Endocrine Pancreas: Histology and Physiology

Dave Bridges, Ph.D.

October 8, 2015

This lecture will cover the roles of insulin and glucagon in normal and pathophyiological states, especially diabetes. These hormones are the major glucose controlling hormones and are both secreted from the pancreas. This lecture will cover pages 429-435 of the textbook[1]

[1] Linda Costanzo. *Physiology*. Saunders, 5th editio edition, 2013. ISBN 978-1455708475

Contents

Learning Objectives

For this lecture, the learning objectives are:

- Name the cell types of the Islets of Langerhans and name the hormones secreted by them.
- Describe the main targets and functions of glucagon.
- List the major factors that stimulate or inhibit glucagon and insulin.
- Describe the important physiological roles of insulin.
- List the major actions of insulin in muscle, adipose tissue, and liver.
- Explain briefly the mechanism of glucose uptake into the muscle.
- Name the tissues in which insulin facilitates glucose uptake and those in which insulin does not facilitate glucose uptake.
- List the major factors that stimulate or inhibit insulin secretion.
- Draw an oral glucose tolerance test (oGTT) (glucose, insulin, and glucagon levels) and explain the conditions and describe what is occurring and why. Explain how the two hormones act to promote glucose homeostasis in the plasma and in the key target tissues for each of these hormones.
- State which nutrient storages are preferably used for short-term regulation of energy metabolism if no nutrients are available from the GI tract.
- Discuss the hormones involved, fuel storage capacity, fuel storage consumption, and glucose (or fatty acid) levels during 1) the postprandial period, 2) the post-absorptive period, 3) fasting.
- List the insulin-counteracting hormones and their roles in glucose homeostasis. Discuss the hormones involved in minute-to-minute regulation and long-term regulation of glucose homeostasis.

Mechanisms of Glucose Regulation

GLUCOSE IS MAINTAINED IN A VERY NARROW RANGE, between 4.4 to 6.1 mmol/L. The major tissues responsible for regulating glucose levels are listed in Table 1 These levels need to be re-established after changes in feeding status, or energy utilization. In general, when glucose levels decrease, glucagon is released from alpha cells of the pancreas to promote glucose production, either from glycogen breakdown or gluconeogenesis. Alternately, after a meal when glucose levels increase, insulin is secreted from beta cells of the pancreas causing glucose levels to decrease.

For the purposes of the acute maintenance of glucose homeostasis, four organs are the most important; the pancreas, liver, muscle and adipose tissue. The pancreas senses changes in glucose levels and responds by releasing either glucagon or insulin.

Table 1: Primary sites for regulation of glucose homeostasis

Process	**Tissue**
Insulin Stimulated Glucose Uptake	Muscle and Fat
Regulated Glucose Oxidation	Muscle
Glycogen Storage/Release	Liver and Muscle
Gluconeogenesis	Liver
Lipid Storage/Release	Adipos and Liver

GLUCOSE ENTERS THE CELL, DOWN A CONCENTRATION GRADIENT via passive transport from the blood into most tissues including liver, pancreas, kidneys and the brain. However, for glucose to enter into muscle and fat tissue, insulin is required. This is accomplished by moving GLUT4 transporters from intracellular storage sites to the plasma membrane, allowing for glucose influx.

GLUCOSE CAN BE STORED IN ESTERIFIED FORM AS GLYCOGEN[2]. To form glycogen, glucose must first be converted through glucose-1-phosphate into UDP-glucose. This activated form of glucose is then added onto existing glycogen chains through the activity of an enzyme named glycogen synthase. In addition to being regulated by protein phosphorylation and sub-cellular location, glycogen synthase is also allosterically activated by glucose-6-phosphate, promoting increased glycogen synthesis when glucose levels in the cell are high.

[2] This is known as glycogenesis

TO LIBERATE GLUCOSE FROM STORED GLYCOGEN, AN ENZYME KNOWN AS GLYCOGEN PHOSPHORYLASE IS ACTIVATED. This enzyme hydrolyses glycogen, releasing glucose-1-phosphate, which can then be dephophorylated into glucose for glycolysis or release into the blood stream. This is the preferred source of short term glucose maintenance. In addition to post-translational modifications and recruitment to the glycogen pellet by accessory proteins, glycogen phosphorylase is allosterically activated by energy stress such as increases in AMP, or negatively by increases in glucose-6-phosphate levels.

