Streptozotocin – induced diabetes, bile-pancreatic secretion and insulo-pancreon-axis interaction

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Summary

The present tests were undertaken in order to analyze in male Wistar rats the changes in the exocrine and endocrine pancreas and on the interactions that normally evolve in the insulo-pancreon-axis. To evaluate this by a single i.p. Boots secretin injection, glycemia (G), amylasemia (A) and lipasemia (L) were determined. In bile-pancreatic secretion, we analyzed, pre and post-secretin, the following parameters: volume (V), bicarbonate output (BO), amylase output (AO) and lipase output (LO). Three groups of tests were done: a) control (C); b) streptozotocin-treated non-diabetic-rats (St-ND) and c) streptozotocin-treated diabetic animals (St-D) which showed morning glycemia values higher than l6.0mmol/l. Four months later, under Tiopental i.p anesthesia, a bile-pancreatic fistula was done. Following a 30min basal period, Boots secretin (20CU/kg) was i.p injected. Bile-pancreatic secretion put in evidence a significant fall of BO in both St-ND and St-D series. In controls, AO revealed a post-secretin increase of 160%, while in the St-D rats showed a depression of 41%. The behavior of L was different, being augmented (+27%) in the C, while in the St-D rats the response was significantly higher (+95%). In bile-pancreatic-secretion, the fall of BO and AO in the St-ND and St- D series in respect to the C, are probably consequence of the diminishing potentiating effects exerted normally by insulin on the secretin-induced water and bicarbonate secretion of the pancreon units. In contrast, the rising of LO in the St-D, an expression of an enhancing pancreocyte's synthesis and secretion of lipase. The blood changes of A (depression) and of L (increase) in respect to the C values, although without reaching significant level, mirror those observed in bile-pancreatic secretion.

Key words. *Streptozotocine, Secretin, Insulo-Pancreon, Axis.*

Eje insulo-pancreonal y su disrupción inducida por la estreptozotocina

Resumen

Los presentes tests tuvieron por objetivo analizar en ratas macho Wistar los cambios a nivel pancreático en su componente exocrino-endocrino y en sus interacciones. La evaluación se efectuó en la secreción basal bilio-pancreática (SBBP) en condiciones previas y subsecuentes a una inyección ip de Secretina Boots (20 CU/kg), ello luego de un período basal de 30 min. Tres grupos de animales fueron analizados: control (C), estreptozotocina no diabéticas (St-ND) y estreptozotocina diabéticas (St-D). Este último reveló valores de glucemia en ayunas superiores a la cifra de 16,0 mmol/l. El examen se realizó 4 meses postestreptozotocina. En las series St-ND y St-D el estudio de la SBBP post-secretina puso en evidencia una caída significativa del débito de bicarbonato tanto en el grupo St-D como, inesperadamente, en el St-ND. En cuanto a la amilasa, los animales C revelaron un incremento post-secretina del 160%, mientras que, por el contrario, las ratas St-D mostraron una depresión significativa del 41%. La lipasa puso en evidencia, en contraposición, un aumento del 27% en los C y de 95% en los animales St-D. En la SBBP la caída del débito de bicarbonato y amilasa, tanto en

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las series St-ND como St-D respecto de las C, es consecuencia de la disminución de las normales influencias potenciadoras ejercidas por la insulina en las acciones de la secretina sobre la secreción hidrelática (agua y bicarbonato) y ecbólica (enzimática) del pancreón. En los animales St-D, el aumento del débito de lipasa es consecuencia del efecto incrementador sobre la enzima que acompaña a la caída del tono insulínico de la glándula.

Palabras claves. *Estreptozotocina – Secretina – Eje Insulo-Pancreonal.*

It has been proved that there exists both functional and trophic interactions between the Langerhans islets and the pancreon units,[1,2] the so called "islet-acinar" or insulo–pancreon–axis.[3-7] Insulin induces the synthesis and secretion of amylase and depresses both the content and secretion of lipase and colipase from the acinar cells.[8-l4]

This is coherent with the finding of a fall of amylase and an increase of lipase in the pancreatic juice of diabetic patients.[l5,l6] The same happens in chemical diabetes, induced in animals by alloxan or streptozotocin. Indeed, the Langerhans islets' depression of insulin is associated with a significant fall of amylase synthesis and secretion by the pancreon's acinar cells.[l7]

