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Physiological Studies of Native and Stem Cell-Derived Islets

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Abstract

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In type 1 and type 2 diabetes, the β -cells of the islets of Langerhans are either destroyed by the immune system or stressed due to peripheral insulin resistance. To improve the life of patients with these diseases, new treatments are needed. This thesis examined the role of irisin and cocaine and amphetamine regulated transcript (CART) in islets of Langerhans and their potential pharmaceutical role in type 2 diabetes. Furthermore, β -cell replacement with stem cell-derived islets of Langerhans (SC-islets) for type 1 diabetes was evaluated for optimal implantation site.

In **paper I**, the physiological role of CART in rat islets was examined. CART was shown to specifically lower islet blood flow, which could be a protective effect in type 2 diabetes. No effect from CART on glucose tolerance or insulin release was seen in rat islets, which highlights species differences.

In **paper II**, the expression of irisin and its effect on hormone secretion and pancreatic blood flow was examined. Irisin was expressed in human islets and was secreted glucose dependently. It also lowered islet blood flow but did not affect glucose-stimulated insulin secretion in isolated human or rat islets. Thus, local secretion of irisin could serve as a protective function by lowering islet blood flow in a high glucose state.

In **paper III**, the expression of irisin in SC-islets and its potential beneficial effects in transplantation was examined. SC-islets were found to express higher levels of irisin than human islets. Irisin treatment had no effects on viability and proliferation in SC-islets, in contrast to previous studies in other species. Thus, irisin signaling likely differs between SC-islets and murine and native human islets.

In **paper IV**, SC-islets were transplanted to multiple sites in mice to find the optimal implantation site in terms of graft maturity, function and composition. The liver proved to be the most favorable site due to its higher expression of islet maturity genes and a higher β -cell function and fraction. This poses a dilemma, as the liver site is the most challenging to biopsy and monitor for safety.

In summary, this thesis uncovered new physiological functions of irisin and CART, potentially offering insights relevant to the treatment of type 2 diabetes. Meanwhile, the role of irisin in transplantation of SC-islets seems limited.

Keywords: Irisin, CART, Islet blood flow, Islets of Langerhans, Stem cell-derived islets

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Till glädjens värn och ära

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I. Drott, C.J., **Norman, D.**, Espes, D. (2021) CART decreases islet blood flow, but has no effect on total pancreatic blood flow and glucose tolerance in anesthetized rats. *Peptides*, 135:170431.
- II. **Norman, D.**, Drott, C.J., Carlsson, P-O., Espes, D. (2022) Irisin – A Pancreatic Islet Hormone. *Biomedicines*, 10(2):258.
- III. **Norman, D.**, Cen, J., Vasylovska, S., Carlsson, P-O., Lau, J. Stem Cell-Derived Islets Express Higher Levels of Irisin/*FNDC5* and Respond Differently to Irisin Treatment than Human and Murine Islets. *Manuscript*.
- IV. **Norman, D.**, Thorngren, J., Vasylovska, S., Carlsson, P-O., Lau, J. Function and Composition of Stem Cell-Derived Islets Transplanted to Multiple Organ Sites. *Manuscript*.

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Abbreviations

T2D	Type 2 diabetes
PP	Pancreatic polypeptide
γ -cell	Gamma cell
ϵ -cell	Epsilon cell
GSIS	Glucose-stimulated insulin secretion
CART	Cocaine and amphetamine regulated transcript
ET-1	Endothelin-1
ETA	Endothelin receptor type A
WAT	White adipose tissue
UCP1	Uncoupling protein 1
FNDC5	Fibronectin type III domain containing protein 5
OGTT	Oral glucose tolerance test
IBMIR	Instant blood-mediated inflammatory reaction
TNF- α	Tumor necrosis factor-alpha
IL-1 β	Interleukin-1 beta
IFN- γ	Interferon-gamma
hESCs	Human embryonic stem cells
iPSCs	Induced pluripotent stem cells
IBF	Islet blood flow
PBF	Pancreatic blood flow
SC	Stem cell
ELISA	Enzyme-linked immunosorbent assay
IVGTT	Intravenous glucose tolerance test
KRBH	Krebs-Ringer bicarbonate buffer
qPCR	Quantitative polymerase Chain reaction
GLP-1	Glucagon-like peptide 1
FDR	False discovery rate

Introduction

Global Perspectives on Diabetes

According to the International Diabetes Foundation Diabetes Atlas, 463 million adults live with diabetes [1]. That is three times as many compared to twenty years ago, and the year 2045, estimates say the number will reach 700 million. This upsurge is not only in type 2 diabetes (T2D) due to changed lifestyle, but also in type 1 diabetes. Considering the major individual health hazards due to diabetes and 10% of global health expenditure being spent on diabetes, advances in the field are necessary. A key to finding new treatments for diseases, and thereby increasing individual health and lowering healthcare costs, is understanding the normal physiology. By investigating hormones and their role in the pancreas, new pharmacological possibilities can be found.

Islets of Langerhans

The islets of Langerhans are small endocrine organs, consisting of multiple hormone-producing cell types, arranged closely to their vascular supply. The cells of the islet are the insulin-producing β -cell, the glucagon-producing α -cell, the somatostatin-producing δ -cell, the pancreatic polypeptide-producing PP-cell, and the ghrelin-producing ϵ -cell. The number of islets in the pancreas varies from 3.6 to 14.8 million, constituting around 2% of the total pancreas volume [2]. There are species differences both regarding cytoarchitecture and function, meaning that findings are not always translatable between species [2, 3].

Type 1 Diabetes

Type 1 diabetes stems from an autoimmune destruction of β -cells, rendering the body unable to produce insulin. Due to this, the glucose metabolism becomes dysregulated, leading to high blood glucose levels followed by polyuria, polydipsia and weight loss. If untreated, it leads to ketoacidosis and coma [4, 5]. The current standard treatment for type 1 diabetes is multiple daily injections of insulin and monitoring blood glucose, either manually by capillary sampling or with a continuous glucose monitor [6].

Type 2 Diabetes

T2D is characterized by increased blood glucose levels due to peripheral insulin resistance and metabolically stressed β -cells not being able to compensate. The disease is tightly associated with the metabolic syndrome, with obesity being the strongest risk factor. Furthermore, many complications arise due to T2D, such as atherosclerosis and kidney and nerve damage. The main drivers in the global rise of T2D are obesity, sedentary lifestyles and high caloric diets [7].

Cocaine and Amphetamine Regulated Transcript

Discovery

In the year 1995, Douglass et al. discovered an unknown mRNA-transcript, upregulated fourfold in the striatum of the brain after administration of the psychomotor stimulants cocaine and amphetamine [8]. They later characterized the gene from which this mRNA was transcribed and named it cocaine and amphetamine regulated transcript (CART) [9]. A fragment of the peptide translated from this mRNA had previously been identified by Spiess et al. [10]. Due to the discovery of CART in the brain in relation to psychostimulants, the initial focus became its role in drug addiction.