GLUCONEOGENESIS IS THE GENERATION OF GLUCOSE FROM NON-CARBOHYDRATE PRECURSOR MOLECULES. These typically include amino acids, lactate and the products of fatty acid oxidation. The vast majority of gluconeogenesis occurs in the liver, and generally is important for glucose production from proteins and lipids after glycogen stores are depleted. This process is similar to reverse glycolysis though in several cases different enzymes are used. The rate limiting enzymes in gluconeogenesis are phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase and glucose-6-phosphatase. These enzymes are under both transcriptional and post-translational control as described below.

WHEN ENERGY IS NEEDED, for example during fasting the primary source is circulating glucose, followed by the the breakdown of glycogen and then the production of new glucose (gluconeogenesis) from proteins and lipids. At the same time, the wasteful synthesis of new glycogen is stopped. When glucose levels are too high, the first response is uptake and oxidation by the muscle, followed by glycogen synthesis and then by lipogenesis. At this time, gluconeogenesis (which is not needed) is stoped.

Pancreatic Cell Types

In order to balance the energy requirements of all tissues, blood glucose is primarily controlled via endocrine and neuroendocrine mechanisms. The primary mediators are insulin and glucagon which are secreted from the pancreas during times of hyper and hypoglycemia respectively. These two peptide hormones are released from two cell types in the pancreas, the alpha-cells which release glucagon and the beta-cells which release insulin. Both cell types are located in the Islets of Langerhans within the pancreas (see Figure 1)[3]

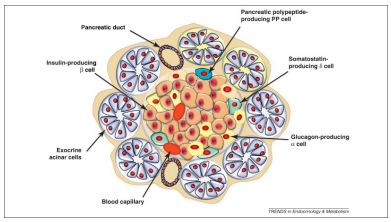

Figure 1: Schematic of a pancreatic islet.

Insulin Promotes Glucose Storage

Insulin was discovered by Frederick Banting and his colleagues at the University of Toronto in 1921. They performed experiments in which they injected extracts from pancreas fractions into dogs which had their pancreas' surgically removed. They showed that a secreted substance from the pancreas lowered blood glucose in these dogs [4]. They were then able to confirm that this treatment was also effective in children with diabetes [5]. This work led to Banting and John Macleod winning the Nobel Prize in Medicine and Physiology in 1923.

When glucose levels are raised, such as after a meal, insulin has four main functions, all of which serve to reduce blood glucose lev-

[3] Shimon Efrat and Holger a. Russ. Making β cells from adult tissues. *Trends in Endocrinology & Metabolism*, 23(6):278–285, 2012. ISSN 10432760. DOI: 10.1016/j.tem.2012.03.005. URL `http://linkinghub.elsevier.com/retrieve/pii/S1043276012000495`

[4] Frederick G. Banting and Charles H. Best. The internal secretion of the pancreas. *Journal of laboratory and clinical medicine*, 7:251–266, 1922

[5] F G Banting, C H Best, J B Collip, W R Campbell, and a a Fletcher. Pancreatic Extracts in the Treatment of Diabetes Mellitus. *Canadian Medical Association journal*, 12(3):141–6, March 1922. ISSN 0008-4409. URL `http://www.ncbi.nlm.nih.gov/pubmed/17580419`

els:

1. Promotes the uptake of glucose from the blood into muscle and adipose tissue.
2. Enhances the synthesis of glycogen and triglycerides in liver, adipose and muscle.
3. Inhibits gluconeogenesis, or the production of glucose from non-glucose precursors such as amino acids and lipids.
4. Promote the breakdown of glucose via glycolysis.

Insulin Release and Insulin Signal Transduction

Beta cells in the pancreas generate insulin and store it in pre-formed secretory granules. Insulin is generated as a pre-pro peptide and then is processed to generate two fragments, mature insulin and C-peptide. Both of these are released from the secretory granules. After the glucose mediated depolarization of beta cells in response to insulin, these secretory granules are exocytosed and their contentes are released into the blood. This happens very quickly and is known as the first phase insulin response. Upon chronic glucose stimulation, new insulin has to be made, packaged and secreted. This is known as second phase and allows insulin to be continually released over a longer time period.

Insulin functions by binding to and activating a receptor tyrosine kinase. This receptor transautophosphorylates itself generating binding sites for phosphotyrosine binding proteins known as insulin receptor substrates.