A feature to take into account is that the islets' hormones: insulin (I), glucagon (Gl), pancreatic polypeptide (PP), somatostatin (SS), calcitonin-gene-related-peptide (CGRP) and PYY, together, with the autonomic nervous system, influence the insulo-pancreon-axis. The latter occurs through the nerve fibers of the vagal, splanchnic-celiac and entero-pancreatic nervous complexes which modulate the intrapancreatic ganglia.[l8-27] In that sense, previous reports have shown that celiac ganglionectomy, superimposed on 95% subtotal pancreatectomy (Foglia), induces a normalization of hyperglycemia, a rising of depressed plasma insulin values and fecal chymotrypsin concentration. Moreover, when celiac ganglionectomy is performed on non-pancreatectomized rats, divergent changes in the blood values of amylase and lipase are elicited. Indeed, as the former increases, the latter drops in respect to control levels. Besides, the isolated islet cells, in *in-vitro* tests, release more insulin than those of the control animals.[27-29]

The aim of the present tests is to gain further insight in the complex interrelationship that normally evolves in the insulo-pancreon-axis. This undertaking has as a main goal to get new basis for a simple, non-invasive examination procedure, especially at clinical level (i.e., amplifying the classical oral glucose tolerance test) that could offer and encompassing view of the two components (exocrine and endocrine) of the pancreatic gland and of what we have described as insulo-pancreon-axis interactions.

Material and methods

The experiments were performed on male Wistar rats. The initial weight of the animals was of approximately 200 g. Six animals were allocated in the control group (C). Fifteen rats were injected i.p with streptozotocin in citrate buffer (PH 4.5) at the dose of 60 mg/kg body weight.

From the tail, the blood glucose level was checked weekly with the oxidase method.[30] Diabetes was diagnosed in those rats that revealed a blood glucose level over 6.5 mmol/l. Out of the l5 animals injected, 6 fell into this category (St-D). The rest were considered as streptozotocin-non-diabetic (St-ND) rats. They were housed four in a cage with a temperature and light controlled environment.

They were fed with an ordinary laboratory diet (Purina, Buenos Aires, Argentina) and water was offered *ad-libitum*. They were followed for 4 months. At this period and before autopsy, after a 3-hour fast a biliary-pancreatic fistula was performed under thiopental anesthesia.[31] Bile-pancreatic secretion (BPS) was collected for 30 min as a basal period and for another 30 min following and i.p injection of Boots secretin at the dose of 20 CU/kg. Blood was drawn from the tail at the beginning of each test. Following i.p secretin, blood testing for glucose, amylase and lipase was carried out at 5 min (orbital puncture) and 30 min (aorta exsanguination). The BPS was collected in ice-chilled tubes. Volume was measured by weight difference (1.0 g = 1.0 ml). Amylase was determined by the Somogyi method[32] and lipase by the titrimetric method of Bang modified by Lehmann et al.[33] Bicarbonate concentration was determined by the titrimetric method of Lehmann et al.[34] Bicarbonate output was calculated and expressed as µEq/30 min. At autopsy, the head of the pancreas (segment of the gland comprised between the inner border of the duodenum and an arbitrary line that follows the superior mesenteric vessels up to the pylorus) and the body-tail segment (rest of the gland) were evaluated separately after carefully removing fat and lymphoid tissue. The gland was blotted between filter paper and the wet weight of each segment was measured. The results were expressed as the mean +/- SEM.

Data were analyzed by the Student t test for non-paired values and a *P* value lower than 0.05 was considered as significant.

Results

Changes in the rats' body weight and the pancreatic gland

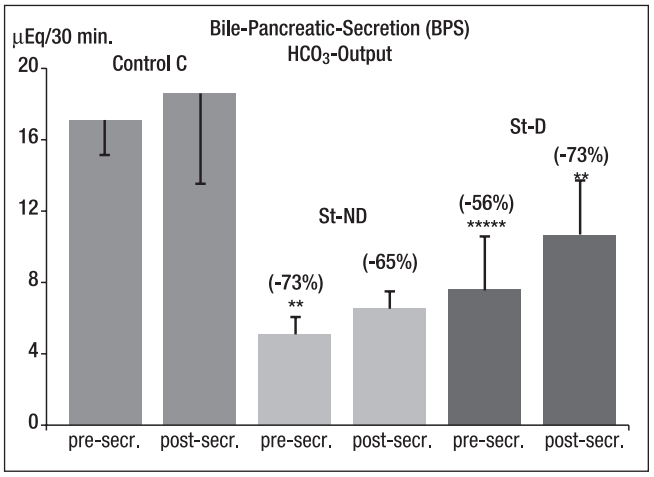
Body weight. At the end of the 4-month post-streptozotocin injection the animals turned diabetic (St-D) and revealed a significant fall in the body weight (Table 1). In contrast, the St-ND animals had the same rate of body weight progression as the controls (C) (Table 1).