The Peptide CART

Splicing of the CART-gene results in two mRNA forms. One translates the short propeptide, proCART 1–89, and one the long, proCART 1–102, with the numbers stating amino acid length. In humans, only the short form is present, while rat has both, but the active amino acids are the same in both forms. Further processing by prohormone convertases results in the peptides CART 42–89 and CART 49–89 in human. In rat, these are also produced, as well as CART 55–102 and CART 62–102, stemming from the long transcription form [11].

CART and the Pancreas

Some years after its discovery, findings regarding CART regulating food intake and body weight were made [12]. Therefore, the field expanded in the metabolic direction, which also led to an interest in CART and the pancreas. Already 1999, CART was shown to be expressed in rat islets of Langerhans – primarily in somatostatin containing δ -cells [13]. Since then, it has been shown that also a subpopulation of β -cells in rat islets contains CART, as well as pancreatic neurons [14]. In humans, CART has been localized to both α -

and β -cells in islets [15]. Furthermore, CART has been shown to be upregulated by glucose in β -cells of human, rat and mouse islets in T2D [14, 15]. A specific mutation in the CART gene has been associated with familial early onset obesity [16]. Taken together, this indicates a role for CART in the pathophysiology of T2D.

In light of this, the effect of CART on glucose-stimulated insulin secretion (GSIS) has been studied, with contradictory results between species. In human islets from healthy and T2D donors, GSIS was increased by CART administration [15]. In mice, GSIS was increased by CART while CART knock out mice had impaired GSIS [17]. In rat, GSIS was unaltered or lowered by CART alone, but raised in the presence of the cAMP elevating agent forskolin [14, 18]. Despite differences between species, CART mainly seems to have an augmentative effect on GSIS, especially in T2D, considering CART being upregulated in β -cells in this condition.

CART and Blood Flow

In the islets of Langerhans, neurons containing CART are found. In those neurons, CART colocalizes with vasodilating vasoactive intestinal peptide (VIP) in mouse, rat and pig [17, 19, 20]. CART is also highly involved in the stress response of the hypothalamic-pituitary-adrenal axis and after hypotensive stress, CART is released into the pituitary portal circulation [11]. CART has previously been shown to mediate vasoconstriction in cerebral circulation and a suggested mediator has been the endothelin system, specifically endothelin-1 (ET-1) acting on the endothelin receptor type A (ETA). ET-1 is a vasoconstrictive peptide derived from endothelium, which acts on the smooth muscle receptor ETA and the endothelial receptor type B [21]. The vasoconstrictive effect via ETA is also present in both the exo- and endocrine pancreas [22]. To study the endothelin system, specific inhibitors are used, e.g., BQ-123, inhibiting the receptor ETA.

Taken together, the upregulation of CART in T2D and its effect on endocrine function along with blood flow, suggests that further research could lead to new pharmacological treatments of T2D.

Irisin

Discovery

In 2012, a new myokine was found to convert white adipose tissue (WAT) into brown-like, increasing thermogenesis and energy expenditure through uncoupling protein 1 (UCP1). It was named irisin and showed to reduce body

weight in obese mice. Furthermore, exercise increased irisin plasma levels and its expression in muscle [23]. This led to an interest in irisin as a treatment for obesity.

Production and Secretion

Irisin is derived from cleavage of the membrane protein fibronectin type III domain containing protein 5 (FNDC5), resulting in a 112 amino acids long hormone [23]. The tissue with the most FNDC5 expression is muscle, but numerous tissues express it in lower amounts [24]. Plasma irisin levels are believed to mostly come from skeletal muscle and in part from adipose tissue [25]. Controversial results have been obtained on plasma levels of irisin being increased during or directly after exercise. A meta-analysis from 2018 concluded that an increase from exercise is seen [26]. The mechanisms of secretion are still to be discovered. However, in mice, a mechanism connecting irisin signaling and exercise has been found. The extracellular heat shock protein 90 α is elevated from muscle at exercise but also activates integrin α V β 5, the proposed receptor for irisin [27], allowing high affinity binding of irisin [28].

Irisin Receptors

So far, irisin signaling through integrin α V β 5 has been shown in multiple tissues, e.g., osteocytes, adipocytes and enterocytes [27, 29-33]. There are no published papers on irisin signaling through α V β 5 in islets, however it is expressed in human islets [34, 35]. Interestingly, in a recent conference abstract by Marrano et al., it was proposed that irisin does not signal through α V β 5 in INS-1E cells but instead signals through being endocytosed [36]. To study irisin signaling through α V β 5, the compound cilengitide is used, blocking α V β 5 but also α V β 3 [31-33].

Controversies

Since the discovery of irisin, there have been several controversies regarding its relevance. Factors contributing to this has especially been the lack of high-quality antibodies, questioning the reliability of ELISAs and measured plasma levels [37]. Another concern has been multiple bands on western blots with FNDC5 antibodies [38]. This has been addressed and was a result of heavy glycosylation of irisin, with the bands corresponding to the molecular weights of glycosylated and unglycosylated irisin [39]. Some concerns regarding different obtained molecular weights still exist [37]. In addition, due to questioning of the existence of circulating irisin, one group used mass spectrophotometry to demonstrate circulating irisin, thus not solving the antibody issue but supporting the existence of circulating irisin [40]. The same study met the

concerns regarding an atypical start codon of the FNDC5 gene [41], i.e., non-AUG start codon, by showing that irisin is mainly expressed from this unusual start codon. This is supported by the fact that many genes resulting in key cellular proteins are non-AUG [42]. Furthermore, considering that a proposed mechanism for signaling through the irisin-receptor $\alpha V\beta 5$ exists and that numerous effects from irisin are seen, even at picomolar concentrations [27], the relevance of further research on irisin persists.

Irisin in Glucose Homeostasis

Due to the metabolic involvement of irisin, its role in glucose homeostasis has also been studied. In organs involved in glucose homeostasis, such as the pancreas and liver, the expression is low compared to muscle rich tissues [24]. Nevertheless, irisin seems to be involved in glucose homeostasis.

In WAT, irisin results in lowered lipid accumulation and increased lipolysis. It also carries out browning of WAT, inducing expression of UCP1. However, only selected depots of WAT is affected by irisin, while brown adipose tissue (BAT) is unaffected [43]. The relevance of irisin affecting WAT in human is unclear since contradictory results have been published [25]. The potential pharmacological effect of irisin on obesity is therefore to be determined.

