

5-2015

DISTRIBUTION OF GHRELIN IN PANCREATIC ISLET CELLS OF NORMAL AND DIABETIC RATS AND ITS EFFECT ON DIABETES MELLITUS

Haba Y. M. El Abadlah

Follow this and additional works at: https://scholarworks.uaeu.ac.ae/all_theses

Part of the [Medicine and Health Sciences Commons](#)

Recommended Citation

M. El Abadlah, Haba Y., "DISTRIBUTION OF GHRELIN IN PANCREATIC ISLET CELLS OF NORMAL AND DIABETIC RATS AND ITS EFFECT ON DIABETES MELLITUS" (2015). *Theses*. 66.
https://scholarworks.uaeu.ac.ae/all_theses/66

This Thesis is brought to you for free and open access by the Electronic Theses and Dissertations at Scholarworks@UAEU. It has been accepted for inclusion in Theses by an authorized administrator of Scholarworks@UAEU. For more information, please contact fadl.musa@uaeu.ac.ae.

United Arab Emirates University
College of Medicine and Health Sciences
Department of Pharmacology

DISTRIBUTION OF GHRELIN IN PANCREATIC ISLET CELLS
OF NORMAL AND DIABETIC RATS AND ITS EFFECT ON
DIABETES MELLITUS

Haba Y. M. El Abadlah

This thesis is submitted in partial fulfillment of the requirements for the degree
of Master of Medical Sciences (Pharmacology and Toxicology)

Under the Supervision of Professor Ernest Adeghate

May 2015

Declaration of Original Work

I, Haba Y. M. El Abadlah, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled “*Distribution of Ghrelin in Pancreatic Islet Cells of Normal and Diabetic Rats and its Effect in Diabetes Mellitus*”, hereby, solemnly declare that this thesis is an original research work that has been done and prepared by me under the supervision of Professor Ernest Adeghate, in the College of Medicine and Health Sciences at UAEU. This work has not been previously formed as the basis for the award of any academic degree, diploma or a similar title at this or any other university. The materials borrowed from other sources and included in my thesis have been properly cited and acknowledged.

Student's Signature _____

Date _____

Copyright © 2015 Haba Y. M. El Abadlah
All Rights Reserved

This Master Thesis is approved by the following Examining Committee Members:

- 1) Advisor (Committee Chair): Ernest Adeghate

Title: Professor

Department of Anatomy

College of Medicine and Health Sciences

Signature _____

Date _____

- 2) Member: Abdu Adem

Title: Professor

Department of Pharmacology and Therapeutics

College of Medicine and Health Sciences

Signature _____

Date _____

- 3) Member: Frank C. Howarth

Title: Professor

Department of Physiology

College of Medicine and Health Sciences

Signature _____

Date _____

- 4) Member (External Examiner): Masayasu Kojima

Title: Professor

Department of Molecular Genetics

Institution: Institute of Life Science, Kurume University, Fukuoka, Japan

Signature _____

Date _____

This Master Thesis is accepted by:

Dean of the College of Medicine and Health Sciences: Professor Dennis Templeton

Signature _____ Date _____

Dean of the College of the Graduate Studies: Professor Nagi T. Wakim

Signature _____ Date _____

Copy ____ of ____

Abstract

Ghrelin, a 28-amino acid peptide, is identified as the endogenous ligand of the orphan growth hormone secretagogue-receptor type 1a. Ghrelin presents a unique modification at Ser3 position essential for its activity. It was first discovered in the stomach of rat in 1999. Aside from ghrelin's role as a potent growth hormone secretagogue and food intake modulator, ghrelin is thought to play a role in insulin and glucagon secretion and in glucose homeostasis. A lot of contradictory data have been reported in the literature regarding ghrelins co-localization with other hormones in the islets of Langerhans, its role in insulin secretion and attenuation of type 2 diabetes mellitus. In this study, we investigated the effect of chronic ghrelin treatment on glucose, body weight and insulin level in normal, STZ-induced diabetic and ghrelin-treated male Wistar rats. We also examined the distribution pattern and co-localization of ghrelin in the pancreatic islet of Langerhans with both pancreatic hormones; insulin and glucagon. In addition, we examined how ghrelin treatment influences liver function in normal and STZ-diabetic Wistar rats. Control groups received intraperitoneal injection of normal saline while treated groups received intraperitoneal injections of 5µg/kg ghrelin on daily basis for duration of four weeks. Our results show that administration of ghrelin increases the serum insulin level in both normal and diabetic rats. We also demonstrated that ghrelin co-localizes with insulin as well as glucagon in the pancreatic islet cells and that the pattern of ghrelin distribution is shown to alter after the onset of diabetes. Moreover, ghrelin treatment increased insulin secretion as a result of increasing insulin-secreting β cells. In conclusion ghrelin co-localizes with both insulin and glucagon in pancreatic islet cells and plays a regulatory role in insulin secretion.

Keywords: Ghrelin, diabetes mellitus, streptozotocin (STZ), immunohistochemistry (IHC), liver functions, insulin secretion.

Title and Abstract (in Arabic)

توزيع الجريلين (ghrelin) في خلايا جزر البنكرياس في الجرذان المصابة بالسكري و
السليمة غير المصابة و تأثيره على مرض السكري

الملخص

الجريلين (ghrelin) هرمون يفرز من المعدة ويتكون من 28 حمض أميني، تم اكتشافه عام 1999 على أنه محفز قوي لافراز هرمون النمو . يتميز الجريلين بخاصية تعديل حمضه الأميني الثالث سيرين (Ser3) ليتحول إلى شكله النشط الذي يرتبط بدوره بمستقبلات افراز هرمون النمو من نوع (GHS-R 1a) a1 ليقوم بلعب دوره في افراز هرمون النمو و دوره كهرمون منظم للشهية و كافة الأنشطة الأخرى المعروفة عنه. من جهة أخرى، يظن الباحثون أن الجريلين يلعب دور في افراز هرمون الانسولين و الجلوكاجون و أيضا في تعديل مستويات السكر في الدم.

نظراً لاختلاف الباحثين بخصوص تأثير الجريلين على افراز هرمون الانسولين وقدرته على تحسين مرض السكر من النوع الثاني ، و تعارض نتائج الأبحاث حول تواجد الجريلين في خلايا بيتا المفرزة للانسولين و خلايا ألفا المفرزة للجلوكاجون في جزر البنكرياس. قمنا بتصميم هذه الدراسة للبحث عن تأثير العلاج بالجريلين على جرذان من نوع Wistar غير المصابين بالسكر و مقارنة نتائجهم بآخرين تم حقنهم بمادة سترپتوزوتوسين (Streptozotocin "STZ") التي تستخدم في الأبحاث للتسبب بمرض السكر لدى القوارض و غيرها.

كما تمت مقارنة نتائج العلاج بالجريلين بنتائج مجموعة أخرى من الجرذان تمت معالجتهم بمحلول ملحي (Normal Saline) بعد 4 أسابيع من العلاج تم خلالها حقن 5 ميكروجرام/كيلوجرام من الجريلين في الصفاق وما يقابلها من المحلول الملحي بشكل يومي.

يكمُن الهدف من هذه الأطروحة في الكشف عن دور الجريلين كعلاج لمرض السكر، حيث تقوم الدراسة بعرض تأثير العلاج بالجريلين على مستويات السكر بالدم وقدرته على إفراز الانسولين ومدى تأثيره على الوزن نظراً لكونه هرمون فاتح للشهية. كما تمت دراسة تأثير العلاج بالجريلين على وظائف الكبد.

بالإضافة إلى ذلك، تمت دراسة نمط توزيع الجريلين في جزر البنكرياس وتواجده في خلايا بيتا المفرزة لهرمون الانسولين و خلايا ألفا المفرزة لهرمون الجلوكاجون. كشف البحث عن تواجد الجريلين في خلايا بيتا و ألفا على حد سواء حيث يختلف التوزيع نسبياً بعد الإصابة بمرض السكر. كما أثبتت الدراسة قدرت العلاج بالجريلين على زيادة نسبة الانسولين في الدم؛ كنتيجة لزيادة عدد الخلايا المفرزة للانسولين في جزر البنكرياس.

مفاهيم البحث الرئيسية: الجريلين (Ghrelin)، مرض السكر، ستريبتوزوتوسين (Streptozotocin “STZ”)، اختبار المناعة الكيمونسيجية (Immunohistochemistry “IHC”)، وظائف الكبد، إفراز هرمون الانسولين.

Acknowledgements

In the name of Allah, most gracious and most merciful. Praise be to Allah, Lord of the worlds for blessing me with the strength and patience I needed to go through this journey, and for surrounding me with amazing people who helped me through difficult times and stood with me every time I felt like giving up.

Foremost, I would like to express my sincere gratitude to Prof. Ernest Adeghate for the continuous support of my MSc study and research and for his understanding and encouragement.

I would also like to thank the members of my thesis committee; Prof. Abdu Adem and Prof. Frank C. Howarth, for their encouragement and insightful comments.

My sincere thanks also go to my external examiner Prof. Masayasu Kojima, for his motivational comments, enthusiasm and most of all for being an inspiration.

I am also grateful to Mr. Rasheed Hameed and Mrs. Crystal Melanie D'Souza. I am extremely thankful to both of them for their sincere help, guidance and constant encouragement.

I would like to take this opportunity to express gratitude to my family for the endless encouragement, unconditional love and support, prayers and patience, and for their understanding. I am grateful to have such a loving family who supported my ambitions by believing in me.

I am also immensely grateful to my best friends Rana, Safa and Reem FarajAllah for their love, care and ultimate support and encouragement. I am blessed to have them in my life. They made me laugh and forget my troubles so I couldn't have done it without them.

Thanks to my dearest friend Amal who was always there for me in every step of this journey. Thanks for really helping me when I needed it the most. Thank you to my lovely friend Salma, for her constant encouragement and her confidence in me. Thanks to Rawan for being a sister at heart, for the sweet memories we shared together and for just being amazing. Finally I would like to thank Deena for her amazing help in my defense, her valuable input and for going out of her way for me.

Dedication

To my beloved parents and family

Table of Contents

Title.....	i
Declaration of Original Work.....	ii
Copyright.....	iii
Approval of the Master Thesis.....	iv
Abstract.....	vi
Title and Abstract (in Arabic).....	vii
Acknowledgements.....	ix
Dedication.....	xi
Table of Contents.....	xii
List of Tables.....	xvii
List of Figures.....	xviii
Chapter 1: Introduction.....	1
1.1 Pancreas.....	1
1.1.1 Exocrine Part of the Pancreas.....	3
1.1.2 Endocrine Part of the Pancreas.....	4
1.1.3 Hormones of the Pancreas.....	5
1.1.3.1 Insulin.....	5
1.1.3.1.1 Discovery of Insulin.....	6
1.1.3.1.2 Synthesis of Insulin.....	8
1.1.3.1.3 Function of Insulin.....	9
1.1.3.1.4 Receptors of Insulin.....	9
1.1.3.1.5 Mechanism of Action Insulin.....	10
1.1.3.2 Glucagon.....	11
1.1.3.3 Somatostatin.....	11

1.1.3.4 Pancreatic Polypeptide.....	12
1.1.4 Diseases of the Pancreas.....	12
1.2 Diabetes Mellitus.....	13
1.2.1 History of Diabetes Mellitus.....	13
1.2.2 Epidemiology and Prevalence of Diabetes Mellitus.....	14
1.2.3 Definition of Diabetes Mellitus.....	15
1.2.4 Classifications of Diabetes Mellitus.....	15
1.2.4.1 Type 1 Diabetes Mellitus.....	16
1.2.4.2 Type 2 Diabetes Mellitus.....	17
1.2.4.3 Gestational Diabetes Mellitus.....	17
1.2.4.4 Other Types of Diabetes Mellitus.....	18
1.2.5 Symptoms of Diabetes Mellitus.....	18
1.2.6 Criteria for Diagnosis of Diabetes Mellitus.....	20
1.2.7 Complications of Diabetes Mellitus.....	20
1.2.8 Management and Treatment of Diabetes Mellitus.....	22
1.2.8.1 Nutrition.....	22
1.2.8.2 Exercise.....	23
1.2.8.3 Pharmacotherapy.....	24
1.2.8.3.1 Insulin Treatment of Diabetes Mellitus.....	24
1.2.8.3.2 Oral Antidiabetic Agents.....	25
1.2.8.3.2.1 Insulin Secretagogues.....	26
1.2.8.3.2.2 Biguanids.....	27
1.2.8.3.2.3 Thiazolidinediones or Glitazones.....	27
1.2.8.3.2.4 α -Glucosidase Inhibitors.....	28
1.2.8.3.2.5 Other Antidiabetic Agents.....	28
1.2.8.4 Surgery.....	29

1.2.8.4.1 Islet and Pancreatic Transplantation.....	30
1.2.8.4.2 Other Types of Treatments.....	31
1.2.8.4.3 Glucagon-like Peptide-1.....	31
1.2.8.4.4 Leptin.....	32
1.2.8.4.5 Substance P.....	32
1.2.8.4.6 Ghrelin.....	33
1.3 Ghrelin	34
1.3.1 Discovery of Ghrelin.....	34
1.3.2 Structure of Ghrelin.....	34
1.3.3 Ghrelin Receptor.....	35
1.3.4 Tissue Distribution of Ghrelin.....	36
1.3.5 Ghrelin Signaling Pathway.....	37
1.3.6 Effect of Ghrelin on Food Intake.....	39
1.3.7 Effect of Ghrelin on Body Weight and Obesity.....	39
1.3.8 Ghrelin in Pancreatic Islet Cells and its Association with Islet Cells Hormones.....	40
1.3.8.1 Ghrelin and Insulin.....	40
1.3.8.2 Ghrelin and Other Islets Hormones.....	41
1.3.9 Ghrelin Effect on Diabetes Mellitus.....	42
1.3.10 Other Functions and Role of Ghrelin in Different Tissues.....	42
1.4 Streptozotocin and its Diabetogenic Effects.....	45
Chapter 2: Hypothesis, Aims and Objectives.....	47
Chapter 3: Materials and Methods.....	48
3.1 Animal Models.....	48
3.2 Induction of Experimental Diabetes Using Streptozotocin.....	48
3.3 Experimental Design.....	48

3.4 Fasting Blood Glucose and Body Weight Measurements.....	50
3.5 Glucose Tolerance Test.....	50
3.6 Blood and Tissue Collection	51
3.7 Tissue Processing	51
3.7.1 Tissue Processing for Immunohistochemistry and Immunofluorescence Study.....	51
3.7.2 Tissue Processing for Electron Microscopy.....	52
3.8 Immunohistochemistry Study: Avidin-Biotin Complex Staining Method for Paraffin Section.....	53
3.9 Double Labeling Immunofluorescence Staining Method for Paraffin Sections.....	54
3.10 Transmission Electron Microscopy Post-embedding Immunogold Double Labeling Protocol.....	55
3.11 Enzyme-Linked Immunosorbent Assay.....	56
3.12 Biochemical Analysis.....	58
3.13 Statistical Analysis.....	58
Chapter 4: Results	59
4.1 Effect of Ghrelin Treatment on Fasting Blood Glucose Level of Normal and Diabetic Wistar Rats.....	59
4.2 Effect of Ghrelin Treatment on Body Weight of Normal and Diabetic Wistar Rats.....	60
4.3 The Effect of Four Weeks of Ghrelin Treatment on Preventing or Delaying the Onset of STZ-induced Diabetes Mellitus in Wistar Rats.....	61
4.4 The Effect of Ghrelin Treatment on Glucose Tolerance Test.....	62
4.5 Summary and Discussion – I.....	63
4.6 Immunohistochemical Localization of Insulin and Ghrelin in Normal and Diabetic Rat Pancreas.....	64
4.6.1 Insulin.....	64
4.6.2 Ghrelin.....	65

4.7 Summary and Discussion – II.....	69
4.8 Effect of Ghrelin on Serum Insulin Level.....	70
4.9 Summary and Discussion – III	71
4.9.1 Co-Localization of Ghrelin with Insulin and Glucagon in Pancreatic Islet Cells.....	72
4.9.1.1 Ghrelin and Insulin.....	72
4.9.1.2 Ghrelin and Glucagon.....	74
4.9.2 Localization of Ghrelin and Insulin in the Secretory Granules of Pancreatic β Cells.....	76
4.9.2.1 Wistar Normal Control Rat.....	76
4.9.2.2 Wistar Normal Treated Rat.....	77
4.9.2.3 Wistar Diabetic Control Rat.....	78
4.9.2.4 Wistar Diabetic Treated Rat.....	79
4.10 Summary and Discussion – IV.....	80
4.11 Effect of Ghrelin Treatment on Liver Function.....	81
4.11.1 Alanine Transaminase.....	81
4.11.2 Aspartate Transaminase.....	82
4.12 Summary and Discussion – VII.....	83
Chapter 5: Summary	84
Chapter 6: Discussion	85
Chapter 7: Conclusion.....	88
Chapter 8: Limitations and Future Prospective.....	89
Bibliography.....	90
List of Publications.....	126

List of Tables

Table 1: The prevalence of diabetes mellitus in the Middle East and North Africa. .	14
Table 2: Classification of different type of insulin injection according to their duration of action.	25
Table 3: List of names and dilutions of the primary and secondary antibodies used in this study	55

List of Figures

Figure 1: Location of the pancreas.....	2
Figure 2: Structure of the pancreas.	2
Figure 3: Pancreatic blood supply and innervations.	3
Figure 4: Distribution of pancreatic endocrine cells.	5
Figure 5: Discoverer of insulin.	7
Figure 6: Structure and formation of insulin.....	8
Figure 7: Insulin receptor.....	10
Figure 8: Mechanism of action of Insulin.....	11
Figure 9: Diabetes mellitus symptoms.....	19
Figure 10: Diabetes mellitus complications.....	21
Figure 11: Sulfonylureas mechanism of action.....	26
Figure 12: Schematic illustration of the mechanism of action of metformin on the liver cell.....	27
Figure 13: Ghrelin synthesis and structure.....	35
Figure 14: Signaling pathway of ghrelin-inducing GH release.	37
Figure 15: Signaling pathway of ghrelin-inducing food intake in the hypothalamus.....	38
Figure 16: Ghrelin signaling in islet β cells.	41
Figure 17: Physiological action of ghrelin.	44
Figure 18: Streptozotocin mechanism in inducing diabetes mellitus.....	46
Figure 19: Schematic drawing describing animal groups in this study.	49
Figure 20: Schematic drawing explaining the study design of pre-diabetic ghrelin-treated group.....	50
Figure 21: Illustration of Mercodia Ultrasensitive Rat Insulin ELISA protocol.	57
Figure 22: Effect of ghrelin treatment on fasting blood glucose level of normal and diabetic wistar rats.....	59
Figure 23: Effect of ghrelin treatment on body weight of normal and diabetic wistar rats.....	60
Figure 24: The effect of four weeks of ghrelin treatment on preventing or delaying the onset of STZ-induced diabetes mellitus in wistar rats.	61
Figure 25: The effect of ghrelin treatment on glucose tolerance test.....	62
Figure 26: Immunohistochemical localization of insulin and ghrelin in normal and diabetic rat pancreas.....	66
Figure 27: Percentage of insulin- and ghrelin-immunopositive cells.	68
Figure 28: Effect of ghrelin on serum insulin level.	70
Figure 29: Co-localization of ghrelin with insulin in pancreatic islet cells.	73
Figure 30: Co-localization of ghrelin with glucagon in pancreatic islet cells.....	75
Figure 31: Localization of ghrelin and insulin in the secretory granules of pancreatic β cells of normal control rats.....	76
Figure 32: Localization of ghrelin and insulin in the secretory granules of pancreatic β cells of normal ghrelin-treated rats.	77

Figure 33: Localization of ghrelin and insulin in the secretory granules of pancreatic β cells of diabetic control rats.	78
Figure 34: Localization of ghrelin and insulin in the secretory granules of pancreatic β cells of diabetic ghrelin-treated rats.	79
Figure 35: Serum Alanine Transaminase levels.....	81
Figure 36: Serum Aspartate Transaminase levels.....	82
Figure 37: Summary of the outcomes in this study.	84
Figure 38: Conclusion of the study	88

Chapter 1: Introduction

1.1 Pancreas

The word pancreas is derived from Greek word “Pan-Kreas” that means all flesh. The pancreas is an elongated, soft, J shaped gland. It is light pink in color, measures approximately 12-15 cm in length and weighs about 70 – 100 g (Tan, 2008). It was the last organ in the abdomen to attract the attention of anatomists, physiologists, physicians and surgeons.

It is located in the abdominal cavity, in the upper part, behind the stomach (Figure 1). The Pancreas is divided into 4 parts: head, neck, body and tail (Figure 2). The head, which is the wider end of the pancreas, lies within the curvature of the duodenum. The rest of the pancreas lies obliquely in the posterior abdomen, with the tail extending as far as the gastric surface of the spleen. The neck connects the head to the main part of the pancreas: the body. The latter is the biggest and main part of the pancreas located to the left of the superior mesenteric blood vessels. The superior mesenteric vein joins the splenic vein behind the neck of the pancreas to form the portal vein (Moore & Dalley, 1999). The pancreas receives its blood supply from the superior and inferior pancreaticoduodenal arteries, while the splenic artery supplies the tail and part of the body of the pancreas (Figure 3) (Faiz & Moffat, 2006).

The pancreas is divided into lobules by connective tissue septae (Guyton, 1981). This organ plays a dual role as a secretory gland (Eroschenko, 2008), with both exocrine and endocrine functions. The exocrine part makes, secretes and releases digestive enzymes into the duodenum. While, the endocrine part, which is embedded within the exocrine tissue is responsible for making and secreting a

variety of pancreatic hormones into the blood stream (Petersen, 1992).

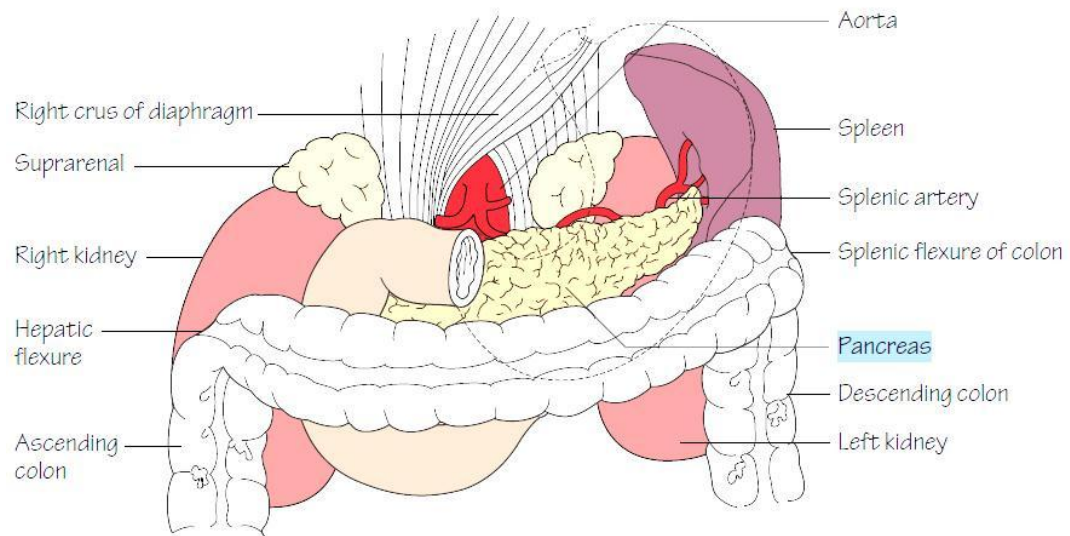


Figure 1: Location of the pancreas.

“Reproduced and edited (Faiz & Moffat, 2006)”.

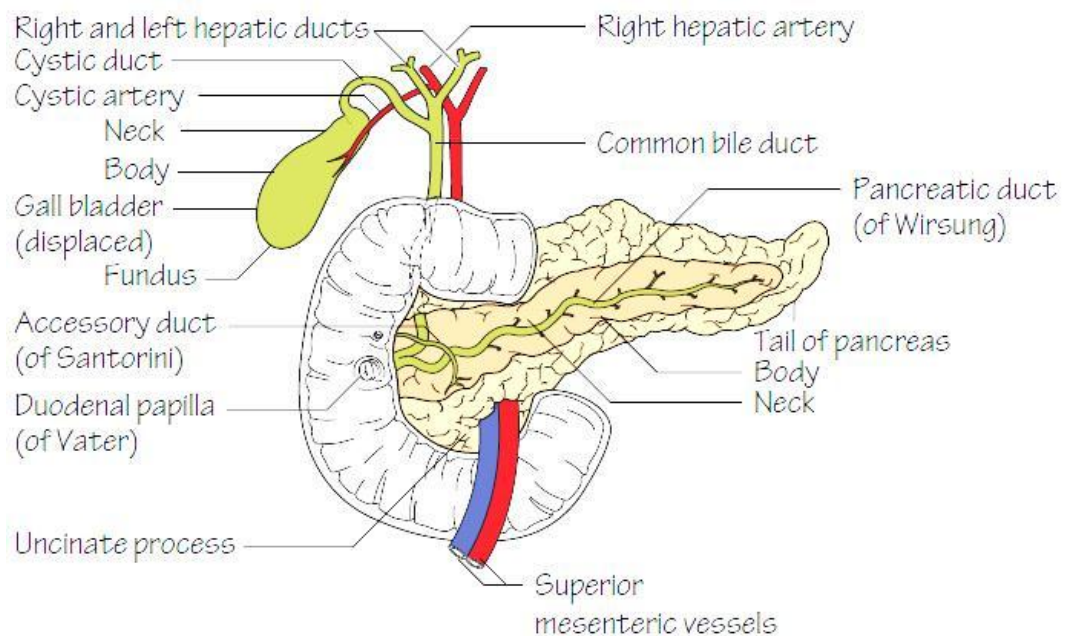


Figure 2: Structure of the pancreas.

“Reproduced and edited (Faiz & Moffat, 2006)”.

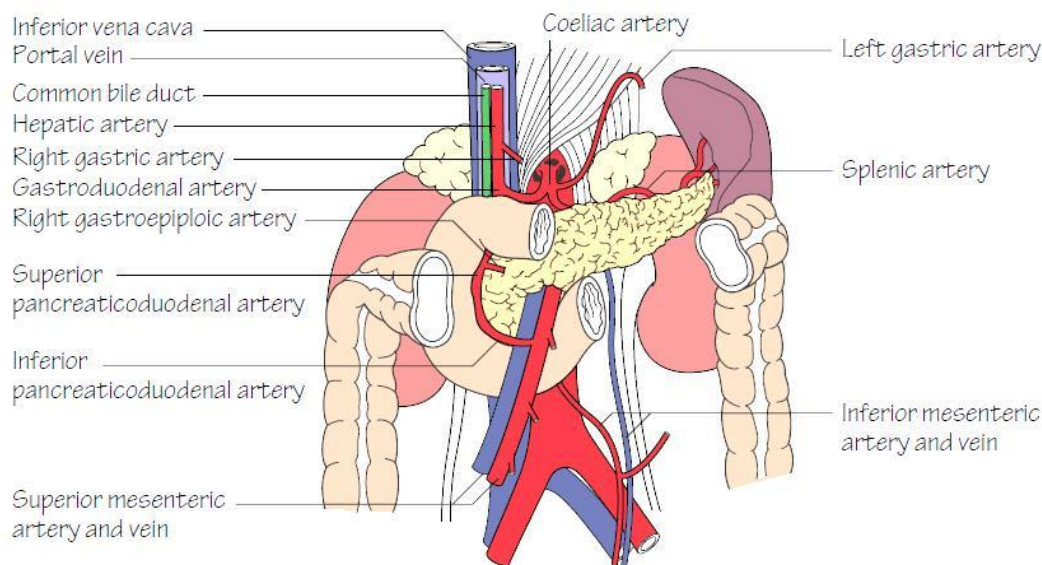


Figure 3: Pancreatic blood supply and innervations.

“Reproduced and edited (Faiz & Moffat, 2006)”.

1.1.1 Exocrine Part of the Pancreas

The exocrine part is a grape-like cluster of acinar cells which comprises more than 95% of the pancreas mass. This portion of the pancreas is responsible for making and secreting the digestive enzymes which are released into the duodenum. It is composed mainly of acinar and ductal cells with associated connective tissue, vessels and nerves. The main pancreatic duct is referred to as “Wirsung”, which begins from the tail and runs to the head of the pancreas to join the common bile duct to form the hepatopancreatic ampulla (Bockman, Büchler, & Beger, 1986). The accessory pancreatic duct, which is referred to as the duct of “Santorini” is superior to the opening of the main duct. The two ducts are responsible for draining the exocrine secretions through the ampulla of Vater into the duodenum (Campbell & Verbeke, 2013; Eroschenko, 2008). Digestive enzymes, bicarbonate, NaCl and water are secreted into the duodenum via pancreatic ducts. These enzymes help in the digestion of carbohydrates, proteins, and fats and neutralizing stomach acid in

the duodenum (Ganong, 2005; Pandol, 2011).

1.1.2 Endocrine Part of the Pancreas

Endocrine cells, limited to about 1-2% of the whole pancreatic tissue, (Barrett, Ghishan, Merchant, Said, & Wood, 2006; Greenstein & Wood, 2011; Tortora & Derrickson, 2011), regulate pancreatic and gastrointestinal functions and regulate energy metabolism of the whole-body. The endocrine part is composed mainly of four types of cells: Alpha (α) (17%), Beta (β) (70%), Delta (δ)(7%) and PP or Gamma (γ) (Figure 4).

These cells together form what is called the islet of Langerhans, first described by the German doctor, Paul Langerhans in 1869 (Karamitsos, 2011; Rosenfeld, 2002a). These cells are richly vascularized and embedded within the exocrine tissue (Ganong, 2005). Recently, a new cell type termed epsilon (ϵ) cells has been discovered in the islets of Langerhans (Ross & Pawlina, 2006). Pancreatic islet cytoarchitecture differs between species. In rodents, β cells are predominantly found as a cluster in the core of the islet, while α , δ and γ cells are scattered in the periphery. On the other hand, human β cells are not clustered and the majority of the β cells are shown to be scattered in the islet in association with α and δ cells (Brissova et al., 2005; Cabrera et al., 2006).

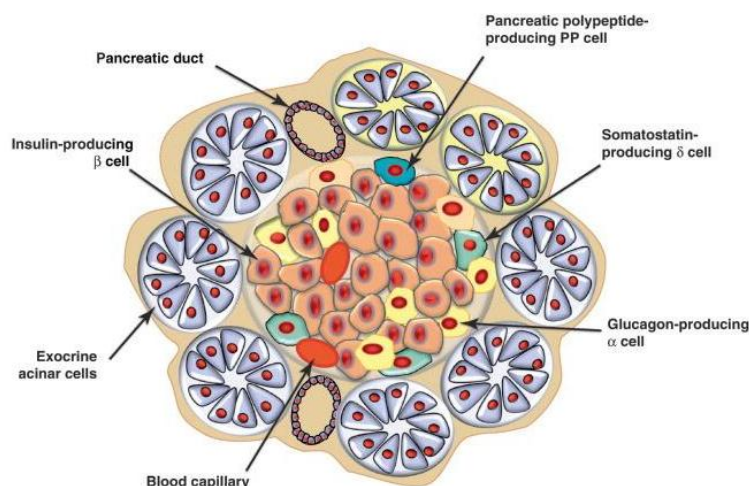


Figure 4: Distribution of pancreatic endocrine cells.

“Modified from Trends Endocrinology and Metabolism, 2012” (Efrat & Russ, 2012).

1.1.3 Hormones of the Pancreas

The endocrine part of the pancreas produces important hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide, which play a key regulatory role in several metabolic functions either systemically, regionally (in the gastrointestinal tract) or locally (in the islet) (Ross & Pawlina, 2006).

1.1.3.1 Insulin

Insulin is a polypeptide hormone composed of 51 amino acids distributed in 2 polypeptide chains, which are linked together by two disulfide bridges [(Figure 5.A); (Harvey & Ferrier, 2011)]. Insulin is the most abundant endocrine polypeptide (Ross & Pawlina, 2006) and the most well-known pancreatic hormone produced by β cells (Tan, 2008). Insulin secretion is stimulated by elevated blood glucose level after a meal or when a person is challenged with glucose (Tortora & Derrickson, 2011).