These proteins are also phosphorylated by the insulin receptor, which creates binding sites for a phosphatidylinositol-3-kinase (PI3K). This kinases generates the key second messenger in insulin signaling, phosphatidylinositol-(3,4,5)-triphosphate (PIP_3). Most known functions of insulin are blocked when PI3K is inhibited [6].

Once PIP_3 is generated by insulin stimulation, it can diffuse along the internal membranes of the cells. This lipid second messenger recruits two important protein kinases, Akt (also referred to as PKB) and PDK1. Both of these proteins have domains called pleckstrin homology domains which recruit the kinases together to the plasma membrane. Once there, PDK1 and another protein kinase called mTORC2 are able to phosphorylate and activate Akt. Once activated, *Akt is the most important protein kinase in mediating insulin function*.

[6] F Kanai, K Ito, M Todaka, H Hayashi, S Kamohara, K Ishii, T Okada, O Hazeki, M Ui, and Y Ebina. Insulin-stimulated GLUT4 translocation is relevant to the phosphorylation of IRS-1 and the activity of PI3-kinase. *Biochemical and biophysical research communications*, 195(2):762–8, September 1993. ISSN 0006-291X. DOI: 10.1006/bbrc.1993.2111. URL http://www.ncbi.nlm.nih.gov/pubmed/8396927

Regulation of Glucose Uptake in Muscle and Adipose Tissue

In fat and muscle tissue, insulin promotes the movement of a facilitative glucose transporter named GLUT4. Normally GLUT4 resides in intracellular compartments, but in response to insulin vesicles from these compartments fuse with the plasma membrane, inserting GLUT4 into the extracellular membrane. This allows for glucose to enter fat and muscle cells.

In both fat and muscle, the PI3K/Akt dependent signaling pathways are absolutely required for insulin stimulated glucose uptake. The major targets of Akt in this signaling pathway are AS160 and RGC1/2, two proteins which regulate the activity of small GTPases involved in GLUT4 translocation. The full mechanisms regulating GLUT4 trafficking are not yet fully understood.

Regulation of Lipid and Glycogen Synthesis in Muscle, Liver and Adipose Tissue

The other functions of insulin, including promoting glucose uptake will provide more UDP-Glucose for glycogen synthase, and will also allosterically activate the enzyme by generating large amounts of glucose-6-phosphate. This is augmented by post-translational activation of glycogenic enzymes.

In addition to these allosteric activators, both glycogen synthase and glycogen phosphorylase are regulated by protein phosphorylation. In the case of glycogen synthase, the phosphorylated form is relatively inactive, and is resistant to allosteric activation by glucose-6-phosphate [7]. Glycogen synthase is phosphorylated by several protein kinases including AMPK and GSK-3 [8]. In addition to inactivating the upstream kinases, insulin also activates a protein phosphatase, which removes the phosphate groups. In a co-ordinated way, protein phosphorylation activates glycogen phosphorylase [9]. This means that when these enzymes are phosphorylated, the balance tips towards glycogenolysis, and when they are dephosphorylated glycogen is synthesized.

Regulation of Gluconeogenesis in the Liver

The activation of glucose uptake and glycolysis leads to increased levels of several glycolytic intermediates which themselves will regulate gluconeogenesis. The most important of these is Fructose-2,6-bisphosphate which is raised during glycolysis and inhibits FBPase, one of the key rate limiting steps in gluconeogenesis.

In addition to these effects, both G6Pase and PEPCK, two other rate limiting enzymes are regulated transcriptionally. Akt phospho-

[7] Daniel L. Friedman and Joe. Larner. Studies on UDP G-Alphaglucantransclucosylase. III. Interconversion of Two forms of Muscle UDP-G-Alphaglucantransglucosylase by a phosphorylation-dephosphorylation reaction sequence. *Biochemistry*, 2: 669–75, 1963. ISSN 0006-2960. URL http://www.ncbi.nlm.nih.gov/pubmed/14075096

[8] Peter J Parker, Noor Embi, F Barry Caudwell, and Philip Cohen. Glycogen synthase from rabbit skeletal muscle. State of phosphorylation of the seven phosphoserine residues in vivo in the presence and absence of adrenaline. *European Journal of Biochemistry*, 124(1): 47–55, May 1982. ISSN 0014-2956. URL http://www.ncbi.nlm.nih.gov/pubmed/6211353