In skeletal muscle, irisin seems to increase glucose and lipid uptake by upregulation of transport genes, while downregulating genes of glycogenolysis and gluconeogenesis [44]. This indicates a function of irisin in muscle during high glucose levels, facilitating glucose use, while stopping usage of glycogen reserves and unnecessary production of more glucose.

In the liver, similar effects as in skeletal muscle is seen. Primary human hepatocytes treated with irisin had downregulated genes of gluconeogenesis and lipogenesis [45]. In the same study, overexpression of irisin in diabetic mice showed improved glucose tolerance and suppressed hepatic steatosis. Another study reproduced this result, showing a lowered gluconeogenesis and increased glycogenesis, improving glucose homeostasis [46]. This, together with the results from skeletal muscle, indicates a role for irisin in glucose abundant states.

In the pancreas, irisin is present in islets of Langerhans and exocrine tissue [47]. It has been shown to increase β -cell proliferation, besides protecting β -cells from glucose induced apoptosis, by modulating the expression of apoptosis proteins in INS-1 cells. These cells also obtained an augmented insulin secretion from irisin after 24h incubation in high glucose. In the same study, diabetic rats treated with irisin had reduced body weight, reduced levels of insulin and fasting glucose, and an improved oral glucose tolerance test

(OGTT) [48]. The antiapoptotic and proliferating properties of irisin on β -cells have been reproduced, with lowered apoptotic rate after treatment with the saturated fatty acid palmitate [49]. Increased GSIS and total insulin content was also observed in human and murine islets [49]. Another study, however at present published as an abstract, showed improved glycemia, GSIS and glucose tolerance in a mouse model for T2D [50]. Glucose tolerance was also improved in mice after 14 days of daily irisin injections [49]. Another study showed improved GSIS in presence of irisin during a 48h incubation with high glucose and palmitic acid [51]. In summary, an improvement of GSIS or glucose tolerance by irisin has mainly been reported during high glucose and palmitic acid levels.

In type 1 diabetes, irisin plasma levels are elevated [52, 53], while meta-analyses have shown lowered levels in T2D [54, 55]. Interestingly, in obese individuals without T2D, levels are instead increased [25].

Irisin and Blood Flow

A previous study suggested that irisin may regulate blood pressure in rats. Effects on blood pressure was seen after both peripheral and central administration, the latter in the 3rd ventricle of the brain [56]. In mice, irisin has been shown to relax mesenteric arteries [57]. Furthermore, irisin has shown to improve endothelial function in the aorta in obese mice [58].

Irisin has also been shown to improve angiogenesis (see below). Interestingly, the irisin receptor $\alpha V\beta 5$ is involved in angiogenesis, potentially being a link between irisin and angiogenesis [59].

Based on the results showing that irisin protects stressed β -cells and improves GSIS, the interest for irisin in the treatment of T2D is apparent, particularly since irisin levels are lowered in this condition. Finding the exact mechanisms and receptors involved, could guide interesting pharmacological treatments.

Irisin and Transplantation

In light of the anti-apoptotic and proliferative effects seen, irisin could be interesting as an adjuvant therapy in transplantation. In fact, irisin has been over-expressed or used as pretreatment for transplanted bone marrow mesenchymal stem cells in two studies, showing improved survival [60, 61]. Also, pretreatment of cardiac progenitor cells with irisin before transplantation to ischemic myocardium promoted cardiac regeneration and function [62]. Other general effects seen from irisin that could be beneficial in transplantation are reduced ischemic damage [63-65], increased angiogenesis [66-68] and reduced

oxidative stress [69, 70]. Thus, investigating if irisin can improve survival and engraftment in islet transplantation is of interest.

Transplantation of Islets

Islet transplantation is a therapy aiming to replace exogenous insulin with donated β -cells. This therapy has progressed from the first sort of islet transplantation in 1893, where a sheep's pancreas was transplanted to a boy with ketoacidosis [71], to the present possibility to infuse isolated human donor islets into the portal vein of patients [72]. The first successful isolation of rodent islets was done by Ballinger and Lacy in 1972, when they also transplanted the islets by intraportal infusion, restoring glycemic control in diabetic rats [73]. A major breakthrough in clinical islet transplantation was the Edmonton protocol, markedly improving outcome [74].

Despite the successes leading up to today's intraportal transplantation, the liver still does not make up an optimal implantation site for pancreatic islets. There is a substantial loss of islet mass within the first hours of transplantation and a major factor causing this is instant blood-mediated inflammatory reaction (IBMIR) [75, 76]. Furthermore, in the context of stem cell-derived islet-like clusters (SC-islets), the inability to retrieve the infused islets does not only complicate research but is also of concern, considering the risk for teratomas. On the upside, the transplantation procedure is minimally invasive and does not require surgery or general anesthesia.

All in all, the liver is to date the only site reliably achieving high rates of insulin independence in human [75]. Meanwhile, one study showing islets transplanted to the omentum resulting in restored glycemic control and insulin independence in humans [77], as well as one in non-human primates [78], have been published.

Most tissues of the body have been tested experimentally as implantation sites [79] but the ones that have made it to human clinical trials are e.g., the spleen [80], omentum [77], liver [75], bone marrow [81] and muscle [82].

The engraftment of islets comes with several challenges, resulting in a profound loss of islet mass, irrespective of site [83]. The isolated islets initially have no blood flow and must rely on diffusion prior to revascularization. The capillary network of donor islets is destroyed in the isolation process, while SC-islets are naturally devoid of capillaries, leading to hypoxia. This in combination with oxidative stress causes apoptosis [76]. Also leading to apoptosis is the cytokine stress induced by the host immune system. Islets are particularly sensitive to the cytokines TNF- α , IL-1 β , and IFN- γ [84], which is utilized

in models of transplantation cytokine stress. Both hypoxia and cytokine stress can trigger endoplasmic reticulum stress in β -cells, leading to apoptosis and poor function [76, 85].

Stem Cell-Derived Islets

Two sources for creating SC-islets are mainly considered, human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). hESCs are obtained from the inner mass of the blastocyst and can derive cell types from all three germ layers [86]. iPSCs are derived from differentiated cells, by being reprogrammed back to a pluripotent stage by retroviral transduction of the transcription factor genes Oct4, Sox2, c-Myc and Klf4 [87]. The benefit of iPSCs is that transplantation could be autologous, reducing the need for immunosuppression, which is the main limiting factor for islet transplantation [72].

Using either hESCs or iPSCs, stem cell-derived β -cells and even α - and δ -cells can be differentiated *in vitro*. In 2014, the two first research groups succeeded in deriving well-functioning β -cells from hESCs [88, 89]. In present protocols, cytokines and signaling molecules are used to guide the signaling pathways towards β -cell differentiation. Controlling concentrations, time and culture environment allows for differentiation of SC-islets [72].

