

# Intravital $\text{Ca}^{2+}$ imaging of pancreatic $\beta$ cell function after bariatric surgery

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## ABSTRACT

Bariatric surgery has long become an appropriate and common treatment for patients with severe obesity and many related conditions such as type 2 diabetes. However, the exact mechanism leading to improved metabolism shortly after surgery, most notably independent of weight loss, has not yet been fully elucidated. Akalestou's recent publication, "Intravital imaging of islet  $\text{Ca}^{2+}$  dynamics reveals enhanced  $\beta$  cell connectivity after bariatric surgery in mice," provides the first detailed insight into the progression of pancreatic islet function after bariatric surgery. By transplanting pancreatic islets equipped with a genetically encoded calcium indicator into the anterior chamber of the eye, improvements in  $\text{Ca}^{2+}$  dynamics and a more potent  $\beta$  cell network were observed over an extended time course after the surgical procedure. In the following sections, we will take the opportunity to briefly outline the association between bariatric surgery and diabetes, highlight the issue of anesthesia during intravital  $\text{Ca}^{2+}$  imaging, and finally comment on some biological relationships related to  $\beta$  cell function.

## 1. Bariatric surgery and type 2 diabetes

Over the past decade, vertical sleeve gastrectomy (VSG) has emerged as the most common procedure for surgical treatment of severe obesity and type 2 diabetes, along with the second most common procedure, Roux-en-Y gastric bypass (RYGB), both of which are used when dietary change and drug treatment are ineffective [1]. Whereas for VSG, a tubular stomach is formed by partial gastrectomy of the greater curvature side, for RYGB, a small gastric pouch is created from the proximal portion of the stomach and connected to the distal end of the small intestine [2]. Bariatric surgery is primarily intended to provide long-term weight loss, which still must be supported by lifestyle changes. Obesity itself is one of the greatest risk factors for the development of type 2 diabetes [3], with the combination of progressive decline in insulin production capacity and increasing insulin resistance resulting in the body's inability to maintain euglycemia [4]. Unfortunately, many anti-diabetes medications such as sulfonylureas, meglitinides, insulin, and thiazolidinediones are associated with weight gain [5], and even weight-negative anti-diabetes treatment options such as glucagon-like peptide-1 receptor agonists (GLP-1) and sodium-glucose transporter-2

inhibitors are often insufficient to treat severe obesity [6,7]. Therefore, it seems rational to break the vicious circle between obesity and type 2 diabetes through a surgical intervention that forces weight loss primarily through caloric restriction and malabsorption. However, over time, it has been recognized that bariatric surgery can ameliorate both obesity and its comorbidities, such as type 2 diabetes, through pleiotropic effects on intestinal physiology, incretin hormone secretion, bile acid metabolism, lipid regulation, microbiome alterations, neuronal signaling, and glucose homeostasis [8–11]. Bariatric surgery has therefore been classified as "metabolic surgery" [2,12]. Although there was no consensus on the definition of diabetes remission in early studies, analysis of data shows that one of the most important metrics after bariatric surgery is the rate of improvement in patients' diabetes [1]. According to clinical studies, in 33–90% of patients undergoing bariatric surgery, diabetes has regressed one year after treatment, compared to 0–39% in patients treated with medication therapy [1]. The fact that improvements in glycemic control in type 2 diabetes patients take place over the course of the first few days after surgery, even before substantial weight loss is achieved, suggests that additional mechanisms contribute to the metabolic amelioration [13]. However, the

