, KOx – potassium oxalate, HTF – human type of food, LCF – low calcium feed

for 3 days before the start of the urine collections during particular experimental periods (P1-P6), which lasted: P1: 51, P2: 3, P3: 3, P4: 7, P5: 78 and P6: 47 days, respectively. Body weights were recorded weekly on Mondays before the morning feed.

The mean body weight of pigs at the beginning of the experiment was 12.6 ± 2.5 kg. The experimental group of 7 pigs (4 in Lund and 3 in Lublin) underwent Roux-en-Y gastric bypass surgery performed by the same surgeon. Another group of 4 pigs (2 in Sweden and 2 in Poland) served as intact, healthy controls. Control pigs became part of the experiment 3 weeks after the Roux-en-Y pigs had fully recovered from surgery. Before surgery and until the end of period 4 (P4) – day 64, all 11 pigs were fed regular feed (RF, tab. 1) at a total of 4% of their body weight divided in two meals (for 3 weeks after surgery pigs obtained feed per day in amount of 2% of their body weight). To mimic high amounts of oxalate in the intestines the regular pig feed was enriched with two levels of potassium oxalate (KOx) (0.5 and 1% of the total weight of feed for 3 days; during period P2 and P3, respectively) between days 52 and 57. After KOx diet enrichment, the pigs were fed RF for the next 7 days (period P4). After 80 days of the experiment, all pigs in Lublin (2 control and 3 Roux-en-Y pigs) and 2 controls and 2 Roux-en-Y pigs in Lund were killed and submitted to post mortem autopsy. Two Roux-en-Y pigs in Sweden continued with the experiment for up to 190 days. From day 65 to day 143 (period P5), pigs were fed with human-type food (HTF) that contained vegetables moderately rich in oxalate (peas, cucumber, cabbage) (19, 21) and bread (carbohydrates 50%, protein 10.7%). During period 6 (day 144 to 190), pigs were fed a low calcium dry feed (LCF, tab. 1). Tap water was provided *ad libitum* during the study, except during urinary collection when 24-hour drinking water intake was recorded.

Table 1. Components of the regular feed (RF) and low calcium feed (LCF).

Nutrients	Regular feed – RF (%)	Low calcium feed – LCF (%)
Protein	15.5	14.1
Fat	4.3	4.7
Fiber	4.7	4.1
Starch	66.4	70.5
Lysine	1.1	1.03
Methionine	0.38	0.49
Ashes	5.9	4.48
Calcium	0.9	0.037
Phosphate	0.7	0.36
Sodium	0.15	0.133

Sample collection and analysis: urine collection and measurement

All blood and sample collections were performed in an identical manner in Sweden and Poland. The 24-hour urine samples were collected from the metabolic cages into a bucket placed underneath the cage, containing 5 to 15 mL of 6N hydrochloric acid, in order to acidify samples and achieve a pH of below 3, which was necessary to maintain all oxalate soluble in the urine. After measurement of total urine volume, 3 mL samples extracted with charcoal were transferred to plastic tubes for future analysis. The 24 hour collections were taken 5 times during period P1, twice during P2, twice during P3, once at the end of period P4, once at the end of P5, and 3 times at the end of P6.

Blood samples were collected before the morning meal from jugular vein catheters on the same day as the 24-hr urine collections, during the respective experimental periods. Blood samples were collected into heparin tubes and centrifuged for 15 minutes at 3000 rpm, and blood plasma was separated into new tubes. Samples were stored at -20°C for further analysis. Creatinine concentration in blood plasma and urine was analyzed using a Mindray BS-130 analyzer. Total oxalate concentration in urine was measured using oxalate reagents (Kit 591D, Trinity Biotech, Ireland) according to the manufacturer's instructions. Urinary Ca^{2+} was determined by means of an ion analyser (BioMaxima, Poland). The creatinine clearance was estimated based on plasma and urine creatinine concentration and as well as diuresis according to the following equation: $C_{Cr} = (U_{Cr} \times V/P_{Cr.})/bwt$ and the results are presented as ml/min/kg b wt. Daily urinary excretion of creatinine, oxalate and Ca^{2+} was calculated based on daily urine volume and urine concentration.