1.1.3.1.1 Discovery of Insulin

In 1920, Frederick Banting had the idea that pancreatic digestive enzymes are harmful to the secretions produced by pancreatic islet of Langerhans. In 1921, Banting took his theory to Professor John Macleod at the University of Toronto. Macleod who was a leading figure in diabetes research in Canada provided Banting with a laboratory, a minimum number of equipment and ten dogs. Charles Best, a medical student joined Banting to assist him in the experiments which was assigned to start in the summer of 1921. They removed the pancreas from one dog and observed that the dog became very weak, thirsty and urinated more often. The dog blood glucose rose and it had developed diabetes. Using another dog, both Banting and Best surgically ligated the pancreas to prevent the flow of nourishment which will result in degeneration of the pancreas. Later on, they isolated the pancreas, froze it, grinded it and filtered it. When the diabetic dog (first dog) was injected with the filtered extract, its blood glucose dropped. Its health appeared to be improving and it was symptoms free with the repetitive administration of the extract, which Banting and Best called “isletin”. In late 1921, the biochemist, Bertram Collip joined Banting and Best, and his task was to help in purifying the pancreatic extract so it can be tested in human. In April, 1922 when Canadian researchers decided on the name of insulin, which had been proposed by a Belgian doctor a few years prior to its isolation. Collip succeeded in purifying the extract and it was not until January 1923 when he witnessed the first administration of “insulin” by intramuscular injected to Leonard Thomson, a 14-year-old boy, who weighed a mere 27 kg. It was a dramatic moment to see his blood glucose level drop from 440 to 320 mg/dl. The observation of Leonard’s health after the insulin injection encouraged the team to introduce the treatment and test it in more diabetic volunteers, who had the same

response as Leonard to the insulin extract. The Nobel Prize was awarded to Banting and Macleod on October 26th, 1923. Banting shared his half with Best, while Macleod split his with Collip (Karamitsos, 2011; Pavel & Sdrobici, 1972; “The Discovery of Insulin,” n.d.) (Figure 5).

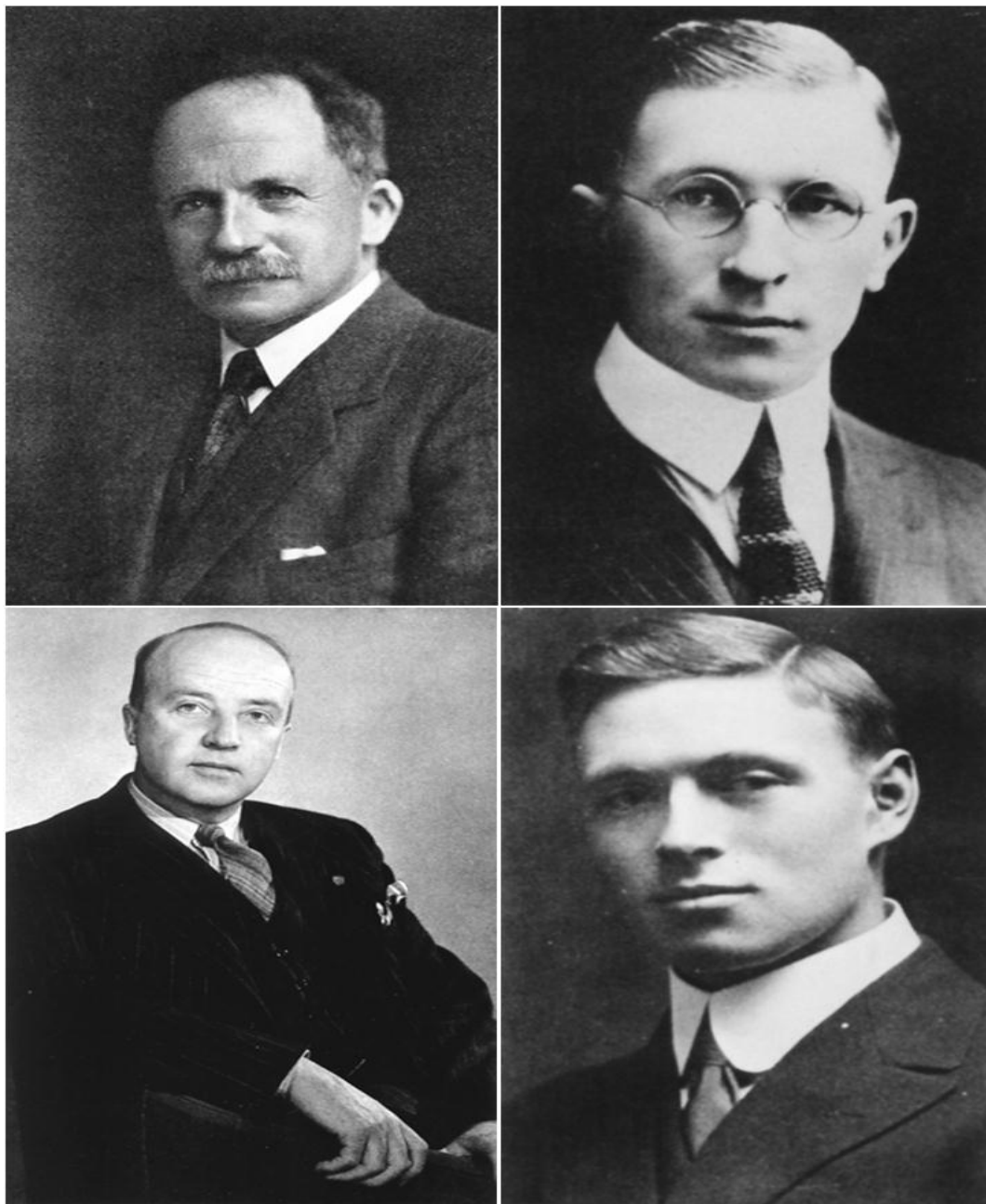


Figure 5: Discoverer of insulin.

From top, J.J.R. Macleod (left), Frederick Banting (right), from bottom, Charles Best (left) and Bertram Collip (right) (Rosenfeld, 2002b).

1.1.3.1.2 Synthesis of Insulin

As shown in Figure 6.B, insulin is the end product of an inactive precursor called preproinsulin. First, “proinsulin” is generated after cleavage of its N-terminal signal peptide during insertion into the endoplasmic reticulum (ER). Proinsulin consists of three domains: an N-terminal B chain, a connecting peptide in the middle known as the C peptide and a C-terminal A chain. Within the ER, proinsulin is exposed to several specific endopeptidases, which excise the C peptide and so yield the mature insulin. Finally, the mature form of insulin and the free C-peptide are packaged in the Golgi apparatus to form secretory granules which accumulate in the cytoplasm. Since C-peptide and insulin are produced at the same rate, C-peptide is a useful marker that indicates the effectiveness of insulin production and secretion in early DM (Harvey & Ferrier, 2011).

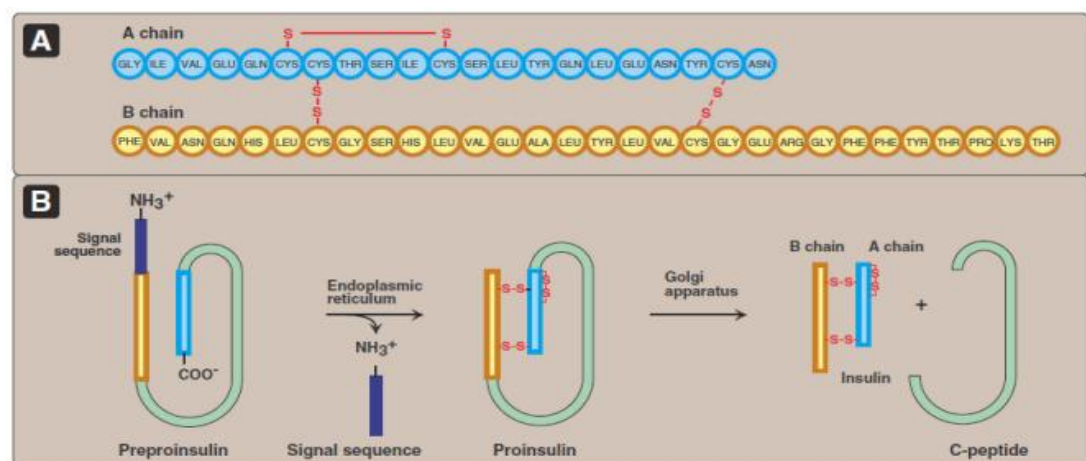


Figure 6: Structure and formation of insulin.

Insulin structure (A) and formation from preproinsulin (B) (Harvey & Ferrier, 2011).

1.1.3.1.3 Function of Insulin

The principal actions of insulin take place in the liver, skeletal muscle and adipose tissue (Ross & Pawlina, 2006). Insulin lowers the glucose level in the blood by accelerating the membrane transport of glucose into the liver, muscles cells and adipose cells. Insulin accelerates the conversion of glucose into glycogen in liver cells (Eroschenko, 2008). Insulin does not only influence glucose metabolism, but also plays a role in protein and fat metabolism (Tan, 2008).

1.1.3.1.4 Receptors of Insulin

The insulin receptor, which belongs to the tyrosine kinase superfamily, is a heterotetrameric glycoprotein that comprises two subunits: α (723 amino acids) and β (620 amino acids). Alpha subunits exist primarily extracellularly, while β subunits are transmembrane proteins. Both subunits are connected together with disulfide bonds (Fig. 7). Binding of insulin molecules to α subunits lead to conformational changes of the receptors resulting in an autophosphorylation of tyrosine residues by enabling ATP to bind to the β subunits in the intracellular domain (De Meyts, 2004; Hubbard, 2013; C. R. Kahn & White, 1988; Kido, Nakae, & Accili, 2001).

Insulin receptors are expressed on virtually all mammalian tissues. The number of receptors varies from one site to another with nearly more than 200,000 receptors on adipocytes and hepatocytes. The brain was initially thought to be an insulin-dependent organ. This was based on the awareness that insulin cannot permeate the blood-brain barrier (C. R. Kahn & White, 1988; Watanabe, Hayasaki, Tamayama, & Shimada, 1998), but the brain has been shown to have insulin receptors (Schulingkamp, Pagano, Hung, & Raffa, 2000).

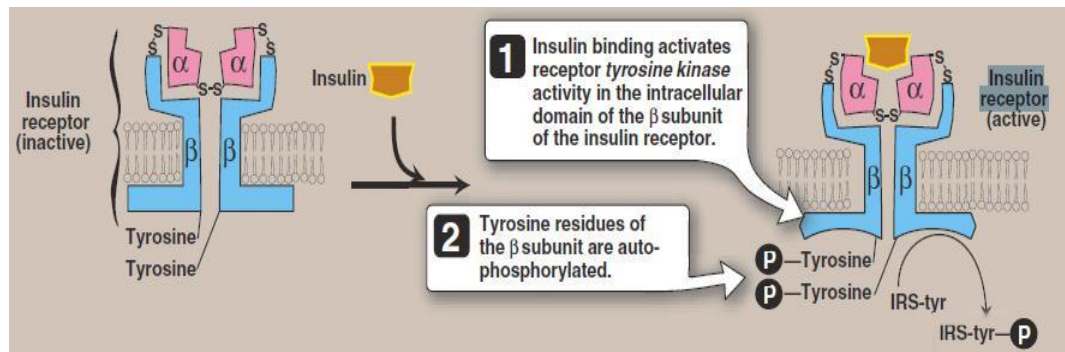


Figure 7: Insulin receptor.

“Reproduced and edited (Harvey & Ferrier, 2011)”.

1.1.3.1.5 Mechanism of Action of Insulin

Once insulin binds to the α -subunits of the receptor, it provokes many reactions like the rapid recruitment of insulin-sensitive glucose transporters (GLUT-4) into adipocytes and skeletal muscle cells from a pool located in intracellular vesicles. After the autophosphorylation of β subunits, which in turn phosphorylate many other proteins such as the insulin receptor substrate proteins (IRS's). IRS's, then activate multiple signaling pathways and lead to many biological effects known to be exerted by insulin like facilitating the availability of glucose receptors and correspondingly the ability to uptake glucose molecules (Fig. 8) (Harvey & Ferrier, 2011).

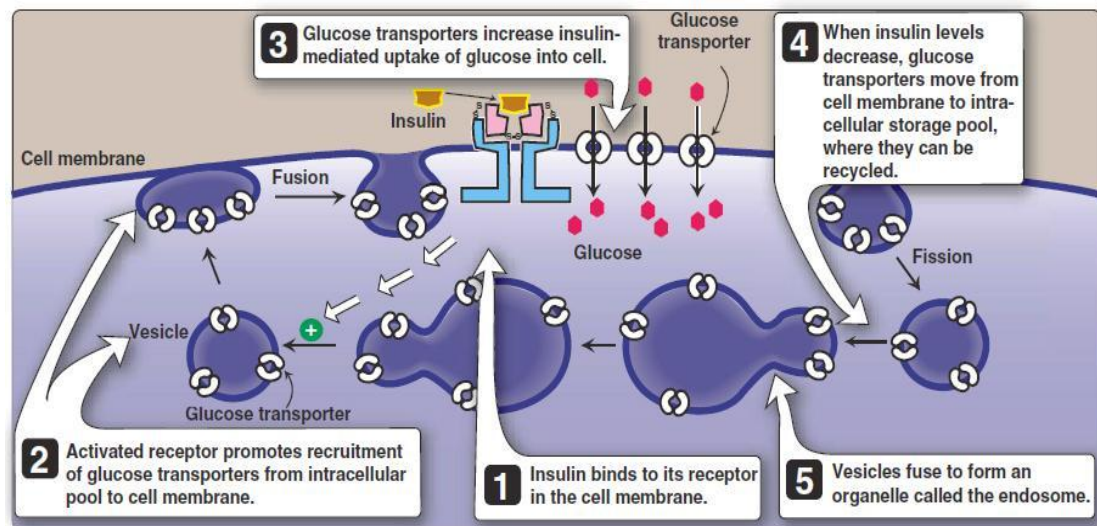


Figure 8: Mechanism of action of Insulin.

“Reproduced and edited (Harvey & Ferrier, 2011)”.

1.1.3.2 Glucagon

Glucagon is the second most important pancreatic endocrine hormone after insulin and it is secreted by α cells. Glucagon is usually released in response to low levels of glucose in the blood (Eroschenko, 2008). Its actions are essentially reciprocal to those of insulin. For example, it stimulates the release of glucose into bloodstream, glycogenesis and glyconeolysis in liver as well as the proteolysis to promote gluconeogenesis, mobilizes fats from adipose tissue cells and stimulates hepatic lipase (Ross & Pawlina, 2006).

1.1.3.3 Somatostatin

Somatostatin, which is produced by δ cells, acts in a paracrine manner to inhibit insulin and glucagon secretion. Moreover, it plays a role in slowing the absorption of nutrients from the gastrointestinal tract, gallbladder and inhibiting GH secretion (Ross & Pawlina, 2006; Tortora & Derrickson, 2011).

1.1.3.4 Pancreatic Polypeptide

PP cells also known as γ cells secrete a pancreatic polypeptide hormone, which inhibits somatostatin secretion, gallbladder contraction and secretion of digestive enzymes by the pancreas (Tortora & Derrickson, 2011). PP has been implicated in the regulation of energy balance (Asakawa et al., 2003).

1.1.4 Diseases of the Pancreas

Many diseases and disorders have been reported to affect the pancreas. In addition to developmental anomalies, and trauma, two distinct types of acute and chronic pancreatitis are the most common conditions along with pancreatic cancer. The pathogenesis of these disorders is not well established, yet all of them share the genetic, immunologic and environmental involvement in the manifestation of each and every one of them. Unfortunately, no cure is available till today. Patient therapy revolves around pain management and delaying the progression by medication, diet, endoscopy and surgery (Baker, 2004; Braganza, Lee, McCloy, & McMahon, 2011; DiMagno & DiMagno, 2012, 2012; Frossard, Steer, & Pastor, 2008, p. -; D. Li, Xie, Wolff, & Abbruzzese, 2004; Mitchell, Byrne, & Baillie, 2003; Spalding & Williamson, 2011; Vincent, Herman, Schulick, Hruban, & Goggins, 2011).

Diabetes Mellitus (DM) is one of the major disorders affecting the pancreas. It attracts a lot of attention because of its complex etiology and its high prevalence (Section 1.2).

1.2 Diabetes Mellitus

1.2.1 History of Diabetes Mellitus

The burden of DM has been growing over the years. DM which is becoming an epidemic disease is not a new phenomenon. DM is thought to have been described for the first time by an Egyptian called Hesy-Ra, who described a rare disease which causes loss of body weight, along with a frequent urge to urinate circa 1552 BC.

Aretaeus (30-90CE), the Greek physician was the one who gave diabetes its name (which in Greek means "flowing through"), when he recorded the symptoms of constant thirst, frequent urination and loss of body weight. Aretaeus thought it was a disease of the kidneys. In his book "Causes and Indications of Acute and Chronic Diseases", Aretaeus distinguished between DM and diabetes insipidus (Ahmed, 2002).

In 1025, in the book, "Canon of Medicine", Avicenna, also known as Ibn Sina (The famous Arabian Physician) described the symptoms and the complications of DM in detail.

The term "diabetes" came from the Greek word "diabainein", which means "to pass through", and later, in 1675 the Latin word "mellitus": i.e. honey-sweet' was added by the British physician and neuroanatomist, Thomas Willis (Howarth, 2013).

At that same time, an Indian physician called "Charaka Samhita", identified diabetes and called it "madhumeha", which means "honey urine", when he noticed that the urine attracts ants and he started to use ants to diagnose his patients with diabetes. Charaka and another Indian physician called Susruta were the first to

identify the difference between the two main types of DM, noticing that thin patients developed diabetes at a younger age compared to obese patients (“dLife Diabetes Museum,” n.d.).

1.2.2 Epidemiology and Prevalence of Diabetes Mellitus

As the human population’s growth, obesity, sedentary lifestyle, urbanization and aging increases, the prevalence of DM also increases (Wild, Roglic, Green, Sicree, & King, 2004). The fast increase of diabetes prevalence and the severity of its complications are complex challenges that researchers face. A lot of effort is invested in understanding the complexity of DM and many studies are searching for ways to cure DM, prevent it or delay its progression.

The sixth edition of the Diabetes Atlas published by the International Diabetes Federation estimated that, in 2014, 36.8 million people were diagnosed with DM in the Middle East and North Africa regions (“Diabetes Atlas | International Diabetes Federation,” n.d.), which corresponds to 9.7% of the population (Table 1).

Diabetes in MENA (20-79 years)	2014	2035
Total population (millions)	656	896
Adult population (millions)	378.3	583.7
Number of people with diabetes (millions)	36.8	67.9
Regional prevalence (%)	9.7	11.6
Comparative prevalence (%)	11.3	11.3
Undiagnosed cases (millions)	17.9	-
Total diabetes-related deaths (thousands)	362.8	-
Deaths under the age of 60 (%)	52.7	-
Total diabetes-related health expenditure (USD billions)	16.8	24.7

Table 1: The prevalence of diabetes mellitus in the Middle East and North Africa.

Modified from International Diabetes Federation, Diabetes Atlas, 6th edition, 2014 (“Diabetes Atlas | International Diabetes Federation,” n.d.).

Epidemiologists have looked into the prevalence of DM from many aspects, including the role that geographical location, ethnicity and gender play. Some reports indicate a higher prevalence of type 1 diabetes mellitus (T1DM) in men, while type 2 diabetes mellitus (T2DM) was reported to be higher in women. However, in another study which was conducted in a Mexican population, T2DM was found to be higher in men than in women. In addition, T1DM was found to be highest in Scandinavian and lowest in Japanese population (Ernest Adeghate, Schattner, & Dunn, 2006a).

An unhealthy diet and lack of exercise played a role in listing some of the six Gulf Cooperation Council Countries among the world's top 10 countries with the highest prevalence of DM (Guariguata et al., 2014). DM prevalence is estimated to be 9% and 25% in Bahrain, 16.1% in Oman, 21.4% in Kuwait, 16.7% in Qatar, 23.7% in Saudi Arabia and 23.3% in the United Arab Emirates (Howarth, 2013).

1.2.3 Definition of Diabetes Mellitus

According to the World Health Organization (WHO) report in 1999, DM is a metabolic disorder of multiple etiologies, characterized by chronic hyperglycemia with disturbance in carbohydrate, fat and protein metabolism, which results from defects in insulin secretion, action or both ("Diagnosis and Classification of Diabetes Mellitus," 2010, "WHO | Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia," n.d.).

1.2.4 Classifications of Diabetes Mellitus

DM was previously classified on the basis of insulin dependent treatment, yet; recently it changed according to the pathology of the disease along with the

physiological and morphological changes. In literature, T1DM and T2DM are known to be the main types of DM. Gestational diabetes (GDM) is another common type of DM which is less prevalent than the other two. Less common classes of DM include Maturity Onset Diabetes of the Young (MODY), pancreatic DM and others (See sections 1.2.4.3 & 1.2.4.4).

1.2.4.1 Type 1 Diabetes Mellitus

T1DM was previously called “Insulin Dependent Diabetes Mellitus (IDDM)” or “juvenile diabetes”; because it occurs in childhood or in young adults.

About 5-10% of cases of DM are T1DM, where the pancreas is either unable to produce insulin or it does but in very small amounts that do not meet the body's need to maintain its function. This may be a consequence of selective pancreatic β cells autoimmune destruction leading to a crucial decrease in the mass of these cells in the islets of Langerhans (E. Adeghate, 2001).

The events that trigger the autoimmune response remain unclear. The pathogenesis of T1DM is known to involve recruitment of CD_{4+} helper and CD_{8+} killer T lymphocytes and infiltration of macrophages causing certain interleukins to attack and destroy pancreatic islets (Gillespie, 2006).

Antibodies against insulin secreting cells, including those against insulin, glutamic acid decarboxylase and against the whole islet cells are known to be involved in the development of T1DM pathogenesis (45) and can be detected at birth or in infancy (Skyler & Sosenko, 2013).

Some researchers suspect a connection between some viruses and T1DM, especially when alpha interferon was found to be expressed in patients at the onset of the disease (Scherbaum, 1992). IgM against viruses like Coxsackie was found in

20 – 30% of patients with T1DM. Likewise, an increase in congenital rubella and mumps incidences (Ernest Adeghate, Schattner, & Dunn, 2006b), as well as enterovirus and rotavirus infections (Gillespie, 2006) were consistent with increased cases of T1DM.

Moreover, patients with T1DM were found to have other autoimmune disorders such as Addison's and autoimmune thyroid (Ernest Adeghate et al., 2006b).

1.2.4.2 Type 2 Diabetes Mellitus

The majority of patients (around 90%) diagnosed with DM have T2DM which previously was called “Non-Insulin Dependent Diabetes Mellitus (NIDDM)”.

T2DM is characterized mainly by insulin resistance with association of relatively reduced insulin secretion (S. E. Kahn, 2000; Stumvoll, Goldstein, & van Haeften, 2005). T2DM predisposition relies on lifestyle and genetics factors (Leahy, 2005). Obesity for instance is the main lifestyle factor which contributes in the manifestation and development of T2DM (S. E. Kahn, Hull, & Utzschneider, 2006; Mokdad AH, Ford ES, Bowman BA, & et al, 2003). Other factors known to increase the risk of developing T2DM are persistent stress and drugs like corticosteroids and oral contraceptives (Ernest Adeghate et al., 2006b).

1.2.4.3 Gestational Diabetes Mellitus

GDM is another common form of DM known to occur in late gestation and is characterized by insulin resistance (Ernest Adeghate et al., 2006b). A study done on Iranian women showed an increased risk of developing GDM in women with polycystic ovarian syndrome (Ashrafi et al., 2014). Women who develop GDM are at risk of developing T2DM (Kleinwechter et al., 2014).

1.2.4.4 Other Types of Diabetes Mellitus

Many cases do not fit into the above classification, yet these cases are considered to be DM. A specific type of DM may be caused by a mutation in the insulin receptor and or by genetic impairment of pancreatic β cells, whereas some may be caused by insufficient insulin release and impaired action. Other cases of DM may be a consequence of toxin-induced DM. Alzheimer's disease, caused by abnormal amyloid protein has been considered by some as the third type of DM (T3DM) (American Diabetes Association, 2014; Pilcher, 2006).

MODY is another form of DM which is inherited in autosomal dominant trait. The hyperglycemia appears after puberty and is detectable before the age of twenty-five years. MODY is treatable without insulin involvement for the first five years of the onset, yet later on it will require insulin injection like T1DM. MODY prevalence varies from one population to another. For example it was found that Blacks and Indian populations have about 10% prevalence, while Caucasians were found to have less than 1%.

1.2.5 Symptoms of Diabetes Mellitus

Diabetic patients may or may not present characteristic symptoms of polyuria, continued thirst 'polydipsia' (Ernest Adeghate et al., 2006b), dramatic loss of body weight and blurred vision. Without adequate management and treatment patients will develop a severe form of ketoacidosis or non-ketotic hyperosmolar state, which will become a life threatening condition when it develops to stupor, coma and eventually death (Figure 9).



Figure 9: Diabetes mellitus symptoms.

"Reproduced and edited ("diabetes symptoms | Pharma Mirror Magazine," n.d.)."

1.2.6 Criteria for Diagnosis of Diabetes Mellitus

According to the American Diabetes Association (ADA), to diagnose DM one of the following three measurements should be positive:

- 1 A fasting plasma glucose (FPG) level ≥ 126 mg/dL (7.0 mmol/L), or
- 2 A 2-hour plasma glucose level ≥ 200 mg/dL (11.1 mmol/L) during a 75 g oral glucose tolerance test (OGTT), or
- 3 A random plasma glucose ≥ 200 mg/dL (11.1 mmol/L) in a patient with classic symptoms of hyperglycemia and or hyperglycemic crises

1.2.7 Complications of Diabetes Mellitus

In DM chronic hyperglycemia eventually will cause damage, dysfunction and failure of various organs like the eyes, kidneys, nerves, heart and blood vessels (Figure 10). Life-threatening and disabling complications have been also reported, especially if DM is poorly controlled (“Diagnosis and Classification of Diabetes Mellitus,” 2010, “WHO | Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia,” n.d.).

Complications differ from patient to patient and vary in severity. Retinopathy, nephropathy, peripheral neuropathy, autonomic neuropathy and sexual dysfunction are all found among patients with DM. Also incidences of cardiac complications have been reported to increase in patients with DM. These complications may be associated with hypertension (American Diabetes Association, 2014).

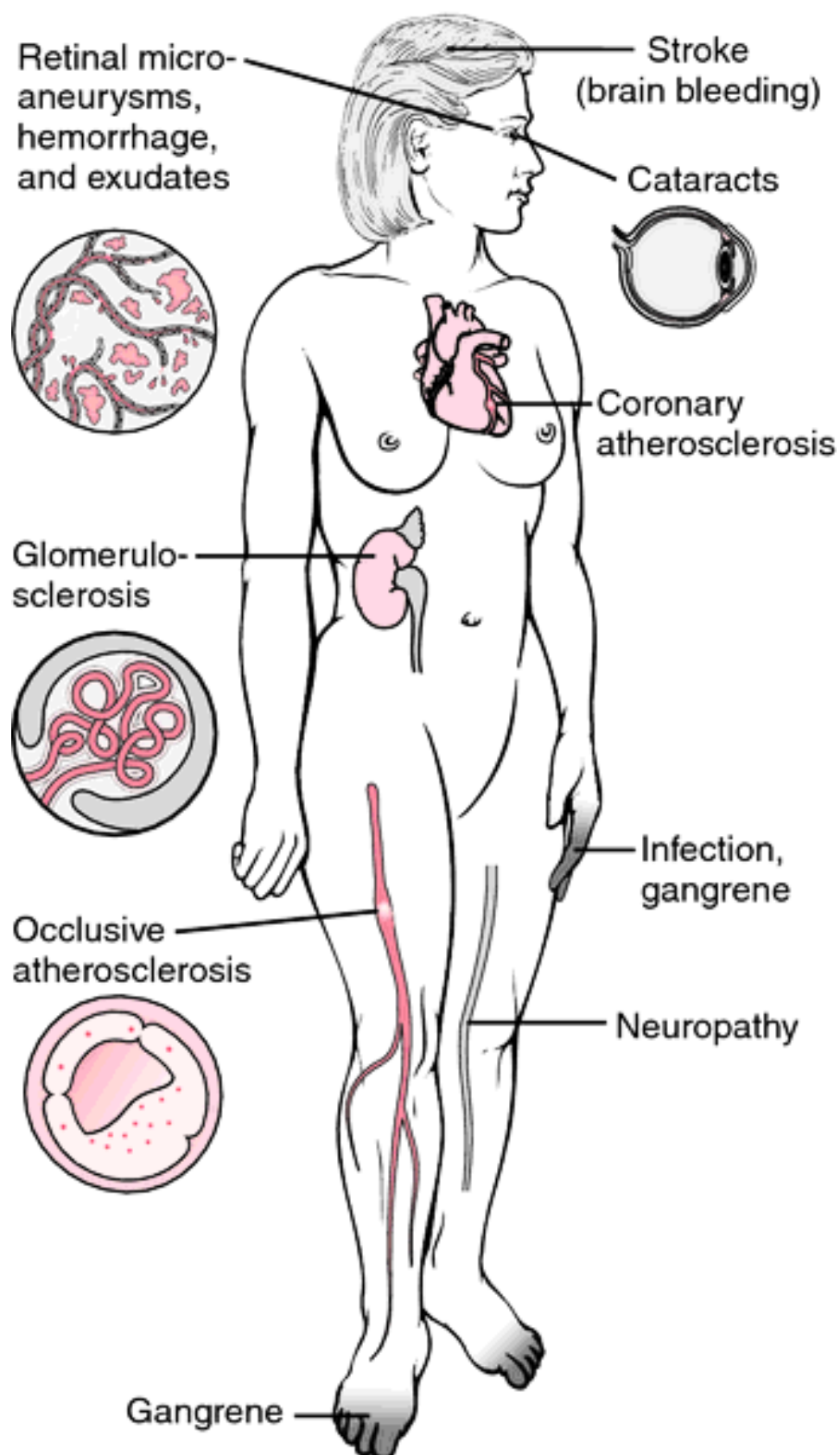


Figure 10: Diabetes mellitus complications.

“Reproduced and edited (“secondary diabetes mellitus,” n.d.)”.

A study conducted in Al Ain City, in the United Arab Emirates showed that the prevalence rates of retinopathy, neuropathy, nephropathy, peripheral vascular diseases and congestive heart disease were 54.2%, 34.7%, 40.8%, 11.1% and 10.5%, respectively (Saadi et al., 2007).

Blindness and end-stage renal damage are highly prevalent among patients with DM. Also poorly controlled DM patients are more susceptible to bacterial and fungal infections, and developing diabetic foot ulcers (E. Adeghate, 2001).

1.2.8 Management and Treatment of Diabetes Mellitus

There are four main aims of DM treatment. First is to enhance the glycemic control, second is to slow the progression of the disease, third is to prevent complications and fourth is to improve quality of life (Shrivastava, Shrivastava, & Ramasamy, 2013). The aims can be achieved with body weight reduction and active lifestyle which are considered to be important factors in treating patients with T2DM, whereas the main corner stone is patient's compliance to the medications regimen (Bonora, 2008).

1.2.8.1 Nutrition

A healthy and balanced diet helps in controlling blood sugar level in diabetic patients. There is no ideal percentage of caloric intakes, typical food plan or eating pattern that works universally. However individualized nutrition therapy depending on patient preferences and metabolic goals should be taken into consideration. A registered dietitian should be assigned to follow up with each and every diabetic patient; to educate them about their diet and how to approach their glycemic control goal.

A gradual and sustained body weight reduction is important for obese patients with DM or at risk of developing it. It has been shown that body weight reduction is effective not only in preventing the onset of the disease and its progression, but also it helps in ameliorating the acute and long-term DM complications (“REDUCTION IN THE INCIDENCE OF TYPE 2 DIABETES WITH LIFESTYLE INTERVENTION OR METFORMIN,” 2002).

1.2.8.2 Exercise

Many researches in the literature reported that exercise can be a cornerstone in the management of DM. Lack of exercise among other lifestyle habits like smoking and alcohol consumption was proven to increase the risk of developing T2DM (Hu et al., 2001). However, Different types of exercise are effective in DM management, and physicians should tailor individualized programs for each DM case depending on the glycemic reading especially for those with T1DM; to meet their therapeutic goals (Colberg & Swain, 2000).

The role of exercise is not as clear in T1DM as it is in T2DM, and fear of hypoglycemia and worsening of the complications in T1DM should be taken into consideration when introducing exercise or any other regular physical activity as one of the management approaches (Horton, 1988). Increasing insulin sensitivity, lowering blood glucose level, enhancing lipid profile, reducing the risk for hypertension and positively affecting the psychological status are well-known effects of exercise on T2DM, whereas with T1DM the fluctuation between hypo- and hyperglycemia can be managed by careful monitoring of blood glucose level, adjusting insulin doses and following appropriate diet (Riddell & Perkins, 2006; White & Sherman, 1999).

1.2.8.3 Pharmacotherapy

In T2DM; Diet, body weight reduction and exercise may not be sufficient to meet the glycemic control and oral glucose-lowering agents will be introduced to the patients. T2DM patients might require insulin injection to approach their glycemic goal; whereas, patients with T1DM depend mainly on insulin for survival.