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physiological pathways that cause this effect are only partially characterized. It has been shown that the function of  $\beta$  cells and the associated insulin secretion directly affect the improvement of blood glucose levels after bariatric surgery by increasing glucose sensitivity of  $\beta$  cells [14]. A key factor for short-term and long-term pleiotropic effects might be the increased release of glucagon-like peptide 1 (GLP-1) after surgery [15]. Rapid and early delivery of nutrients to the distal small intestine amplifies the secretion of distal intestinal peptides, primarily glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) [16]. The hormone GLP-1 stimulates the  $\beta$  cells to produce insulin, increases  $\beta$  cell mass and glucose-stimulated insulin secretion [17], blocks glucagon release from pancreatic  $\alpha$  cells via somatostatin [18], slows down gastric emptying [19], suppresses appetite by affecting hypothalamus and amygdala [20], improves peripheral glucose tolerance [21], and exerts a protective effect against glucolipotoxicity [22]. Interestingly, Akalestou's study also establishes a link between increased GLP-1 release and enhanced  $\beta$  cell network, which may serve as an explanation for the increased glucose-stimulated insulin secretion and improved glucose homeostasis [23]. Especially because we are dealing with a multifactorial process, the study of  $\text{Ca}^{2+}$  activity as an indicator of pancreatic islet cell function seems to be an important element in assessing the overall picture that attempts to explain the remission of diabetes and the recovery of islet cell function after bariatric surgery.