Wet weight of the pancreatic gland. There were not significant changes between the experimental groups and the control series. Only when the index g/kg was considered, the whole pancreatic gland wet weight of the St-D series showed a significant difference with the C group (Table 1)

	Control (n= 7)	St-N-D (n= 9)	St- D (n= 6)
Body Weight (grs.)	361 (± 9.64)	338 (± 18.41)	242 * (±19.58)
	PANCREATIC GLAND		
Cephalic Segment (mg)	547 (± 57.55)	543 (± 79.86)	425 (± 38.17)
Index g/Kg.	1.53 (± 0.16)	1.62 (± 0.23)	1.84 (± 0.25)
Body-Tail Segment	786 (± 76.62)	958 (± 139)	756 (± 66.12)
Index g/Kg	2.17 (± 0.20)	2.78 (± 0.34)	3.17 (± 0.27)
Total Gland (mg)	1333 (± 126)	1495 (± 198)	1181 (± 63.08)
Index g/kg	3.70 (± 0.34)	4.40 (± 0.49)	5.02 * (± 0.41)

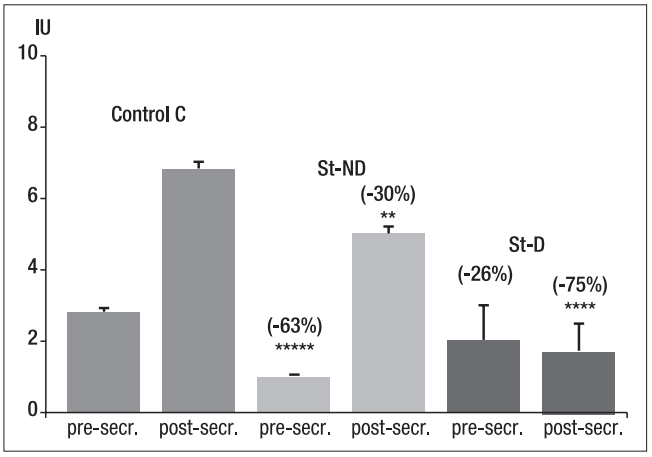
Values are expressed as the mean ± SEM
* p <0.05 in respect to Control value

Figure 1. *Bicarbonate output (μEq/30 min) in the bile-pancreatic-secretion (BPS) in both the 30-min basal and 30-min post i.p Boots secretin (20 CU/kg) in the rats of the (C), (St-ND) and (St-D).*



In brackets the percentage change in respect to the control series in both the basal and post-secretin periods. Statistical analysis with the Student "t" test: * $P < 0.05$, ** $P < 0.025$, *** $P < 0.01$, **** $P < 0.005$, ***** $P < 0.001$. Statistical significance in respect to the same period of the control series.

Figure 2. *Bile-pancreatic-secretion, (BPS) amylase output.*



* $P < 0.05$, ** $P < 0.025$, *** $P < 0.01$, **** $P < 0.005$, ***** $P < 0.001$. Statistical significance in respect to the same period of the control series.

Bile-pancreatic secretion (BPS) changes

The analysis of the hydrelatic response (water and bicarbonate) in the pre and post secretin period of both the St-ND and the St-D groups showed, a significant depression of bicarbonate output at the expense of its concentration when compared to control values (Figure 1).