1.2.8.3.1 Insulin Treatment of Diabetes Mellitus

Insulin is not administered orally because as a peptide hormone it will rapidly be broken down or digested by the acids in the stomach or by proteolytic enzyme, respectively; hence insulin is not administered orally. The most common route of administration for insulin is subcutaneous. Many forms of insulin are available and it is common practice to use combination therapy of at least two forms of insulin to treat patients with T1DM.

Insulin forms differ in their origin, onset and duration of action. Aspart, Lispro and Glulisin are known to be rapid acting insulin with fast onset (15 - 30 minutes), while regular insulin is a short acting type of insulin. Isophane insulin, insulin zinc suspension and protamine zinc insulin are intermediate acting types of insulin with onset of action of approximately 1 – 2 hours, while glargine and detemir are long acting analogues of human insulin (Committee, 2014) (Table 2).

Another form of insulin is Exubera which was approved by the Food and Drug Administration (FDA) was the first inhaled form of insulin (Wells et al., 2008). This drug has now been withdrawn because of concerns of developing lung cancer (Heinemann, 2010) but a new inhalable insulin called Afrezza was approved by the FDA in June 2014.

Type of insulin	Onset (Hours)	Peak (Hours)	Duration (Hours)
Rapid-acting			
Aspart	13-30 min	1-2	3-5
Lispro	13-30 min	1-2	3-4
Glulisine	13-30 min	1-2	3-4
Short-acting			
Regular	0.5-1.0	2-3	3-6
Intermediate-acting			
NPH	2-4	4-6	8-12
Lente	3-4	6-12	12-18
Long-acting			
Ultralente	6-10	10-16	18-20
Glargine	4-5	/	22-24

Table 2: Classification of different type of insulin injection according to their duration of action.

“Reproduced and edited (DiPiro et al., 2011)”

1.2.8.3.2 Oral Antidiabetic Agents

The FDA has approved six classes of antidiabetic agents to treat T2DM; each class of antidiabetic agent has a different mechanism of action.

1. Insulin secretagogues
 1. Sulfonylureas
 2. Meglitinide
2. Biguanides
3. Thiazolidinediones (TZD'S) or glitazones
4. Alpha glucosidase inhibitors
5. Other antidiabetic agents
 1. Incretin mimetics
 2. Dipeptidyl peptidase – IV inhibitors (DPP4)
 3. Synthetic amylin analog

1.2.8.3.2.1 Insulin Secretagogues

Sulfonylureas and meglitinide analogs act by enhancing the release of insulin. Sulfonylureas differ in potency (second generation agents are more potent), potential side effects and plasma protein binding affinity. As shown in Figure 11, sulfonylureas act predominantly through blocking of the ATP-sensitive K^+ channel leading to depolarization of the β cell membrane and Ca^{2+} influx, resulting in exocytosis of insulin granules.

Meglitinide analogs like repaglinide, a benzoic acid derivative and nateglinide, a phenylalanine amino acid derivative are both considered to be short acting insulin secretagogues. Both necessitate the availability of glucose to exert their effect, which is similar to sulfonylureas.

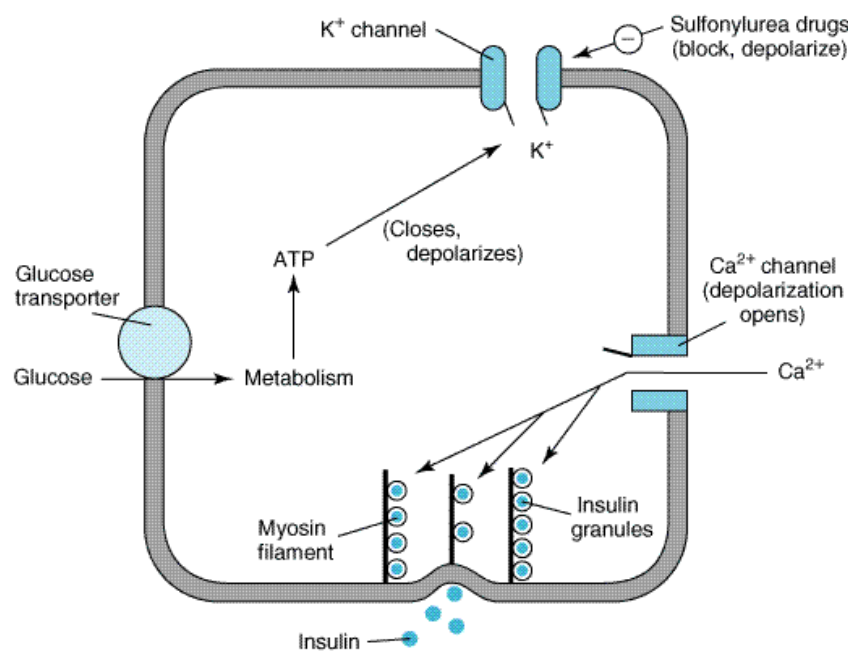


Figure 11: Sulfonylureas mechanism of action.

“Reproduced and edited (Katzung, 2007)”.

1.2.8.3.2.2 Biguanides

Metformin is the only agent available in this class and achieves its action not by directly acting on β cells but rather by augmenting insulin sensitivity of both hepatic and peripheral (muscle) tissues leading to increased uptake of glucose (Figure 12).

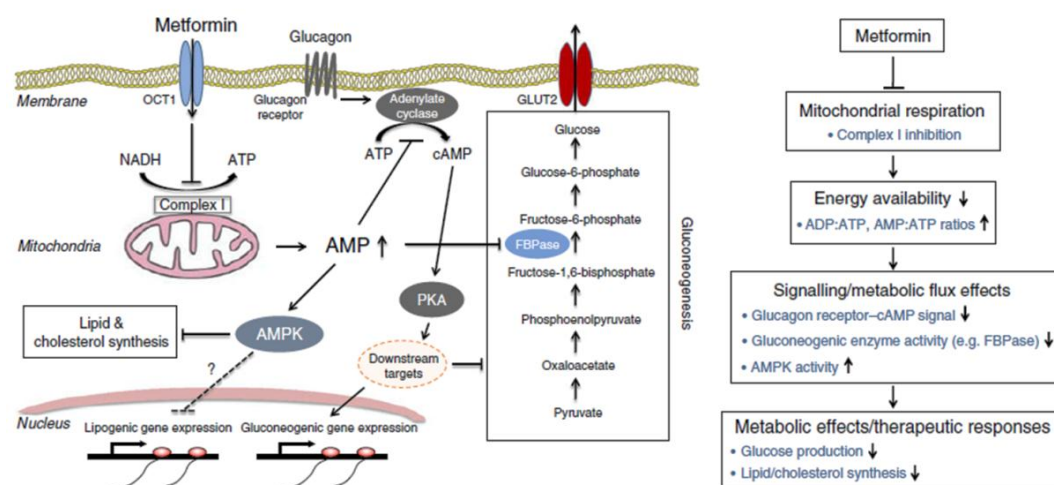


Figure 12: Schematic illustration of the mechanism of action of metformin on the liver cell.

“Reproduced and edited (Rena, Pearson, & Sakamoto, 2013)”

It was shown that pre-gestational use of metformin reduces the risk of developing GDM (Ashrafi et al., 2014). Metformin has also been used to treat polycystic ovarian syndrome, which can contribute to GDM (Reyes-Muñoz et al., 2012; Weerakiet et al., 2004).

1.2.8.3.2.3 Thiazolidinediones or Glitazones

Thiazolidinediones (TZD's) or glitazones were introduced as a treatment of T2DM in 1996, when troglitazone was approved by the FDA. Unfortunately troglitazone was withdrawn from the market due to its side effect (idiosyncratic

hepatotoxicity) in 2000(Kendall, 2006). Other agents such as pioglitazone and rosiglitazone were approved as treatment for T2DM. Glitazones act by inhibiting peroxisome proliferator activator receptor γ (PPAR γ) which is located in vascular and fat cells. This class of drugs enhances insulin sensitivity in muscle, liver as well as fat tissues and results in increased the level of high density lipoproteins (HDL) with a slight reduction in blood glucose level. It is a regular clinical procedure to initially perform a liver function test before start the treatment with any of the glitazones and to repeat it periodically thereafter.

1.2.8.3.2.4 α -Glucosidase Inhibitors

Acarbose and miglitol, reversibly bind to and inhibit α -glucosidase in the membrane of the intestinal brush border. Both are taken at the beginning of meals to exert their effects by delaying the digestion of carbohydrates resulting in lower postprandial glucose level. They also inhibit pancreatic amylase and interfere with the breakdown of starch. Since α -glucosidase inhibitors have no effect whatsoever on insulin secretion and insulin function, it will not result in hypoglycemia if it is used as a monotherapy. Side effects of this drug may include flatulence, abdominal cramps and diarrhea, along with complete inhibition of some enzymes such as, maltase, iso-maltase and glucoamylase which will interfere with absorption of these nutrients.

1.2.8.3.2.5 Other Antidiabetic Agents

Another two classes of hypoglycemic agents are the incretin mimetics (Exenatide) and Dipeptidyl Peptidase–IV Inhibitors (DPP4; Vildagliptin and Sitagliptin). Both classes improve glucose dependent insulin secretion, they also slow gastric emptying time, reduce food intake, decrease postprandial glucagon

secretion and promote β cell proliferation. Exenatide, a 39 amino acid polypeptide, is 50% homologous to GLP-1, yet unlike GLP-1, increases gastric secretion (Lotfy, Singh, Kalász, Tekes, & Adeghate, 2011)).

Synthetic Amylin Analog (Pramlintide) is used in the treatment of both T1DM and T2DM patients (Ernest Adeghate & Kalász, 2011) . Pramlintide exerts effects similar to those of incretin mimetic agents with the additional favorable effect of improving satiety (Chapman et al., 2005).

Simultaneous administration of pramlintide and insulin led to 50% reduction in insulin dose and a reduced risk of hypoglycemia (Clark, Harvey, Finkel, Rey, & Whalen, 2011; DiPiro et al., 2011; Wells et al., 2008).

1.2.8.4 Surgery

Surgical intervention is usually the last resort for any disease especially if it is manageable or curable by other approaches. Yet, in DM a surgical approach is becoming more common and is usually the first choice to either eliminate risk factors of developing T2DM such as in obese people, or to prevent the progression of the disease and its complications.

Studies revealed that 83% of patients with T2DM who have undergone gastric bypass maintained normal blood glucose level and HbA1c concentration independently from body weight loss. The astonishing remission of T2DM by gastric bypass is thought to be multifactorial and that gut hormones play a significant role, especially when a rise in postprandial GLP-1 and peptide YY concentrations were reported along with reduction in the basal ghrelin level (Tahrani, Bailey, Del Prato, & Barnett, 2011a, 2011b). It is worth noting, however,

that gastric bypass surgery has its own shortcomings including the life-long use of supplements (Padoin et al., 2009)

1.2.8.4.1 Islet and Pancreatic Transplantation

The first known attempt of islet transplantation was reported in 1893 (McCall & Shapiro, 2014), followed by years of trials until success was met (Robertson, 2004). Moreover, pancreatic transplantation showed persistent success over the years not only in reducing mortality rates in diabetic patients but also in ameliorating the complications associated with it (Kelly, Lillehei, Merkel, Idezuki, & Goetz, 1967).

Patients with T1DM who suffer from a history of severe hyperglycemia and metabolic instability may receive islet transplantation from multiple donors in conjunction with immunosuppressive drugs to prevent islet rejection. Islet transplantation has resulted in insulin independence with exceptional metabolic, normoglycemia (Warnock et al., 1991) and improved renal function in patients who received islet cells transplantation alone or in combination with kidney transplantation (Fiorina et al., 2003).

Patients who are eligible for this procedure mostly have diabetes with end-stage renal failure or frequent episodes of hypoglycemia (Gruessner & Sutherland, 2005).

Pancreatic organ transplantation is performed simultaneously with kidney transplantation with a favor of a living donor for the pancreas if possible; to maintain the full function of the organ (Farney et al., 2000).

Post-transplantation complications are common and include organ rejection, which is the reason behind the lifelong use of immunosuppressive therapy in patients with organ transplants.

1.2.8.4.2 Other Types of Treatments

There has always been a connection between DM and peptides especially gut peptides which mainly affect postprandial insulin secretion. These peptides include incretins such as glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) (Ahrén, 2003). Ghrelin which possess an opposite effect to leptin regarding hunger state (D. E. Cummings, Frayo, Marmonier, Aubert, & Chapelot, 2004; Higgins, Gueorguiev, & Korbonits, 2007; Hotta et al., 2009), also plays a role in diabetes especially in T2DM (Katsuki et al., 2004; Østergård et al., 2003; Pöykkö et al., 2003).

1.2.8.4.3 Glucagon-like Peptide-1

Glucagon-Like Peptide-1 (GLP-1), is a gut-derived incretin hormone known to stimulate insulin and suppress glucagon. GLP-1 affects food intake and appetite reversibly. Using incretin enhancers and/or mimetics benefited patients with T2DM not only due to body weight regulation, but also by reducing fasting and postprandial blood glucose (Drucker & Nauck, 2006). GLP-1, aside from stimulating insulin secretion, inhibits glucagon release and regulates glycogen synthesis in adipose and muscle tissues (Drucker, 1998).

A study where 10 patients were assigned to receive a continuous subcutaneous infusion of GLP-1 for a period of 6 weeks showed a reduction in fasting glucose level, HbA1c, fructosamine and free fatty acid and body weight

along with an increase in insulin sensitivity and improvement in β cell function (Zander, Madsbad, Madsen, & Holst, 2002).

GLP-1 also inhibits pancreatic β cell apoptosis and enhances proliferation and differentiation of insulin secreting β cells (Farilla et al., 2003; Perfetti, Zhou, Doyle, & Egan, 2000; Xu, Stoffers, Habener, & Bonner-Weir, 1999).

1.2.8.4.4 Leptin

Leptin or “satiety hormone”, is produced by fat cells in order to regulate fat storage in the body. Yet, in obese subjects it was noticed that leptin concentration is high and correlates to the percentage of body fats (Hickey et al., 1996), which could be a result of leptin-resistance, in the same manner as insulin resistance (Considine et al., 1996).

Over the years, a correlation between leptin insufficiency and DM was studied and reported to account leptin as one of etiological factors in the pathogenesis of DM, however, with the presence of a feedback regulatory system between insulin and leptin in regulating glucose homeostasis, leptin was thought to be the best nominated therapeutic agent to replace insulin injection (B. P. Cummings, 2013; Kalra, 2013).

1.2.8.4.5 Substance P

Substance P (SP), is a neuropeptide that functions either as a neuromodulator or neurotransmitter (Datar, Srivastava, Coutinho, & Govil, 2004; Harrison & Geppetti, 2001). Some reports have shown that SP can reverse diabetes in mice (Tsui, Razavi, Chan, Yantha, & Dosch, 2007), yet in humans, it decreased insulin secretion and causes fluctuations in blood glucose level (Brown & Vale, 1976).

Furthermore, patients with T1DM were found to have low level of serum SP especially those suffering from diabetic neuropathy (Kunt et al., 2000).

There is contradictory data in the literature about the effect of SP on insulin secretion. Some studies have reported that a low dose of SP stimulates insulin release and at a high dose the effect was abolished (Lundquist, Sundler, Ahrén, Alumets, & Håkanson, 1979), whereas others have shown the effects of SP on insulin release is dose-dependent (Hermansen, 1980).

1.2.8.4.6 Ghrelin

Ghrelin, a novel peptide was recently discovered and reported to play a role in insulin secretion (Ernest Adeghate & Ponery, 2004) and thought to influence DM (Zhang, Zhang, Zhang, Li, & Jiang, 2013) will be further discussed next (section 1.3).

1.3 Ghrelin

1.3.1 Discovery of Ghrelin

“If one of our team members, Kenji Kangawa, Hiroshi Hosoda, or I, were not involved, ghrelin would not have been discovered.” said, Masayasu Kojima.

After years of peptide hunting, Kangawa and his team finally succeeded in discovering a novel peptide named “ghrelin” from a few mg of stomach extract in 1999 (M. Kojima et al., 1999; Masayasu Kojima, 2008; Masayasu Kojima & Kangawa, 2011).

1.3.2 Structure of Ghrelin

Ghrelin, a word derived from “ghre” which means “grow” in Proto-Indo-European languages and “relin” which means release (Walker, Gong, Park, Zigman, & Sakata, 2013) to indicate its role in stimulating growth hormone release (Riley, Hirano, & Grau, 2002; Takaya et al., 2000). Ghrelin is a 28-amino-acid peptide secreted by the stomach (from that was termed X/A-like cell in rats and P/D1 cell in humans) (M. Kojima et al., 1999; Stengel & Taché, 2012), in which Ser3 is modified by n-octanoic acid which is a unique acyl modification and is essential for ghrelin’s activity. Ghrelin is encoded in prepro-ghrelin which on splicing yields three peptides; a single peptide, a mature peptide and a C-terminal peptide (Figure 13) (Sato et al., 2012).

In humans, at least there are four different forms of ghrelin: a non-acylated form, an n-octanoylated form bound to the serine-3 residue of the peptide, a decanoylated form, and lastly a decenoylated form (Hiroshi Hosoda, Kojima, Mizushima, Shimizu, & Kangawa, 2003). N-octanoylated ghrelin is thought to be

the only active form and exerts different biological effects in different tissues (Masayasu Kojima, Hosoda, & Kangawa, 2012). However, decanoyl, hexanoyl, butynoyl and other lipid-modified forms of ghrelin showed some activity (Riley, 2013; Thompson et al., 2004).

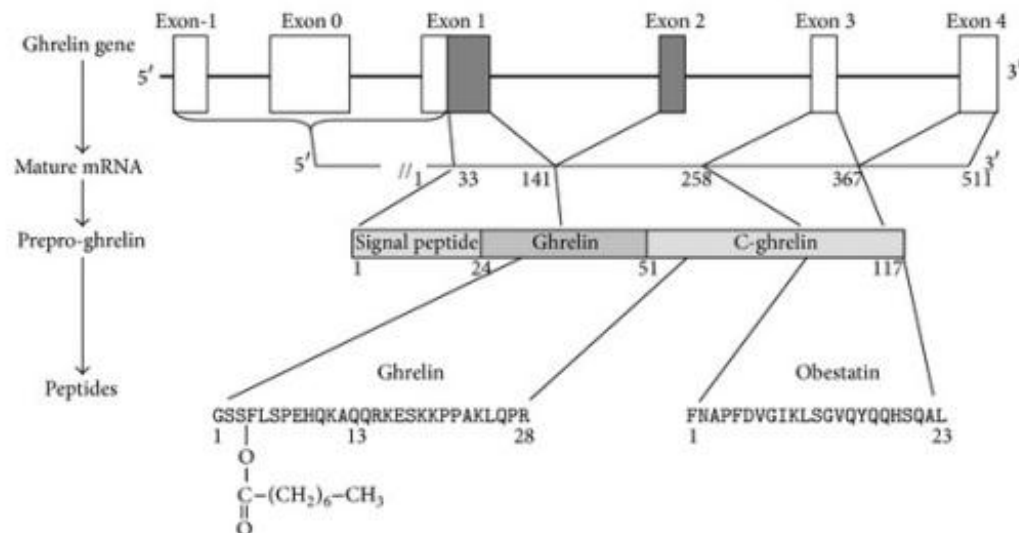


Figure 13: Ghrelin synthesis and structure.

“Reproduced and edited (Delporte, 2013)”.

1.3.3 Ghrelin receptor

Ghrelin receptor or growth hormone secretagogue receptor belongs to the G Protein-coupled receptor family that binds to ghrelin (Howard et al., 2001). In human, ghrelin receptor is encoded by a single-copy *GHSR* gene which is located on chromosome 3 (Wajnrajch, Ten, Gertner, & Leibel, 2011). *GHSR* gene generates two isoforms which differ in their carboxyl-terminal, GHS-R1a and GHS-R1b. GHS-R1a has seven transmembrane domains while GHS-R1b lacks the transmembrane domains 6 and 7. Isoform 1a is the active receptor which binds ghrelin and exerts signal transduction in the cell; however, the function of 1b receptor remains unclear (Gahete et al., 2014; S, 2002; Unniappan & Peter, 2005).

GHS-R is located in both the central nervous system (CNS) as well as the peripheral nervous system (PNS). It was found in multi hypothalamic nuclei, pituitary gland (Reichenbach, Steyn, Sleeman, & Andrews, 2012), in CA2 and CA3 regions of the hippocampus, the substantia nigra, ventral tegmental area, and dorsal and median raphe nuclei (Davenport et al., 2005). GHS-R is highly expressed in dopaminergic, cholecystokinin-containing neurons of the substantia nigra and ventral tegmental area of the midbrain (Zigman, Jones, Lee, Saper, & Elmquist, 2006).

GHS-R was also found to be expressed in the thyroid gland, pancreas, spleen, myocardium and adrenal gland, stomach, small and large intestines, liver, lung and adipose tissue, indicating the numerous roles of ghrelin (Gnanapavan et al., 2002; Masayasu Kojima & Kangawa, 2005).

1.3.4 Tissue Distribution of Ghrelin

Ghrelin is expressed predominantly in the fundus of the stomach (Lee, Wang, Englander, Kojima, & Greeley, 2002), also in kidney glomerulus (Mori et al., 2000), intestine (H. Hosoda, Kojima, Matsuo, & Kangawa, 2000), human placenta (Gualillo et al., 2001) and in human T cells, B cells and neutrophils (Hattori et al., 2001). Furthermore, ghrelin was detected in human pancreas (Papotti et al., 2000) where many studies showed ghrelin co-localizes with insulin in β cells (Volante et al., 2002). Others revealed the presence of ghrelin in α cells indicating its role in regulating insulin secretion (Yukari Date et al., 2002). However, it was reported that a new pancreatic cells known as epsilon-cells contain ghrelin (Andralojc et al., 2009a).

1.3.5 Ghrelin Signaling Pathway

Ghrelin was found to stimulate the pituitary gland to release GH differently than GHRH. Binding of ghrelin to its receptor leads to the activation of phospholipase C1 which in turn leads to an increase in intracellular Ca^{2+} level via inositol 1,4,5-triphosphate (IP_3) signal transduction and diacylglycerol (Figure 14) (Malagón et al., 2003).

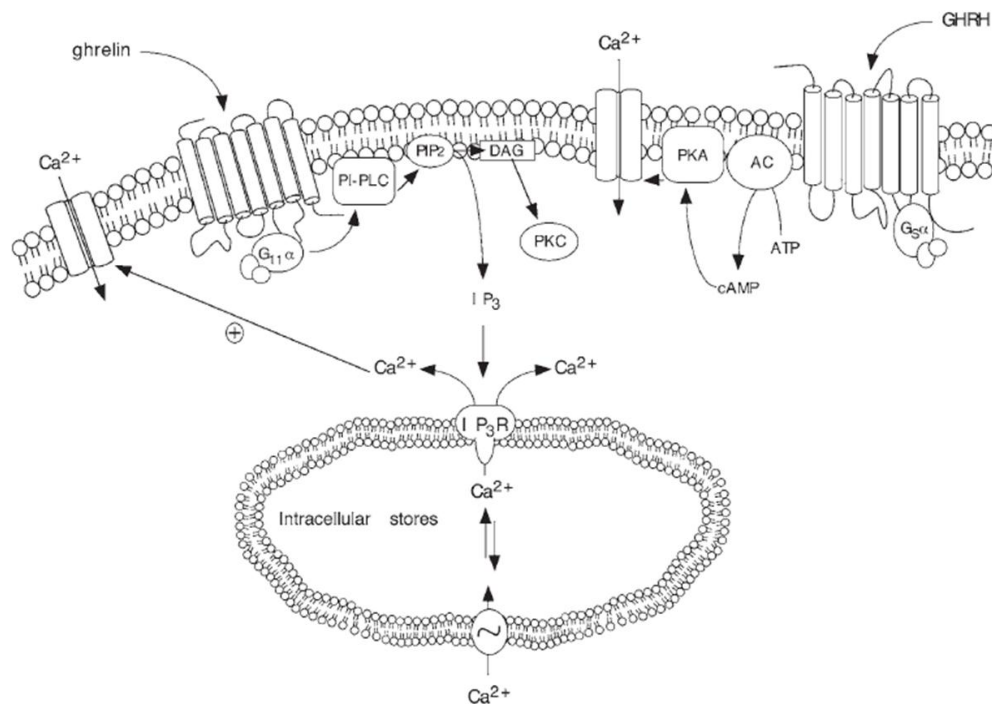


Figure 14: Signaling pathway of ghrelin-inducing GH release.
“Reproduced and edited (Camiña et al., 2003)”.

The orexigenic effect of ghrelin is mediated by raising $[\text{Ca}^{2+}]_i$ via adenosine monophosphate-activated protein kinase (AMPK) in neuropeptide Y (NPY) (Kohno, Sone, Minokoshi, & Yada, 2008) and agouti-related peptide (AgRP) neurons in the hypothalamus (Figure 15) (Seoane et al., 2003).

Another way for ghrelin to exert its orexigenic effect is by triggering AMPK phosphorylation to inhibit mechanistic target of rapamycin complex 1 (mTORC1),

which is known to have an anorexigenic effect aside from being one of the major mediators in insulin signaling pathway (Figure 15) (Martin et al., 2007; Veilleux, Houde, Bellmann, & Marette, 2010).

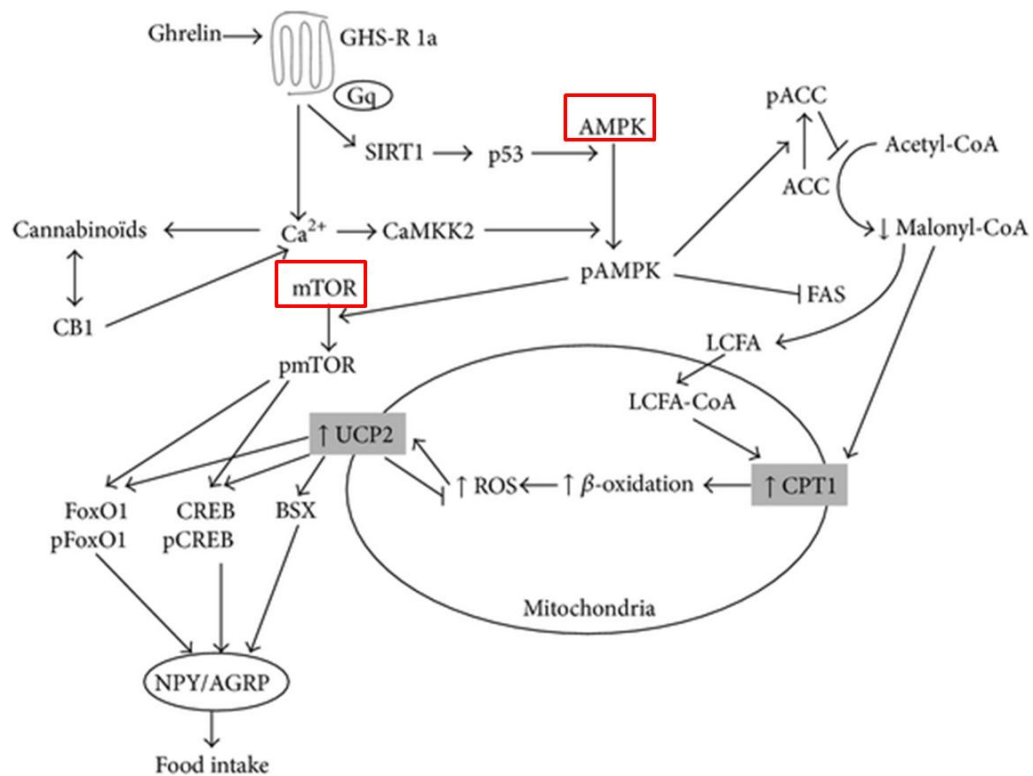


Figure 15: Signaling pathway of ghrelin-inducing food intake in the hypothalamus. “Modified from Hindawi Publishing Corporation, Scientifica” (Delparte, 2013)

The vagus nerve was shown to mediate ghrelin effects by transmitting its signals to the brain (Yukari Date, 2012), aside from ghrelin’s own autocrine, endocrine and paracrine effects (Avau et al., 2013; De Vriese, Grégoire, De Neef, Robberecht, & Delparte, 2005; Jeffery, Herington, & Chopin, 2003; Miller et al., 2005).

1.3.6 Effect of Ghrelin on Food Intake

Plasma ghrelin level have been shown to increase during fasting and before meals and decrease after meal, yet in obese humans, ghrelin was not suppressed after food consumption (English, Ghatei, Malik, Bloom, & Wilding, 2002).

Ghrelin level was also shown to differ dramatically when equal amounts of calories were served from sugar, protein and fat (Beck, Musse, & Stricker-Krongrad, 2002).

Centrally administered ghrelin led to a significant increase in food consumption (Lawrence, Snape, Baudoin, & Luckman, 2002) and NPY and AgRP expression (Goto et al., 2006; Kamegai et al., 2000) independently from its GH releasing effect (Kamegai et al., 2001; M. Nakazato et al., 2001; M. Tschöp, Smiley, & Heiman, 2000). Ghrelin also stimulates growth hormone releasing hormone directly to alter feeding activity (Osterstock et al., 2010).

Ghrelin is thought to play a role in meal initiation (D. E. Cummings et al., 2001; Sugino et al., 2002), while lacking ghrelin receptor led to less food consumption (Zigman et al., 2005).

Patients with bulimia nervosa, an eating disorder showed no difference in ghrelin serum level than the their aged-matched controls (Michiko Nakazato et al., 2004).

1.3.7 Effect of Ghrelin on Body Weight and Obesity

The dramatic increase in the prevalence of obesity and its consequent metabolic complications is becoming a burden and a threat to health and life span of affected individuals or those with a high risk of becoming obese.

In obese people circulating ghrelin level decrease (Matthias Tschöp et al., 2001) and correlate negatively with body mass index (BMI) (Shiia et al., 2002). Moreover, body weight loss is associated with elevated circulating ghrelin level (Hansen et al., 2002) implying the possibility of recruiting ghrelin to be used as a preventive measure to curb the obesity rise.

Whereas, administration of ghrelin caused weight gain (M. Tschöp et al., 2000), in a dose-dependent manner (Matthias Tschöp, Statnick, Suter, & Heiman, 2002), a study showed that absence of GHS-R1a can protect against developing diet-induced obesity, indicating a direct effect of ghrelin on body weight (Zigman et al., 2005).

1.3.8 Ghrelin and Pancreatic Islet Cells and its Association with Islet Cells Hormones

Ghrelin is expressed in pancreatic tissue along with its receptor (Volante et al., 2002) and interestingly fetal pancreas highly expresses ghrelin than fetal stomach in the first two weeks postnatal life (Chanoine & Wong, 2004). However, ghrelin was also found to be secreted from the pancreas (Dezaki et al., 2006).

1.3.8.1 Ghrelin and Insulin

Ghrelin and insulin were found to react correspondingly with preprandial increase and postprandial decrease (D. E. Cummings et al., 2001; Shiia et al., 2002). Moreover, glucose-induced insulin secretion was enhanced by ghrelin infusion (Takahashi et al., 2006).

In vitro studies have shown that ghrelin induces insulin secretion differently in normal and in diabetic pancreas (E. Adeghate & Ponery, 2002) and that ghrelin concentration influences insulin secretion differently (Salehi, Dornonville de la

Cour, Håkanson, & Lundquist, 2004). In another study, ghrelin failed to stimulate basal insulin release or to increase cytosolic free calcium concentration in rat β cells in the presence of 2.8 mmol/l glucose, which is contradictory to its effect at 8.2 mmol/l glucose level (Yukari Date et al., 2002). In a study done by Dezaki K et al, (2011), ghrelin suppresses glucose-induced insulin release via attenuating cAMP pathway in β cells (Figure 16) (Dezaki et al., 2011; Wang et al., 2010). Additionally, ghrelin was found to influence glucose homeostasis through its effect on insulin secretion and insulin receptor signaling (Yada et al., 2014).