Differentiated SC-islets show a lower function than native islets *in vitro* but SC-islets keep maturing *in vivo* to more closely represent native islets [90, 91]. They have shown to improve glycemic control in mice as well as non-human primates [76].

Now, SC-islets have been transplanted to humans in clinical trials. One was conducted using a microencapsulation device, showing a meal response in C-peptide [92], while another ongoing trial (NCT04786262, <http://clinicaltrials.gov>) implanted the SC-islet product VX-880, showing promising results – albeit not having had their results peer reviewed yet [93].

Just as in native islets, SC-islets face challenges when transplanted. It has been shown that cytokine stress induces apoptosis and impairs GSIS *in vitro* [94] and that SC-islets respond with similar mechanisms as native islets when exposed to cytokines [95]. Thus, SC-islets would benefit from improvements in implantation through e.g., adjuvant therapy.

Aims

Paper I

To test the hypothesis that CART regulates pancreatic blood flow, and if so, if it is through the endothelin system. In addition, to investigate the direct effect of CART on insulin secretion both *in vivo* and *in vitro*.

Paper II

To investigate the expression and secretion of irisin in islets, as well as its effect on hormone secretion, hormone content and pancreatic blood flow.

Paper III

To elucidate if irisin is expressed in SC-islets and if it exerts effects that would be beneficial in transplantation of stem cell-derived islets, such as reduced stress from cytokines and increased proliferation.

Paper IV

To find the most beneficial transplantation site for stem cell-derived islets with regard to transcriptional maturity, function and composition.

Method and Materials

Animals (I–IV)

Male Sprague Dawley rats (Taconic, Ry, Denmark) were used in paper I and II. C57 BL/6 mice (M&B, Ry, Denmark) were used in paper II. Paper III used no animals. For paper IV, both male and female NOD.Cg-Prkdcscid112rgtm1Sug (NOG) mice (Taconic M&B, Ejby, Denmark) were used. All experiments were approved by the Animal Ethical Committee in Uppsala, Sweden.

Human Donor Islets (II–IV)

Human islets were provided by the Nordic Network for Clinical Islet Transplantation and used in paper II–IV. Use was approved by the regional ethical committee in Uppsala.

Stem Cell-Derived Islet Differentiation (III, IV)

SC-islets were derived from the human embryonic stem cell line H1 (Wicell®, Madison, WI, USA). Briefly, H1-cells were propagated on laminin coated plates in a CO₂ incubator in mTeSR-Plus medium (#100-0274/100-0275, STEMCELL Technologies) with 10 μM ROCK inhibitor Y-27632 (#72304, STEMCELL Technologies) before commencing the seven-stage differentiation protocol as previously described by Balboa et al [96]. Experiments utilizing SC-islets were performed in CMRL1066 media (15-110-CVR, Corning, Wiesbaden, Germany).

Intravenous Glucose Tolerance Test (I, IV)

In paper I, anesthetized Sprague-Dawley rats (n=9) were subject to an intravenous glucose tolerance test (IVGTT) by injecting glucose (300 mg/ml, 2 g/kg). Starting 30 minutes before the glucose injection, 25 μg CART dissolved in saline, or only saline, was infused for one hour, thus ending 30 minutes after the glucose injection. The left femoral vein was used for infusions and the left

femoral artery for arterial blood samples. Arterial samples were taken at -30, 0, 5 and 30 minutes after the glucose injection. Tail vein blood glucose was measured at -30, 0, 15, 30, 60 and 120 minutes. Arterial samples were analyzed for insulin and CART (Insulin ELISA, Mercodia, Uppsala, Sweden and CART EIA, Sigma Aldrich, Steinheim, Germany, respectively).

In paper IV, a glucose stimulatory test was performed in NOG-mice at one and three months post SC-islet transplantation by injection of 6.67 μl of 300 mg/ml glucose-solution per g bodyweight (2 g/kg). At 10 minutes, a blood sample was taken from the saphenous vein to measure stimulated C-peptide.

Blood Flow Measurements (I, II)

Detailed descriptions of the method used for blood flow measurements have previously been published [97, 98]. Explained briefly, Sprague-Dawley rats were injected with microspheres into the ascending aorta and a reference arterial sample was taken from the femoral artery. The number of microspheres in different organs was counted histologically and blood flow was calculated through a formula using the number of microspheres and blood flow in the arterial reference sample. Blood samples were taken from the femoral artery and vein to measure blood glucose and levels of CART (paper I) or irisin (paper II). A difference of less than 15% (paper II) or 20% (paper I) in left and right adrenal gland blood flow was used as an internal quality control.

In paper I, groups were infused with saline (1 ml in 1 h), glucose (2 g/kg; 1 ml bolus at 57 min) or BQ-123 (100 $\mu\text{g}/\text{kg}$; 0.5 ml in 0.5h or 1 ml in 1 h) with or without CART (25 $\mu\text{g}/\text{h}$, 1 ml/h). See table 1 in paper I for details.

In paper II, irisin in saline (6.25 $\mu\text{g}/\text{ml}$; 2 ml in 1 h) or saline alone was infused for one hour.

Glucose-Stimulated Insulin Secretion (I, II, III)

For rat islets (paper I and II), collagenase based isolation of islets was performed as previously described [99]. Human islets (paper II) were obtained from the Nordic Network for Clinical Islet Transplantation (Rudbeck Laboratory, Uppsala University Hospital, Sweden). Islets were incubated for 48–72h in supplemented cell medium (see respective paper for details) prior to experiments. GSIS was then performed, with triplicates of ten islets being incubated in supplemented Krebs-Ringer bicarbonate buffer (KRBH), first with 1.67 mmol/l for one hour and then 16.7 mmol/l for a second hour (paper I, II). The incubation medium was saved after each incubation and measured

for insulin (Insulin ELISA, Mercodia) and glucagon (Glucagon ELISA, Mercodia; paper II).

In paper I, the islets were incubated with CART (100 nmol/l), either one hour before GSIS or during, in both the low and high glucose medium.

In paper II, after the initial 48–72h incubation, islets were incubated 24h with or without irisin (100 nmol/l). GSIS was then performed with or without irisin (100 nmol/l) or an irisin neutralizing antibody (Abcam, Recombinant Anti-FNDC5 antibody, ab174833). A table of the five groups can be seen in paper II, Table 1. Islets within the triplicates were then sonicated in distilled water and added to 95% acid ethanol, and total insulin and glucagon content was measured (Insulin and glucagon ELISA for human and rat, Mercodia, Uppsala, Sweden).