## 2. Isoflurane anesthesia during intravital $\text{Ca}^{2+}$ imaging

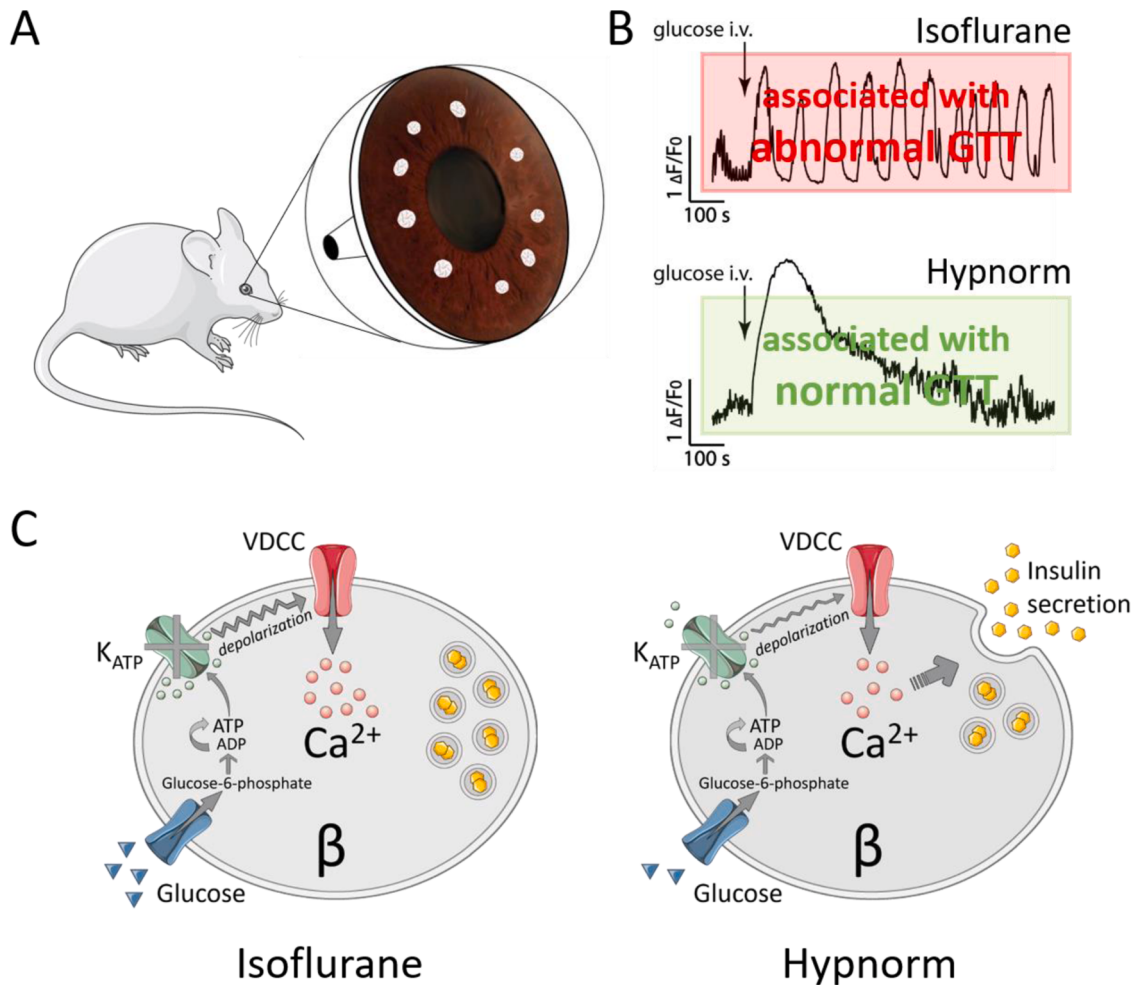
In their *in vivo* study, Akalestou and colleagues visualize for the first time that diabetes remission after vertical sleeve gastrectomy can be directly associated with increased pancreatic  $\beta$  cell function and improved intra-islet connectivity [23]. The use of GCaMP-positive sensor islets transplanted into the anterior chamber of the eye provides an excellent opportunity to monitor the change in  $\text{Ca}^{2+}$  dynamics in pancreatic  $\beta$  cells after bariatric surgery. The experimental design of the study and the dietary animal model are ideally chosen to reveal the physiological relationships between surgical intervention and pancreatic islet cell function. In particular, the characterization of  $\text{Ca}^{2+}$  waves in pancreatic islets by spinning disk confocal microscopy is a compelling technical approach, even though the full implications of the 3D structure of the  $\beta$  cell network is not considered. We appreciate that the authors have pointed out potential limitations of their study. In this context, the issue of isoflurane anesthesia seems worthy of comment Figure 1. In our intravital microscopy study, we demonstrated that when isoflurane is used as a volatile anesthetic in animal experiments, intracellular  $\text{Ca}^{2+}$  activity in  $\beta$  cells appears to be uncoupled from insulin secretion [24]. In mice anesthetized with isoflurane, insulin secretion was completely disrupted, resulting in persistently elevated blood glucose levels.  $\text{Ca}^{2+}$  imaging under the same anesthetic conditions showed uniformly oscillating intracellular  $\text{Ca}^{2+}$  concentrations in pancreatic  $\beta$  cells. Since the characterization of  $\text{Ca}^{2+}$  dynamics is intended to represent the secretory activity of pancreatic  $\beta$  cells, the correlation under these conditions no longer reflects the physiological state. We circumvented this problem by using fentanyl/fluanisone (trade name Hypnorm) as an anesthetic during intravital microscopy and showed that glucose homeostasis was equivalent to that of non-anesthetized animals.  $\text{Ca}^{2+}$  dynamics recorded under Hypnorm anesthesia was characterized by a sharp increase in intracellular  $\text{Ca}^{2+}$  concentration after glucose stimulation, which was then followed by a steady oscillatory decrease, resembling the profile of biphasic insulin release [25]. In the Akalestou et al. study, it should be noted that the examination of improved glucose homeostasis and diabetes remission was performed without isoflurane, whereas the characterization of  $\beta$  cell activity and intra-islet connectivity was performed under the influence of this anesthetic. Hence, the two data sets may not be fully comparable reflecting the true physiological situation.

It is well known that volatile anesthetics such as isoflurane impair insulin secretion and glucose homeostasis [26], which is also the case in humans [27]. To our knowledge, the effect of isoflurane on  $\text{Ca}^{2+}$