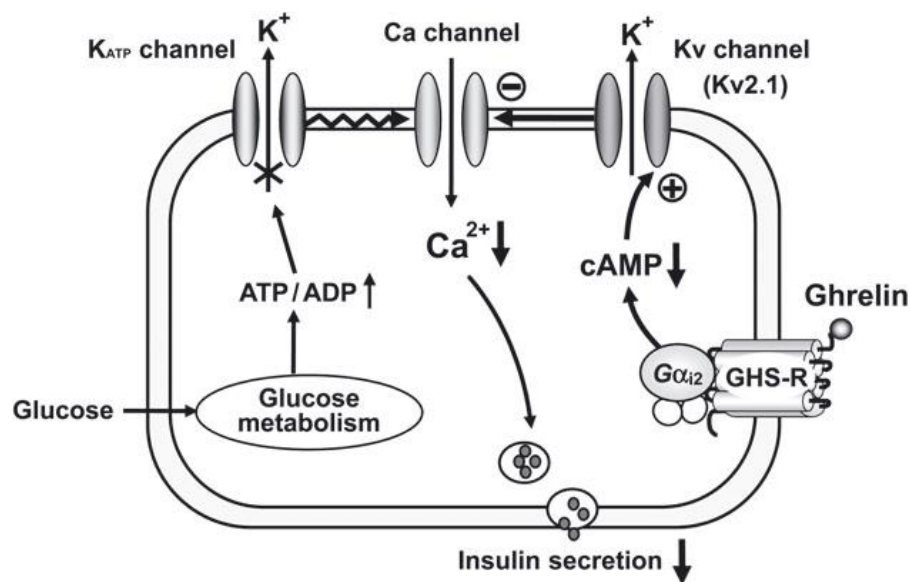


Figure 16: Ghrelin signaling in islet β cells.

“Reproduced and edited (Yada et al., 2014)”.

1.3.8.2 Ghrelin and Other Islets Hormones

Ghrelin co-localized with glucagon in pancreatic α cells (Yukari Date et al., 2002). However, a study showed that ghrelin is synthesized and secreted from α cells (Kageyama et al., 2005a). Another study showed that ghrelin induces glucagon release in diabetic pancreas but not in normal pancreas (Ernest Adeghate & Parvez,

2002), which might be in part mediated by neuronal constitutive nitric oxide synthase (Qader, Lundquist, Ekelund, Håkanson, & Salehi, 2005).

Ghrelin was found to significantly increase the circulating levels of both somatostatin and pancreatic polypeptide in human (Arosio et al., 2003). Whereas, somatostatin suppresses ghrelin secretion from the stomach (Shimada et al., 2003), and reduces circulating ghrelin level independently of GH status (Nørrelund et al., 2002).

1.3.9 Ghrelin Effect on Diabetes Mellitus

Ghrelin was implicated in the development of insulin resistance (Zhang et al., 2013) and β cell insufficiency (Lin, Chen, Lin, & Duan, 2009) in T2DM (Pöykkö et al., 2003). Independently from the sedentary life style and obesity, ghrelin level were found to be reduced in T2DM patients and in insulin-resistant obese adults (McLaughlin, Abbasi, Lamendola, Frayo, & Cummings, 2004), while in T2DM women aerobic exercise with the absence of substantial body weight loss influenced ghrelin level positively (Kadoglou et al., 2012). Furthermore, treatment of T2DM with metformin raised plasma ghrelin level (Doogue et al., 2009) and postprandial fall in ghrelin concentrations was prolonged (English et al., 2007).

1.3.10 Other Functions and Role of Ghrelin in Different Tissues

Ghrelin plays a role in many systems (Figure 17). For example, in the gastrointestinal system, ghrelin acts by increasing gastric acid secretion and stimulating gastric motility (Y. Date et al., 2001; Masuda et al., 2000). In the cardiovascular system, ghrelin has potent cardioprotective actions (Nagaya & Kangawa, 2004) where it plays a role in preventing heart failure (Yang, Liu, Liu, & Yang, 2014), and associated cardiac dysfunction and remodeling (Du et al.,

2014). Plasma ghrelin was found to be elevated in advanced renal failure (Tritos, Kissinger, Manning, & Danias, 2004), yet it decreases significantly in advanced cancer (Legakis, Stathopoulos, Matzouridis, & Stathopoulos, 2009).

Additionally, ghrelin is thought to be involved in the growth and progression of tumors (Omoto et al., 2014), whereas ghrelin variants (In1-ghrelin, which lacks exon 3-4) have been detected in human breast cancer tissue (Gahete et al., 2011). Ghrelin and ghrelin receptor were also highly expressed in prostate cancer (Lanfranco et al., 2008).

Ghrelin is involved in many other biological functions like learning (Bellar, Glickman, Judge, & Gunstad, 2013; E. Li et al., 2013; Tóth, László, & Lénárd, 2010; Zhu et al., 2013), memory (Goshadrour, Kermani, Ronaghi, & Sajjadi, 2013; Zhao et al., 2014), sleeping (Al-Disi et al., 2010; Figueiro, Plitnick, & Rea, 2012; García-García, Juárez-Aguilar, Santiago-García, & Cardinali, 2014; Steiger, Dresler, Schüssler, & Kluge, 2011), depression (Ozsoy, Besirli, Abdulrezzak, & Basturk, 2014; Poretti et al., 2015) and behavior (Carlini et al., 2002; Gastón, Schiöth, De Barioglio, & Salvatierra, 2015).

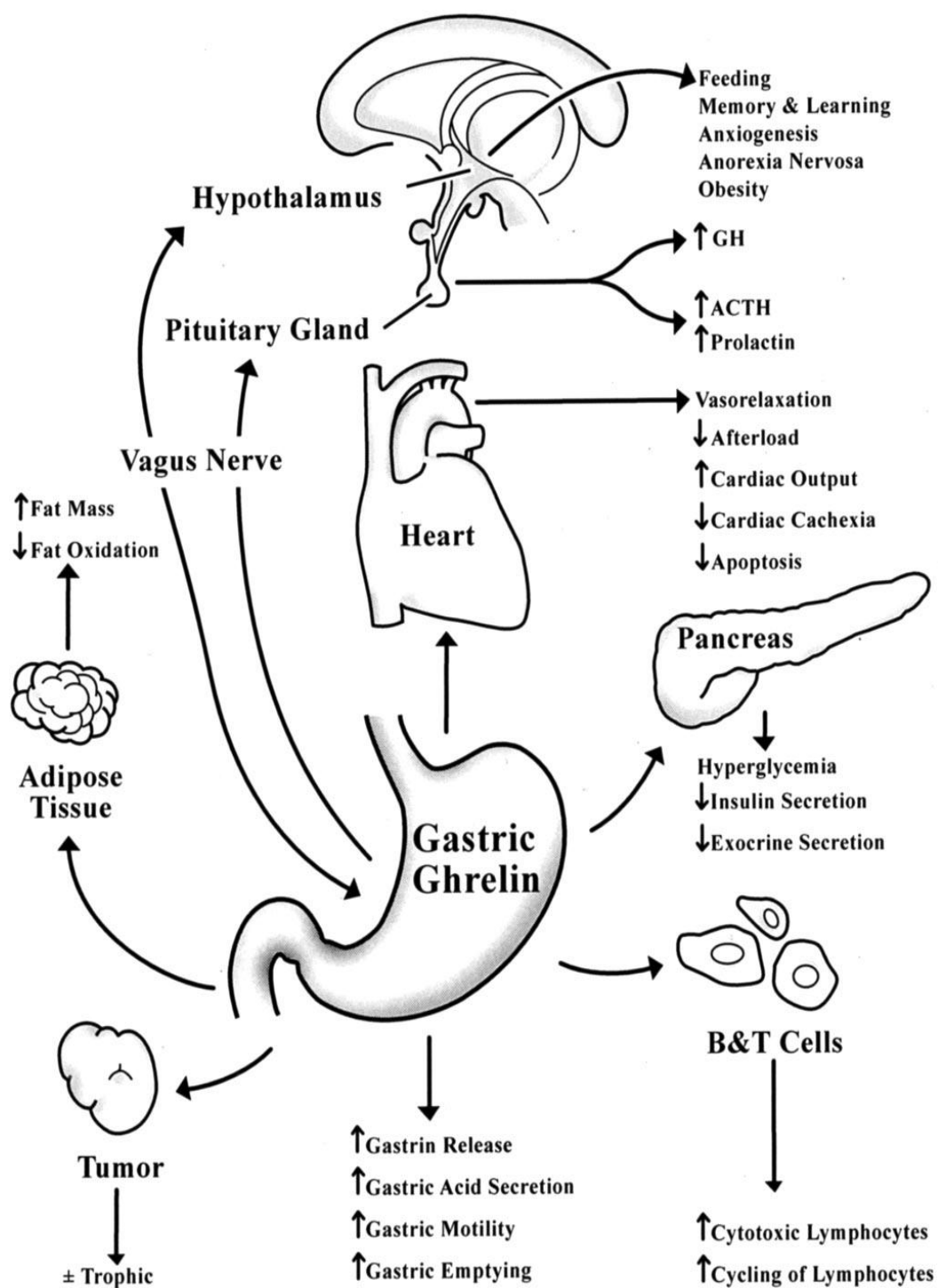


Figure 17: Physiological action of ghrelin.

“Reproduced and edited (Wu & Kral, 2004)”.

1.4 Streptozotocin and its Diabetogenic Effects

Streptozotocin (STZ) is an antibiotic used in chemotherapy an alkylating agent in the treatment of metastatic cancer of the pancreatic islet cells. In 1963 it was reported that STZ is diabetogenic. Ever since STZ has been used by many researchers to induce experimental models of DM. STZ is known to be particularly toxic to insulin-secreting β cells in the pancreas. Due its chemical structure which is similar to glucose, STZ is easily transported into the cell by the glucose transport protein GLUT2 (Akbarzadeh et al., 2007; Damasceno et al., 2014; Lenzen, 2008; Szkudelski, 2001).

Figure 18, describes the mechanism of action by which STZ induces DM. In β cells STZ causes alkylation of DNA which will induce activation of poly ADP-ribosylation, a process known to be more important for the diabetogenicity of STZ. Poly ADP-ribosylation leads to cellular NAD^+ and ATP depletion which will provide a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. STZ will also release nitric oxide leading to inhibition of aconitase activity and finally participates in DNA damage. As a result β cells will be destroyed by necrosis (Szkudelski, 2001).

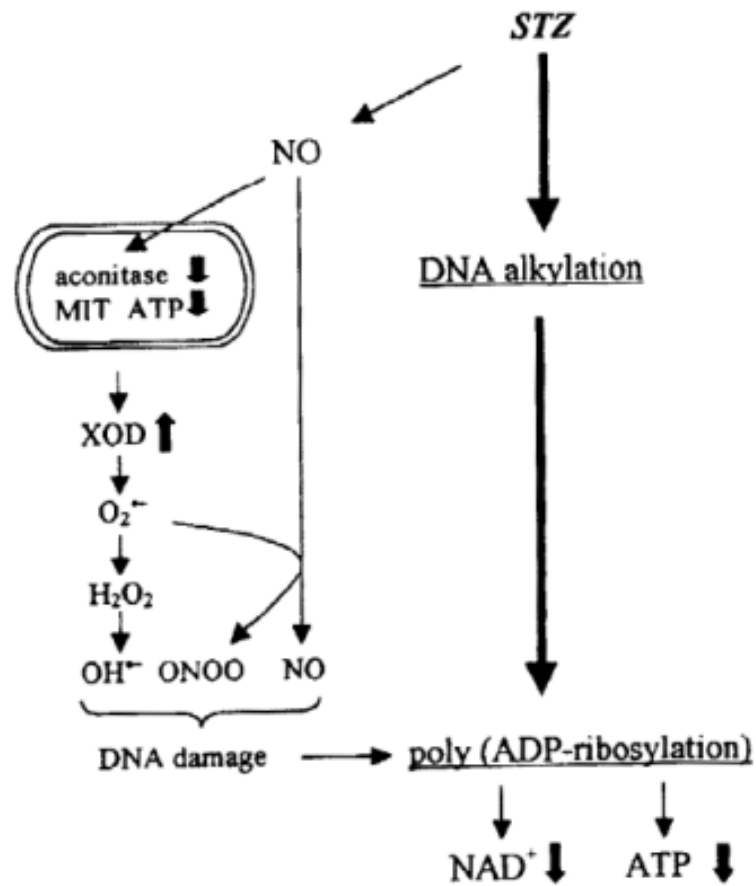


Figure 18: Streptozotocin mechanism in inducing diabetes mellitus.

“Reproduced and edited (Szkudelski, 2001)”.

Chapter 2: Hypothesis, Aims and Objectives

In literature, ghrelin was not only reported to be expressed in the pancreas but also to be secreted from it. Based on this we hypothesized that ghrelin co-localizes with insulin-secreting β cells of the pancreatic islet of Langerhans and that it increases insulin secretion which will result in subsequent low level of glucose in the blood. We also hypothesized that ghrelin can prevent or delay the onset of DM.

The primary focus of this study was to investigate the effect of ghrelin treatment on fasting blood glucose, body weight, insulin secretion and liver functions in normal and STZ-diabetic compared to age-matched control rats. We also examined the pattern of distribution and co-localization of ghrelin in the pancreatic islet of Langerhans.

Specific objectives of the study are:

1. To study the effect of chronic ghrelin treatment on fasting blood glucose level, body weight, glucose handling and insulin secretion in ghrelin-treated rats in comparison to age-matched controls.
2. To examine the distribution pattern and co-localization of ghrelin with insulin and glucagon in pancreatic islet cells.
3. To investigate the co-localization of ghrelin and insulin in secretory granules of β cells.

Chapter 3: Materials and Methods

3.1 Animal Models

Male Wistar rats aged 7–8 weeks and weighing 200 – 300 g were used in this study. All rats were acquired from the College of Medicine and Health Sciences, United Arab Emirates University. Rats were housed in the Animal House Facility which was maintained at 22-25 °C, along with a 12 hours light/dark cycle. A standard pellet diet with tap water was provided *ad libitum*. Diabetic rats were kept in metal cages and normal (non-diabetic) rats were kept in regular polycarbonate cages with wood chips bedding.

3.2 Induction of Experimental Diabetes Using Streptozotocin

Streptozotocin (STZ) was used to induce diabetes in rats by a single intraperitoneal injection at a dose of 60 mg/kg body weight (Akbarzadeh et al., 2007). STZ was freshly prepared by dissolving it in citrate buffer (0.5M, pH 4.5). Rats were considered diabetic if their fasting blood glucose level were above 126 mg/dl.

3.3 Experimental Design

As shown in Figure 18, the rats were divided into two groups: normal and diabetic. Diabetic group received intraperitoneal injection of freshly prepared STZ (section 2.2) to induce DM. Two days after STZ injection, fasting blood glucose (FBG) level was measured and rats having FGB level less than 126 mg/dL were discarded from the study. The two main groups were further subdivided into another two groups, where one group received normal saline (NS) “as control” and the other

5 $\mu\text{g/kg}$ body weight of ghrelin “as treated”. Both normal saline and ghrelin were injected intraperitoneally on a daily basis and for duration of four weeks. At the end of the fourth week, all rats were sacrificed except some rats from normal treated group that were subjected to STZ injection to induce diabetes after one week from stopping ghrelin treatment; in order to investigate the ability of ghrelin to prevent or delay the onset of DM. FBG and body weight were measured after one week of STZ injection (Figure 19).

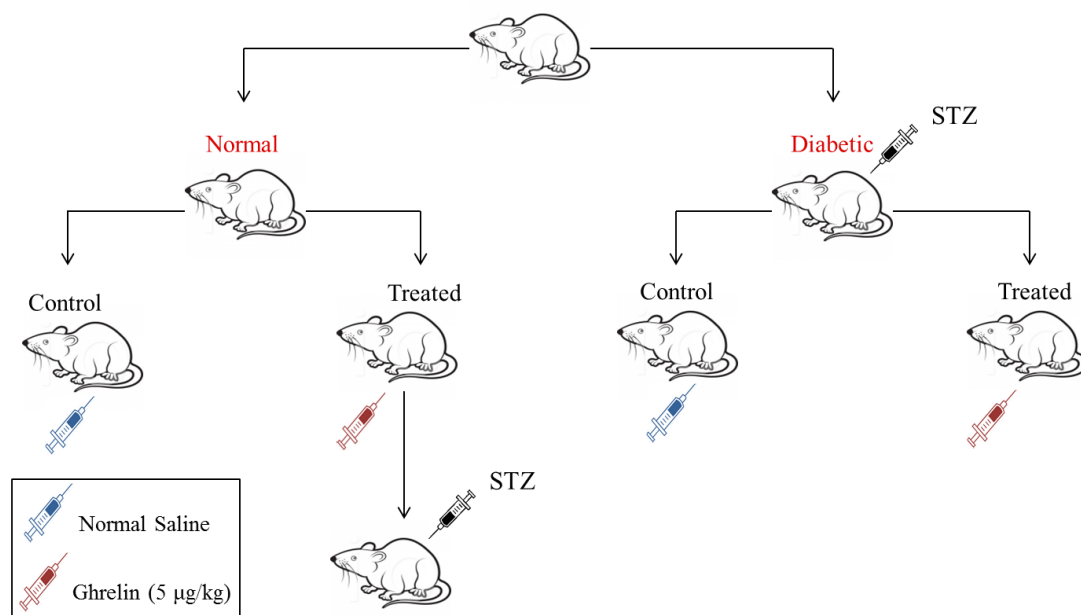


Figure 19: Schematic drawing describing animal groups in this study.

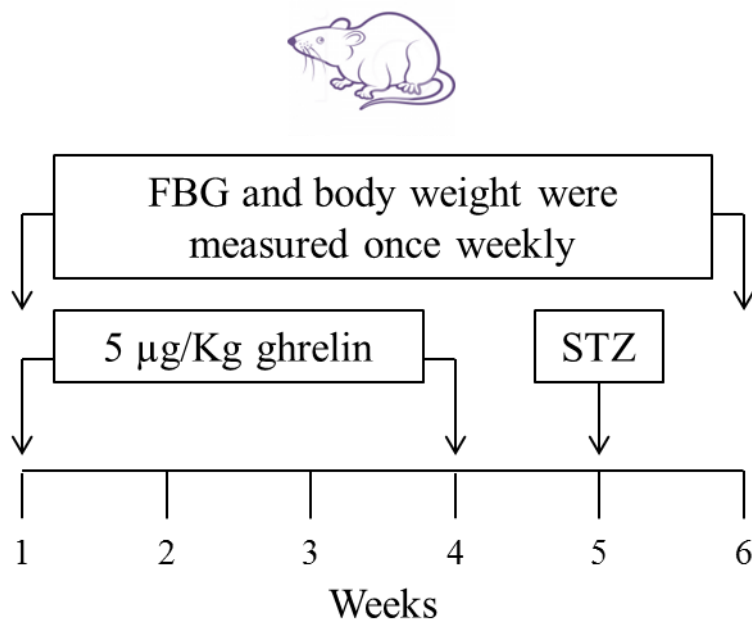


Figure 20: Schematic drawing explaining the study design of pre-diabetic ghrelin-treated group.

3.4 Fasting Blood Glucose and Body Weight Measurements

Body weight was measured once weekly along with FBG level. Animals were fasted for 12 hours and blood samples were collected from the tail vein in order to measure FBG using OneTouch® Ultra®2 Glucometer (LifeScan, Inc., Milpitas, CA, USA).

3.5 Glucose Tolerance Test

Glucose tolerance test (GTT) was performed at the end of the experiment (four weeks, except pre-diabetic ghrelin-treated group where it was performed after six weeks). Rats were fasted overnight (12 hours) and each rat received an intraperitoneal injection of 10 mg/kg/wt glucose. Blood samples were collected from the tail vein of the rats and blood glucose level was measured at 0 and 30, 60, 120 minutes after glucose load.

3.6 Blood and Tissue Collection

After the GTT, all rats were anesthetized with ether. A clean incision through the abdominal wall was made to expose the organ of interest. Blood was obtained from the inferior vena cava and kept in two separate tubes (yellow top: gel tube that helps splitting the serum from the rest of the blood sample components, purple top: contain EDTA).

Pancreatic tissue was collected from all the animals and cut into two parts: one part for immunohistochemistry and immunofluorescence studies and the second for electron microscopy.

3.7 Tissue Processing

Pancreatic tissues were embedded in Zamboni's fixative for overnight fixation prior to immunohistochemistry and immunofluorescence studies using light microscopy. However, for electron microscopy, pancreatic tissues were fixed overnight in McDowell solution.

3.7.1 Tissue Processing for Immunohistochemistry and Immunofluorescence Study

After overnight fixation in Zamboni's fixative pancreatic tissues were dehydrated in a series of graded ethanol concentrations ascending from 70% to 95% and "two changes" in 100% ethanol for a minimum of 2 hours per step. Specimens were then exposed to xylene "two changes" and subsequently paraffin wax at 55 °C (three changes). Tissues were embedded in paraffin blocks and sections of 6 µm thickness were sliced using a microtome (Shandon AS325, City, USA), and incubated in a water bath for a few seconds at 40-42 °C. Tissues were placed on

gelatin-coated slides, and kept on a hot plate to dry for a minimum of 2 hours to enhance the attachment of the sections.

3.7.2 Tissue Processing for Electron Microscopy

Some of the pancreatic tissues that were collected at the end of the experiment, were cut into small pieces, and immersed in McDowell's fixative for overnight fixation at 4 °C after trimming off fats and connective tissue. The specimens were later washed three times with 0.1 phosphate buffer (15 minutes each) and post-fixed in 1% osmium tetroxide for one hour. The samples were then dehydrated in ascending concentrations of ethanol (30%, 50%, 70%, 95%, and 100% "two changes", 15 minutes each). Two changes of propylene oxide was used for clearing the specimens for 15 minutes each. The samples were then transferred and exposed to different ratios of propylene oxide and resin (1:1, 1:2 and 1:3) for one hour each before infiltration with pure resin for overnight at 4 °C. Specimens were embedded in the resin using molds and kept in the oven to polymerize for overnight at 60 °C. Blocks of resin were trimmed and 1µm semi-thin sections were cut using glass knives on the ultra-microtome. Sections were transferred into a drop of water on the microscope slides using watchmaker's forceps and toluidine blue was used to stain the tissues. Toluidine blue-stained tissues were later examined with the microscope to locate the islets of Langerhans using light microscopy examination. Using a diamond knife, resin blocks were further trimmed for ultra-thin sectioning and placed on copper grids using a wire loop. The grids were placed on filter paper to dry and left to dry.

3.8 Immunohistochemistry Study: Avidin-Biotin Complex Staining Method for Paraffin Section

Pancreatic tissues slides were rinsed for 5 minutes in xylene solution (two changes) to remove the paraffin wax, followed by rehydration step using a series of descending concentration of ethanol solution (100% ethanol “two changes, 5 minutes each”, 95%, 70% and 50% “3 minutes each”), followed by washing with phosphate buffer solution (PBS) for 5 minutes before retrieval of antigen using citrate buffer (pH 6) treatment, where the slides were incubated for 1 min in the microwave using power 10, followed by 10 minutes incubation at power 1, and finally 20 minutes incubation at room temperature. The slides were washed with PBS (three changes, 5 minutes each) before marking the section with a Dako pen. Slides were incubated with 0.3% hydrogen peroxidase - methanol solution for 30 minutes to block endogenous peroxidase activity. The tissue sections were blocked using protein block for 45 minutes at room temperature, followed by overnight incubation with corresponding primary antibody (insulin anti-guinea pig or ghrelin anti-rabbit) at 4°C. Next day, the slides were kept at room temperature for an hour then washed with PBS (three changes, 5 minutes each), followed by incubation with the secondary biotinylated antibody (anti-guinea pig IgG for insulin and anti-rabbit IgG for ghrelin) at room temperature for 1 hour. The secondary antibody was washed off with PBS (three changes, for 5 minutes each), and the section were then incubated with streptavidin peroxidase conjugated for an hour. To reveal peroxidase activity, sections were washed with PBS (three changes) for 5 minutes each followed by 3,3-diaminobenzidine tetrahydrochloride DAB (Sigma) in PBS incubation at room temperature for 5 minutes.

Finally, the sections were washed for 5 minutes using distilled water and dehydrated in a series of ascending concentration of ethanol 50%, 70%, 95% and 100% (two changes) followed by xylene (two changes) for 5 minutes each. The slides were finally mounted with DPX . AxioCam HRc digital camera with AxioVision 3.0 software was used to capture images of stained sections (Carl Zeiss, Oberkochen, Germany). Images were then adjusted for contrast and brightness using image J 1.47V.

3.9 Double Labeling Immunofluorescence Staining Method for Paraffin Sections

Sections of pancreas, 6 μm thickness, were immersed twice in xylene for 5 minutes at room temperature to remove paraffin wax followed by rehydration in descending concentrations of ethanol for 3 minutes each. Slides were then incubated for 5 minutes in distilled water before treating the slides with citrate buffer for antigen retrieval in the microwave as described previously in section 2.8. Slides were then washed three times in PBS after marking the sections of interest with a Dako pen. Slides were incubated first with the blocking reagent at room temperature for 45 minutes followed by an overnight incubation with primary antibody (Table 2) (insulin anti-guinea pig, ghrelin anti-rabbit/ anti mouse and glucagon anti-mouse) at 4 °C. On next day, the slides were incubated at room temperature for one hour followed by PBS wash (three changes, for 5 minutes each) followed by one hour incubation at room temperature with the secondary antibody (ghrelin: anti-rabbit / anti-mouse RRX, insulin: anti-guinea pig FITC and glucagon: anti-mouse FITC) (Table 3). Finally slides were washed with PBS (three changes, 5 minutes each) before mounting with CTTI-Fluore mounting media. Slides were examined using a

fluorescence microscopy. Sections were examined using AxioCam HRc digital camera with AxioVision 3.0 software (Carl Zeiss, Oberkochen, Germany) fixed with z-plane fluorescence. Sections contrast and brightness were adjusted images were merged using image J 1.47V.

Primary	Dilution	Secondary	Dilution
Ghrelin (Phoenix Pharmaceuticals, Burlingame, CA, USA)	1:100	RRX (Jackson laboratories, Bar Harbor, USA)	1:100
Insulin (Dako, Glostrup, Denmark)	1:1000	FITC (Jackson laboratories, Bar Harbor, USA)	1:100
Glucagon (Dako, Glostrup, Denmark)	1:1000	FITC (Jackson laboratories, Bar Harbor, USA)	1:100

Table 3: List of names and dilutions of the primary and secondary antibodies used in this study

3.10 Transmission Electron Microscopy Post-embedding Immunogold Double Labeling Protocol

All grids were jet-washed with deionized water (5 mL each), and were incubated with 25 μ L of 10% H_2O_2 in water for 10 minutes. Grids were again washed with deionized water and immersed in 0.5 M NH_4Cl in 0.01 M PBS (pH 7.3) for 20 minutes before washing them again with PBS ‘washing buffer’ (1% BSA and 0.1% Tween-20).

Tissues were blocked by 20% normal goat serum (NGS) diluted in washing buffer ‘blocking buffer’ for 10 minutes followed by overnight incubation at 4 °C with primary antibody (Ghrelin anti-rabbit 1:100 diluted in PBS, pH 7.3, containing 1% BSA, 0.1% Tween-20 and 5% NGS). Next day, the grids were kept at room

temperature for an hour before washing them with PBS three times and again blocking buffer incubation at room temperature for 20 minutes. Grids were further incubated in goat anti-rabbit IgG conjugated to 15 nm gold particles (diluted 1:20 in antibody buffer) at room temperature for two hours.

Grids were rinsed again with PBS three times before repeating the whole process again but with insulin as second primary antibody (insulin anti-mouse (1:100) diluted in antibody buffer (PBS, pH 7.3, containing 1% BSA, 0.1% Tween-20 and 5% NGS)) which was labeled with 5 nm gold particles diluted (1:20 in antibody buffer) at room temperature for two hours before fixing the grids with glutaraldehyde (2.5% aqueous) for 5 minutes, then washing with deionized water and left on filter paper to dry for one hour. Contrasting the grids was done with uranyl acetate and lead citrate for 15 and 7 minutes, respectively. They were later washed with deionized water full 10 mL syringes each before leaving the grids to dry on filter paper. The sections were later viewed with a Philips TEM.

3.11 Enzyme-Linked Immunosorbent Assay

For the quantitative determination of insulin in serum samples, Enzyme-Linked Immunosorbent Assay (ELISA) was performed using Mercodia Ultrasensitive Rat Insulin ELISA kit and protocol as shown in Figure 21.

In wells 25 μ L of Calibrators (standards, 0, ...,6) were added, 25 μ L of samples were added in the rest of the wells followed by the addition of 100 μ L of enzyme conjugate 1X solution into each well, then the plate was incubated in the shaker (700-900 rpm) for 2 hours at room temperature (18-25°C). Later on, the plate was wash 6 times with 700 μ L wash buffer 1X solution per well using an automatic plate washer with overflow-wash function. After that a 350 μ L washing solution is

added to each well and discarded. Finally, 200 μL Substrate TMB was added into each well and was incubate 15 minutes at room temperature (18-25°C) before adding 50 μL Stop Solution to each well. The plate was placed on the shaker for approximately 5 seconds to ensure mixing and then the results were read by Mercodia software using optical density of 450 nm.

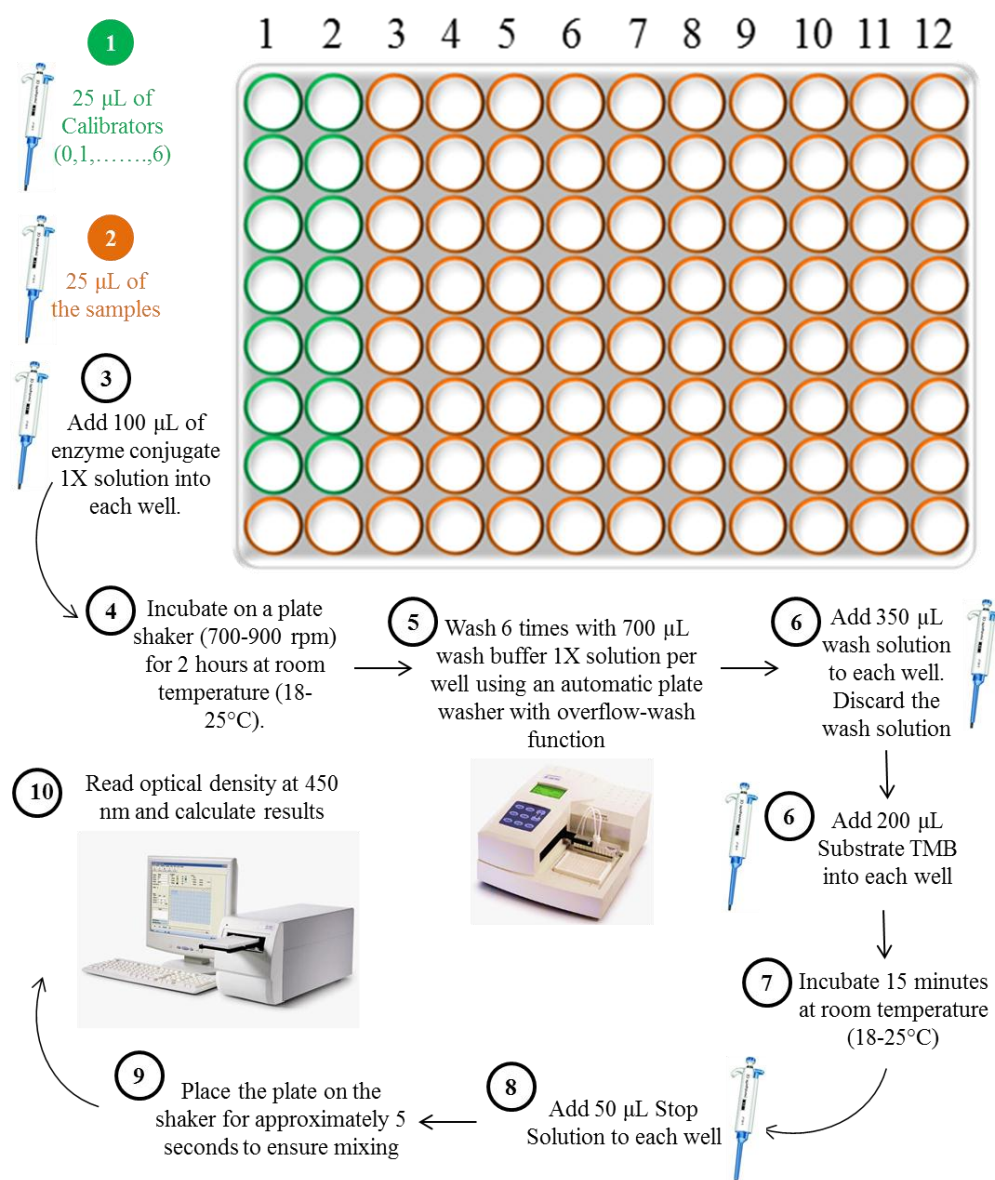


Figure 21: Illustration of Mercodia Ultrasensitive Rat Insulin ELISA protocol.

3.12 Biochemical Analysis

Serum samples were used to detect the levels of Alanine Aminotransferase (ALT) and Aspartate transaminase (AST) using a Beckman Coulter Synchron Lx20 PRO clinical system at Al Qatara Veterinary Laboratory.