In paper III, SC-islets were incubated for 24h with 100 nM irisin or control, as well as two additional groups with the cytokines TNF- α , IL-1 β , and IFN- γ (1000, 50 and 1000 U/ml, respectively; PeproTech, London, UK) in the media, with or without 100 nM irisin. The SC-islets were then equilibrated in 2.8 mM glucose Krebs-Ringer-buffer (KRB) for 90 minutes. After a wash in KRB, SC-islets were incubated sequentially for 30 minutes each in 2.8, 16.8 mM and 2.8 mM with potassium chloride. Supernatants were saved after each incubation. SC-islets were saved for total insulin content analysis and DNA normalization.

Histology (II, III, IV)

In paper II and III, stainings for irisin, insulin, glucagon and nuclei were carried out on pancreatic sections from human, rat, and mouse or SC-islets, respectively. Images were acquired with a confocal microscope. For antibodies and detailed protocols, see the respective papers.

Transplanted SC-islets were stained for islet antigen 2 (IA-2), used to determine total endocrine cell area for calculations, as well as insulin, glucagon, and pimonidazole (hypoxia marker). ImageJ 1.53q was used for image quantifications.

Quantitative Polymerase Chain Reaction (II, III)

In paper II, extraction of total RNA from isolated human and rat islets was carried out with RNeasy Plus Micro Kit (Qiagen, Hilden, Germany). Extractions had OD 260/280 in the range of 1.9–2.1, measured with NanoDrop

2000C spectrophotometer (Thermo Scientific, Wilmington, USA). Skeletal muscle total RNA was used as a reference and was bought from Ambion (Invitrogen, Life Technologies, Stockholm, Sweden). The genes *FNDC5*, *GAPDH* and *RPS7* were analyzed using qPCR. Further details are found in paper II.

To evaluate *FNDC5* expression in paper III, total RNA was extracted from SC- and human islets using RNeasy Plus Micro Kit (Qiagen, Hilden, Germany). A total RNA human skeletal muscle reference sample was purchased (#636534, Takara Bio, Kusatsu, Shiga, Japan). Separately, SC-islets were incubated for 6 and 24h with or without irisin (100nM, #067-29A, Phoenix Peptides Europe GmbH, Karlsruhe, Germany). Synthesis of cDNA was carried out using SuperScript First-Strand Synthesis SuperMix (Invitrogen, Waltham, MA, USA). RT-qPCR was performed using PowerUp SYBR Green Master Mix (Applied Biosystems, Waltham, Massachusetts, USA) on QuantStudio 5 Real-Time PCR Systems (Applied Biosystems, Waltham, Massachusetts, USA). *ACTB* and *RPS7* were used as housekeeping genes. Relative gene expression was calculated by subtracting the geometric mean Ct-value of the housekeeping genes and the $2^{-\Delta\Delta C_t}$ method. Primers are available in paper III.

Perifusion (II)

Human islets were perfused with supplemented KRBH with low (3.3 mmol/L) and high (33.3 mmol/L) glucose concentration, in groups of 50 size matched islets. The perifusion started with low glucose for 30 minutes, followed by low, high, and low glucose concentration. A separate experiment with addition of forskolin, an insulin release potentiating substance, was carried out. Irisin levels in the perfusates were measured (Irisin EIA, Phoenix Peptides Europe GmbH, Germany, #EK-067-29). In light of the problems with measuring irisin as described in the introduction, best ELISA at the time was chosen [25].

Cytokine Stress Model (III)

SC-islets were treated with 100 nM irisin, irisin and 10 μ M cilengitide, cilengitide only or control while incubating in media containing TNF- α , IL-1 β , and IFN- γ (1000, 50 and 1000 U/ml, respectively; PeproTech, London, UK). After 24h of incubation, SC-islets were stained with propidium iodide (PI) and bisbenzimidazole (BIS) for 30 minutes in 37 °C. PI stains DNA but only passes the cell membrane in apoptotic or necrotic cells, and therefore works as a viability marker. Images were acquired in a Zeiss LSM 780 confocal microscope and percentage of PI-positive nuclei were quantified using Image J 1.53q.

Proliferation Assay (III)

SC-islets were incubated for 24h in groups treated with irisin, irisin and cilengitide, cilengitide only or control. EdU (10 μ M) was added to each media, which binds replicating DNA. EdU was labeled with a click-reaction to a fluorophore. Images were acquired using a Zeiss LSM 780 confocal microscope. Percentage of EdU-labeled nuclei was calculated using Image J 1.53q.

Transplantation and Retrieval of SC-islets (IV)

SC-islets (700–800 islet equivalents) were transplanted to each site. A polyethylene tube packed with SC-islets at the tip was used for implantation of the tissue under the renal capsule or a surgically created omental pouch. Injection of SC-islets into the liver through the portal vein, splenic part of the pancreas and the abdominal muscle was performed with a 25-gauge butterfly needle. At three months post-transplantation, grafts were harvested, embedded in OCT and frozen in isopentane in liquid nitrogen and stored at -80 °C.

Laser Capture Microdissection (IV)

Grafts were sectioned 10 μ m thick and put on RNase-free membrane slides to be stained with cresyl violet followed by dehydration. Simultaneously, sections were put on regular glass slides for immunohistochemistry. Multiple graft levels were obtained by alternating membrane slides and regular slides.

Using a Leica LMD6000B microscope (Leica Microsystems), grafts were laser microdissected into 50 μ l lysis buffer (RNeasy Plus Micro Kit, Qiagen, Hilden, Germany) containing 2 M dithiothreitol and 1:40 RNase inhibitor (RiboLock RNase inhibitor, #10389109, Fisher Scientific, Gothenburg, Sweden). RNA was then extracted according to the manufacturers protocol (RNeasy Plus Micro Kit, Qiagen, Hilden, Germany).

RNA-sequencing (IV)

Samples were quality controlled using the Agilent 5400 Fragment Analyzer (Agilent, Santa Clara, CA, United States). Since amounts RNA obtained from LCM often are low, samples were amplified using the SMART-Seq V4 Ultra Low Input RNA kit for Sequencing (Cat No. 634893, Takara Bio Inc, Shiga, Japan). Then, library preparation was performed by purifying mRNA, synthesizing cDNA and preparing the cDNA for sequencing (see details in paper IV). The Illumina NovaSeq 6000 S4 flowcell with PE150 (Illumina, San

Diego, CA, USA) was used for sequencing. The above was performed by NovoGene Co., Ltd (UK sequencing center, Cambridge, United Kingdom).

Transplanted samples were batch corrected using the limma package version 3.56.2 [100], due to a batch effect from time seen in a principal component analysis of the transplanted samples. Differential gene expression analysis was performed in edgeR package version 3.42.4, where all sites were compared to liver as a reference. False discovery rate limit, i.e., p-values adjusted with the Benjamini-Hochberg method, was set to 0.05.

Statistics (I–IV)

All calculations were performed using the software Prism (versions 7–10, GraphPad Software, San Diego, CA, USA). For specific statistical methods, see respective paper.