[9] E. G. Krebs and E H Fischer. Phosphorylase and related enzymes of glycogen metabolism. *Vitamins and hormones*, 22: 399–410, January 1964. ISSN 0083-6729. DOI: 10.1016/S0083-6729(08)60345-3. URL http://www.ncbi.nlm.nih.gov/pubmed/14284112

rylates and inactivates the transcription factor FOXO which would normally drive the expression of these enzymes. Therefore when insulin activate the PI3K/Akt cascade, FOXO mediated transcription of G6Pase and PEPCK is decreased and the levels of these enzymes are reduced, decreasing gluconeogenesis.

Glucagon Promotes Glucose Elevation

When glucose levels are low, glucagon is released from alpha cells in the pancreas. This promotes the breakdown of glycogen stores in liver and muscle, and the generation of glucose from gluconeogenic precursors. Glucagon receptors exist mainly in the liver, so glucagon does not exert its main catabolic effects on either adipose or muscle tissue.

The mechanisms which underlie hypoglycemia induced glucagon release are incompletely understood. What is clear however, is that when blood glucose levels decrease, glucagon is released from the alpha cells of the pancreas into the portal vein.

Glucagon Signal Transduction

Adrenergic-receptor coupled mediated cAMP synthesis was the first example of a hormonal second messenger. Earl Sutherland was interested in the regulation of glycogenolysis and he noticed that if he added adrenaline to intact cells, he could accelerate glycogen breakdown, but if he added it to lysed cells he could not. In his key experiment he treated one set of livers with adrenaline, then lysed them. He then added that lysate to a second set of livers which had already been broken. He found that there was an internal factor (later identified as cAMP) in the stimulated tissues, that could accelerate glycogenolysis in the other tissues [10]. For this work, Sutherland won the Nobel Prize in Medicine and Physiology in 1971.

In metabolism, the main effector of cAMP in cells is Protein Kinase A (PKA). This protein kinase is allosterically activated by cAMP and phosphorylates a wide variety of important metabolic substrates. The identification of PKA and its role in carbohydrate homeostasis led to Fisher and Krebs winning the Nobel Prize in Medicine and Physiology in 1992. The major role of glucagon is to increase blood glucose, both by mobilizing glycogen stores and inducing gluconeogenesis. The mechanisms for this are identical to those for adrenaline, as both of these hormones activate Gs-linked receptors and result in PKA activation in the liver.

[10] T W Rall, E W Sutherland, and W D Wosilait. The relationship of epinephrine and glucagon to liver phosphorylase. III. Reactivation of liver phosphorylase in slices and in extracts. *The Journal of biological chemistry*, 218(1): 483–95, January 1956. ISSN 0021-9258. URL `http://www.ncbi.nlm.nih.gov/pubmed/13278355`

The Primary Target of Glucagon is the Liver

As described above, glucagon stimulates the breakdown of glycogen. This proceeds via protein phosphorylation of both glycogen phosphorylase (which activates the enzyme) and glycogen synthase (which inactivates the enzyme). In combination, this leads to a breakdown of glycogen into glucose.

PKA is the primary mediator of the activation of glycogen phosphorylase. Once activated by adrenergic signaling, PKA phosphorylates and activates glycogen phosphorylase kinase. This kinase in turn, phosphorylates and activates glycogen phosphorylase[11]. PKA also directly phosphorylates glycogen synthase, which in concert with the activation of the other glycogen synthase kinases (notably GSK3 and AMPK) leads to increased phosphorylation and inactivation of glycogen synthase.

In addition to the activation of these protein kinases, there is a reduction of glycogen associated protein phosphatase activity. As a balance, this leads to more highly phosphorylated and therefore more glycogenolytic activities.

[11] E. G. Krebs and E H Fischer. The phosphorylase b to a converting enzyme of rabbit skeletal muscle. *Biochimica et biophysica acta*, 20(1):150–7, April 1956. ISSN 0006-3002. URL `http://www.ncbi.nlm.nih.gov/pubmed/13315361`

GLUCAGON PROMOTES GLUCONEOGENESIS. In addition to the decreased flux of glycolytic intermediates which allosterically activate gluconeogenesis, there are both post-translational and transcriptional mechanisms by which adrenergic signaling promotes gluconeogenesis.