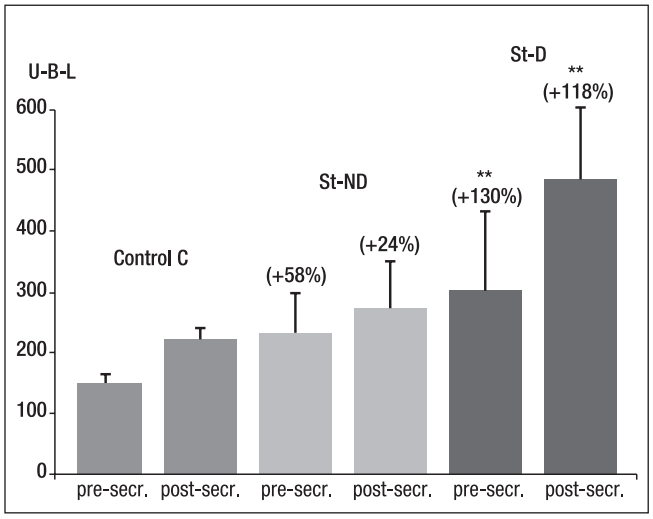
The evaluation of the ecbolic response (enzymes) revealed that amylase, in both the St-ND and St-D groups and in both the pre and post-secretin periods, was significantly depressed in respect to the C animals. In the St-D rats, amylase was further decreased following secretin injection (Figure 2).

In contrast to amylase, lipase output showed non-significant raised values in the St-ND and primarily in the St-D series, compared to the values of the C group, in the pre as well as in the post-secretin periods (Figure 3). The percentage changes post vs. pre-secretin values were higher in the St-ND and the St-D groups than in the C rats.

Glycemia and changes of pancreatic enzyme values in blood

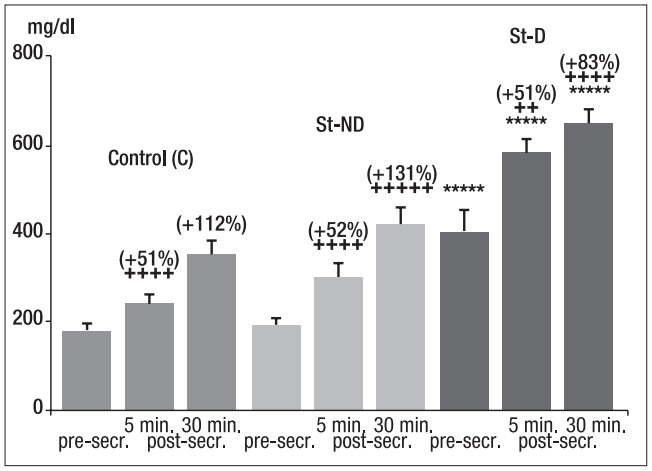
In the three series, C, St-ND and St-D, at 5 and 30 min post i.p Boots secretin samples, the basal glycemia values were significantly raised. The degree of increase,

Figure 3. *Bile-pancreatic-secretion, (BPS) lipase output.*



* $P < 0.05$, ** $P < 0.025$, *** $P < 0.01$, **** $P < 0.005$, ***** $P < 0.001$.
Statistical significance in respect to the same period of the control series.

Figure 4. *Blood glucose.*



* $P < 0.05$, ** $P < 0.025$, *** $P < 0.01$, **** $P < 0.005$, ***** $P < 0.001$. Statistical significance in respect to the same period of the control series.+ $P < 0.05$, ++ $P < 0.025$, +++ $P < 0.01$, ++++ $P < 0.005$, +++++ $P < 0.001$.
Statistical significance in respect to the pre-secretin period.

expressed as percentage, was closely similar in the three groups (Figure 4).

The amylase and lipase enzymes, revealed, following the i.p secretin, comparing the St-ND and the St-D series results with those observed in the C rats, a tendency to an opposite behavior. Indeed, as post-secretin amylase non-significantly dropped in respect to the pre-secretin period results, especially in the St-D group (Figure 5), the reversal was shown by lipase which showed, in both experimental series, a non-significant enhancement of its blood level (Figure 6).