Post-mortem analysis and histopathology

At the end of the experiment, pigs were euthanized and submitted to post-mortem examination. Histopathological hematoxylin and eosin analysis of kidney sections were performed.

Statistical analysis

Statistical analysis was performed on the data generated from the study using one-way analysis of variance and multiple range tests. Differences were considered significant if $P \leq 0.05$. Data are expressed as the mean ± SD or mean ± SEM.

RESULTS

The experiment performed in two different sites (Lund and Lublin), on two different types of pigs, confirm the applicability and repeatability of the model and at the same time limiting the number of animals allocated to the experiment. Pigs at both the Lublin experimental facility and the Lund farm stayed in good health throughout the experimental procedure. There were no significant differences in daily water intake and urine production between treated and control pigs during period P1. The mean feed consumption after 3 weeks restriction during P1, did not differ between bariatric pigs and intact, control pigs. However, after 3 weeks restriction period reduced food intake in the Roux-en-Y group was observed, compared to that of the controls. Bariat-

ric pigs did not grow during the first 3 weeks following surgery. Over time, bariatric pigs gained significantly less weight compared to controls; however, a significant body weight increase ($P < 0.05$) was observed at week 12 after surgery in RYGB pigs (from 12.5 ± 2.5 to 36.8 ± 8.5 kg) and control pigs (from 11.6 ± 1.3 to 50.5 ± 6.4 kg). Moreover, in healthy control pigs, a significant increase (24.5 ± 5.1 kg, $P < 0.05$) in body weight was observed already at week 7 (fig. 2).

Post-mortem gross examination and histopathological analysis of the kidneys did not show any changes in both control and RYGB pigs.

Blood and urine creatinine levels, as well as oxalate and calcium urinary excretion are presented in table 2. Plasma creatinine concentrations were within the normal physiological range and measured between 1.3 ± 0.04 and 1.8 ± 0.06 mg/dl, with no significant differences observed between control and RYGB pigs. Similarly, urinary creatinine excretion in control pigs was not significantly different from the values obtained in RYGB pigs. Creatinine clearance showed a tendency toward higher values during the experimental periods in the RYGB pigs compared to the control pigs, and was significantly different between groups during the 1% KOx dietary enrichment (RYGP – 2.19 ± 0.64 vs. control – 0.90 ± 0.04 ml/min/kg b wt., $P < 0.05$).

The mean oxalate excretion in Roux-en-Y pigs and unoperated, control pigs during P1 fluctuated within a narrow range of approximately 20 mg ± 10.6/24 h on day 51 and it was treated as the baseline for both groups. Enrichment of feed with 0.5% KOx during P2 raised urinary oxalate excretion levels on day 52 to 43.7 ± 32.1 mg/24 h and 23.2 ± 18.4 mg/24 h and on day 54 to 51.4 ± 20.5 mg/24 h and 48.7 ± 39.2 mg/24 h in the RYGB and control groups, respectively. An increase in KOx dietary enrichment to 1% resulted in higher levels of urinary oxalate excretion, which reached 77.1 ± 40.8 mg/24 h and 51.2 ± 40.8 mg/24 h on day 55 and 83.2 ± 38.4 mg/24 h and 62.6 ± 40.0 mg/24 h on day 57 in the RYGB and control groups, respectively. These changes were significantly different ($P < 0.05$) in operated pigs but not in unoperated, control pigs when compared to the baseline (fig. 3). After one week of KOx challenge, and then it removal and feeding with regular feed (P4), the urinary oxalate excretion remained at a higher level of 51.9 ± 19.5 mg/24 h in bariatric pigs, whereas in controls it dropped to levels similar to baseline, 30 ± 24.1 mg/24 h.

Two bariatric pigs in Sweden continued with the experiment for an additional 4 months. Urine collections were made during P5 on day 143, where pigs were consuming a human-type diet and during P6 on day 190, after adaptation to a low calcium diet (tab. 2). Urinary oxalate excretion measured 51.9 ± 19.5 mg/24 h and 78.8 ± 18.2 mg/24 h on day 143 and day 190, respectively. The increase in daily urinary excretion of oxalate on day 190 following surgery was significantly different to that observed during P1 (fig. 3).