3.13 Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 5.01. Data were presented as mean \pm Standard Deviation (SD). $p < 0.05$ were considered to be statistically significant. One-way ANOVA was used between experimental groups and corresponding control groups, followed by post-hoc Bonferroni. Unpaired t-test was used to analyze the significance of differences between mean values within the group between two different time points.

Chapter 4: Results

4.1 Effect of Ghrelin Treatment on Fasting Blood Glucose Level of Normal and Diabetic Wistar Rats

Figure 22 shows the effect of ghrelin treatment on fasting blood glucose level of normal and diabetic rats compared to normal saline treatment in control groups. Ghrelin treatment did not appear to have an influence on fasting blood glucose of normal treated rats when compared to normal control. Despite the slight elevation in fasting blood glucose level of diabetic rats treated with ghrelin after two weeks from the beginning of the treatment, this group showed lower fasting blood glucose level when compared to diabetic control group treated with normal saline at week three and four.

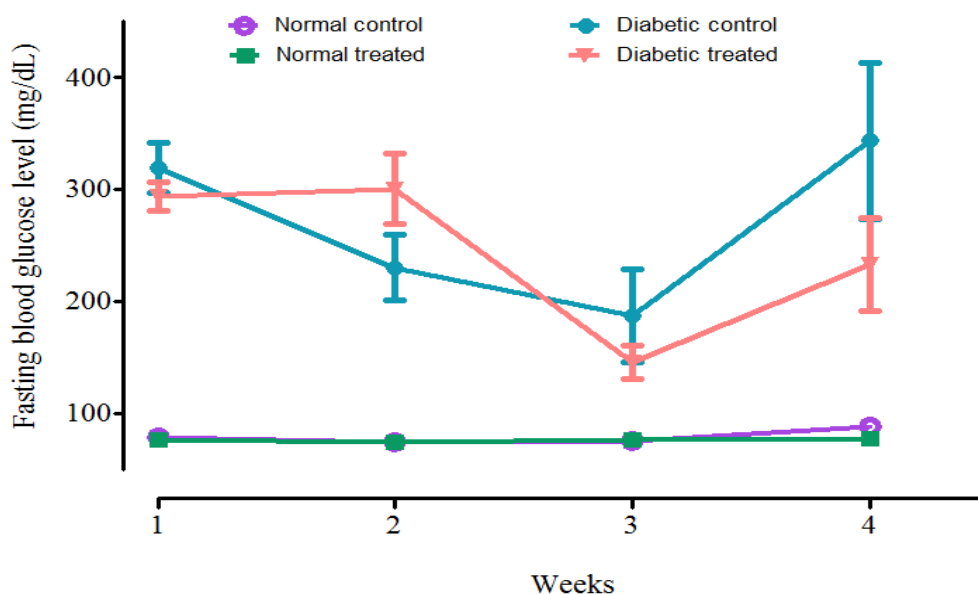


Figure 22: Effect of ghrelin treatment on fasting blood glucose level of normal and diabetic wistar rats.

Fasting blood glucose level (mg/dL) in normal and diabetic rats treated with either ghrelin or normal saline (NS), during a course of four weeks treatment. (n=4-7).

Results are expressed as mean \pm SD.

4.2 Effect of Ghrelin Treatment on Body Weight of Normal and Diabetic Wistar Rats

Figure 23 shows the effect of ghrelin treatment on body weight of normal and diabetic rats in comparison to age-matched controls treated with normal saline.

After two weeks from the beginning of the treatment, normal and diabetic groups treated with ghrelin had higher body weight than other rats treated with normal saline. However, after four weeks, ghrelin-treated rats showed lower body weight than their corresponding controls.

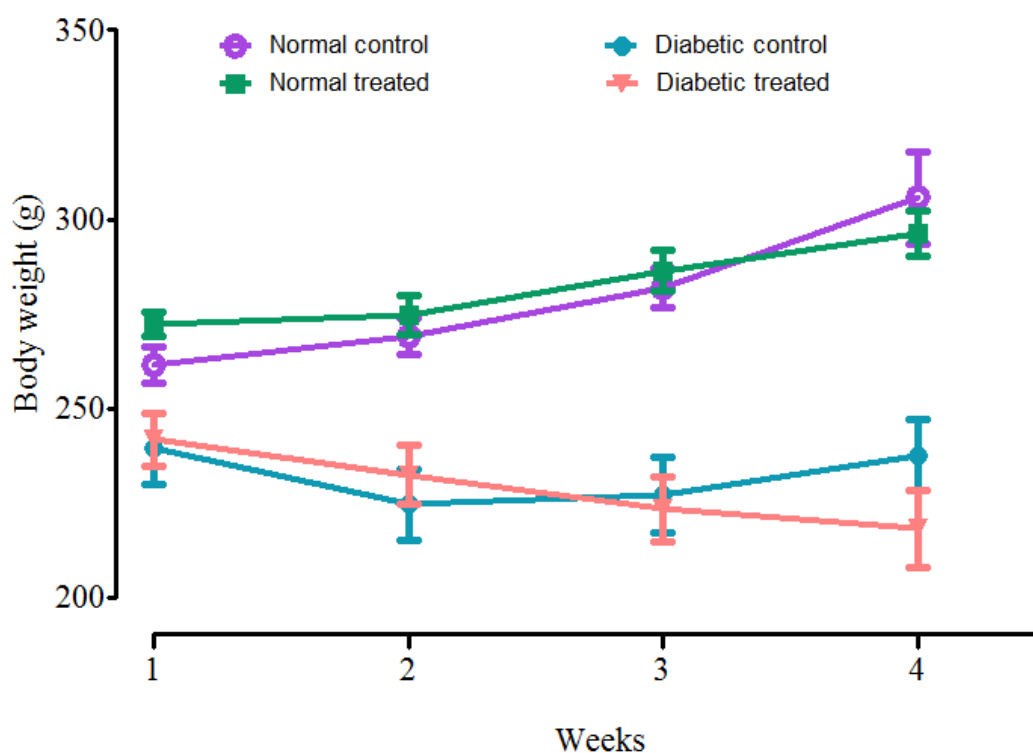


Figure 23: Effect of ghrelin treatment on body weight of normal and diabetic wistar rats.

Body weight (g) in normal and diabetic rats treated with either ghrelin or normal saline (NS), during a course of four weeks treatment. (n=3-7). Results are expressed as mean \pm SD.

4.3 The Effect of Four Weeks of Ghrelin Treatment on Preventing or Delaying the Onset of STZ-induced Diabetes Mellitus in Wistar Rats

Figure 24 shows the effect of ghrelin treatment before and after the induction of DM on fasting blood glucose level and body weight. Body weight (red line) was increasing upon ghrelin treatment, while fasting blood glucose level (blue line) was maintained within the normal range. Ghrelin treatment was stopped after four weeks. The rats received a single intraperitoneal STZ injection to chemically induce diabetes on week after stopping ghrelin treatment. One week from STZ injection, body weight showed a significant reduction, whereas fasting blood glucose level was increased significantly.

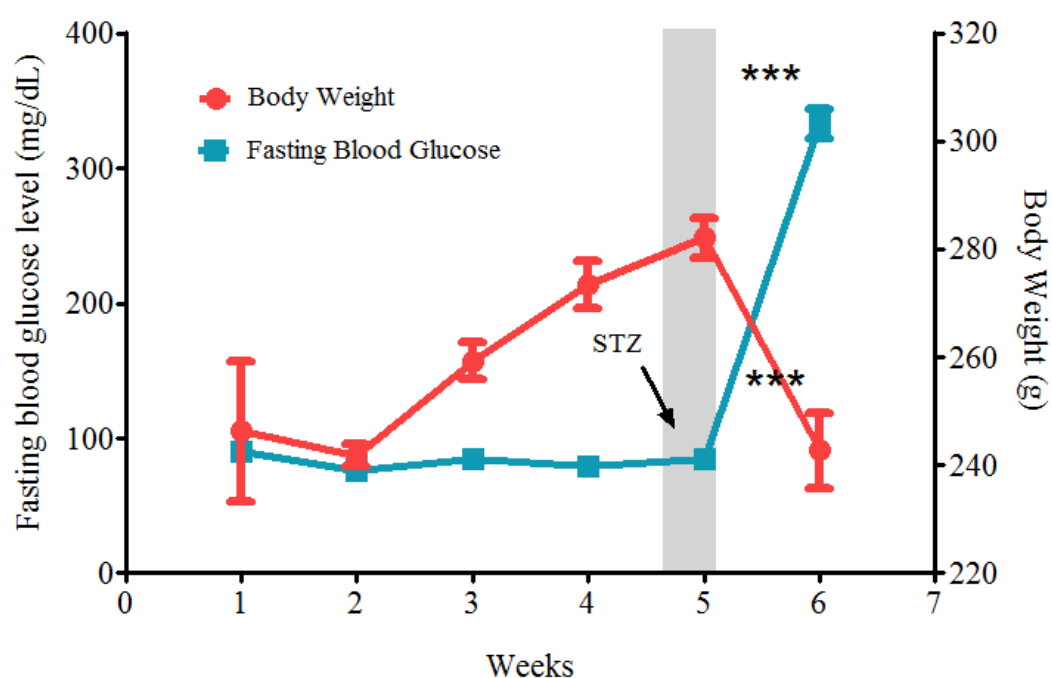


Figure 24: The effect of four weeks of ghrelin treatment on preventing or delaying the onset of STZ-induced diabetes mellitus in wistar rats.

Fasting blood glucose level “mg/dL” (blue line) and body weight “g” (red line) in normal rats treated with ghrelin before the administration of STZ to chemically induce DM in the fifth week of the study (arrow). (n=7). Results are expressed as mean \pm SD. (***) $P \leq 0.001$

4.4 The Effect of Ghrelin Treatment on Glucose Tolerance Test

Figure 25 shows blood glucose values after glucose challenge in normal and diabetic rats treated with ghrelin compared to age-matched controls treated with normal saline.

Ghrelin treatment showed no effect on the acute elevation of blood glucose level in normal group compared to NS treatment in normal control. On the other hand, though it is not statistically significant ($p>0.05$), diabetic rats treated with ghrelin exhibited a slight improvement in responding to glucose challenge when compared to diabetic rats treated with normal saline at 60 and 120 minutes after glucose load.

Furthermore, despite the fact that pre-diabetic ghrelin-treated group showed elevated blood glucose baseline when compared to the other two diabetic groups which can be explained by the new onset of diabetes, the group handled glucose challenge well at 120 minutes after glucose loading.

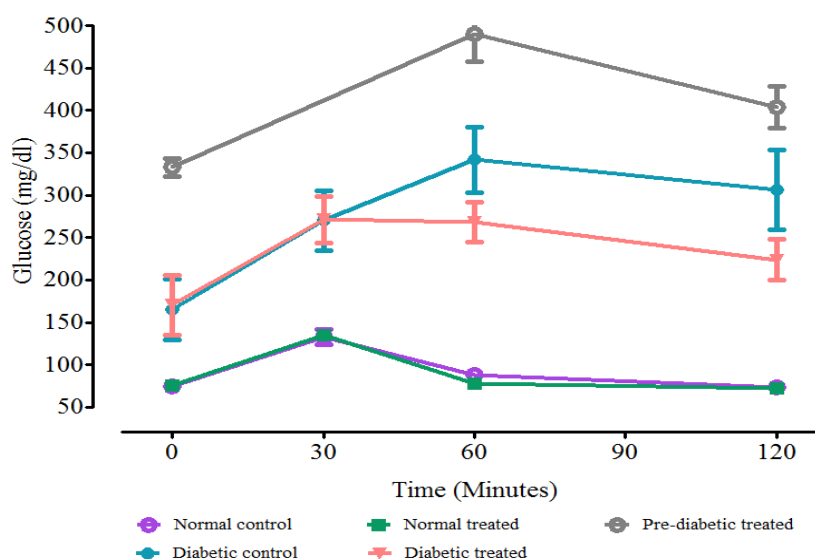


Figure 25: The effect of ghrelin treatment on glucose tolerance test.

Intraperitoneal glucose tolerance in normal and diabetic groups treated with either ghrelin (before and after diabetes onset) or normal saline (NS), after a course of four weeks treatment. (n=3-7). Results are expressed as mean \pm SD.

4.5 Summary and Discussion – I

- Although it was not statistically significant, but four weeks of ghrelin treatment succeeded in lowering fasting blood glucose level slightly in diabetic group.
- No differences in the body weight were observed between ghrelin-treated rats and their age-matched controls treated with normal saline.
- Pretreatment of rats with ghrelin did not prevent nor delay the onset of diabetes mellitus.
- Diabetic rats treated with ghrelin whether before or after the onset of DM showed better glucose handling when compared with diabetic rats treated with normal saline.

The above outcomes of ghrelin treatment seem promising and might be enhanced if one (or all) of the parameters of this study changed. For example increasing the dose of ghrelin for instance can result in either significant reduction of fasting blood glucose level or in increasing the body weight since many researches showed that the biological effects of ghrelin are dose-dependent (Akamizu et al., 2004; Carlini et al., 2002; Saito et al., 2005; ThidarMyint, Yoshida, Ito, & Kuwayama, 2006). Longer duration of ghrelin treatment may also yield a better outcome in diabetic rats.

Since it has been reported that pancreatic cells express ghrelin receptor (Andralojc et al., 2009b; Chanoine & Wong, 2004; Kageyama et al., 2005b) and that the pancreas can also secrete ghrelin (Dezaki et al., 2006), coupled with the findings of this study, we decided to further study the pattern of distribution in the rat pancreas and its association with insulin-secretion cells.

4.6 Immunohistochemical Localization of Insulin and Ghrelin in Normal and Diabetic Rat Pancreas

4.6.1 Insulin

Figure 26 shows the distribution pattern of insulin-secreting cells in the pancreatic tissue of normal control (A), normal treated (B), diabetic control (C) diabetic treated (D) and pre-diabetic ghrelin-treated (E) rats.

Figure 26 shows insulin-immunopositive cells (black arrows) in the central and peripheral regions of the islets of normal control rats (A). Whereas in normal rats treated with ghrelin, this pattern is slightly disrupted (B) and insulin-immunopositive cells (black arrows) are shown to be predominant in the central region of the islet. In diabetic control group insulin-immunopositive cells are hardly seen in the islet (C) as a result of STZ selective destruction to insulin-secreting β cells (Szkudelski, 2001).

On the other hand, a higher number of insulin-immunopositive cells (black arrows) can be observed randomly in both the center and peripheral regions of islet in diabetic rats treated with ghrelin before and after the onset of DM, (E) and (D), respectively.

4.6.2 Ghrelin

Figure 26 also shows the pattern of distribution of ghrelin-containing cells in the pancreatic tissue of normal control (F), normal treated (G), diabetic control (H) diabetic treated (I) and pre-diabetic ghrelin-treated (J) rats.

In normal control rats (F), ghrelin-containing cells (black arrows) are observed in the central and peripheral regions of pancreatic islets where insulin-secreting cells are usually found.

Whereas, in normal rats treated with ghrelin (G) the distribution pattern of ghrelin-immunopositive cells (black arrows) was found to be disrupted as insulin-immunopositive cells of the same group (B).

It was noticed that not only the number of insulin-secreting cells are affected by DM (C), but also the number of ghrelin-containing cells (H) which appear to be reduced when compared to normal control (F).

Diabetic group treated with ghrelin (I) showed a well preserved distribution pattern of ghrelin-containing cells (black arrow) in normal control (F). While ghrelin-immunopositive cells (black arrow) are randomly distributed in the pancreatic islet of pre-diabetic ghrelin-treated rats which is similar to what was observed in diabetic control group (H).

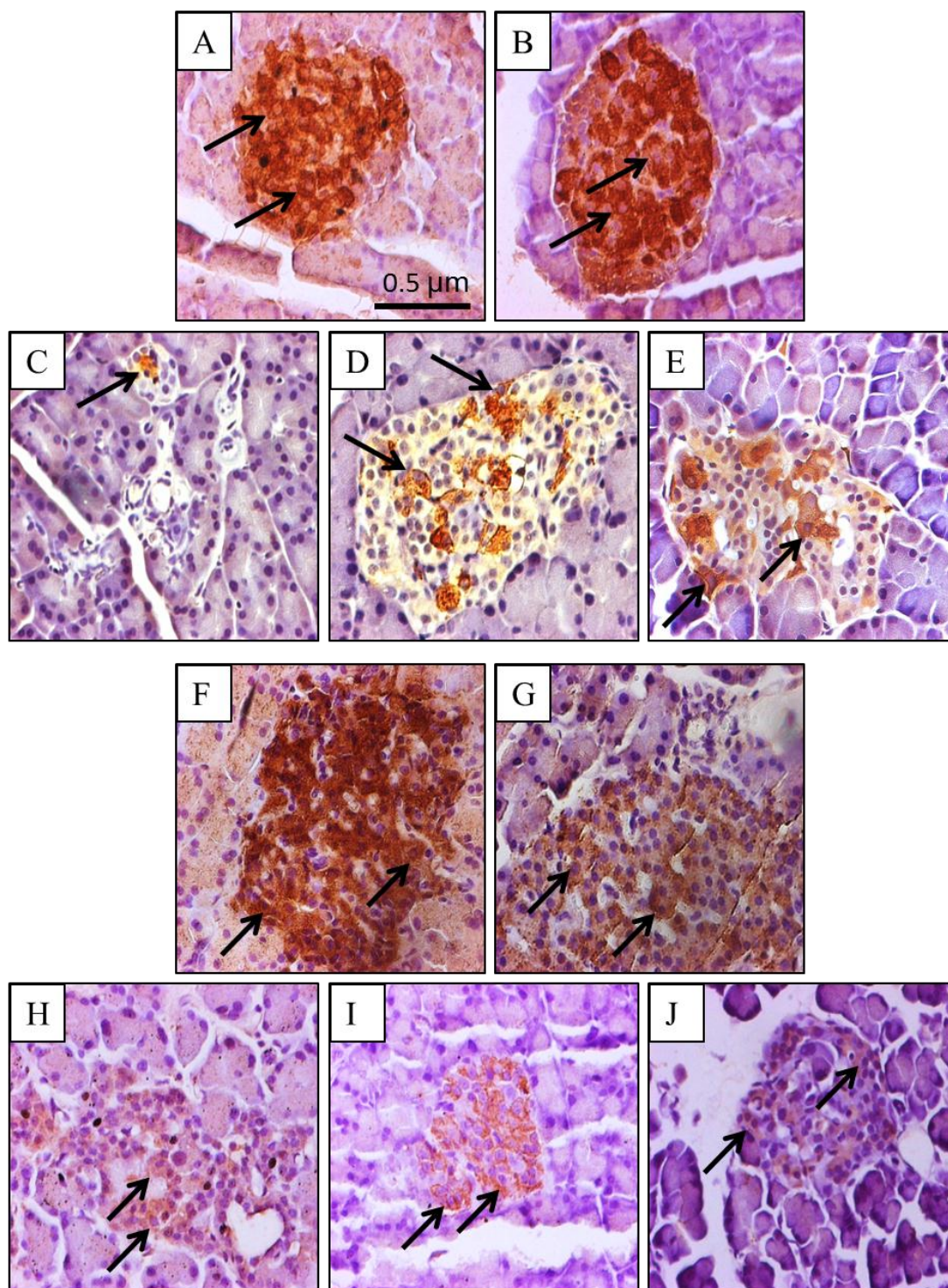


Figure 26: Immunohistochemical localization of insulin and ghrelin in normal and diabetic rat pancreas.

Distribution pattern of insulin-secreting cells (A, B, C, D, and E) and ghrelin-secreting cells (F, G, H, I, and J) in the pancreatic tissue. Normal control (A and F), normal treated (B and G), diabetic control (C and H), diabetic treated (D and I) and pre-diabetic ghrelin-treated (E and J). Arrows shows insulin- and ghrelin-immunopositive cells. Data are typical of those for 4 different animals in each group. Magnification, X400.

In order to quantitative the results of ABC staining, both insulin- and ghrelin-immunopositive cells were counted in correlation to the total number of islet cells and presented as a percentage, as shown in Figure 27.

In Figure 27-A, the percentage of ghrelin-immunopositive cells is the same in normal control and normal treated rats. On the other hand, a slight reduction is noticed in the percentage of ghrelin-immunopositive cells after the onset of DM, whereas diabetic treated and pre-diabetic treated groups displayed a higher percentage of ghrelin-positive cells when compared to diabetic control.

The percentage of insulin-immunopositive cells shown in Figure 27-B is increased in normal treated compared to normal control rats. However, in diabetic control the percentage of insulin-containing cells showed dramatic reduction compared to normal control. Moreover, ghrelin treatment in diabetic and pre-diabetic groups resulted in significant increase in insulin-immunopositive cells, 50% and 69.3%, respectively, compared to diabetic control.

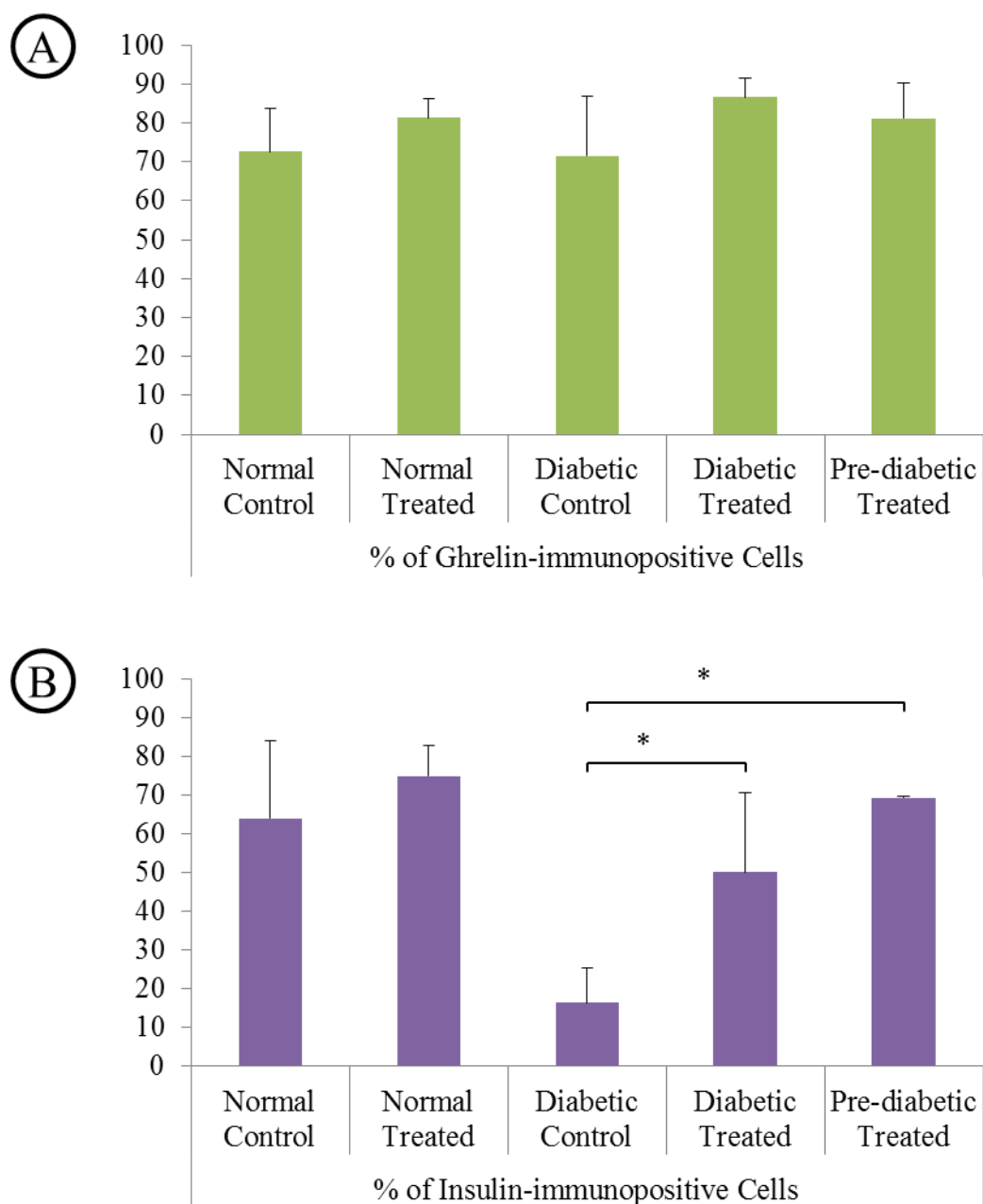


Figure 27: Percentage of insulin- and ghrelin-immunopositive cells.

The relative-percentage of ghrelin-immunopositive cells (green bars) and insulin-immunopositive cells (purple bars) in ghrelin treated normal and diabetic groups and their age-matched controls to total islet cells number. Results are expressed as mean \pm SD. * $p < 0.006$. One-way ANOVA.

4.7 Summary and Discussion – II

- Ghrelin-immunopositive cells in the pancreas share the same distribution pattern with insulin-secreting cells in normal and diabetic groups.
- The distribution pattern of insulin- and ghrelin-containing cells in the pancreatic islet was shown to be disrupted by the onset of DM in diabetic control rats and by ghrelin treatment in normal treated rats.
- Ghrelin treatment increased the number of insulin-containing cells significantly in both diabetic and in pre-diabetic groups.
- The percentages of insulin- and ghrelin-immunopositive cells in the pancreatic tissue of diabetic control rats were shown to decrease.

Ghrelin treatment rescued the insulin-secreting cells from STZ selective destruction in diabetic rats. While in the normal treated group, ghrelin treatment appears to enhance either the proliferation or the differentiation of insulin-secreting cells which resulted in a higher percentage of those cells.

Although ghrelin cells in the pancreas (epsilon cells) have been reported to represent about 1% of total islet cells, our study showed that the percentage of ghrelin-immunopositive cells is higher in all groups. This indicates that ghrelin is expressed by other types of cells in the islet. Moreover, inducing DM via STZ resulted not only in reducing insulin-immunopositive cells but also ghrelin-immunopositive cells which led to the assumption that either epsilon cells are also sensitive to STZ or that some of the β cells that were destroyed contained both insulin and ghrelin.

The significant increase in number of insulin-secreting cells observed in this study has prompted us to perform further experiments to determining serum insulin level and to examine the co-localization of ghrelin with insulin-secreting cells.

4.8 Effect of Ghrelin on Serum Insulin Level

Figure 28, shows serum insulin level in normal, diabetic and ghrelin-treated rats using Mercodia Ultrasensitive Rat Insulin ELISA kit. A clear elevation in serum level of insulin was observed in all ghrelin-treated groups in comparison to their corresponding controls. A significant ($p < 0.02$) decrease in serum insulin level of diabetic control rats was observed when compared to normal control rats. Furthermore, normal ghrelin-treated rats showed significant ($p < 0.04$) increase in insulin level compared to their age-matched control. Moreover, ghrelin treatment increased serum insulin level in diabetic groups compared to diabetic control; however it was significant only in pre-diabetic ghrelin-treated group.

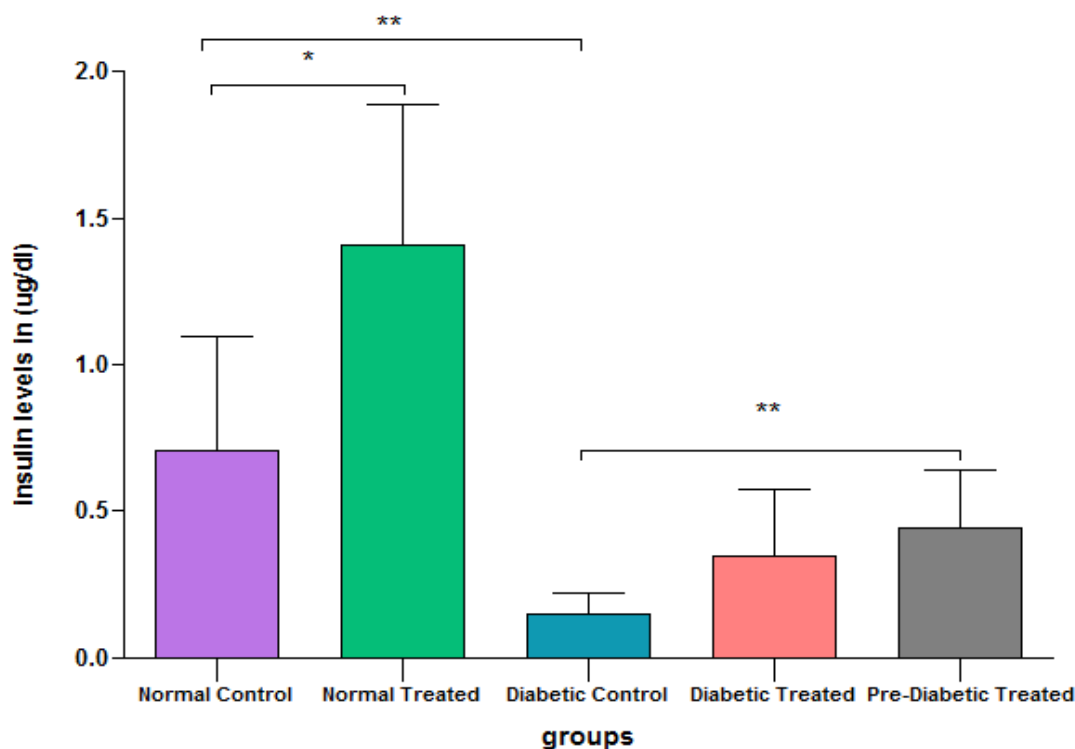


Figure 28: Effect of ghrelin on serum insulin level.

Quantitative determination of insulin level in the serum of normal and diabetic groups treated with either ghrelin (before and after diabetes onset) or normal saline (NS), for a course of four weeks. ($n = 4-7$). Results are expressed as mean \pm S.D. * $p < 0.04$ and ** $p < 0.02$. Unpaired t -test.

4.9 Summary and Discussion – III

- Ghrelin treatment increased insulin secretion in normal and diabetic groups of rats.
- Despite the new onset of DM in pre-diabetic treated group, ghrelin pre-treatment showed significant increase in the serum insulin level.

This result suggests that ghrelin treatment did not only increase the percentage of insulin-secreting cells significantly in diabetic groups (Figure 27) but also enhanced their secretory function which resulted in increasing serum insulin level (Figure 28). This suggests that ghrelin treatment rescued β cells from destruction by STZ.

Moreover, ghrelin treatment impact on insulin secretion can be recruited as a new approach in DM management to either reduce insulin dose or delay insulin treatment in T1DM and T2DM, respectively.

4.9.1 Co-Localization of Ghrelin with Insulin and Glucagon in Pancreatic Islet Cells

4.9.1.1 Ghrelin and Insulin

Figure 29 shows ghrelin-containing cells (A) and insulin-containing cells (B) and the co-localization of ghrelin with insulin (C).

It is clearly shown that in normal control rats, ghrelin localizes in both the central and peripheral portions of the islet where insulin-secreting cells are usually found. Merging of the two figures shows that ghrelin is co-localized with insulin (white arrows).

In normal ghrelin-treated group, ghrelin-positive cells are observed to concentrate in the periphery where glucagon secreting cells are found, however few cells showed co-localization of ghrelin with insulin.

In diabetic groups, ghrelin-positive cells appear numerous in the pancreatic islet, in contrast to insulin-positive cells which are observed to be fewer. Moreover, the distribution pattern of insulin secreting cells is disrupted due to DM. On the other hand, ghrelin-positive cells localize mainly in the central portion of the pancreatic islet. On merging, very few cells showed co-localization between ghrelin and insulin (white arrows).

It is noteworthy that in diabetic and pre-diabetic rats treated with ghrelin, insulin secreting cells were shown to be increased in number compared to diabetic control, which resulted in more co-localization between ghrelin and insulin (white arrows).