Figure 5. *Blood amylase.*

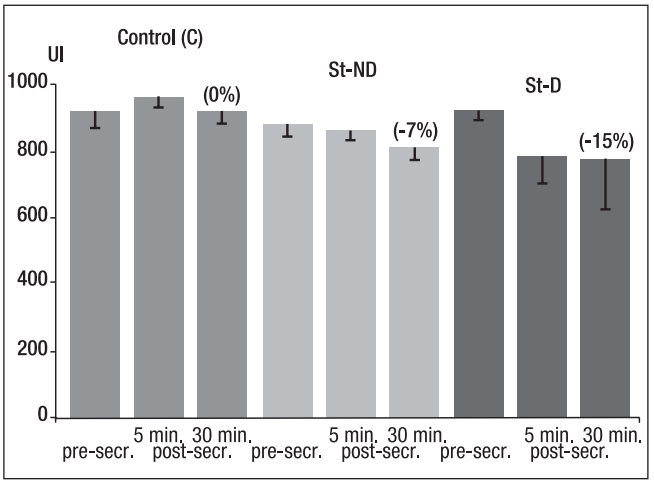
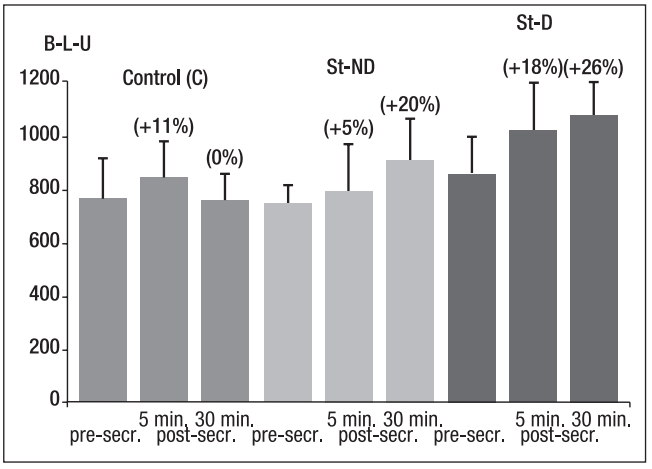


Figure 6. *Blood lipase.*



Discussion

We managed, as a preliminary step, a simple non-invasive test that could provide an accurate assessment of the exocrine and endocrine pancreas and of their interactions (insulo-pancreon-axis), to analyze in rats the probable modifications induced by a chemical evoked diabetes, both in blood and bile-pancreatic secretion (BPS). To accomplish the above purpose, the streptozotocin method was chosen. As with alloxan, the latter beta cell toxin trigger the DNA strand breaks. The damaged DNA results in the depression of islet functions, including insulin synthesis.[11,14,17,39,42-44]

In the analysis of diabetic patients, the literature reveals that in this metabolic disease the exocrine pancreatic secretion response to secretin is impaired. This

has also been our experience with the secretin test, both in type 1 and type 2 diabetes.[2,16] Undoubtedly insulin influences the exocrine pancreas. It stimulates acinar protein synthesis and potentiates, in rats, CCK or secretin-stimulated exocrine pancreatic secretion.[37,38] In diabetic rat pancreata, the depressed pancreatic secretion stimulated by secretin or CCK is reversed by insulin treatment.[36,37] Chey et al have shown, in conscious rats, that the administration of anti-insulin serum resulted in depression of post-prandial exocrine pancreatic secretion, including volume, bicarbonate and amylase.[9] Furthermore, the anti-insulin serum completely blocked the secretin and CCK-stimulated pancreatic secretion and even the basal values were lower, suggesting that insulin may exert a tonic effect on basal pancreatic secretion in rats.

According to the group of Chey,[9] secretin and CCK, in physiologic doses, can exert their action on the exocrine pancreas only in the presence of insulin in the circulation.[9] Thus endogenous insulin plays an important role in the physiology of the exocrine pancreas, including acini, ductular and duct cells.

In tests like the present with streptozotocin, besides insulinopenia, plasma or serum levels and tissue contents of other islet hormones (SS, Gl, PP) have been reported to be increased. These latter peptides are known to inhibit exocrine pancreatic secretion stimulated by secretin and/or CCK, or a mixed meal.[25,26] Then, it is quite possible that SS, Gl, PP and other islet peptides, like galanin (Gal) and PYY, play a significant inhibitory role on the pancreatic secretion in the present streptozotocin-induced diabetic rats. We are also tempted to consider as very probable that this mechanism is also operative in the streptozotocin-injected rats in which this chemical agent failed to elicit a significant increase of the blood glucose level (St-ND series).

The changes observed in the present series are the consequence of the streptozotocin-induced modifications on the insulo-pancreon-axis. Duan et al [12,13] have found that insulin depresses the incorporation of cystine into lipase and colipase in rats while, in contrast, induces an opposite influence on amylase synthesis.[12,13] The inhibition of insulin on lipase synthesis results from a pre-translational regulation while the enhancement of amylase results from a stimulation of translation.