The urinary oxalate/creatinine ratio (Ox/Cr) amounted to 13.5 ± 3.3 mg/g in the RYGB pigs and was not different from that observed control pigs (19.4 ± 12.4 mg/g) on the 51st day following surgery (P1), when fed a regular feed. Ox/Cr increased significantly during dietary enrichment with 0.5% (P2) and 1.0% (P3) KOx. both in RYGB and control pigs. A significantly lower Ox/Cr (11.7 ± 5.4 mg/g) was observed in control pigs one week after KOx was removed from the diet (P4). In contrast, Ox/Cr remained at a significantly high level in RYGB pigs (54.0 ± 20.7 mg/g, $P < 0.05$). During the period where pigs were fed a human type food, either

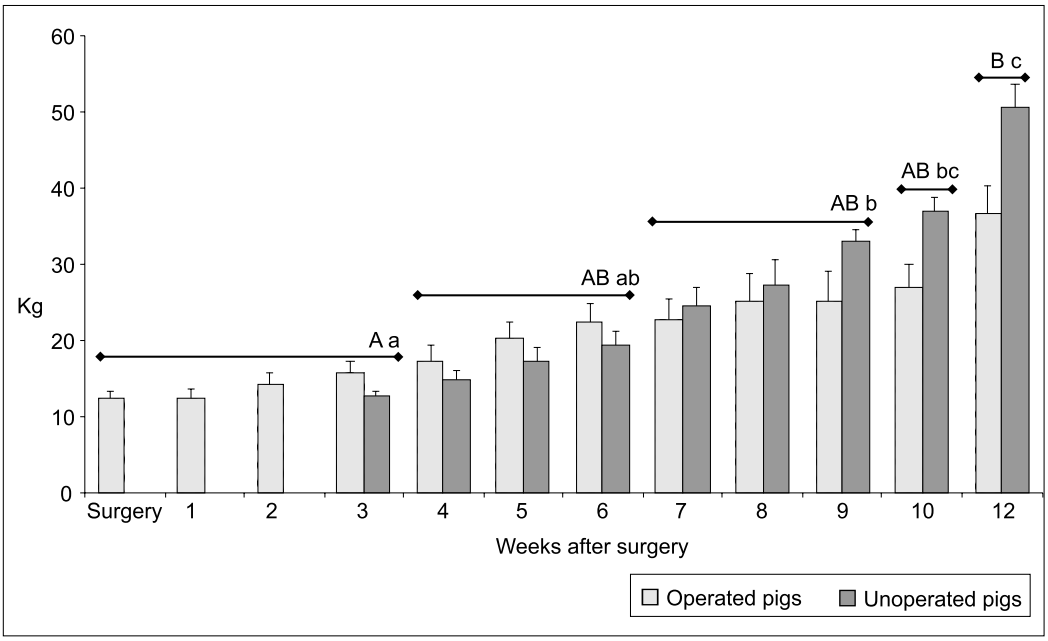

Fig. 2. Body weight of unoperated, control pigs (n = 4) and Roux-en-Y gastric bypass operated (RYGB) (bariatric) pigs (n = 7) during the 12 weeks post-surgery. Data are expressed in kg as means ± SEM. Unoperated pigs were incorporated into the study 3 weeks after RYGP pigs. Different capital letters given with the results describe significant differences between body weight in bariatric pigs when $P < 0.05$. Different lower case letters given with results describe significant differences between body weight in intact (control) pigs when $P < 0.05$.

Table 2. Concentration of creatinine in blood plasma, creatinine clearance and urinary excretion of creatinine, calcium ions and oxalate/creatinine ratio in control (intact) and Roux-en-Y gastric bypass (RYGB) pigs (values represent means ± SEM).