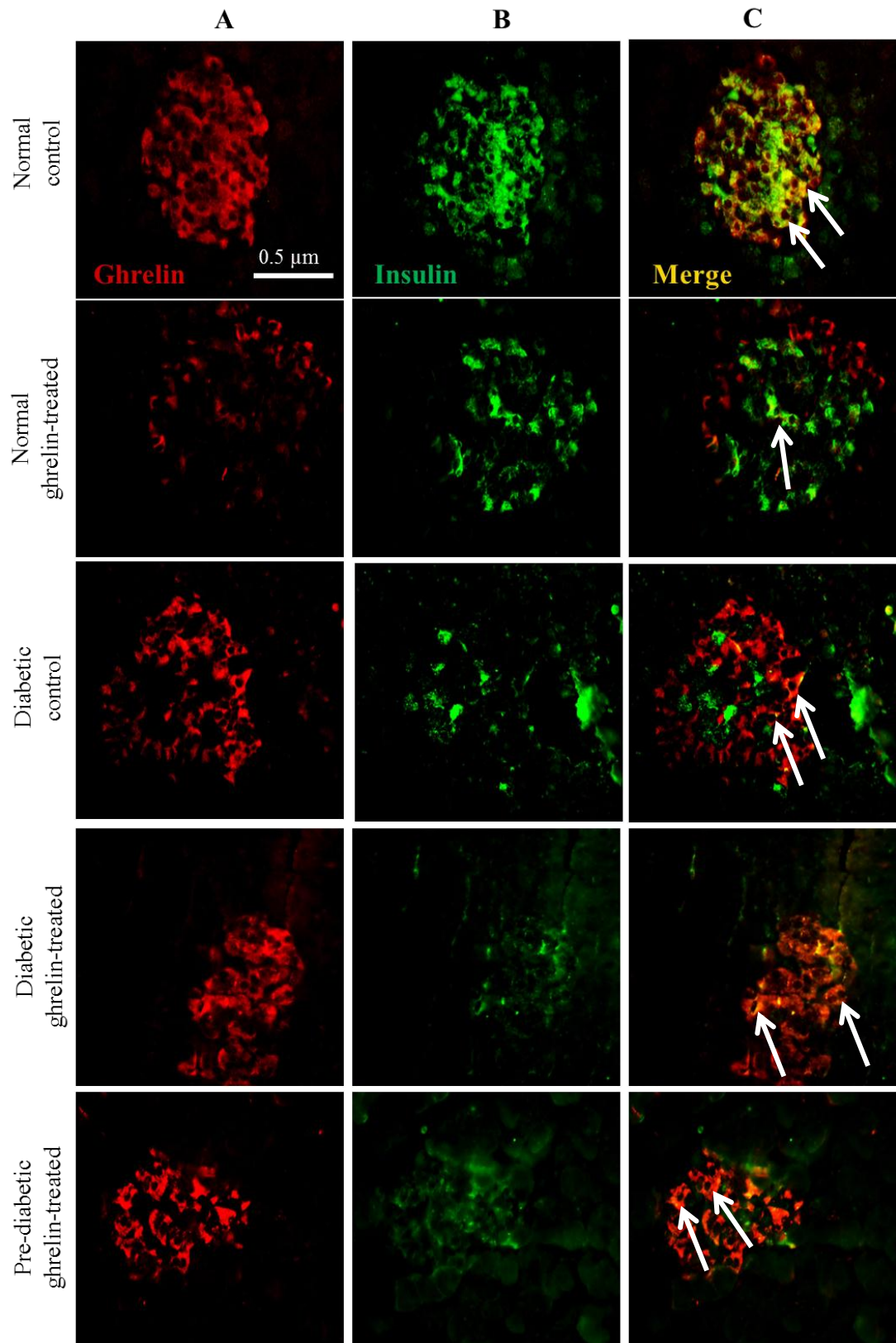


Figure 29: Co-localization of ghrelin with insulin in pancreatic islet cells.

Ghrelin-containing cells (A), Insulin-containing cells (B) and the merged image (C) where co-localization of ghrelin and insulin (white arrows). Magnification: X 400

4.9.1.2 Ghrelin and Glucagon

Figure 30 shows ghrelin-containing cells (A) and glucagon-containing cells (B) and the co-localization of ghrelin with glucagon (C).

In the pancreatic islet of normal control rats, ghrelin is observed to localize in the central as well as the peripheral portions, whereas, glucagon is seen primarily in the peripheral region. On merging, ghrelin was observed to co-localize with glucagon (white arrow); however, many ghrelin-immunoreactive cells do not contain glucagon.

Moreover, ghrelin was found to concentrate in the periphery of the pancreatic islet of normal ghrelin-treated group which resulted in a predominant co-localization of ghrelin with glucagon (white arrow).

Figure 30 also shows that ghrelin co-localizes predominantly with glucagon (white arrow) in diabetic control, diabetic and pre-diabetic treated rats.

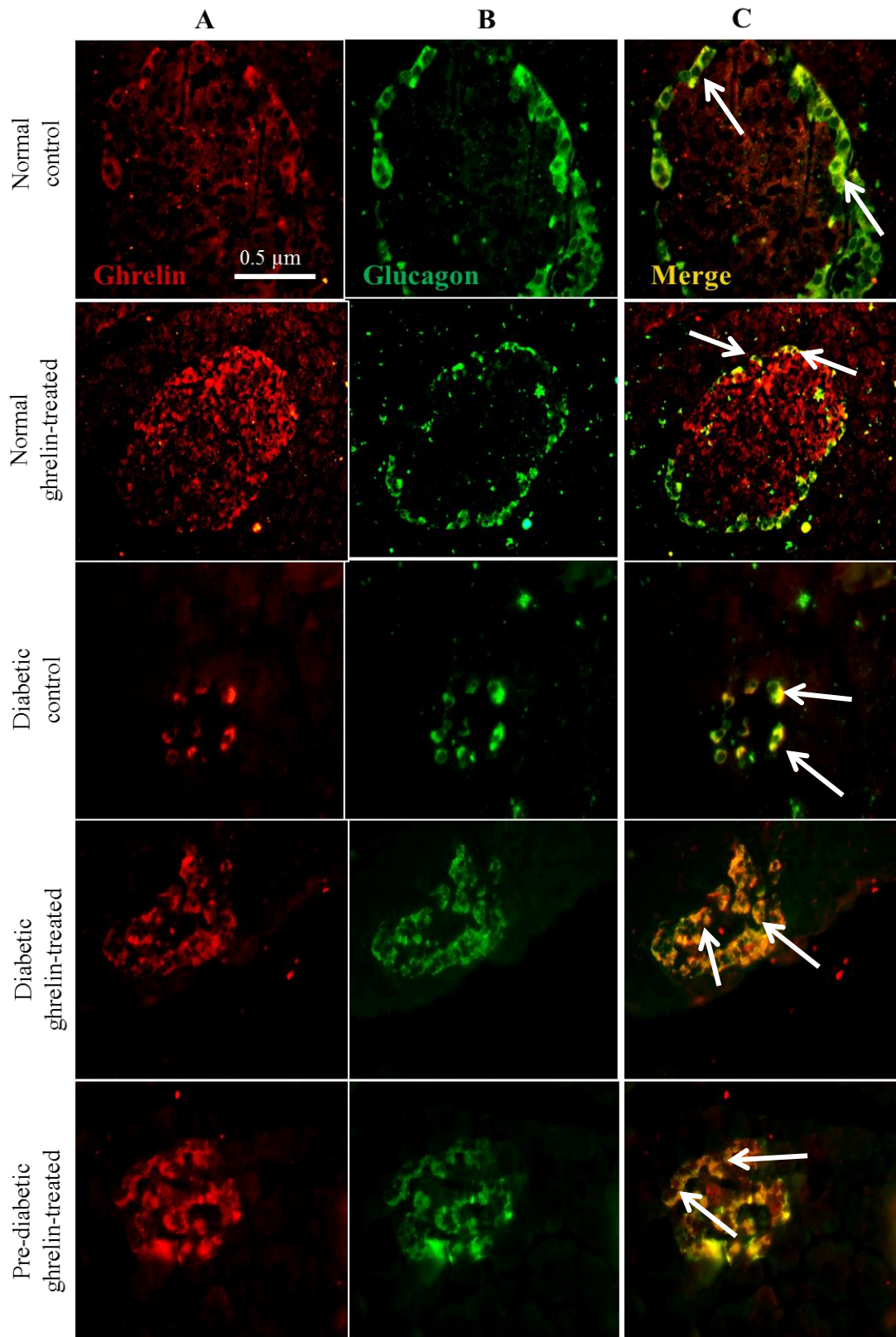


Figure 30: Co-localization of ghrelin with glucagon in pancreatic islet cells.

Ghrelin-containing cells (A), Insulin-containing cells (B) and the merged image (C) where co-localization of ghrelin and glucagon (white arrows). Magnification: X 400

4.9.2 Localization of Ghrelin and Insulin in the Secretory Granules of Pancreatic β Cells

4.9.2.1 Wistar Normal Control Rat

In Figure 31, shows the localization of ghrelin and insulin in β cells secretory granules in normal control group.

The majority of secretory granules contain insulin (solid arrow) alone, whereas ghrelin (dashed arrow) is observed alone in some. Moreover, ghrelin and insulin were found to share the same secretory granules in normal control (circle★).

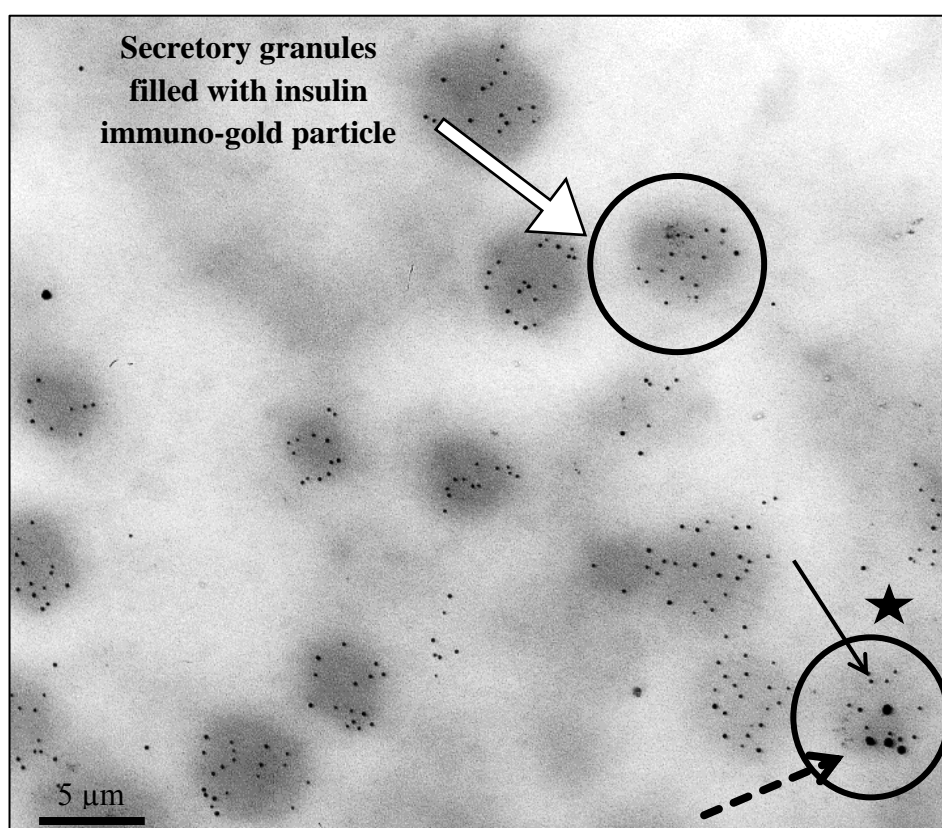


Figure 31: Localization of ghrelin and insulin in the secretory granules of pancreatic β cells of normal control rats.

Electron micrograph showing immunogold labeling of ghrelin (15 nm gold particles) and insulin (5 nm gold particles) in the secretory granules of normal control Wistar rats. Ghrelin (dashed arrow) and insulin (solid arrow) in the secretory granules (circle★). Magnification: X27500.

4.9.2.2 Wistar Normal Treated Rat

Figure 32, shows the localization of ghrelin and insulin in β cells secretory granules in normal ghrelin-treated group.

Most of the secretory granules of pancreatic β cells contain ghrelin (dashed arrow) in contrast to normal control group (Figure 31). Furthermore, many secretory granules are found to contain both ghrelin and insulin (circle★).

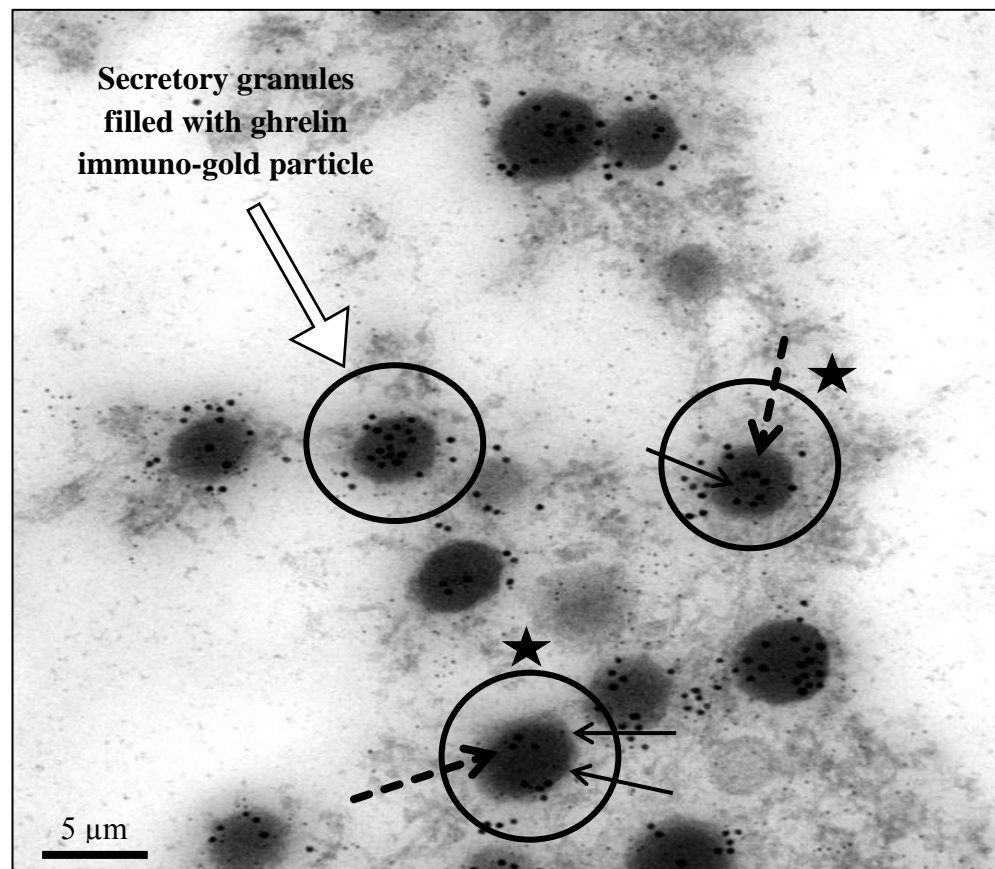


Figure 32: Localization of ghrelin and insulin in the secretory granules of pancreatic β cells of normal ghrelin-treated rats.

Electron micrograph showing immunogold labeling of ghrelin (15 nm gold particles) and insulin (5 nm gold particles) in the secretory granules of normal Wistar rats treated with ghrelin. Ghrelin (dashed arrow) and insulin (solid arrow) in the secretory granules (circle★). Magnification: X27500.

4.9.2.3 Wistar Diabetic Control Rat

Figure 33 shows the localization of ghrelin and insulin in β cells secretory granules in diabetic control group.

In diabetic control group, the secretory granules can be seen to reduce in number in diabetic control in comparison to normal control (31) as well as insulin-immunopositive gold-conjugates (solid arrow). In addition, ghrelin (dashed arrow) was found to co-localize with insulin in some of the secretory granules (circle★).

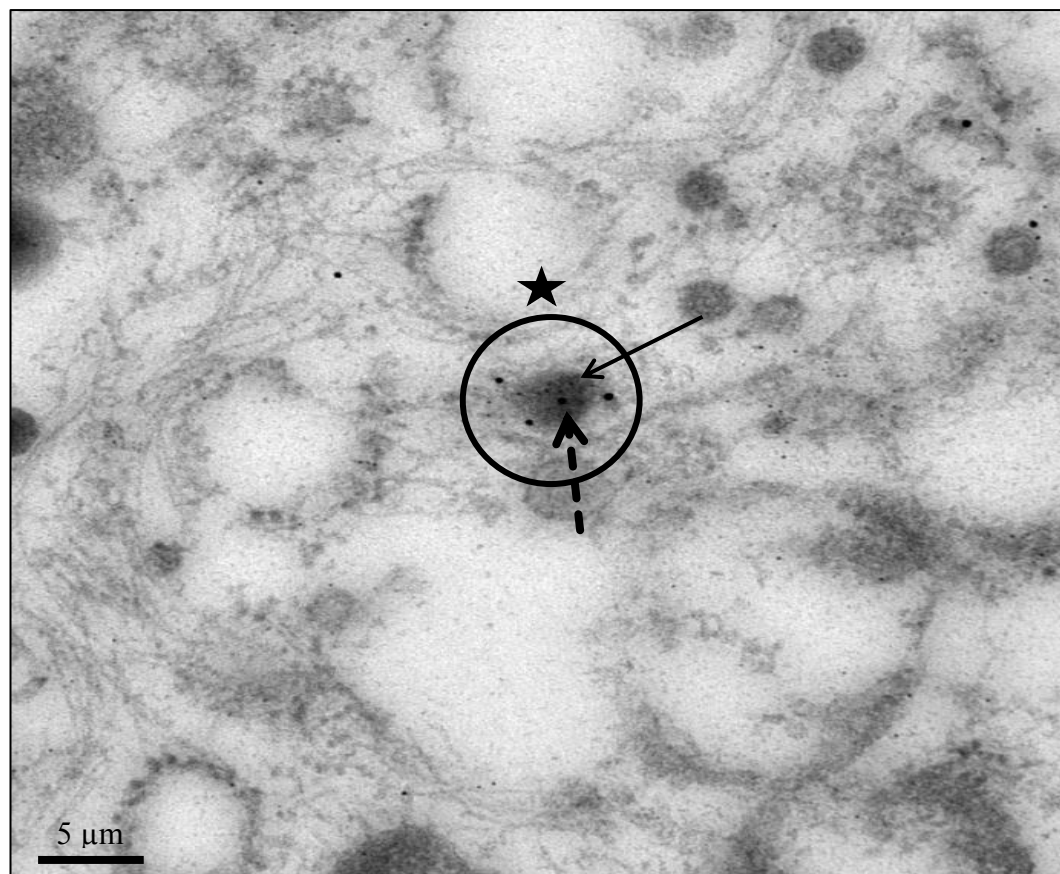


Figure 33: Localization of ghrelin and insulin in the secretory granules of pancreatic β cells of diabetic control rats.

Electron micrograph showing immunogold labeling of ghrelin (15 nm gold particles) and insulin (5 nm gold particles) in the secretory granules of untreated diabetic rats. Ghrelin (dashed arrow) and insulin (solid arrow) in the secretory granules (circle★). Magnification: X27500.

4.9.2.4 Wistar Diabetic Treated Rat

Figure 34 shows the localization of ghrelin and insulin in β cells secretory granules in diabetic group treated with ghrelin.

Ghrelin treatment resulted in increasing both ghrelin (dashed arrow) and insulin (solid arrow) in the diabetic group compared to their age-matched control (Figure 33). Moreover, ghrelin immunopositive gold-conjugates are seen to share secretory granules in pancreatic β cells with insulin (circle★).

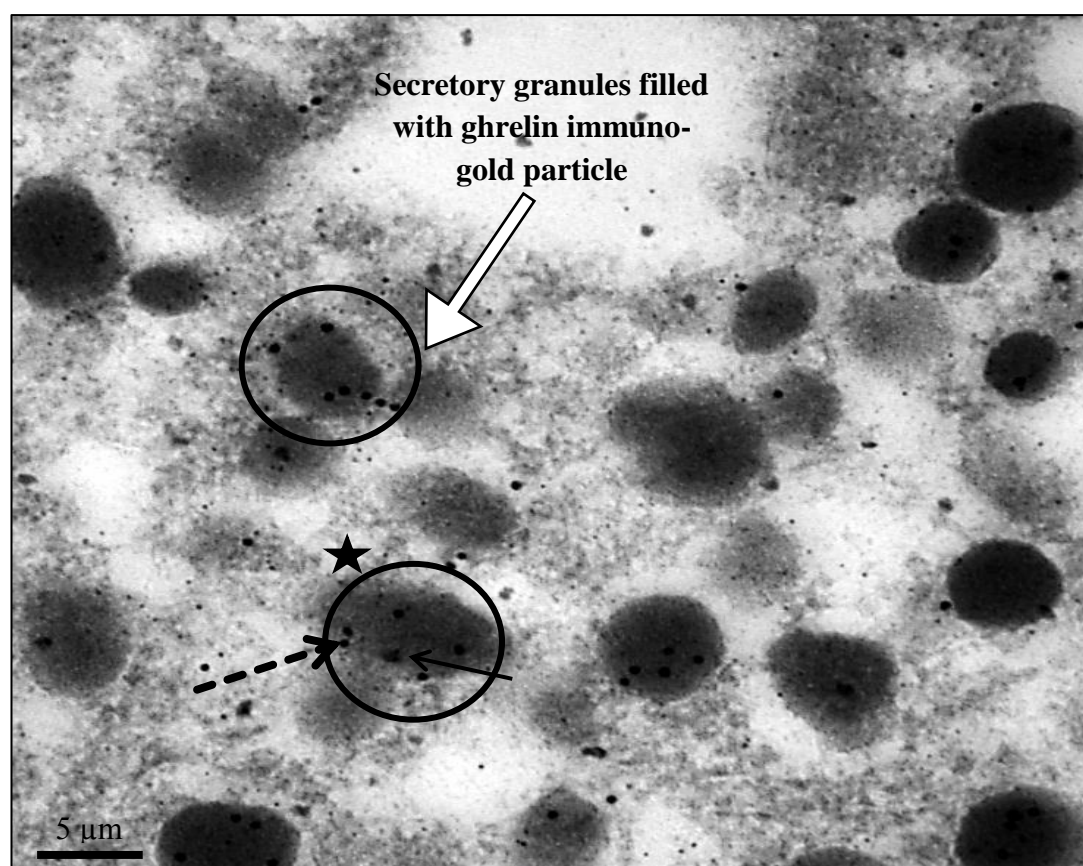


Figure 34: Localization of ghrelin and insulin in the secretory granules of pancreatic β cells of diabetic ghrelin-treated rats.

Electron micrograph showing immunogold labeling of ghrelin (15 nm gold particles) and insulin (5 nm gold particles) in the secretory granules of diabetic rats treated with ghrelin. Ghrelin (dashed arrow) and insulin (solid arrow) in the secretory granules (circle★). Magnification: X27500.

4.10 Summary and Discussion – IV

- This study showed that ghrelin co-localizes with both insulin and glucagon.
- We revealed that ghrelin co-localization pattern is disrupted after the onset of DM (diabetic control).
- We demonstrated that ghrelin co-localizes with insulin in the secretory granules of pancreatic β cells.

Investigating the co-localization of ghrelin and insulin in secretory granules of pancreatic β cells revealed that in ghrelin-treated groups both ghrelin and insulin immunopositive gold-conjugates were observed to be higher when compared to age-matched controls.

Furthermore, in ghrelin-treated group many secretory granules contain both ghrelin and insulin; whereas in normal and diabetic control groups few secretory granules were shown to contain both of them together.

In addition, a previous study (Kageyama et al., 2005c) showed that GHS-R localizes with glucagon in the periphery of the islet. Also the same study showed that both ghrelin and GHS-R are located in the periphery of the pancreatic islets where glucagon cells are found. Along with the outcomes of this study, we concluded that ghrelin may enhance insulin secretion directly and also indirect through either inhibiting the effect of glucagon or by inhibiting its secretion.

4.11 Effect of Ghrelin Treatment on Liver Function

4.11.1 Alanine Transaminase

Figure 35, shows serum Alanine Transaminase (ALT) level in normal, diabetic and ghrelin-treated groups.

The serum ALT level was elevated in control and ghrelin-treated groups. However, in diabetic groups treated with ghrelin, ALT level was further elevated.

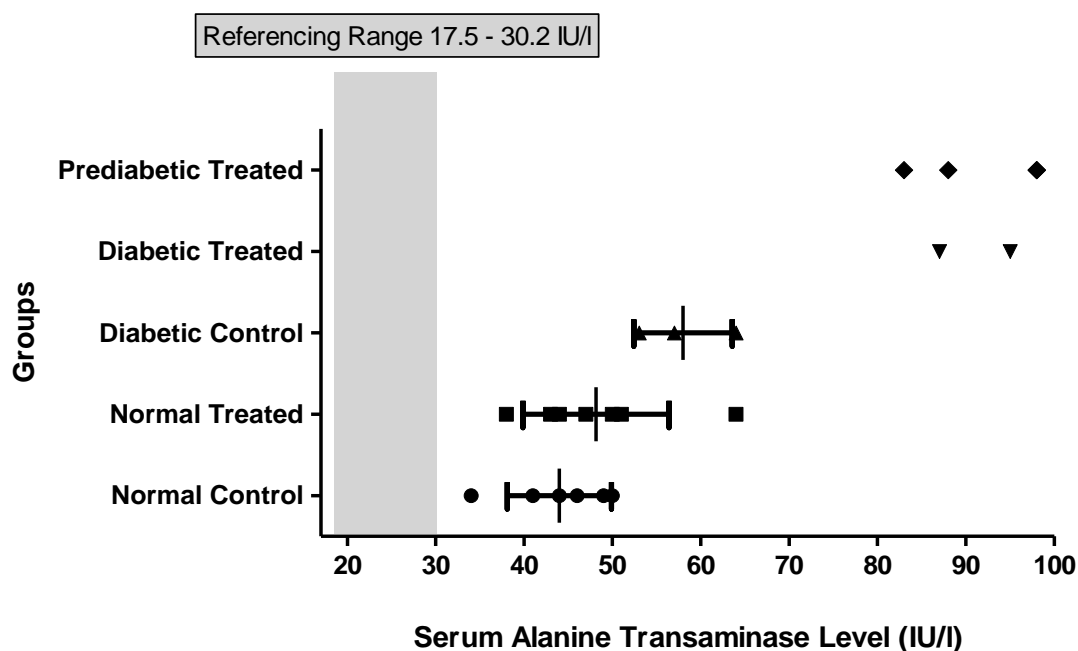


Figure 35: Serum Alanine Transaminase levels.

Serum ALT level of normal, diabetic and ghrelin-treated rats (ref. range: 17.5 – 30.2 IU/l). (n=3-7).

4.11.2 Aspartate Transaminase

Figure 36, shows serum Aspartate Transaminase (AST) level in normal, diabetic and ghrelin-treated groups.

Serum AST level was also elevated in control groups as well as ghrelin-treated groups. However AST level was higher in diabetic groups treated with ghrelin.

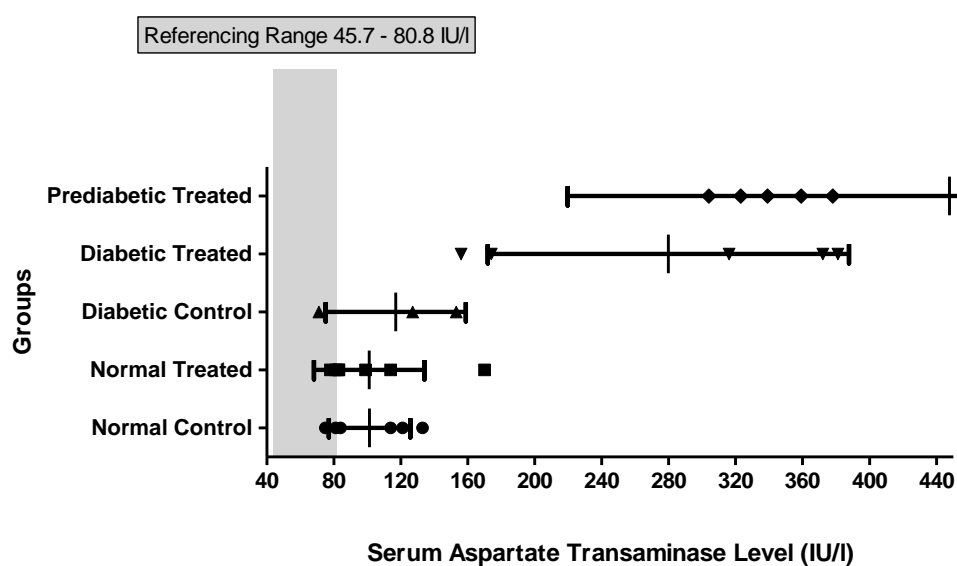


Figure 36: Serum Aspartate Transaminase levels.

Serum AST level of normal, diabetic and ghrelin-treated rats (ref. range: 45.7-80.8 IU/l). (n=3-7).

4.12 Summary and Discussion – VII

In this study we tried to determine the effect of ghrelin chronic treatment on liver function by measuring the level of Alanine Transaminase (ALT) and AST in the blood serum of normal, diabetic and ghrelin-treated rats.

ALT and AST are enzymes mainly found in liver, but also in small amount in the heart, pancreas, muscle and kidneys. Elevated level of ALT or AST or both indicates presence of liver damage.

Control and ghrelin-treated groups showed elevated serum levels of ALT and AST. However, ghrelin treatment and pre-treatment in diabetic rats resulted in much higher levels of both enzymes than any other group which indicates liver damage.

Our results of the harmful effect of ghrelin on liver correlate with De Vriese *et al*, who suggested the involvement of ghrelin in the pathogenesis of liver diseases (Delhanty & van der Lely, 2013; Estep et al., 2011; Mykhalchyshyn, Kobylak, & Bodnar, 2015). Furthermore, ghrelin was reported to modulate liver function (Okamatsu et al., 2009).

Chapter 5: Summary

1. Even though FBG level was not reduced significantly in diabetic ghrelin-treated group than diabetic control group.

And ghrelin treatment did not prevent nor delay the onset of diabetes mellitus (pre-diabetic group).

Diabetic ghrelin-treated groups showed better handling of glucose than diabetic control.

2. Pancreatic ghrelin distribution pattern was shown to be disrupted after ghrelin treatment in normal group and also in diabetic groups when compared to normal control.

However, ghrelin-treated groups showed higher percentage of insulin-immunopositive cells than their controls.

Explaining the elevated serum insulin level which was shown in ghrelin-treated groups and was significant in normal and pre-diabetic ghrelin-treated groups.

3. Ghrelin co-localizes with both insulin and glucagon secreting cells.

Ghrelin co-localized with insulin in secretory granules of β cell.

Indicating ghrelin direct role in insulin secretion.

Figure 37: Summary of the outcomes in this study.

Chapter 6: Discussion

- Maintenance of glucose homeostasis is a very complicated process. Many neuroendocrine hormones are involved in regulating glucose uptake, storage and release.

- Our results imply that ghrelin has an important role in glucose homeostasis which correspond to Heppner KM et al, report (Heppner, Müller, Tong, & Tschöp, 2012). However, the controversy about the exact effects of ghrelin on blood glucose level remain unresolved (Chabot, Caron, Laplante, & St-Pierre, 2014).

- In literature, it was reported that ghrelin level correlates positively with insulin resistance (Purnell, Weigle, Breen, & Cummings, 2003). However Pöykkö SM et al, reported that in T2DM ghrelin concentration was negatively associated with insulin resistance (Pöykkö et al., 2003). However, in this study ghrelin-treated rats handled glucose challenge well indicating that ghrelin enhances tissues response to insulin.

- Ghrelin is known as a “hunger hormone” (Higgins et al., 2007) and was reported to increase food consumption (Kamegai et al., 2001). Moreover, while many studies showed that ghrelin treatment positively induces weight gain (Salfen, Carroll, Keisler, & Strauch, 2004), a study on Japanese quail showed that the effect of ghrelin on food intake correlates negatively with the dose of ghrelin (Shousha et al., 2005). However our observation correlates with those of Gelling RW *et al*, which revealed that food intake of STZ-diabetic rats was increased despite their weight reduction (Gelling et al., 2004). Unfortunately, no

measurement was taken to calculate the food consumption in this study and the reasons behind the slight reduction in the body weight are not clear.

- Altering one or two or all of the treatment criteria such as dose, duration and frequency of the treatment, may further influence our outcomes. Moreover, the difference between our results and those available in the literature may be due to one of more of the factors outlined above.

- We have demonstrated that the distribution pattern of ghrelin-containing cells is similar to that of insulin-containing cells and that both patterns were disrupted after the onset of DM. Moreover, in a study investigating the cellular distribution of ghrelin in the pancreas of diabetic rats it was shown that DM is associated with a slight reduction in the expression of ghrelin in pancreatic islet cells (Yildirim, Sundler, & Bolkent, 2007). This observation corroborates data reported in our study.

- We also revealed that ghrelin treatment and pre-treatment resulted in significant increase of insulin-containing cells. This finding is in agreement of that of Turk *et al.*, who reported that the number of insulin-positive cells increase after the administration of 100 µg of ghrelin per day for four weeks (Turk, Dağıstanlı, Sacan, Yanardag, & Bolkent, 2012).

- Moreover, ghrelin treatment resulted in elevated level of serum insulin, indicating the role of ghrelin in enhancing insulin secretion. This suggests the possibility of recruiting ghrelin as a potential therapeutic approach in the management of DM.

- Additionally, ghrelin was observed to co-localize with both insulin and glucagon in pancreatic islet. The co-localization of ghrelin with insulin was disrupted in diabetic control group and in normal ghrelin-treated group.