Results and Discussion

Pancreatic Blood Flow Regulation

Blood Flow and CART

Total pancreatic blood flow (PBF) was unchanged by CART alone but increased by glucose. However, when CART was added to the glucose group, the previously seen increase in PBF disappeared (Fig 1a, paper I). BQ-123 with or without CART did not affect PBF (Fig 1b, paper I).

In contrast, islet blood flow (IBF) was notably lowered by CART (Fig 1c, paper I; $p < 0.05$). The increase in IBF from glucose was lowered by CART (Fig 1d, paper I; $p < 0.05$). BQ-123 lowered IBF, but CART did not additionally affect blood flow in that group (Fig 1f, paper I). The percentage of PBF going to the islets was unchanged in control, glucose, glucose combined with CART and BQ-123, but CART alone lowered this percentage (Fig 1g–h, paper I). All in all, although CART also prevented the glucose-induced increase of PBF, CART seems predominantly to have effects on the islet vasculature in the pancreas. This opens the possibility that CART exerts local effects in islets, where it is also produced.

If the vasoconstriction from CART would be mediated through EtA, an effect on PBF should be seen since the receptor is expressed in the exocrine pancreas as well [22]. However, since CART alone did not lower PBF, it is less likely that EtA is the mediator. On the other hand, BQ-123 previously did not prevent lowering of blood flow through EtA in the pancreas [22]. Although, since CART lowered blood flow despite EtA being blocked by BQ-123, CART may work through another or many receptors. For unknown reasons, BQ-123 alone paradoxically also lowered blood flow as much as CART, when the effect should theoretically be the opposite, since BQ-123 inhibits EtA and thereby should prevent vasoconstriction. In summary, there is evidence that CART lowers IBF, but it is inconclusive if this is mediated partly through the receptor EtA.

Despite the lowering of IBF by CART, glucose tolerance assessed by IVGTT was unchanged by CART. If CART would have a major effect on decreasing

IBF, this should affect insulin secretion [101]. On the other hand, the increase of IBF from glucose could compensate for the decrease by CART.

Blood Flow and Irisin

One hour of irisin infusion lowered IBF and blood flow in WAT (Fig 6a and c, paper II; $p < 0.01$ and $p < 0.05$ respectively) but not PBF (Fig 6b, paper II; $p = 0.074$). Blood flow in the duodenum, colon, kidneys and skeletal muscle was unaffected by irisin (data not shown). Thus, irisin specifically regulates IBF, which could be a protective effect since irisin was found to be secreted in response to high glucose levels in human islets (see section “Irisin and Glucose Control”). As discussed below, irisin often seems to exert effects in hyperglycemia. The blood flow regulation from irisin was seen in normoglycemia but a reduction in blood flow as a protective measure during hyperglycemia is still plausible, which would go hand in hand with irisin being secreted locally in islets in response to higher glucose levels.

CART and Glucose Control

In the IVGTT, an increased p-CART was confirmed in CART-infused rats. During the IVGTT, CART-infusion did not affect insulin or glucose levels compared to saline infusion (Fig 2a–b, paper I). Furthermore, GSIS was not altered by preincubation with CART or adding CART to the low and high release media (Fig 3a–b, paper I). Thus, CART did neither affect insulin secretion from rat islets *in vitro*, nor in rats *in vivo*. In human and mouse islets, an increase in GSIS from CART has previously been reported [15, 17]. Previous studies in rat have not shown an increase, except for in combination with cAMP elevating agents [14, 18]. Therefore, the present observation is in line with previous results and emphasizes the existence of species differences, which is also supported by the differences in localization of CART in the islets, as discussed in the introduction. Considering that CART is upregulated in T2D, its role in the islets could be to potentiate GSIS during long standing hyperglycemia. However, a lowering of IBF from increased CART concentrations in T2D would not aid GSIS but could lower the stress on the islets as a protective measure. Although CART is expressed in the islets, and even more so during T2D, it is to be determined if it is secreted locally. Moreover, there are CART-containing neurons in the islets, also containing VIP, which could regulate blood flow. The colocalization with the vasoactive VIP supports this latter notion.

Taken together, the potentially protective lowering of IBF from upregulated CART in T2D, while still potentiating GSIS, makes CART interesting for pharmacological treatment of T2D.

Irisin and Glucose Control

Irisin or an anti-irisin antibody did not affect insulin or glucagon secretion from human or rat islets (Fig 4a–d, paper II). Neither was total insulin or glucagon content changed by 24h irisin incubation. However, human islets secreted irisin which was increased in response to increased glucose (Fig 3a–b, paper II).

This study added the pancreas as a source of secreted irisin, in addition to the primary source, skeletal muscle. The amount secreted from islets is unlikely to affect plasma levels, which is supported by plasma levels not being increased during an OGTT in obese women [102]. The production and secretion of irisin locally in islets is supported by irisin being expressed in the islets, as seen histologically and by qPCR. The function of this local secretion could be to protect β -cells during high glucose and saturated fatty acid levels, two conditions often coinciding in T2D, where irisin has shown to increase β -cell proliferation and reduce β -cell apoptosis [48, 49].

In both the liver and skeletal muscle, irisin has effects indicating an important function during high glucose conditions, e.g., by increasing glycogenesis and decreased gluconeogenesis. A previous study showed increased GSIS by irisin in INS-1E cells and human and murine islets [49]. That study also showed increased total insulin content after one hour of irisin incubation, an effect we could not reproduce. Many other studies showing improved GSIS, did so after 24–48h of incubation with high glucose and/or palmitic acid [48, 51]. If this is due to anti-apoptotic effects of irisin, thus improving β -cell survival, or a direct function is unclear. In support of an indirect effect, our study could not show any direct effect from irisin on GSIS during normal conditions. Differences between the studies are the incubation medium and brands of irisin peptide.

In the case of SC-islets, irisin treatment seemingly has no effect on GSIS (Figure 8, paper III), in contrast to previous data on INS-1E cells and human and murine islets [49].

Interestingly, many of the discussed effects are shared with glucagon-like peptide 1 (GLP-1) analogues, already successfully used in the treatment of T2D [103]. Even a decrease of IBF, as seen in this study, has previously been shown by GLP-1 [104].

In summary, the potential beneficial effects of irisin are to restrict blood flow locally, thereby protecting the islets from stress, as well as likely augmenting GSIS, directly or indirectly. Further research is needed to clarify whether irisin treatment could be of future interest for patients with T2D.

Human and SC-islet Expression of Irisin

Histology

In pancreatic sections from human, rat and mouse, most of the pancreatic islet and spots of the exocrine pancreas stained for *FNDC5*, the precursor of irisin (Figure 1 and 2, paper II). In SC-islets, most of the islet was stained for *FNDC5* (Figure 5, paper III).