handling in the pancreatic  $\beta$  cell has only been documented in our study [24]. The exact molecular mechanisms whereby isoflurane affects  $\text{Ca}^{2+}$  activity in the pancreatic  $\beta$  cell have therefore not been elucidated, although in other tissues it has been reported to engage intracellular  $\text{Ca}^{2+}$  mobilization [28,29]. Studies by Tanaka and colleagues have attempted to provide an explanation for the isoflurane-dependent interruption of insulin secretion. Isoflurane has been shown to decrease the ATP sensitivity of pancreatic  $\text{K}_{\text{ATP}}$  channels, preventing depolarization of the plasma membrane at high glucose concentrations [30]. Since  $\text{K}_{\text{ATP}}$  channel closure couples glucose sensing to the opening of voltage-dependent  $\text{Ca}^{2+}$  channels by depolarization, intracellular  $\text{Ca}^{2+}$  dynamics were hypothesized to be the distorted link between impaired  $\text{K}_{\text{ATP}}$  channel regulation and impaired glucose-stimulated insulin release [31]. Our *in vivo* data contradict this theory by showing that glucose-stimulated  $\text{Ca}^{2+}$  activity and elevation in  $\beta$  cells can be unambiguously measured under the influence of isoflurane, even though the  $\text{Ca}^{2+}$  profile no longer corresponds to the biphasic insulin response to glucose because of impaired insulin secretion and constantly elevated blood glucose levels [24]. The fact that  $\text{Ca}^{2+}$  can be stimulated while insulin secretion is blocked suggests that isoflurane more directly disrupts late steps in the exocytotic pathway in the  $\beta$  cell. As there are no studies on this particular topic, we speculate that the administration of lipophilic anesthetics such as isoflurane may lead to disorganization of the lipid bilayer, resulting in disruption of the vesicle docking mechanism in  $\beta$  cells. This is supported by previous studies showing that isoflurane can affect the compactness and lateral organization of lipid bilayers on the one hand [32], and that lipid-induced dissociation of  $\text{Ca}^{2+}$  channels from secretory vesicles can prevent insulin secretion on the other [33]. Under normal conditions, voltage-dependent  $\text{Ca}^{2+}$  channels are organized into separate membrane microdomains in which brief membrane depolarizations provoke high levels of  $\text{Ca}^{2+}$  influx and subsequent exocytosis of closely associated insulin granules [33]. Although isoflurane appears to interfere only with the late steps of the stimulus-secretion coupling and not the  $\text{Ca}^{2+}$  response to the stimulus, there is an isoflurane effect that might affect the overall  $\text{Ca}^{2+}$  dynamics of the electrically coupled  $\beta$  cell network. Studies on connexin-36, the major gap junction protein of the  $\beta$  cell network, showed that isoflurane can bind to specific docking sites of connexin-36 and has a stimulatory effect on the gap junction conductance [34]. This may result in biased measurements of intra-islet connectivity. Together with the interrupted insulin secretion and the disturbed glucose homeostasis in the experimental animals during intravital microscopy, this suggests that we are not dealing with a physiological state of  $\beta$  cell function under isoflurane anesthesia. Hence, the perfect model for intravital microscopy should dispense with anesthesia altogether in order to visualize cell function and survival without affecting the overall physiology of the organism.

## 3. Effect of incretin hormones on the $\beta$ cell network

It would be interesting to further elucidate mechanistically the increased intra-islet connectivity after bariatric surgery, which was characterized by “superwaves” depending on electrical coupling of the  $\beta$  cell network. Since incretins were earlier found to play a role in maintaining a highly coordinated subnetwork of  $\beta$  cells, synchronized islet activity, and normoglycemia in mice exposed to a high-fat diet [35], a correlation is plausible and was also experimentally demonstrated in the study by Akalestou and colleagues [23]. Nevertheless, simply reducing intake of the high-fat diet after surgery could have influenced diabetes remission and thus restored normal islet function. Detailed recordings of caloric intake would provide clarity in this case. The actual influence of GLP-1 could be further highlighted by considering the GLP-1-induced secondary messenger cAMP pathways and their intracellular effects via activation of protein kinase A (PKA) or guanine nucleotide exchange protein 2A (Epac2A) [36]. PKA and Epac2A are implicated in  $\beta$  cell-to-cell coupling by mediating the cAMP regulation of connexin-36 (Cx36), the most prominent gap junction protein in  $\beta$  cells [37]. It has