However, ghrelin and glucagon co-localization was not affected in either diabetic control or normal ghrelin-treated groups. Ghrelin localization in pancreatic islet cells is controversial. Our observation implies that ghrelin predominantly co-localizes with glucagon in pancreatic islet, which corroborates with those of Date, et al. (Yukari Date et al., 2002), who showed that ghrelin is located in pancreatic α cells. However, another studies revealed that ghrelin co-localizes with β cells (Volante et al., 2002), while some have even reported that ghrelin has its own cells (Wierup, Yang, McEvilly, Mulder, & Sundler, 2004).

- Furthermore, our study demonstrated that ghrelin co-localizes with insulin in the secretory granules of pancreatic β .

- Elevated serum levels of AST and ALT indicates liver injury (Nkosi, Opoku, & Terblanche, 2006; Senior, 2012). Moreover, it is known that the serum level of ALT increases after the onset of diabetes (Aragon & Younossi, 2010). Our investigation of ghrelin effect on liver function revealed that in DM, ghrelin treatment increase AST and ALT enzymes indicating that ghrelin may be involved in the pathogenesis of liver injury.

Chapter 7: Conclusion

We addressed our hypothesis and demonstrated that ghrelin co-localizes with insulin-secreting β cells. We also revealed the involvement of ghrelin in insulin secretion and its effect on lowering blood glucose level. Unfortunately, ghrelin failed to prevent/delay DM which was the second hypothesis of this study.

The outcomes of this investigation suggest that ghrelin can be a new approach in DM management (Figure 38).

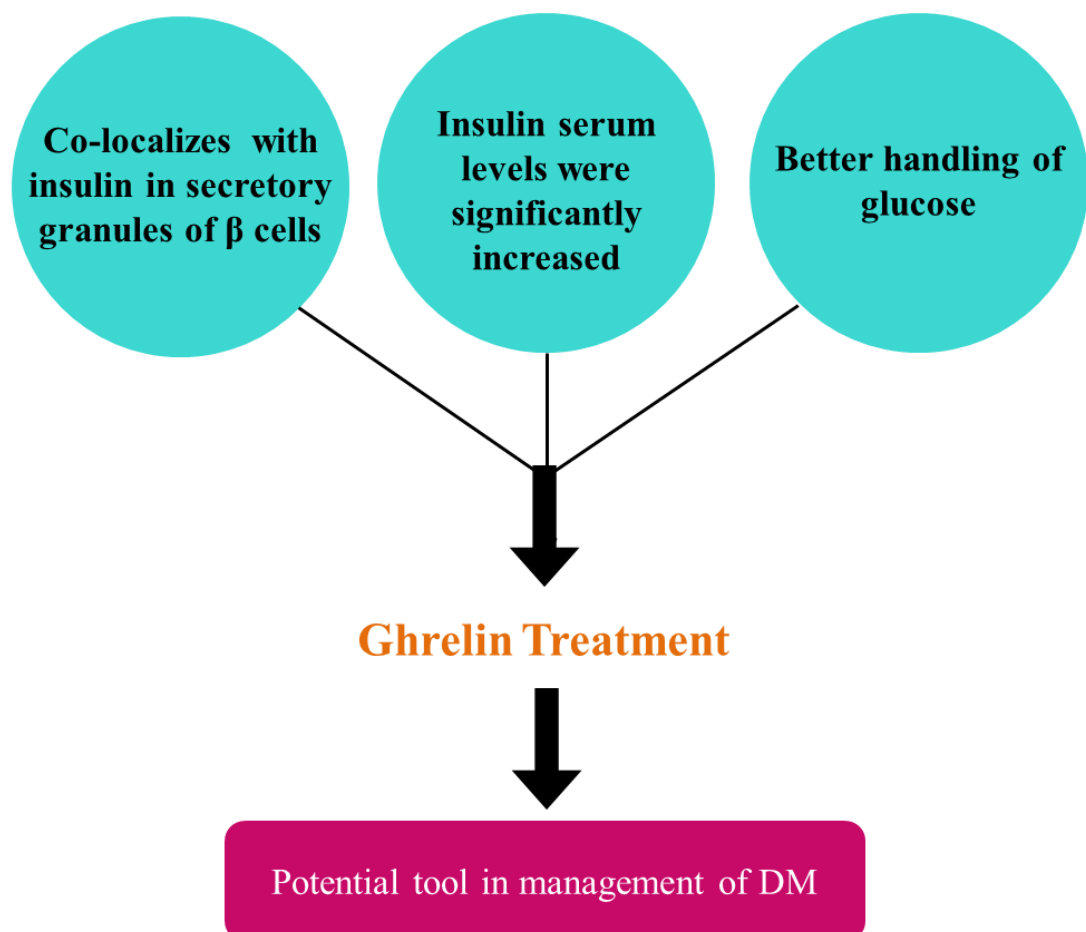


Figure 38: Conclusion of the study

Chapter 8: Limitations and Future Prospective

- The number of rats was low in some groups which resulted in higher Standard Deviation.
- The duration of treatment was only four weeks and we studied only one dose, which is considered to be relatively low.
- Our data represents the effect of ghrelin treatment in only one model of DM (T1DM).
- We only studied the co-localization of ghrelin with insulin in pancreatic β cells secretory granules. The study of the co-localization of ghrelin with glucagon in the secretory granules of α cells is the next step; to address whether ghrelin influence on insulin secretion is a result of ghrelin hypothesized effect on glucagon secretion/inhibitory effect on insulin, in addition to measuring glucagon level in blood serum.
- Studying the effect of ghrelin treatment at a younger age (birth!).
- Determining the amount of food consumption and examining the effect of ghrelin on different caloric intake.
- Finally, more investigations are required to examine the reason behind the slight reduction in body weight by studying the possibility of feedback inhibition via measuring circulating ghrelin level.

Bibliography

- Adeghate, E. (2001). Diabetes mellitus - multifactorial in aetiology and global in prevalence. *Archives of Physiology and Biochemistry*, 109(3), 197–199.
<http://doi.org/10.1076/apab.109.3.197.11588>
- Adeghate, E., & Kalász, H. (2011). Amylin analogues in the treatment of diabetes mellitus: medicinal chemistry and structural basis of its function. *The Open Medicinal Chemistry Journal*, 5(Suppl 2), 78–81.
<http://doi.org/10.2174/1874104501105010078>
- Adeghate, E., & Parvez, H. (2002). Mechanism of ghrelin-evoked glucagon secretion from the pancreas of diabetic rats. *Neuro Endocrinology Letters*, 23(5-6), 432–436.
- Adeghate, E., & Ponery, A. S. (2002). Ghrelin stimulates insulin secretion from the pancreas of normal and diabetic rats. *Journal of Neuroendocrinology*, 14(7), 555–560.
- Adeghate, E., & Ponery, A. S. (2004). Mechanism of ipamorelin-evoked insulin release from the pancreas of normal and diabetic rats. *Neuro Endocrinology Letters*, 25(6), 403–406.
- Adeghate, E., Schattner, P., & Dunn, E. (2006a). An update on the etiology and epidemiology of diabetes mellitus. *Annals of the New York Academy of Sciences*, 1084, 1–29. <http://doi.org/10.1196/annals.1372.029>
- Adeghate, E., Schattner, P., & Dunn, E. (2006b). An update on the etiology and epidemiology of diabetes mellitus. *Annals of the New York Academy of Sciences*, 1084, 1–29. <http://doi.org/10.1196/annals.1372.029>
- Ahmed, A. M. (2002). History of diabetes mellitus. *Saudi Medical Journal*, 23(4), 373–378.

- Ahrén, B. (2003). Gut peptides and type 2 diabetes mellitus treatment. *Current Diabetes Reports*, 3(5), 365–372. <http://doi.org/10.1007/s11892-003-0079-9>
- Akamizu, T., Takaya, K., Irako, T., Hosoda, H., Teramukai, S., Matsuyama, A., ... Kangawa, K. (2004). Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. *European Journal of Endocrinology*, 150(4), 447–455. <http://doi.org/10.1530/eje.0.1500447>
- Akbarzadeh, A., Norouzian, D., Mehrabi, M. R., Jamshidi, S., Farhangi, A., Verdi, A. A., ... Rad, B. L. (2007). Induction of diabetes by Streptozotocin in rats. *Indian Journal of Clinical Biochemistry: IJCB*, 22(2), 60–64. <http://doi.org/10.1007/BF02913315>
- Al-Disi, D., Al-Daghri, N., Khanam, L., Al-Othman, A., Al-Saif, M., Sabico, S., & Chrousos, G. (2010). Subjective sleep duration and quality influence diet composition and circulating adipocytokines and ghrelin levels in teen-age girls. *Endocrine Journal*, 57(10), 915–923.
- American Diabetes Association. (2014). Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, 37(Supplement_1), S81–S90. <http://doi.org/10.2337/dc14-S081>
- Andralojc, K. M., Mercalli, A., Nowak, K. W., Albarello, L., Calcagno, R., Luzi, L., ... Piemonti, L. (2009a). Ghrelin-producing epsilon cells in the developing and adult human pancreas. *Diabetologia*, 52(3), 486–493. <http://doi.org/10.1007/s00125-008-1238-y>
- Andralojc, K. M., Mercalli, A., Nowak, K. W., Albarello, L., Calcagno, R., Luzi, L., ... Piemonti, L. (2009b). Ghrelin-producing epsilon cells in the developing

and adult human pancreas. *Diabetologia*, 52(3), 486–493.

<http://doi.org/10.1007/s00125-008-1238-y>

Aragon, G., & Younossi, Z. M. (2010). When and how to evaluate mildly elevated liver enzymes in apparently healthy patients. *Cleveland Clinic Journal of Medicine*, 77(3), 195–204. <http://doi.org/10.3949/ccjm.77a.09064>

Arosio, M., Ronchi, C. L., Gebbia, C., Cappiello, V., Beck-Peccoz, P., & Peracchi, M. (2003). Stimulatory effects of ghrelin on circulating somatostatin and pancreatic polypeptide levels. *The Journal of Clinical Endocrinology and Metabolism*, 88(2), 701–704. <http://doi.org/10.1210/jc.2002-021161>

Asakawa, A., Inui, A., Yuzuriha, H., Ueno, N., Katsuura, G., Fujimiya, M., ... Kasuga, M. (2003). Characterization of the effects of pancreatic polypeptide in the regulation of energy balance. *Gastroenterology*, 124(5), 1325–1336.

Ashrafi, M., Sheikhan, F., Arabipoor, A., Hosseini, R., Nourbakhsh, F., & Zolfaghari, Z. (2014). Gestational diabetes mellitus risk factors in women with polycystic ovary syndrome (PCOS). *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, 181C, 195–199. <http://doi.org/10.1016/j.ejogrb.2014.07.043>

Avau, B., De Smet, B., Thijs, T., Geuzens, A., Tack, J., Vanden Berghe, P., & Depoortere, I. (2013). Ghrelin is involved in the paracrine communication between neurons and glial cells. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society*, 25(9), e599–608. <http://doi.org/10.1111/nmo.12171>

Baker, S. (2004). Diagnosis and management of acute pancreatitis. *Critical Care and Resuscitation: Journal of the Australasian Academy of Critical Care Medicine*, 6(1), 17–27.

Barrett, K. E., Ghishan, F. K., Merchant, J. L., Said, H. M., & Wood, J. D. (2006).

Physiology of the Gastrointestinal Tract. Academic Press.

Beck, B., Musse, N., & Stricker-Krongrad, A. (2002). Ghrelin, macronutrient intake and dietary preferences in long-evans rats. *Biochemical and Biophysical Research Communications*, 292(4), 1031–1035.

<http://doi.org/10.1006/bbrc.2002.6737>

Bellar, D., Glickman, E. L., Judge, L. W., & Gunstad, J. (2013). Serum ghrelin is associated with verbal learning and adiposity in a sample of healthy, fit older adults. *BioMed Research International*, 2013, 202757.

<http://doi.org/10.1155/2013/202757>

Bockman, D. E., Büchler, M., & Beger, H. G. (1986). Structure and function of specialized cilia in the exocrine pancreas. *International Journal of Pancreatology: Official Journal of the International Association of Pancreatology*, 1(1), 21–28. <http://doi.org/10.1007/BF02795236>

Bonora, E. (2008). Protection of pancreatic beta-cells: is it feasible? *Nutrition, Metabolism, and Cardiovascular Diseases: NMCD*, 18(1), 74–83.

<http://doi.org/10.1016/j.numecd.2007.05.004>

Braganza, J. M., Lee, S. H., McCloy, R. F., & McMahon, M. J. (2011). Chronic pancreatitis. *The Lancet*, 377(9772), 1184–1197.

[http://doi.org/10.1016/S0140-6736\(10\)61852-1](http://doi.org/10.1016/S0140-6736(10)61852-1)

Brissova, M., Fowler, M. J., Nicholson, W. E., Chu, A., Hirshberg, B., Harlan, D. M., & Powers, A. C. (2005). Assessment of Human Pancreatic Islet Architecture and Composition by Laser Scanning Confocal Microscopy. *Journal of Histochemistry & Cytochemistry*, 53(9), 1087–1097.

<http://doi.org/10.1369/jhc.5C6684.2005>

- Brown, M., & Vale, W. (1976). Effects of neurotensin and substance p on plasma insulin, glucagon and glucose levels. *Endocrinology*, 98(3), 819–822.
<http://doi.org/10.1210/endo-98-3-819>
- Cabrera, O., Berman, D. M., Kenyon, N. S., Ricordi, C., Berggren, P.-O., & Caicedo, A. (2006). The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proceedings of the National Academy of Sciences of the United States of America*, 103(7), 2334–2339.
<http://doi.org/10.1073/pnas.0510790103>
- Camiña, J. P., Carreira, M. C., Micic, D., Pombo, M., Kelestimur, F., Dieguez, C., & Casanueva, P. F. F. (2003). Regulation of ghrelin secretion and action. *Endocrine*, 22(1), 5–12. <http://doi.org/10.1385/ENDO:22:1:5>
- Campbell, F., & Verbeke, C. S. (2013). *Pathology of the Pancreas: A Practical Approach*. Springer Science & Business Media.
- Carlini, V. P., Monzón, M. E., Varas, M. M., Cragnolini, A. B., Schiöth, H. B., Scimonelli, T. N., & de Barioglio, S. R. (2002). Ghrelin increases anxiety-like behavior and memory retention in rats. *Biochemical and Biophysical Research Communications*, 299(5), 739–743.
- Chabot, F., Caron, A., Laplante, M., & St-Pierre, D. H. (2014). Interrelationships between ghrelin, insulin and glucose homeostasis: Physiological relevance. *World Journal of Diabetes*, 5(3), 328–341.
<http://doi.org/10.4239/wjd.v5.i3.328>
- Chanoine, J.-P., & Wong, A. C. K. (2004). Ghrelin gene expression is markedly higher in fetal pancreas compared with fetal stomach: effect of maternal fasting. *Endocrinology*, 145(8), 3813–3820. <http://doi.org/10.1210/en.2004-0053>

- Chapman, I., Parker, B., Doran, S., Feinle-Bisset, C., Wishart, J., Strobel, S., ... Horowitz, M. (2005). Effect of pramlintide on satiety and food intake in obese subjects and subjects with type 2 diabetes. *Diabetologia*, 48(5), 838–848. <http://doi.org/10.1007/s00125-005-1732-4>
- Clark, M. A., Harvey, R. A., Finkel, R., Rey, J. A., & Whalen, K. (2011). *Pharmacology*. Lippincott Williams & Wilkins.
- Colberg, S., & Swain, D. (2000). Exercise and Diabetes Control A Winning Combination. *The Physician and Sportsmedicine*, 28(4), 0. <http://doi.org/10.3810/psm.2000.04.843>
- Committee, J. F. (2014). *British National Formulary (BNF) 67*. Pharmaceutical Press.
- Considine, R. V., Sinha, M. K., Heiman, M. L., Kriauciunas, A., Stephens, T. W., Nyce, M. R., ... Caro, J. F. (1996). Serum Immunoreactive-Leptin Concentrations in Normal-Weight and Obese Humans. *New England Journal of Medicine*, 334(5), 292–295. <http://doi.org/10.1056/NEJM199602013340503>
- Cummings, B. P. (2013). Leptin therapy in type 2 diabetes. *Diabetes, Obesity and Metabolism*, 15(7), 607–612. <http://doi.org/10.1111/dom.12048>
- Cummings, D. E., Frayo, R. S., Marmonier, C., Aubert, R., & Chapelot, D. (2004). Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *American Journal of Physiology - Endocrinology and Metabolism*, 287(2), E297–E304. <http://doi.org/10.1152/ajpendo.00582.2003>

- Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E., & Weigle, D. S. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*, *50*(8), 1714–1719.
- Damasceno, D. C., Netto, A. O., Iessi, I. L., Gallego, F. Q., Corvino, S. B., Dallaqua, B., ... Rudge, M. V. C. (2014). Streptozotocin-Induced Diabetes Models: Pathophysiological Mechanisms and Fetal Outcomes. *BioMed Research International*, *2014*, e819065. <http://doi.org/10.1155/2014/819065>
- Datar, P., Srivastava, S., Coutinho, E., & Govil, G. (2004). Substance P: Structure, Function, and Therapeutics. *Current Topics in Medicinal Chemistry*, *4*(1), 75–103. <http://doi.org/10.2174/1568026043451636>
- Date, Y. (2012). Ghrelin and the vagus nerve. *Methods in Enzymology*, *514*, 261–269. <http://doi.org/10.1016/B978-0-12-381272-8.00016-7>
- Date, Y., Nakazato, M., Hashiguchi, S., Dezaki, K., Mondal, M. S., Hosoda, H., ... Matsukura, S. (2002). Ghrelin Is Present in Pancreatic α -Cells of Humans and Rats and Stimulates Insulin Secretion. *Diabetes*, *51*(1), 124–129. <http://doi.org/10.2337/diabetes.51.1.124>
- Date, Y., Nakazato, M., Murakami, N., Kojima, M., Kangawa, K., & Matsukura, S. (2001). Ghrelin acts in the central nervous system to stimulate gastric acid secretion. *Biochemical and Biophysical Research Communications*, *280*(3), 904–907. <http://doi.org/10.1006/bbrc.2000.4212>
- Davenport, A. P., Bonner, T. I., Foord, S. M., Harmar, A. J., Neubig, R. R., Pin, J.-P., ... Kangawa, K. (2005). International Union of Pharmacology. LVI. Ghrelin Receptor Nomenclature, Distribution, and Function. *Pharmacological Reviews*, *57*(4), 541–546. <http://doi.org/10.1124/pr.57.4.1>

- Delhanty, P. J. D., & van der Lely, A. J. (2013). Ghrelin: a new treatment for non-alcoholic fatty liver disease? *Endocrine*, 43(2), 247–248.
<http://doi.org/10.1007/s12020-012-9800-2>
- Delporte, C. (2013). Structure and physiological actions of ghrelin. *Scientifica*, 2013, 518909. <http://doi.org/10.1155/2013/518909>
- De Meyts, P. (2004). Insulin and its receptor: structure, function and evolution. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, 26(12), 1351–1362. <http://doi.org/10.1002/bies.20151>
- De Vriese, C., Grégoire, F., De Neef, P., Robberecht, P., & Delporte, C. (2005). Ghrelin is produced by the human erythroleukemic HEL cell line and involved in an autocrine pathway leading to cell proliferation. *Endocrinology*, 146(3), 1514–1522. <http://doi.org/10.1210/en.2004-0964>
- Dezaki, K., Damdindorj, B., Sone, H., Dyachok, O., Tengholm, A., Gylfe, E., ... Yada, T. (2011). Ghrelin attenuates cAMP-PKA signaling to evoke insulinostatic cascade in islet β -cells. *Diabetes*, 60(9), 2315–2324.
<http://doi.org/10.2337/db11-0368>
- Dezaki, K., Sone, H., Koizumi, M., Nakata, M., Kakei, M., Nagai, H., ... Yada, T. (2006). Blockade of pancreatic islet-derived ghrelin enhances insulin secretion to prevent high-fat diet-induced glucose intolerance. *Diabetes*, 55(12), 3486–3493. <http://doi.org/10.2337/db06-0878>
- Diabetes Atlas | International Diabetes Federation. (n.d.). Retrieved July 11, 2014, from <http://www.idf.org/diabetesatlas>
- diabetes symptoms | Pharma Mirror Magazine. (n.d.). Retrieved from <https://pharmamirror.wordpress.com/tag/diabetes-symptoms/>

- Diagnosis and Classification of Diabetes Mellitus. (2010). *Diabetes Care*, 33(Suppl 1), S62–S69. <http://doi.org/10.2337/dc10-S062>
- DiMagno, M. J., & DiMagno, E. P. (2012). Chronic pancreatitis. *Current Opinion in Gastroenterology*, 28(5), 523–531.
<http://doi.org/10.1097/MOG.0b013e3283567dea>
- DiPiro, J. T., Talbert, R. L., Yee, G. C., Matzke, G. R., Wells, B. G., & Posey, L. M. (2011). *Pharmacotherapy: A Pathophysiologic Approach, Eighth Edition*. McGraw Hill Professional.
- dLife Diabetes Museum. (n.d.). Retrieved July 4, 2014, from
<http://www.dlife.com/files/Timeline/>
- Doogue, M. P., Begg, E. J., Moore, M. P., Lunt, H., Pemberton, C. J., & Zhang, M. (2009). Metformin increases plasma ghrelin in Type 2 diabetes. *British Journal of Clinical Pharmacology*, 68(6), 875–882.
<http://doi.org/10.1111/j.1365-2125.2009.03372.x>
- Drucker, D. J. (1998). Glucagon-Like Peptides. *Diabetes*, 47(2), 159–169.
<http://doi.org/10.2337/diab.47.2.159>
- Drucker, D. J., & Nauck, M. A. (2006). The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *The Lancet*, 368(9548), 1696–1705. [http://doi.org/10.1016/S0140-6736\(06\)69705-5](http://doi.org/10.1016/S0140-6736(06)69705-5)
- Du, C.-K., Zhan, D.-Y., Morimoto, S., Akiyama, T., Schwenke, D. O., Hosoda, H., ... Shirai, M. (2014). Survival benefit of ghrelin in the heart failure due to dilated cardiomyopathy. *Pharmacology Research & Perspectives*, 2(5), e00064. <http://doi.org/10.1002/prp2.64>

- Efrat, S., & Russ, H. A. (2012). Making β cells from adult tissues. *Trends in Endocrinology & Metabolism*, 23(6), 278–285.
<http://doi.org/10.1016/j.tem.2012.03.005>
- English, P. J., Ashcroft, A., Patterson, M., Dovey, T. M., Halford, J. C. G., Harrison, J., ... Wilding, J. P. H. (2007). Metformin prolongs the postprandial fall in plasma ghrelin concentrations in type 2 diabetes. *Diabetes/Metabolism Research and Reviews*, 23(4), 299–303. <http://doi.org/10.1002/dmrr.681>
- English, P. J., Ghatei, M. A., Malik, I. A., Bloom, S. R., & Wilding, J. P. H. (2002). Food fails to suppress ghrelin levels in obese humans. *The Journal of Clinical Endocrinology and Metabolism*, 87(6), 2984.
<http://doi.org/10.1210/jcem.87.6.8738>
- Eroschenko, V. P. (2008). *DiFiore's Atlas of Histology with Functional Correlations*. Lippincott Williams & Wilkins.
- Estep, M., Abawi, M., Jarrar, M., Wang, L., Stepanova, M., Elariny, H., ... Younossi, Z. M. (2011). Association of obestatin, ghrelin, and inflammatory cytokines in obese patients with non-alcoholic fatty liver disease. *Obesity Surgery*, 21(11), 1750–1757. <http://doi.org/10.1007/s11695-011-0475-1>
- Faiz, O., & Moffat, D. (2006). *Anatomy at a Glance*. Wiley.
- Farilla, L., Bulotta, A., Hirshberg, B., Li Calzi, S., Khoury, N., Noushmehr, H., ... Perfetti, R. (2003). Glucagon-Like Peptide 1 Inhibits Cell Apoptosis and Improves Glucose Responsiveness of Freshly Isolated Human Islets. *Endocrinology*, 144(12), 5149–5158. <http://doi.org/10.1210/en.2003-0323>
- Farney, A. C., Cho, E., Schweitzer, E. J., Dunkin, B., Philosophe, B., Colonna, J., ... Bartlett, S. T. (2000). Simultaneous Cadaver Pancreas Living-Donor Kidney

Transplantation: A New Approach for the Type 1 Diabetic Uremic Patient.

Annals of Surgery, 232(5), 696–703.

Figueiro, M. G., Plitnick, B., & Rea, M. S. (2012). Light modulates leptin and ghrelin in sleep-restricted adults. *International Journal of Endocrinology*, 2012, 530726. <http://doi.org/10.1155/2012/530726>

Fiorina, P., Folli, F., Zerbini, G., Maffi, P., Gremizzi, C., Carlo, V. D., ... Secchi, A. (2003). Islet Transplantation Is Associated with Improvement of Renal Function among Uremic Patients with Type I Diabetes Mellitus and Kidney Transplants. *Journal of the American Society of Nephrology*, 14(8), 2150–2158. <http://doi.org/10.1097/01.ASN.0000077339.20759.A3>

Frossard, J.-L., Steer, M. L., & Pastor, C. M. (2008). Acute pancreatitis. *Lancet*, 371(9607), 143–152. [http://doi.org/10.1016/S0140-6736\(08\)60107-5](http://doi.org/10.1016/S0140-6736(08)60107-5)

Gahete, M. D., Córdoba-Chacón, J., Hergueta-Redondo, M., Martínez-Fuentes, A. J., Kineman, R. D., Moreno-Bueno, G., ... Castaño, J. P. (2011). A Novel Human Ghrelin Variant (In1-Ghrelin) and Ghrelin-O-Acyltransferase Are Overexpressed in Breast Cancer: Potential Pathophysiological Relevance. *PLoS ONE*, 6(8), e23302. <http://doi.org/10.1371/journal.pone.0023302>

Gahete, M. D., Rincón-Fernández, D., Villa-Osaba, A., Hormaechea-Agulla, D., Ibáñez-Costa, A., Martínez-Fuentes, A. J., ... Luque, R. M. (2014). Ghrelin gene products, receptors, and GOAT enzyme: biological and pathophysiological insight. *The Journal of Endocrinology*, 220(1), R1–24. <http://doi.org/10.1530/JOE-13-0391>

Ganong, W. (2005). *Review of Medical Physiology*. McGraw Hill Professional.

García-García, F., Juárez-Aguilar, E., Santiago-García, J., & Cardinali, D. P. (2014). Ghrelin and its interactions with growth hormone, leptin and orexins:

- implications for the sleep-wake cycle and metabolism. *Sleep Medicine Reviews*, 18(1), 89–97. <http://doi.org/10.1016/j.smr.2013.04.003>
- Gastón, M. S., Schiöth, H. B., De Barioglio, S. R., & Salvatierra, N. A. (2015). Gabaergic control of anxiety-like behavior, but not food intake, induced by ghrelin in the intermediate medial mesopallium of the neonatal chick. *Hormones and Behavior*, 67, 66–72. <http://doi.org/10.1016/j.yhbeh.2014.11.015>
- Gelling, R. W., Overduin, J., Morrison, C. D., Morton, G. J., Frayo, R. S., Cummings, D. E., & Schwartz, M. W. (2004). Effect of uncontrolled diabetes on plasma ghrelin concentrations and ghrelin-induced feeding. *Endocrinology*, 145(10), 4575–4582. <http://doi.org/10.1210/en.2004-0605>
- Gillespie, K. M. (2006). Type 1 diabetes: pathogenesis and prevention. *CMAJ: Canadian Medical Association Journal = Journal de l'Association Médicale Canadienne*, 175(2), 165–170. <http://doi.org/10.1503/cmaj.060244>
- Gnanapavan, S., Kola, B., Bustin, S. A., Morris, D. G., McGee, P., Fairclough, P., ... Korbonits, M. (2002). The Tissue Distribution of the mRNA of Ghrelin and Subtypes of Its Receptor, GHS-R, in Humans. *The Journal of Clinical Endocrinology & Metabolism*, 87(6), 2988–2988. <http://doi.org/10.1210/jcem.87.6.8739>
- Goshadrou, F., Kermani, M., Ronaghi, A., & Sajjadi, S. (2013). The effect of ghrelin on MK-801 induced memory impairment in rats. *Peptides*, 44, 60–65. <http://doi.org/10.1016/j.peptides.2013.03.022>
- Goto, M., Arima, H., Watanabe, M., Hayashi, M., Banno, R., Sato, I., ... Oiso, Y. (2006). Ghrelin increases neuropeptide Y and agouti-related peptide gene

expression in the arcuate nucleus in rat hypothalamic organotypic cultures.

Endocrinology, 147(11), 5102–5109. <http://doi.org/10.1210/en.2006-0104>

Greenstein, B., & Wood, D. (2011). *The Endocrine System at a Glance*. Wiley.

Gruessner, A. C., & Sutherland, D. E. R. (2005). Pancreas transplant outcomes for United States (US) and non-US cases as reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR) as of June 2004. *Clinical Transplantation*, 19(4), 433–455.

<http://doi.org/10.1111/j.1399-0012.2005.00378.x>

Gualillo, O., Caminos, J., Blanco, M., García-Caballero, T., Kojima, M., Kangawa, K., ... Casanueva, F. (2001). Ghrelin, a novel placental-derived hormone.

Endocrinology, 142(2), 788–794. <http://doi.org/10.1210/endo.142.2.7987>

Guariguata, L., Whiting, D. R., Hambleton, I., Beagley, J., Linnenkamp, U., & Shaw, J. E. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Research and Clinical Practice*, 103(2), 137–149. <http://doi.org/10.1016/j.diabres.2013.11.002>

Hansen, T. K., Dall, R., Hosoda, H., Kojima, M., Kangawa, K., Christiansen, J. S., & Jørgensen, J. O. L. (2002). Weight loss increases circulating levels of ghrelin in human obesity. *Clinical Endocrinology*, 56(2), 203–206.

<http://doi.org/10.1046/j.0300-0664.2001.01456.x>

Harrison, S., & Geppetti, P. (2001). Substance P. *The International Journal of Biochemistry & Cell Biology*, 33(6), 555–576. [http://doi.org/10.1016/S1357-2725\(01\)00031-0](http://doi.org/10.1016/S1357-2725(01)00031-0)

Harvey, R. A., & Ferrier, D. R. (2011). *Biochemistry*. Lippincott Williams & Wilkins.

- Hattori, N., Saito, T., Yagyu, T., Jiang, B.-H., Kitagawa, K., & Inagaki, C. (2001). GH, GH Receptor, GH Secretagogue Receptor, and Ghrelin Expression in Human T Cells, B Cells, and Neutrophils. *The Journal of Clinical Endocrinology & Metabolism*, 86(9), 4284–4291. <http://doi.org/10.1210/jcem.86.9.7866>
- Heinemann, L. (2010). New ways of insulin delivery. *International Journal of Clinical Practice. Supplement*, (166), 29–40. <http://doi.org/10.1111/j.1742-1241.2009.02276.x>
- Heppner, K. M., Müller, T. D., Tong, J., & Tschöp, M. H. (2012). Ghrelin in the control of energy, lipid, and glucose metabolism. *Methods in Enzymology*, 514, 249–260. <http://doi.org/10.1016/B978-0-12-381272-8.00015-5>
- Hermansen, K. (1980). Effects of Substance P and Other Peptides on the Release of Somatostatin, Insulin, and Glucagon in Vitro. *Endocrinology*, 107(1), 256–261. <http://doi.org/10.1210/endo-107-1-256>
- Hickey, M. S., Considine, R. V., Israel, R. G., Mahar, T. L., McCammon, M. R., Tyndall, G. L., ... Caro, J. F. (1996). Leptin is related to body fat content in male distance runners. *The American Journal of Physiology*, 271(5 Pt 1), E938–940.
- Higgins, S. C., Gueorguiev, M., & Korbonits, M. (2007). Ghrelin, the peripheral hunger hormone. *Annals of Medicine*, 39(2), 116–136. <http://doi.org/10.1080/07853890601149179>
- Horton, E. S. (1988). Role and Management of Exercise in Diabetes Mellitus. *Diabetes Care*, 11(2), 201–211. <http://doi.org/10.2337/diacare.11.2.201>
- Hosoda, H., Kojima, M., Matsuo, H., & Kangawa, K. (2000). Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue.

Biochemical and Biophysical Research Communications, 279(3), 909–913.

<http://doi.org/10.1006/bbrc.2000.4039>

Hosoda, H., Kojima, M., Mizushima, T., Shimizu, S., & Kangawa, K. (2003).

Structural divergence of human ghrelin. Identification of multiple ghrelin-derived molecules produced by post-translational processing. *The Journal of Biological Chemistry*, 278(1), 64–70.