Item	Pigs group	Regular feed (P1)	Regular feed + 0.5% KOX (P2)	Regular feed+ 1% KOX (P3)	Regular feed (P4)	Human type food reg. Ca (P5)	Human type food low Ca (P6)
Creatinine in blood plasma (mg/dl)	RYGB	1.3 ± 0.04	1.5 ± 0.16	1.3 ± 0.10	1.4 ± 0.23	na	na
	Control	1.5 ± 0.12	1.5 ± 0.15	1.5 ± 0.17	1.8 ± 0.06	na	na
Creatinine Clearance (ml/min/kg b wt.)	RYGB	2.58 ± 0.54	2.62 ± 0.56	2.19 ± 0.64	1.42 ± 0.16	na	na
	Control	1.64 ± 0.07	1.57 ± 0.11	0.90 ± 0.04*	1.34 ± 0.45	na	na
Creatinine excretion in urine (g/24h)	RYGB	2.9± 1.52	2.9 ± 0.76	2.9 ± 1.54	2.3 ± 1.03	2.0 ± 0.31	3.9 ± 0.26
	Control	3.6 ± 1.43	4.0 ± 1.73	2.6 ± 0.96	3.8 ± 0.93	na	na
Oxalate/Creatinine ratio (mg/g)	RYGB	13.5 ± 3.3[a]	24.1 ± 5.3[a,b]	52.2 ± 11.6[b]	54.0 ± 20.7[b]	17.2 ± 2.8[a]	20.0 ± 1.1[a]
	Control	19.4 ± 12.4[a]	40.3 ± 23.4[a,b]	52.2 ± 26.5[b]	11.7 ± 5.4[a,*]	na	na
Calcium excretion in urine (mmol/24 h)	RYGB	nd	nd	nd	nd	na	na
	Control	12.7 ± 8.8[a]	6.2 ± 2.8[b]	4.5 ± 3.1[b]	15.0 ± 11.18[a]	na	na

[a, b]Different letters describe significant differences between the same group in different experimental periods at the level of $P < 0.05$
*Describe significant difference between RYGB and control groups at the same time period at the level of $P < 0.05$
nd – not detected, na – not analyzed

with a regular or low calcium content, the RYGB pigs showed slightly higher but not significant Ox/Cr values compared to that measured during P1 (tab. 2).

During the first 2 weeks following surgery, urinary Ca^{2+} decreased significantly in the bariatric pigs, reaching a non-detectable concentration during the rest of the experimental period. A significant drop in urine Ca^{2+} concentration was also observed in the control group during P2 and P3, when pigs were fed the diet supplemented with 0.5% and 1% KOx (tab. 1). Nevertheless, after removing the KOx additive from the feed, the Ca^{2+} concentration normalized in the intact, control pigs.

DISCUSSION

This paper describes an attempt to induce a state of enteric hyperoxaluri, well-known as a complication of EPI condition of various etiology, a in pigs after a Roux-en-Y gastric bypass procedure (RYGB), which is often performed to treat morbid obesity in humans. The surgical procedure was expected to create similar metabolic disturbances (including hyperoxaluria) to