FNDC5 Expression

The mRNA expression of *FNDC5* in human islets ranged from 1–48.5% of that in skeletal muscle, while it ranged from 0.6–8.9% in rat islets (Fig 2, paper II). In paper III, *FNDC5* was shown to be expressed in both SC-islets and human donor islets with mean values of 8.4 and 0.5% of the expression in human skeletal muscle, respectively. SC-islets had a higher expression than human islets ($p < 0.0001$; Figure 1, paper III).

From the sequencing data in paper IV, expression of *FNDC5* in transplanted and non-transplanted SC-islets and human donor control islets was analyzed separately from the paper. The same pattern of expression as in paper III was seen, with SC-islets having a higher expression than human islets (FDR-adjusted p-value: 3^{-8}). Furthermore, expression of *FNDC5* was lowered after transplantation (FDR-adjusted p-value: 0.023).

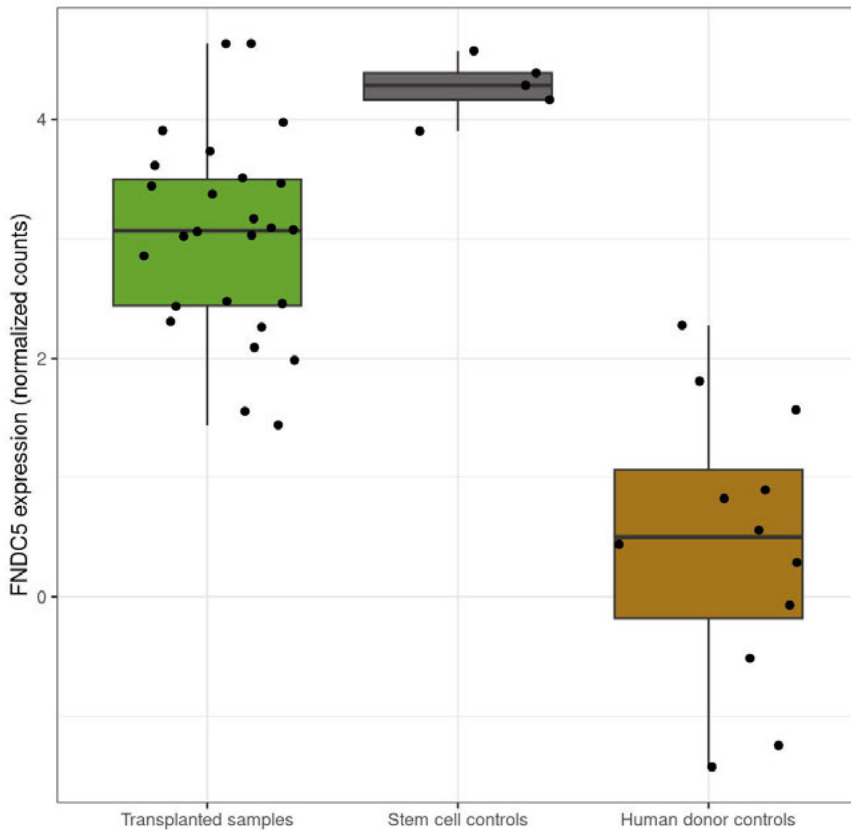


Figure 1. Expression levels of *FNDC5* in transplanted stem cell-derived islets, non-transplanted stem cell-derived islets and human donor islets, derived from the RNA-sequencing data in paper IV.

Protecting SC-islets through Irisin Treatment

Cytokine Stress

In the cytokine stress experiment, viability was assessed after cytokine treatment. Adding irisin or irisin with the irisin-inhibiting cilengitide did not alter viability compared to cytokine treated control, while the cytokine-free control exhibited an improved viability (Figure 6, paper III). This contrasts previous studies, which have indicated an anti-apoptotic effect from irisin in both INS-1 cells and human islets [48, 49]. When analyzing expression of apoptosis related genes, our results in SC-islets differ from those in INS-1 cells, indicating a difference in apoptosis signaling (see paper III for gene details).

Proliferation

Interestingly, despite previous studies showing increased proliferation with irisin treatment in various models, including INS-1E cells, rats, and mice [48, 49], no such effect was observed in SC-islets in our study (Figure 7, paper III). This is despite our duration and dosage being consistent with the *in vitro* data on INS-1E cells. Furthermore, incubating SC-islets for 24h with irisin had no effect on the expression of the proliferation-related genes *PCNA* and *CCND1* (Figure 4, paper III). The absence of effects on proliferation and viability raises the question of translatability of previously seen effects to SC-islets.

Expressional Maturity in Transplanted SC-islet Grafts

Comparing the five different implantation sites pancreas, liver, kidney capsule, omentum and abdominal muscle, no distinct clustering for each site was seen in the PCA of all genes, but liver and muscle exhibited the furthest separation among implantation sites (Figure 3, paper IV). Performing DEG analysis with liver as a reference, only one significantly upregulated gene, *SERF1A*, was seen in the liver compared to the pancreas. Possible reasons for the lack of significant genes are e.g., sample size, high variability within each group and similarity between groups. Looking instead at a heatmap of islet maturity genes (Figure 6, paper IV), it was notable that liver grafts had a higher expression of maturity genes compared to other sites. In line with this, the liver had a lower expression of proliferation related genes compared to other sites, suggesting a more mature state. However, no differences in stage-sorted maturity genes were observed between sites (Figure 7, paper IV).

Comparing transplanted and untransplanted SC-islets and human islets in a PCA for all genes (Figure 5, paper IV) revealed that untransplanted SC-islets were more similar to human donor islets than transplanted SC-islets. This is likely due to changes *in vivo*, but of note, transplanted samples were harvested in a different fashion than untransplanted SC-islets and human islets.

Analysing stage-sorted islet maturity genes in a heatmap, also including untransplanted SC-islets and human donor islets, it was seen that human donor islet had a higher expression of late-stage maturity genes compared to untransplanted and transplanted SC-islets (Figure 8, paper IV). Furthermore, performing DEG analysis comparing untransplanted and transplanted SC-islets revealed an upregulation of the islet maturity genes *FXYD2*, *PDX1*, *SIX2*, *NKX6.1* in untransplanted SC-islets and upregulation of *UCN3* and *MAFA* in transplanted SC-islets (Figure 9, paper IV). Interestingly, upregulated genes in untransplanted SC-islets were markers of earlier stages of maturity, while

transplanted SC-islets had upregulation of genes from later maturation stages. This shows a maturation *in vivo* after transplantation.