**Fig. 1.** Effect of isoflurane on pancreatic  $\beta$  cell function [A] Illustration of pancreatic islets transplanted to the iris of the anterior chamber of the mouse eye. [B] Exemplary intracellular  $\text{Ca}^{2+}$  dynamics of eye-transplanted pancreatic islets using either isoflurane or Hypnorm (fentanyl/fluanisone) to anesthetize the recipient animals. Arrows indicate the timing of intravenous injection of glucose solution into the tail vein to stimulate  $\beta$  cell secretory activity. Under isoflurane anesthesia, blood glucose levels are permanently elevated while blood insulin levels remain unchanged low. Under Hypnorm anesthesia, the glucose tolerance test (GTT) corresponds to that of control animals without anesthesia. [C] Model of isoflurane influence on stimulus-secretion coupling in  $\beta$  cells based on intravital microscopy study [24]. Under Hypnorm anesthesia, glucose uptake leads to an increase in ATP concentration in the  $\beta$  cell, which closes the ATP-sensitive  $\text{K}_{\text{ATP}}$  channels, whereupon membrane depolarization opens the voltage-dependent  $\text{Ca}^{2+}$  channels (VDCC) and the increased  $\text{Ca}^{2+}$  concentration triggers vesicle exocytosis and thereby insulin secretion. Under isoflurane anesthesia, higher blood glucose levels and the subsequent increased uptake of glucose by the  $\beta$  cell may lead to enhanced depolarization of the cell membrane. Although increased  $\text{Ca}^{2+}$  influx leads to higher intracellular  $\text{Ca}^{2+}$  concentrations, insulin secretion is no longer triggered by  $\text{Ca}^{2+}$  and appears to be blocked, which in turn leads to increased blood glucose levels.

been suggested that PKA regulates Cx36 coupling via fast mechanisms such as Cx36 phosphorylation and channel gating, whereas Epac2A regulates Cx36 coupling via slower mechanisms such as trafficking, assembly, or turnover of Cx36 channels [37]. This incretin-dependent association between realignment of gap junction proteins and improved  $\text{Ca}^{2+}$  dynamics was also evidenced by the activation of cAMP signaling through forskolin, which not only increases  $\beta$  cell activity but also improves synchronicity and the coordination of intercellular signals [38]. Increase in cytosolic cAMP during stimulation of pancreatic islets with glucose leads to enhanced synchronization, which is reflected by a higher average correlation coefficient and multicellular dynamics dominated by global and well-aligned  $\text{Ca}^{2+}$  waves [38]. Deviating from this,  $\text{Ca}^{2+}$  activity patterns are more erratic after stimulation with glucose alone [38]. As mentioned before, a demonstration of the correlation between elevated GLP-1 levels and increased intra-islet connectivity, for example by examining the patterning of gap junction proteins and the cAMP signaling pathway, would be insightful to properly assess the direct influence of incretin hormones on the  $\beta$  cell network in terms of bariatric surgery.

#### 4. Long-term effects of increased stimulation by incretin hormones

As a final consideration, we would like to point out potential long-term effects caused by incretin hormones. Although incretin mimetic therapy is suitable for the therapeutic treatment of type 2 diabetes, medical studies inconsistently report about potential side effects such as acute pancreatitis [39,40]. Studies in human islets transplanted into the eye of mice have shown that chronic activation by sustained daily liraglutide treatment is associated with an initial improvement in function that progressively worsens over time [41]. Under diabetic conditions, excessive activation with incretin mimetics may lead to exhaustion of already metabolically stressed  $\beta$  cells and ultimately impaired glucose homeostasis [41]. Hence, if GLP-1 is to be responsible for the sustained enhancement of intracellular  $\text{Ca}^{2+}$  dynamics and intra-islet connectivity after bariatric surgery, the question arises whether a similar overload of  $\beta$  cell activity may occur in the long term after this clinical procedure.

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## 6.

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