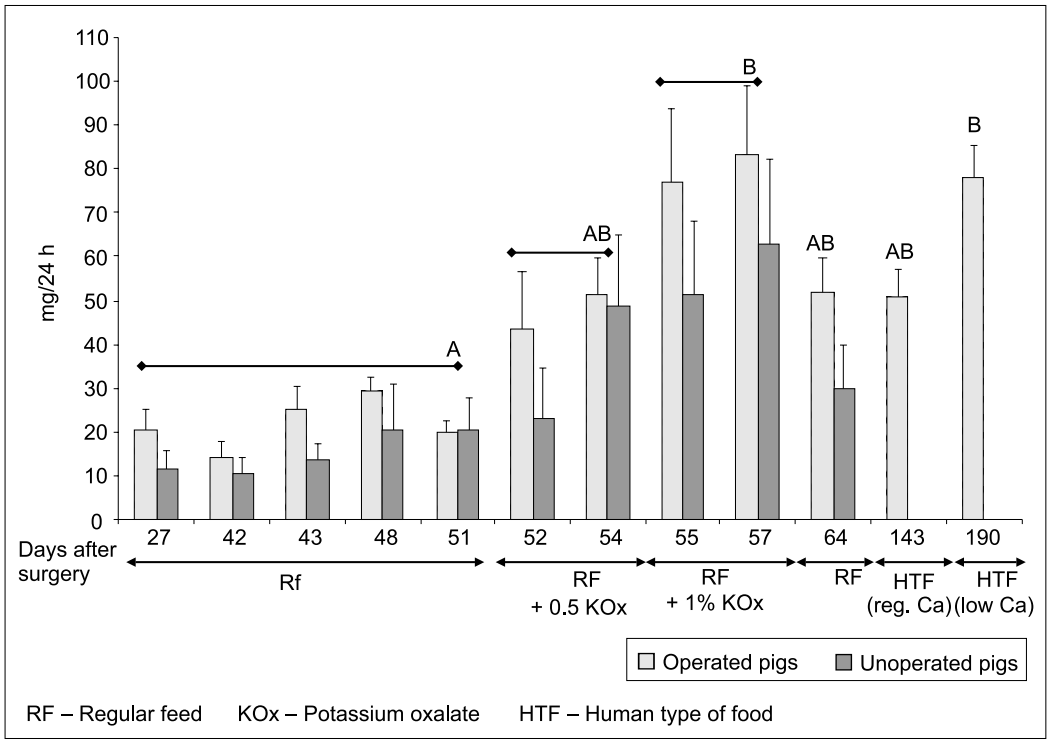

Fig. 3. Urinary oxalate excretion in unoperated, control pigs (n = 4) and Roux-en-Y gastric bypass operated (RYGB) (bariatric) pigs (n = 7) during the 64 days post-surgery. The experiment was continued until day 190 following surgery, on 2 RYGB pigs. Data are expressed in mg per 24-hour urine collection (means ± SEM). Significant differences from the base line (51st day after surgery) were found only in Roux-en-Y pigs after 1% of KOx load ($P < 0.05$).
Different capital letters given with results for particular periods describe significant differences between oxalate excretion in bariatric pigs.

those normally observed in 10-20% of patients after the Roux-en-Y surgical procedure (6, 29-31). Dietary intake of oxalate and Ca^{2+}, as well, as amount of dietary fat, are among several factors that influence the degree of hyperoxaluria development in humans.

The physical limitation of the gastric pouch in operated pigs very likely caused restriction of food intake, 6 weeks after the surgical procedure. The decreased food intake resulted in a consequent decrease in body weight gain during the entire experimental period. However, a significant body weight increase in bariatric pigs was observed at week 12 after surgery and intact, control pigs displayed a significant increase in their body weight earlier than RYGB pigs, at week 7 following surgery. After 140 days following RYGB surgery the body weight of pigs measured 49.3 ± 29.0 kg. This indicates that the RYGB procedure effectively retarded the regular body weight gain in growing pigs due to the malabsorption of essential nutrients, it is worth noticing that described effect could be provided only through the self-digestion of pancreatic enzymes.

Significant increases in plasma creatinine, following RYGB surgery, were not observed in our model irrespective of whether the pigs were receiving regular feed or the oxalate enriched feed. Interestingly, the creatinine clearance tended to be higher in the RYGB pigs throughout the entire experimental period, which may explain the slightly lower creatinine concentration in the blood plasma of this group of pigs. On the other hand significantly lower creatinine clearance was observed in the intact, control pigs which could suggest a lower glomerular filtration rate, induced by dietary enrichment with 1% KOx. Our results in control pigs are in agreement with the creatinine clearance values observed by Kumar et al. (32), in pigs fed a normal protein diet. These authors observed elevated creatinine clearance in pigs fed a high protein diet for 6 months, similar to that observed for the RGBP pigs in our study. Glomerular hyper filtration is associated with early signs of kidney disease and is observed in overweight individuals and metabolic syndrome population groups, together with an increased risk of development of renal disease (6, 33). In our study kidney histolopathological analysis did not show any morphological changes within the kidney, which might be due to the short time period following RYGB surgery.