Post-translationally, the best studied route by which PKA activates gluconeogenesis is through inactivation of phosphofructokinase-2. PFK-2 normally generates the carbohydrate Fructose-2,6,-bisphosphate which is a positive regulator of glycolysis and a negative regulator of gluconeogenesis. The alleviation of this inhibition allows for promotion of the gluconeogenic metabolism.

Transcriptionally, the transcription factor CREB is phosphorylated by PKA where it plays a role in transcriptionally activating the rate limiting gluconeogenic enzymes PEPCK, FPBase and G6Pase.

Other Glucoregulatory Hormones

Since glucagon works primarily on liver tissue, different hormonal messengers function to stimulate catabolism of lipid in muscle and fat tissue. A key difference from adrenaline and glucagon, is that adrenaline also has major effects on fat and muscle tissues, as well as glycogen. Therefore, in addition to simulating hepatic gluconeogenesis and glycogenolysis, adrenaline also promotes lipid release and muscle glucose oxidation. Both adrenaline and glucagon func-

tion by stimulating adrenergic signaling and cAMP-dependent PKA activation

In adipose tissue, adrenaline induces lipolysis, via phosphorylation and activation of Hormone Sensitive Lipase (HSL), Perilipin and Adipocyte Triglyceride Lipase (ATGL). These proteins function to mobilize triglycerides into free fatty acids for use in other tissues, especially muscle. For more information on the regulation of lipolysis, see [12]. At an acute level, these do not contribute much to glucose homeostasis but are extremely important for lipid metabolism.

[12] Stephen G Young and Rudolf Zechner. Biochemistry and pathophysiology of intravascular and intracellular lipolysis. *Genes & development*, 27(5):459–84, March 2013. ISSN 1549-5477. DOI: 10.1101/gad.209296.112. URL http://dx.doi.org/10.1101/gad.209296.112

LONGER TERM GLUCOSE CONTROL IS REGULATED BY TWO OTHER HORMONES PREVIOUSLY DISCUSSED, GROWTH HORMONE AND CORTISOL. These hormones are elevated during times of growth or stress where it is important to keep circulating glucose available for other functions. During a prolonged fast, both GH and cortisol can be released, causing longer-lasting changes which ensure adequate blood supply to the brain.

INCRETINS ARE ENHANCERES OF INSULIN RELEASE, typically released from the gut. They were first described when it was noted that when equal amounts of glucose are provided either through the gut, or intravenously, the gut-supplied glucose leads to a more robust insulin secretion effect. Eventually to gut-derived peptide hormones, GLP-1 and GIP1[13] were described. Both of these peptides are degraded by an enzyme called DPP-4, and inhibitors of this process have provided an exciting new potential therapeutic mechanism for enhancing glucose control.

[13] glucagon-like peptide 1 and gastric inhibitory peptide respectively

Pathophysiology Related to Glucose Control

Type I Diabetes Mellitus

Type I Diabetes is typically caused by autoimmune destruction of pancreatic beta cells. Without these cells, the pancreas is unable to produce insulin and without careful monitoring and exogenous insulin, blood glucose levels will rise.

Insulin Resistance and Type II Diabetes Mellitus

Type II diabetes occurs as a result of a multi-step process starting with negative feedback loops on insulin signaling. As more nutrients are stored, for example in obesity metabolic tissues become resistant to the effects of insulin, likely as a way to protect against excessive lipid storage.

As tissues become more insulin resistant, more insulin must be secreted by the pancreas to maintain normoglycemia. If insulin resistance proceeds, more and more insulin will need to be produced and secreted by beta cells. Eventually the beta cells will be unable to keep up with this demand and glucose levels will rise as the amount of endogenous or exogenous insulin is less and less effective.

These concepts and the complications of diabetes will be covered in the next several lectures by Drs. Dagogo-Jack, Steinberg, Gosmanov and Gupta. Dr. Cook will describe some of the common medications for diabetes on Friday.

References

F G Banting, C H Best, J B Collip, W R Campbell, and a a Fletcher. Pancreatic Extracts in the Treatment of Diabetes Mellitus. *Canadian Medical Association journal*, 12(3):141–6, March 1922. ISSN 0008-4409. URL http://www.ncbi.nlm.nih.gov/pubmed/17580419.

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