Also, following stimulation of endogenous release of insulin by glucose or glibenclamide, an increase of amylase and a decrease of lipase secretion is observed. In the insulin-deficient rats both lipase and colipase are significantly increased, with a corresponding augmentation of lipase mRNA.[12,39-41]

The blood exocrine pancreatic enzymes changes following secretin injection reflect the interactions that develop within the insulo-pancreon-axis. Thus, the tendency to raised post-secretin lipase values both in the St-ND and St-D animals results from the diminished restraint influences normally exerted by the islets insulin content on the synthesis and secretion of this enzyme. In contrast the tendency to a fall of the post-secretin amylasemia level in the same group of rats reflect the drop of insulin secretion.

As to the significant hyperglycemic effect of Boots secretin in the three series of animals (C, St-ND and D), it is noteworthy that the percentage change between the pre- and post-secretin values (5 and 30 min) was of approximately the same degree in the three group of animals (Figure 4). This finding was unexpected. Indeed, being secretin a hormone closely related to GIP, which is recognized as the main physiologic incretin, it would have been expected either no effect or an opposite response with the secretin test.[42] However, it should be mentioned that the incretin action of secretin is still controversial. From what is known at present, it seems that secretin is a releaser of insulin only when administered at pharmacological doses.[42]

We speculate that the secretin-evoked blood glucose changes of the present tests are explained by the fact that Boots secretin is an impure preparation due to contamination with small amounts of CCK, a peptide that is recognized as having an islet-alfa-cell-tropism.[42-44] Nevertheless, it should be emphasized that in recent and still unpublished tests performed with Sigma secretin, closely similar to the present ones, a preparation also contaminated with CCK but injected intravenously, we found consistently that the post i.v glycemia values are lower than those of the basal sample in the C animals, in contrast to the St-ND and D rats. Undoubtedly, all seems to point out that the hormone route of administration, i.p or i.v, plays a crucial role.

Regarding the pancreon hydrelatic response (water and bicarbonate), the fall of bicarbonate output both basally and post-secretin is noteworthy not only in the St-D series but also in those animals of the St-ND group.[1,2,23-26] This fact might reflect a lessened potentiating effect of insulin on the pancreon secretin-induced secretory effects in the St-ND animals. This as the result of a direct toxic effect of streptozotocin on the centroacinar-ductal segment of the "pancreon" units, as it has been described by our group with alloxan in dogs,[45,46] either as a consequence of the modification in the intraislets peptides (SS, PP, PYY)[2,43-48] or of its autonomic innervation.

It is evident that even in those animals with normal blood glucose level (St-ND) the impaired bicarbonate secretion hints to the existence of either some otherwise unsuspected Langerhans islet change and/or the impairment of the pancreon units themselves.

The rise of the pancreatic wet weight index in the streptozotocin-evoked diabetic rats is primarily a reflection of a diabetes-induced loss of the carcass mass relative to that of the pancreatic gland which keeps its total wet weight closely similar to that of the C rats.

Taking into account what is known from autopsy studies in insulin-dependent diabetic patients one might have predicted a decrease of the total wet weight of the pancreas, namely of its body-tail segment. In this sense it should be recalled that Gepts has postulated a trophic influence of PP on the pancreon units.[18] The predominant pancreatic cephalic location of this peptide might explain the sparing of this segment of the gland in the diabetes-elicited hypotrophic process observed in the autopsy of long-term diabetic patients.

Related to the forementioned speculation is the fact that in streptozotocin-induced diabetic animals there is a suggestive hyperplasia of PP cells in the pancreatic gland.[39] Although not particularly evaluated in the present experiments, it is more than probable that this peptide has had a significant influence on the mechanisms impinging upon the insulo-pancreon-axis interactions, primary those with secretin, CCK and the intrapancreatic cholinergic tone.[39-44,47-52]

Prompted by the present experimental findings, we have considered an appropiate approach to try to obtain in certain clinical conditions an encompassing view of insulo–pancreon-axis interactions resorting to our proposed amplified 2-hour oral glucose tolerance test, in which we analyze the 2-hour cumulative values of glucose, amylase, pancreatic isoamylase, lipase, insuline, and several indexes such as insulin/glucose, insulin/isoamylase, and insulin/lipase.

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