The similar urinary excretion of oxalate in both control and RYGB pigs, fed regular feed (RF) for 51 days after surgery, suggests that the pigs did not develop hyperoxaluria during this short time period after RYGB surgery, under standard feeding conditions. Although, feed supplementation with potassium oxalate (KOx) for 3 days at the level of 0.5% (P2) and 1.0% (P3) substantially increased urinary oxalate excretion in a dose dependent manner. Moreover, excretion levels were significantly higher in bariatric pigs compared to intact control pigs. Notably, the urinary oxalate excretion of intact control pigs did not increase after the first day of KOx enrichment as it did in RYGB pigs. This data demonstrate that after the oxalate load an increased intestinal uptake of oxalate takes place, early in the period post bariatric surgery in pigs, suggesting increased intestinal permeability, but the mechanism responsible for this change needs to be elucidated. Froeder et al. (34) revealed that urinary oxalate excretion peaked 2-4 hours after an oxalate load and that an oxalate meal was able to induce a temporary state of hyperoxaluria in human patients after gastric bypass procedures, which may not be detectable in 24-hour urine samples.

Despite removing KOx from the feed, the oxalate excretion in the urine of bariatric pigs tended to remain at higher levels than that of the controls after 7 days of ingestion of the regular feed (P4). This finding showed a tendency for a delayed high concentration of oxalate in urine over time in the bariatric pigs, which might be a result of impaired renal elimination or increased absorption of oxalate. To investigate whether the increase in question would remain constant, 2 operated pigs were left alive to continue with the study. During P5, pigs were fed human-type food, containing vegetables and bread; during P6, Ca^{2+} was reduced to 0.04% in the daily nutritional dose. The 24-hour urine of operated pigs on the low Ca^{2+} diet showed urinary oxalate excretion levels comparable to the highest levels observed during P2 and P3, when the feed was supplemented with KOx. Compared to their baseline levels, RYGB pigs displayed a significantly increased (more than 5 times higher) level of urinary oxalate excretion.

Clinical studies on human patients demonstrate that the increase in oxalate excretion from baseline, 3 months after Roux-en-Y surgery, is moderate and below the level indicative of an increased risk for the development of kidney stones (< 45 mg/d) (35). Other data indicate that Roux-en-Y surgery is associated with an increase in urinary oxalate excretion only 1-2 years after surgery; where the mean excretion of oxalate was twice as high as that measured at baseline (64 ± 30 mg/d vs. 33 ± 9 mg/d) (36).

Although commonly accepted guidelines have not yet been established, the prevention strategies for hyperoxaluria following RYGB surgery, besides that of increased water intake, also include oral calcium supplementation. The rationale is that the Ca^{2+} ions form a complex with free oxalate, which limits oxalate absorption. Moreover, due to the fact that bariatric surgery is associated with both vitamin D and calcium deficiency, this may be of great importance for the prevention of bone loss in this particular group of patients (37). It has been mentioned that for the EPI-related hyperoxaluria the best results are connected with pancreatic-like enzymes replacement therapy.

A decrease in urine Ca^{2+} concentration of unprecedented dimensions was observed in RYGB pigs. It is a well-known fact that urine Ca^{2+} concentrations significantly decrease following the bariatric procedures of Roux-en-Y in humans and in rat models of gastric bypass.

Elimination of Ca^{2+} from urine in experimental Roux-en-Y models should be evaluated, in association with the development of osteoporosis in humans undergoing similar surgery. Calcium deficiency and subsequent osteopenia have been reported after fundectomy and gastrectomy (38, 39). Calcium deficiency can be also a consequence of EPI condition (40). Moreover, studies in rats indicate that fundectomy may alter calcium and bone metabolism, thus influencing the physical properties of the limb bones (41).

In light of the results presented above, we postulate that the pig model of gastric bypass is an appropriate, sensitive model for the study of the adverse effects associated with Roux-en-Y gastric bypass surgery in humans, especially the mechanisms of the development of functional (lack of the active enzymes in the gut chime) exocrine pancreatic insufficiency and enteric hyperoxaluria, although it needs further investigations. The postoperative observation time should be extended to 6-9 months on bigger number of animals.

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