β -cell Function in Transplanted SC-islets

Analysing stimulated C-peptide levels from SC-islets, a two- to sevenfold increase was seen between one and three months post transplantation at the different sites (Figure 13, paper IV). Thus, it is evident that the SC-islet grafts matured and increased function *in vivo*. Comparing different graft sites, liver transplants had the highest individual C-peptide values at one and three months, as well as a higher C-peptide level than pancreas and omentum at one month (Figure 12, paper IV). At three months, no difference was seen, suggesting that the other sites matured to reach the function of the liver site. Interestingly, previous data with syngeneic islets transplanted to mice showed superior function in omentum and striated muscle compared to liver [105, 106]. This highlights the possibility that SC-islets react differently to the local environment of the sites, which in turn could be due to SC-islets maturing *in vivo*, which is not the case for isolated islets.

SC-islet Graft Composition

The cell composition of the grafts ranged from a mean of 18–33% β -cells and 22–24% α -cells. The β -cell fraction was higher in liver compared to all sites except for kidney, while the α -cell fraction did not differ between sites (Figure 2, paper IV). Thus, the larger fraction of β -cells in the liver did not come at the expense of α -cells. A possible reason is that the liver site had a lower fraction of immature islet cells, having derived the additional β -cells from immature graft cells. Furthermore, the larger β -cell fraction could be one of the reasons for the observed superior β -cell function.

Conclusions

Paper I

CART causes a lowering of IBF, which could be a protective effect during long-standing hyperglycemia. No effect of CART on GSIS or glucose tolerance in rat was seen, but an effect on human islets is still plausible. Further research on CART as a pharmacological candidate in T2D is of interest.

Paper II

Irisin is secreted glucose dependently from human islets, is produced in islets, and lowers islet blood flow. These results add to the hypothesis of irisin being protective for the islets. The results on GSIS contradict previous results and therefore create a need to further clarify these discrepant findings. Irisin shares many features with GLP-1 analogues and remains of potential interest in the treatment of T2D.

Paper III

Stem cell-derived islets express higher levels of *FNDC5* than human donor islets and irisin treatment has limited effects on cell viability, proliferation and β -cell function in SC-islets. This highlights possible differences in irisin signaling in SC-islets compared to murine and human donor islets.

Paper IV

The liver is the preferable site for SC-islet implantation due to having a higher β -cell function and fraction, as well as a higher expression of islet maturity genes than other sites. This poses a dilemma since the liver site is also the most challenging to monitor and research.

Populärvetenskaplig sammanfattning

I avhandlingen har två hormoner och deras relation till bukspottskörtelns hormonella funktion utforskats. Dess hormonella funktion sköts av små ansamlingar av hormonproducerande celler som kallas Langerhanska öar. De två hormonen heter irisin och Cocaine and amphetamine regulated transcript (CART) och hittades båda inom forskning på andra delar av kroppen än de som direkt rör diabetes. Därefter har deras inblandning i bukspottskörtelns funktioner hittats. Avhandlingens ingående delarbeten har byggt vidare på dessa insikter och sökt svara på om hormonen skulle kunna komma till gagn för diabetespatienter.

I arbetena har djurmodeller och donerade mänskliga Langerhanska öar använts. Langerhanska öar kan plockas ut från bukspottskörteln genom att bryta ner den vävnad som ligger omkring öarna, så att isolerade öar kan studeras. I två av arbetena har även Langerhanska öar som odlats fram från mänskliga stamceller använts, vilket inte kräver en avliden donator. Det som har undersökts med hjälp av dessa modeller är till exempel effekten av irisin eller CART på de Langerhanska öarnas blodflöde och förmåga att utsöndra insulin – det sockerreglerande hormonet som saknas vid typ 1-diabetes. I det sista delarbetet har Langerhanska öar som odlats fram från mänskliga stamceller transplanterats till flertalet organ i möss för att jämföra vilket mottagarorgan som fungerar bäst. Denna typ av stamcells-öar har redan visat lovande effekter i en klinisk studie på människor med typ 1-diabetes eftersom de kan producera insulin för att kontrollera blodsockret.

I första artikeln i avhandlingen undersöktes effekten av CART på bukspottskörtelns blodflöde samt blodsockerkontroll och insulinutsöndring hos råttor. Resultatet visade att CART sänker blodflödet i öarna men påverkar inte blodsockerkontrollen i levande råttor eller insulinutsöndring i isolerade Langerhanska öar.

I andra artikeln undersöktes om irisin produceras i Langerhanska öar och om irisin har någon effekt på insulinutsöndring eller blodflöde. Det visade sig att irisin produceras i öar, vilket gick att se både genom att genen för irisin uttrycktes, såväl som själva proteinet. Emellertid sågs ingen effekt av irisin på insulinutsöndring från isolerade Langerhanska öar från människa eller råtta.

Däremot sänkte irisin blodflödet lokalt i öarna hos råttor. Artikeln visar på att irisin är ett lokalt hormon med effekter i öarna.

I den tredje artikeln undersöktes ifall irisin skulle kunna vara hjälpsamt vid transplantation av Langerhanska öar som odlats fram från stamceller. Det finns nämligen flera utmaningar för den transplanterade vävnaden, såsom låg syresättning, stress för cellerna och celldöd. Det som undersöktes var dels om irisin produceras i stamcells-öar och dels om irisin har några skyddande effekter som kan vara gynnsamma vid transplantation, såsom minskad inflammatorisk stress, ökad celledelning och förbättrad insulinutsöndring. Irisin visade sig finnas i stamcells-öar men trots att tidigare studier i andra arter visat effekter på de undersökta funktionerna sågs ingen effekt på stamcells-öar. En möjlig förklaring är att stamcells-öar kan ha en annan hantering av signaler som skickas av irisin, då mängden irisin visade sig vara betydligt högre än i Langerhanska öar från råttor och mänskliga donatorer.

Den fjärde artikeln undersökte vilket mottagarorgan som fungerar bäst vid transplantation av stamcells-öar. De undersökta mottagarorganen var bukspottskörteln, levern, bukhinnan, under njurens bindvävskapsel och magmuskeln. Det visade sig att levern främjade stamcells-öarnas funktion. Till exempel utsöndrade stamcells-öar som transplanterats till levern mest C-peptid (som motsvarar insulin), var mognast sett till genuttryck och hade större andel insulinproducerande celler än andra mottagarorgan. Resultatet är väglädande för kommande studier och pekar på ett dilemma: levern verkar fungera bäst som mottagarorgan, men är svårast ur säkerhetsaspekt eftersom transplantaten inte kan plockas ut ur levern eller övervakas genom att ta vävnadsprov.

Vid sammanfattning av artiklarnas resultat kan konstateras att både CART och irisin har specifika funktioner i Langerhanska öar men att effekterna från irisin möjligen inte är desamma i djurmodeller och vanliga mänskliga öar som i stamcells-öar.

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