<http://doi.org/10.1074/jbc.M205366200>

Hotta, M., Ohwada, R., Akamizu, T., Shibasaki, T., Takano, K., & Kangawa, K.

(2009). Ghrelin Increases Hunger and Food Intake in Patients with Restricting-type Anorexia Nervosa: A Pilot Study. *Endocrine Journal*, 56(9), 1119–1128. <http://doi.org/10.1507/endocrj.K09E-168>

Howard, A. D., McAllister, G., Feighner, S. D., Liu, Q., Nargund, R. P., Van der

Ploeg, L. H. T., & Patchett, A. A. (2001). Orphan G-protein-coupled receptors and natural ligand discovery. *Trends in Pharmacological Sciences*, 22(3), 132–140. [http://doi.org/10.1016/S0165-6147\(00\)01636-9](http://doi.org/10.1016/S0165-6147(00)01636-9)

Howarth, F. C. (2013). Epidemiology of Diabetes Mellitus – A Global and Regional Perspective. *HAMDAN MEDICAL JOURNAL*, 6(1).

<http://doi.org/10.7707/hmj.v6i1.149>

Hubbard, S. R. (2013). Structural biology: Insulin meets its receptor. *Nature*,

493(7431), 171–172. <http://doi.org/10.1038/493171a>

Hu, F. B., Manson, J. E., Stampfer, M. J., Colditz, G., Liu, S., Solomon, C. G., &

Willett, W. C. (2001). Diet, Lifestyle, and the Risk of Type 2 Diabetes Mellitus in Women. *New England Journal of Medicine*, 345(11), 790–797.

<http://doi.org/10.1056/NEJMoa010492>

- Jeffery, P. L., Herington, A. C., & Chopin, L. K. (2003). The potential autocrine/paracrine roles of ghrelin and its receptor in hormone-dependent cancer. *Cytokine & Growth Factor Reviews*, 14(2), 113–122.
- Kadoglou, N. P. E., Vrabas, I. S., Kapelouzou, A., Lampropoulos, S., Sailer, N., Kostakis, A., ... Angelopoulou, N. (2012). The impact of aerobic exercise training on novel adipokines, apelin and ghrelin, in patients with type 2 diabetes. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, 18(5), CR290–295.
- Kageyama, H., Funahashi, H., Hirayama, M., Takenoya, F., Kita, T., Kato, S., ... Shioda, S. (2005a). Morphological analysis of ghrelin and its receptor distribution in the rat pancreas. *Regulatory Peptides*, 126(1-2), 67–71. <http://doi.org/10.1016/j.regpep.2004.08.031>
- Kageyama, H., Funahashi, H., Hirayama, M., Takenoya, F., Kita, T., Kato, S., ... Shioda, S. (2005b). Morphological analysis of ghrelin and its receptor distribution in the rat pancreas. *Regulatory Peptides*, 126(1-2), 67–71. <http://doi.org/10.1016/j.regpep.2004.08.031>
- Kageyama, H., Funahashi, H., Hirayama, M., Takenoya, F., Kita, T., Kato, S., ... Shioda, S. (2005c). Morphological analysis of ghrelin and its receptor distribution in the rat pancreas. *Regulatory Peptides*, 126(1-2), 67–71. <http://doi.org/10.1016/j.regpep.2004.08.031>
- Kahn, C. R., & White, M. F. (1988). The insulin receptor and the molecular mechanism of insulin action. *The Journal of Clinical Investigation*, 82(4), 1151–1156. <http://doi.org/10.1172/JCI113711>

- Kahn, S. E. (2000). The importance of the β -cell in the pathogenesis of type 2 diabetes mellitus. *The American Journal of Medicine*, 108(6, Supplement 1), 2–8. [http://doi.org/10.1016/S0002-9343\(00\)00336-3](http://doi.org/10.1016/S0002-9343(00)00336-3)
- Kahn, S. E., Hull, R. L., & Utzschneider, K. M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*, 444(7121), 840–846. <http://doi.org/10.1038/nature05482>
- Kalra, S. P. (2013). Should leptin replace insulin as a lifetime monotherapy for diabetes type 1 and 2? *Indian Journal of Endocrinology and Metabolism*, 17(Suppl1), S23–S24. <http://doi.org/10.4103/2230-8210.119496>
- Kamegai, J., Tamura, H., Shimizu, T., Ishii, S., Sugihara, H., & Wakabayashi, I. (2000). Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinology*, 141(12), 4797–4800. <http://doi.org/10.1210/endo.141.12.7920>
- Kamegai, J., Tamura, H., Shimizu, T., Ishii, S., Sugihara, H., & Wakabayashi, I. (2001). Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. *Diabetes*, 50(11), 2438–2443.
- Karamitsos, D. T. (2011). The story of insulin discovery. *Diabetes Research and Clinical Practice*, 93, Supplement 1, S2–S8. [http://doi.org/10.1016/S0168-8227\(11\)70007-9](http://doi.org/10.1016/S0168-8227(11)70007-9)
- Katsuki, A., Urakawa, H., Gabazza, E. C., Murashima, S., Nakatani, K., Togashi, K., ... Sumida, Y. (2004). Circulating levels of active ghrelin is associated with abdominal adiposity, hyperinsulinemia and insulin resistance in patients with type 2 diabetes mellitus. *European Journal of Endocrinology*, 151(5), 573–577. <http://doi.org/10.1530/eje.0.1510573>

- Katzung, B. G. (2007). *Basic & Clinical Pharmacology*. McGraw-Hill Medical.
- Kelly, W. D., Lillehei, R. C., Merkel, F. K., Idezuki, Y., & Goetz, F. C. (1967). Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. *Surgery*, 61(6), 827–837.
- Kendall, D. M. (2006). Thiazolidinediones The case for early use. *Diabetes Care*, 29(1), 154–157. <http://doi.org/10.2337/diacare.29.01.06.dc05-0711>
- Kido, Y., Nakae, J., & Accili, D. (2001). Clinical review 125: The insulin receptor and its cellular targets. *The Journal of Clinical Endocrinology and Metabolism*, 86(3), 972–979. <http://doi.org/10.1210/jcem.86.3.7306>
- Kleinwechter, H., Schäfer-Graf, U., Bühner, C., Hoesli, I., Kainer, F., Kautzky-Willer, A., ... Sorger, M. (2014). Gestational diabetes mellitus (GDM) diagnosis, therapy and follow-up care: Practice Guideline of the German Diabetes Association(DDG) and the German Association for Gynaecologyand Obstetrics (DGGG). *Experimental and Clinical Endocrinology & Diabetes: Official Journal, German Society of Endocrinology [and] German Diabetes Association*, 122(7), 395–405. <http://doi.org/10.1055/s-0034-1366412>
- Kohno, D., Sone, H., Minokoshi, Y., & Yada, T. (2008). Ghrelin raises $[Ca^{2+}]_i$ via AMPK in hypothalamic arcuate nucleus NPY neurons. *Biochemical and Biophysical Research Communications*, 366(2), 388–392. <http://doi.org/10.1016/j.bbrc.2007.11.166>
- Kojima, M. (2008). The discovery of ghrelin--a personal memory. *Regulatory Peptides*, 145(1-3), 2–6. <http://doi.org/10.1016/j.regpep.2007.09.023>

- Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, *402*(6762), 656–660. <http://doi.org/10.1038/45230>
- Kojima, M., Hosoda, H., & Kangawa, K. (2012). Purification of rat and human ghrelins. *Methods in Enzymology*, *514*, 45–61. <http://doi.org/10.1016/B978-0-12-381272-8.00003-9>
- Kojima, M., & Kangawa, K. (2005). Ghrelin: Structure and Function. *Physiological Reviews*, *85*(2), 495–522. <http://doi.org/10.1152/physrev.00012.2004>
- Kojima, M., & Kangawa, K. (2011). The discovery of ghrelin: with a little luck and great passion. Preface. *Peptides*, *32*(11), 2153–2154. <http://doi.org/10.1016/j.peptides.2011.10.016>
- Kunt, T., Forst, T., Schmidt, S., Pfützner, A., Schneider, S., Harzer, O., ... Beyer, J. (2000). Serum levels of substance P are decreased in patients with type 1 diabetes. *Experimental and Clinical Endocrinology & Diabetes*, *108*(3), 164–167. <http://doi.org/10.1055/s-2000-7738>
- Lanfranco, F., Baldi, M., Cassoni, P., Bosco, M., Ghé, C., & Muccioli, G. (2008). Ghrelin and prostate cancer. *Vitamins and Hormones*, *77*, 301–324. [http://doi.org/10.1016/S0083-6729\(06\)77013-3](http://doi.org/10.1016/S0083-6729(06)77013-3)
- Lawrence, C. B., Snape, A. C., Baudoin, F. M.-H., & Luckman, S. M. (2002). Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. *Endocrinology*, *143*(1), 155–162. <http://doi.org/10.1210/endo.143.1.8561>
- Leahy, J. L. (2005). Pathogenesis of Type 2 Diabetes Mellitus. *Archives of Medical Research*, *36*(3), 197–209. <http://doi.org/10.1016/j.arcmed.2005.01.003>

Lee, H.-M., Wang, G., Englander, E. W., Kojima, M., & Greeley, G. H. (2002).

Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. *Endocrinology*, 143(1), 185–190.

<http://doi.org/10.1210/endo.143.1.8602>

Legakis, I., Stathopoulos, J., Matzouridis, T., & Stathopoulos, G. P. (2009).

Decreased plasma ghrelin levels in patients with advanced cancer and weight loss in comparison to healthy individuals. *Anticancer Research*, 29(10), 3949–3952.

Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes.

Diabetologia, 51(2), 216–226. <http://doi.org/10.1007/s00125-007-0886-7>

Li, D., Xie, K., Wolff, R., & Abbruzzese, J. L. (2004). Pancreatic cancer. *The*

Lancet, 363(9414), 1049–1057. [http://doi.org/10.1016/S0140-6736\(04\)15841-8](http://doi.org/10.1016/S0140-6736(04)15841-8)

Li, E., Chung, H., Kim, Y., Kim, D. H., Ryu, J. H., Sato, T., ... Park, S. (2013).

Ghrelin directly stimulates adult hippocampal neurogenesis: implications for learning and memory. *Endocrine Journal*, 60(6), 781–789.

Lin, S., Chen, Z., Lin, C., & Duan, H. (2009). [Effects of glucagon on plasma

ghrelin level in type 2 diabetes mellitus]. *Zhonghua Yi Xue Za Zhi*, 89(14), 967–969.

Lotfy, M., Singh, J., Kalász, H., Tekes, K., & Adeghate, E. (2011). Medicinal

Chemistry and Applications of Incretins and DPP-4 Inhibitors in the Treatment of Type 2 Diabetes Mellitus. *The Open Medicinal Chemistry Journal*, 5(Suppl 2), 82–92. <http://doi.org/10.2174/1874104501105010082>

- Lundquist, I., Sundler, F., Ahren, B., Alumets, J., & Håkanson, R. (1979). Somatostatin, pancreatic polypeptide, substance P, and neurotensin: cellular distribution and effects on stimulated insulin secretion in the mouse. *Endocrinology*, 104(3), 832–838. <http://doi.org/10.1210/endo-104-3-832>
- Malagón, M. M., Luque, R. M., Ruiz-Guerrero, E., Rodríguez-Pacheco, F., García-Navarro, S., Casanueva, F. F., ... Castaño, J. P. (2003). Intracellular signaling mechanisms mediating ghrelin-stimulated growth hormone release in somatotropes. *Endocrinology*, 144(12), 5372–5380. <http://doi.org/10.1210/en.2003-0723>
- Martin, K. A., Merenick, B. L., Ding, M., Fetalvero, K. M., Rzucidlo, E. M., Kozul, C. D., ... Powell, R. J. (2007). Rapamycin promotes vascular smooth muscle cell differentiation through insulin receptor substrate-1/phosphatidylinositol 3-kinase/Akt2 feedback signaling. *The Journal of Biological Chemistry*, 282(49), 36112–36120. <http://doi.org/10.1074/jbc.M703914200>
- Masuda, Y., Tanaka, T., Inomata, N., Ohnuma, N., Tanaka, S., Itoh, Z., ... Kangawa, K. (2000). Ghrelin Stimulates Gastric Acid Secretion and Motility in Rats. *Biochemical and Biophysical Research Communications*, 276(3), 905–908. <http://doi.org/10.1006/bbrc.2000.3568>
- McCall, M., & Shapiro, A. M. J. (2014). Islet cell transplantation. *Seminars in Pediatric Surgery*, 23(2), 83–90. <http://doi.org/10.1053/j.sempedsurg.2014.03.006>
- McLaughlin, T., Abbasi, F., Lamendola, C., Frayo, R. S., & Cummings, D. E. (2004). Plasma ghrelin concentrations are decreased in insulin-resistant obese adults relative to equally obese insulin-sensitive controls. *The Journal*

of Clinical Endocrinology and Metabolism, 89(4), 1630–1635.

<http://doi.org/10.1210/jc.2003-031572>

Miller, D. W., Harrison, J. L., Brown, Y. A., Doyle, U., Lindsay, A., Adam, C. L., & Lea, R. G. (2005). Immunohistochemical evidence for an endocrine/paracrine role for ghrelin in the reproductive tissues of sheep.

Reproductive Biology and Endocrinology: RB&E, 3, 60.

<http://doi.org/10.1186/1477-7827-3-60>

Mitchell, R. M. S., Byrne, M. F., & Baillie, J. (2003). Pancreatitis. *Lancet*, 361(9367), 1447–1455.

Mokdad AH, Ford ES, Bowman BA, & et al. (2003). PRevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA*, 289(1), 76–79.

<http://doi.org/10.1001/jama.289.1.76>

Moore, K. L., & Dalley, A. F. (1999). *Clinically Oriented Anatomy*. Lippincott Williams & Wilkins.

Mori, K., Yoshimoto, A., Takaya, K., Hosoda, K., Ariyasu, H., Yahata, K., ...

Nakao, K. (2000). Kidney produces a novel acylated peptide, ghrelin. *FEBS Letters*, 486(3), 213–216. [http://doi.org/10.1016/S0014-5793\(00\)02308-5](http://doi.org/10.1016/S0014-5793(00)02308-5)

Mykhalchyshyn, G., Kobylak, N., & Bodnar, P. (2015). Diagnostic accuracy of acyl-ghrelin and it association with non-alcoholic fatty liver disease in type 2 diabetic patients. *Journal of Diabetes and Metabolic Disorders*, 14, 44.

<http://doi.org/10.1186/s40200-015-0170-1>

Nagaya, N., & Kangawa, K. (2004). [Ghrelin in the treatment of cardiovascular disease]. *Nihon Rinsho. Japanese Journal of Clinical Medicine*, 62 Suppl 9, 430–434.

- Nakazato, M., Hashimoto, K., Shiina, A., Koizumi, H., Mitsumoti, M., Imai, M., ... Iyo, M. (2004). No changes in serum ghrelin levels in female patients with bulimia nervosa. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 28(7), 1181–1184. <http://doi.org/10.1016/j.pnpbp.2004.06.012>
- Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., & Matsukura, S. (2001). A role for ghrelin in the central regulation of feeding. *Nature*, 409(6817), 194–198. <http://doi.org/10.1038/35051587>
- Nkosi, C. Z., Opoku, A. R., & Terblanche, S. E. (2006). In Vitro antioxidative activity of pumpkin seed (*Cucurbita pepo*) protein isolate and its In Vivo effect on alanine transaminase and aspartate transaminase in acetaminophen-induced liver injury in low protein fed rats. *Phytotherapy Research: PTR*, 20(9), 780–783. <http://doi.org/10.1002/ptr.1958>
- Nørrelund, H., Hansen, T. K., Ørskov, H., Hosoda, H., Kojima, M., Kangawa, K., ... Jørgensen, J. O. L. (2002). Ghrelin immunoreactivity in human plasma is suppressed by somatostatin. *Clinical Endocrinology*, 57(4), 539–546.
- Okamatsu, Y., Matsuda, K., Hiramoto, I., Tani, H., Kimura, K., Yada, Y., ... Matsuishi, T. (2009). Ghrelin and leptin modulate immunity and liver function in overweight children. *Pediatrics International*, 51(1), 9–13. <http://doi.org/10.1111/j.1442-200X.2008.02647.x>
- Omoto, I., Matsumoto, M., Uchikado, Y., Kita, Y., Sakurai, T., Sasaki, K., ... Natsugoe, S. (2014). Immunohistochemical evidence of association between ghrelin expression and tumor growth in esophageal carcinoma. *Anticancer Research*, 34(6), 2727–2733.
- Østergård, T., Hansen, T., Nyholm, B., Gravholt, C., Djurhuus, C., Hosoda, H., ... Schmitz, O. (2003). Circulating ghrelin concentrations are reduced in healthy

- offspring of Type 2 diabetic subjects, and are increased in women independent of a family history of Type 2 diabetes. *Diabetologia*, 46(1), 134–136. <http://doi.org/10.1007/s00125-002-0985-4>
- Osterstock, G., Escobar, P., Mitutsova, V., Gouty-Colomer, L.-A., Fontanaud, P., Molino, F., ... Méry, P.-F. (2010). Ghrelin stimulation of growth hormone-releasing hormone neurons is direct in the arcuate nucleus. *PloS One*, 5(2), e9159. <http://doi.org/10.1371/journal.pone.0009159>
- Ozsoy, S., Besirli, A., Abdulrezzak, U., & Basturk, M. (2014). Serum ghrelin and leptin levels in patients with depression and the effects of treatment. *Psychiatry Investigation*, 11(2), 167–172. <http://doi.org/10.4306/pi.2014.11.2.167>
- Padoin, A. V., Galvão Neto, M., Moretto, M., Barancelli, F., Schroer, C. E., & Mottin, C. C. (2009). Obese patients with type 2 diabetes submitted to banded gastric bypass: greater incidence of dumping syndrome. *Obesity Surgery*, 19(11), 1481–1484. <http://doi.org/10.1007/s11695-009-9943-2>
- Pandol, S. J. (2011). *The Exocrine Pancreas*. Morgan & Claypool Publishers.
- Papotti, M., Ghè, C., Cassoni, P., Catapano, F., Deghenghi, R., Ghigo, E., & Muccioli, G. (2000). Growth hormone secretagogue binding sites in peripheral human tissues. *The Journal of Clinical Endocrinology and Metabolism*, 85(10), 3803–3807. <http://doi.org/10.1210/jcem.85.10.6846>
- Pavel, I., & Sdrobici, D. (1972). [N. Paulescu, discoverer of insulin. 50th anniversary of the discovery of that hormone]. *Medicină Internă*, 24(11), 1285–1294.
- Perfetti, R., Zhou, J., Doyle, M. E., & Egan, J. M. (2000). Glucagon-Like Peptide-1 Induces Cell Proliferation and Pancreatic-Duodenum Homeobox-1

Expression and Increases Endocrine Cell Mass in the Pancreas of Old, Glucose-Intolerant Rats. *Endocrinology*, 141(12), 4600–4605.

<http://doi.org/10.1210/endo.141.12.7806>

Petersen, O. H. (1992). Stimulus-secretion coupling: cytoplasmic calcium signals and the control of ion channels in exocrine acinar cells. *The Journal of Physiology*, 448, 1–51.

Pilcher, H. (2006). Alzheimer's disease could be "type 3 diabetes." *The Lancet Neurology*, 5(5), 388–389. [http://doi.org/10.1016/S1474-4422\(06\)70434-3](http://doi.org/10.1016/S1474-4422(06)70434-3)

Piñero-Piloña, A., & Raskin, P. (2001). Idiopathic Type 1 diabetes. *Journal of Diabetes and Its Complications*, 15(6), 328–335.

[http://doi.org/10.1016/S1056-8727\(01\)00172-6](http://doi.org/10.1016/S1056-8727(01)00172-6)

Poretti, M. B., Rask-Andersen, M., Kumar, P., Rubiales de Barioglio, S., Fiol de Cuneo, M., Schiöth, H. B., & Carlini, V. P. (2015). Ghrelin effects expression of several genes associated with depression-like behavior. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 56, 227–234. <http://doi.org/10.1016/j.pnpbp.2014.09.012>

Pöykkö, S. M., Kellokoski, E., Hökkö, S., Kauma, H., Kesäniemi, Y. A., & Ukkola, O. (2003). Low Plasma Ghrelin Is Associated With Insulin Resistance, Hypertension, and the Prevalence of Type 2. *Diabetes*, 52(10), 2546–2553. <http://doi.org/10.2337/diabetes.52.10.2546>

Purnell, J. Q., Weigle, D. S., Breen, P., & Cummings, D. E. (2003). Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. *The Journal of Clinical Endocrinology and Metabolism*, 88(12), 5747–5752. <http://doi.org/10.1210/jc.2003-030513>

Qader, S. S., Lundquist, I., Ekelund, M., Håkanson, R., & Salehi, A. (2005). Ghrelin activates neuronal constitutive nitric oxide synthase in pancreatic islet cells while inhibiting insulin release and stimulating glucagon release. *Regulatory Peptides*, 128(1), 51–56. <http://doi.org/10.1016/j.regpep.2004.12.018>

REDUCTION IN THE INCIDENCE OF TYPE 2 DIABETES WITH LIFESTYLE INTERVENTION OR METFORMIN. (2002). *The New England Journal of Medicine*, 346(6), 393–403. <http://doi.org/10.1056/NEJMoa012512>

Reichenbach, A., Steyn, F. J., Sleeman, M. W., & Andrews, Z. B. (2012). Ghrelin receptor expression and colocalization with anterior pituitary hormones using a GHSR-GFP mouse line. *Endocrinology*, 153(11), 5452–5466. <http://doi.org/10.1210/en.2012-1622>

Rena, G., Pearson, E. R., & Sakamoto, K. (2013). Molecular mechanism of action of metformin: old or new insights? *Diabetologia*, 56(9), 1898–1906. <http://doi.org/10.1007/s00125-013-2991-0>

Reyes-Muñoz, E., Castellanos-Barroso, G., Ramírez-Eugenio, B. Y., Ortega-González, C., Parra, A., Castillo-Mora, A., & De la Jara-Díaz, J. F. (2012). The risk of gestational diabetes mellitus among Mexican women with a history of infertility and polycystic ovary syndrome. *Fertility and Sterility*, 97(6), 1467–1471. <http://doi.org/10.1016/j.fertnstert.2012.03.023>

Riddell, M. C., & Perkins, B. A. (2006). Type 1 Diabetes and Vigorous Exercise: Applications of Exercise Physiology to Patient Management. *Canadian Journal of Diabetes*, 30(1), 63–71. [http://doi.org/10.1016/S1499-2671\(06\)01010-0](http://doi.org/10.1016/S1499-2671(06)01010-0)

- Riley, L. G. (2013). Different forms of ghrelin exhibit distinct biological roles in tilapia. *Frontiers in Endocrinology*, 4, 118.
<http://doi.org/10.3389/fendo.2013.00118>
- Riley, L. G., Hirano, T., & Grau, E. G. (2002). Rat ghrelin stimulates growth hormone and prolactin release in the tilapia, *Oreochromis mossambicus*. *Zoological Science*, 19(7), 797–800.
- Robertson, R. P. (2004). Islet Transplantation as a Treatment for Diabetes — A Work in Progress. *New England Journal of Medicine*, 350(7), 694–705.
<http://doi.org/10.1056/NEJMra032425>
- Rosenfeld, L. (2002a). Insulin: Discovery and Controversy. *Clinical Chemistry*, 48(12), 2270–2288.
- Rosenfeld, L. (2002b). Insulin: Discovery and Controversy. *Clinical Chemistry*, 48(12), 2270–2288.
- Ross, M. H., & Pawlina, W. (2006). *Histology*. Lippincott Williams & Wilkins.
- Saadi, H., Carruthers, S. G., Nagelkerke, N., Al-Maskari, F., Afandi, B., Reed, R., ... Benedict, S. (2007). Prevalence of diabetes mellitus and its complications in a population-based sample in Al Ain, United Arab Emirates. *Diabetes Research and Clinical Practice*, 78(3), 369–377.
<http://doi.org/10.1016/j.diabres.2007.04.008>
- Saito, E.-S., Kaiya, H., Tachibana, T., Tomonaga, S., Denbow, D. M., Kangawa, K., & Furuse, M. (2005). Inhibitory effect of ghrelin on food intake is mediated by the corticotropin-releasing factor system in neonatal chicks. *Regulatory Peptides*, 125(1–3), 201–208. <http://doi.org/10.1016/j.regpep.2004.09.003>
- Salehi, A., Dornonville de la Cour, C., Håkanson, R., & Lundquist, I. (2004). Effects of ghrelin on insulin and glucagon secretion: a study of isolated pancreatic

islets and intact mice. *Regulatory Peptides*, 118(3), 143–150.

<http://doi.org/10.1016/j.regpep.2003.12.001>

Salfen, B. E., Carroll, J. A., Keisler, D. H., & Strauch, T. A. (2004). Effects of exogenous ghrelin on feed intake, weight gain, behavior, and endocrine responses in weanling pigs. *Journal of Animal Science*, 82(7), 1957–1966.

Sato, T., Nakamura, Y., Shiimura, Y., Ohgusu, H., Kangawa, K., & Kojima, M. (2012). Structure, regulation and function of ghrelin. *Journal of Biochemistry*, 151(2), 119–128. <http://doi.org/10.1093/jb/mvr134>

Scherbaum, W. A. (1992). Etiology and pathogenesis of type 1 diabetes. *Hormone and Metabolic Research. Supplement Series*, 26, 111–116.

Schulinkamp, R. J., Pagano, T. C., Hung, D., & Raffa, R. B. (2000). Insulin receptors and insulin action in the brain: review and clinical implications. *Neuroscience and Biobehavioral Reviews*, 24(8), 855–872.

secondary diabetes mellitus. (n.d.). Retrieved February 23, 2015, from

<http://medical-dictionary.thefreedictionary.com/secondary+diabetes+mellitus>

Senior, J. R. (2012). Alanine aminotransferase: a clinical and regulatory tool for detecting liver injury-past, present, and future. *Clinical Pharmacology and Therapeutics*, 92(3), 332–339. <http://doi.org/10.1038/clpt.2012.108>

Seoane, L. M., López, M., Tovar, S., Casanueva, F. F., Señarís, R., & Diéguez, C. (2003). Agouti-related peptide, neuropeptide Y, and somatostatin-producing neurons are targets for ghrelin actions in the rat hypothalamus. *Endocrinology*, 144(2), 544–551. <http://doi.org/10.1210/en.2002-220795>

Shiia, T., Nakazato, M., Mizuta, M., Date, Y., Mondal, M. S., Tanaka, M., ... Matsukura, S. (2002). Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *The Journal of Clinical*

Endocrinology and Metabolism, 87(1), 240–244.

<http://doi.org/10.1210/jcem.87.1.8129>

Shimada, M., Date, Y., Mondal, M. S., Toshinai, K., Shimbara, T., Fukunaga, K., ...

Nakazato, M. (2003). Somatostatin suppresses ghrelin secretion from the rat stomach. *Biochemical and Biophysical Research Communications*, 302(3), 520–525.

Shousha, S., Nakahara, K., Kojima, M., Miyazato, M., Hosoda, H., Kangawa, K., & Murakami, N. (2005). Different effects of peripheral and central ghrelin on regulation of food intake in the Japanese quail. *General and Comparative Endocrinology*, 141(2), 178–183. <http://doi.org/10.1016/j.ygcen.2004.12.021>

Shrivastava, S. R., Shrivastava, P. S., & Ramasamy, J. (2013). Role of self-care in management of diabetes mellitus. *Journal of Diabetes and Metabolic Disorders*, 12(1), 14. <http://doi.org/10.1186/2251-6581-12-14>

Skyler, J. S., & Sosenko, J. M. (2013). The evolution of type 1 diabetes. *JAMA*, 309(23), 2491–2492. <http://doi.org/10.1001/jama.2013.6286>

S, P. (2002). Structure and regulation of the growth hormone secretagogue receptor. *Minerva Endocrinologica*, 27(4), 243–256.

Spalding, D., & Williamson, R. C. N. (2011). Pancreatic cancer. *Medicine*, 39(5), 274–278. <http://doi.org/10.1016/j.mpmed.2011.02.011>

Steiger, A., Dresler, M., Schüssler, P., & Kluge, M. (2011). Ghrelin in mental health, sleep, memory. *Molecular and Cellular Endocrinology*, 340(1), 88–96. <http://doi.org/10.1016/j.mce.2011.02.013>

Stengel, A., & Taché, Y. (2012). Ghrelin - a pleiotropic hormone secreted from endocrine x/a-like cells of the stomach. *Frontiers in Neuroscience*, 6, 24. <http://doi.org/10.3389/fnins.2012.00024>

- Stumvoll, M., Goldstein, B. J., & van Haeften, T. W. (2005). Type 2 diabetes: principles of pathogenesis and therapy. *The Lancet*, 365(9467), 1333–1346. [http://doi.org/10.1016/S0140-6736\(05\)61032-X](http://doi.org/10.1016/S0140-6736(05)61032-X)
- Sugino, T., Hasegawa, Y., Kikkawa, Y., Yamaura, J., Yamagishi, M., Kurose, Y., ... Terashima, Y. (2002). A transient ghrelin surge occurs just before feeding in a scheduled meal-fed sheep. *Biochemical and Biophysical Research Communications*, 295(2), 255–260.
- Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research / Academia Scientiarum Bohemoslovaca*, 50(6), 537–546.
- Tahrani, A. A., Bailey, C. J., Del Prato, S., & Barnett, A. H. (2011a). Management of type 2 diabetes: new and future developments in treatment. *The Lancet*, 378(9786), 182–197. [http://doi.org/10.1016/S0140-6736\(11\)60207-9](http://doi.org/10.1016/S0140-6736(11)60207-9)
- Tahrani, A. A., Bailey, C. J., Del Prato, S., & Barnett, A. H. (2011b). Management of type 2 diabetes: new and future developments in treatment. *The Lancet*, 378(9786), 182–197. [http://doi.org/10.1016/S0140-6736\(11\)60207-9](http://doi.org/10.1016/S0140-6736(11)60207-9)
- Takahashi, H., Kurose, Y., Kobayashi, S., Sugino, T., Kojima, M., Kangawa, K., ... Terashima, Y. (2006). Ghrelin enhances glucose-induced insulin secretion in scheduled meal-fed sheep. *The Journal of Endocrinology*, 189(1), 67–75. <http://doi.org/10.1677/joe.1.06310>
- Takaya, K., Ariyasu, H., Kanamoto, N., Iwakura, H., Yoshimoto, A., Harada, M., ... Nakao, K. (2000). Ghrelin strongly stimulates growth hormone release in humans. *The Journal of Clinical Endocrinology and Metabolism*, 85(12), 4908–4911. <http://doi.org/10.1210/jcem.85.12.7167>

- Tan, G. D. (2008). The pancreas. *Anaesthesia & Intensive Care Medicine*, 9(10), 424–427. <http://doi.org/10.1016/j.mpaic.2008.07.016>
- The Discovery of Insulin. (n.d.). Retrieved May 18, 2015, from <http://www.nobelprize.org/educational/medicine/insulin/discovery-insulin.html>
- ThidarMyint, H., Yoshida, H., Ito, T., & Kuwayama, H. (2006). Dose-dependent response of plasma ghrelin and growth hormone concentrations to bovine ghrelin in Holstein heifers. *Journal of Endocrinology*, 189(3), 655–664. <http://doi.org/10.1677/joe.1.06746>
- Thompson, N. M., Gill, D. A. S., Davies, R., Loveridge, N., Houston, P. A., Robinson, I. C. A. F., & Wells, T. (2004). Ghrelin and des-octanoyl ghrelin promote adipogenesis directly in vivo by a mechanism independent of the type 1a growth hormone secretagogue receptor. *Endocrinology*, 145(1), 234–242. <http://doi.org/10.1210/en.2003-0899>
- Tortora, G. J., & Derrickson, B. H. (2011). *Principles of Anatomy and Physiology*. Wiley.
- Tóth, K., László, K., & Lénárd, L. (2010). Role of intraamygdaloid acylated-ghrelin in spatial learning. *Brain Research Bulletin*, 81(1), 33–37. <http://doi.org/10.1016/j.brainresbull.2009.10.003>
- Tritos, N. A., Kissinger, K. V., Manning, W. J., & Danias, P. G. (2004). Association between ghrelin and cardiovascular indexes in healthy obese and lean men. *Clinical Endocrinology*, 60(1), 60–66.
- Tschöp, M., Smiley, D. L., & Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature*, 407(6806), 908–913. <http://doi.org/10.1038/35038090>

- Tschöp, M., Statnick, M. A., Suter, T. M., & Heiman, M. L. (2002). GH-releasing peptide-2 increases fat mass in mice lacking NPY: indication for a crucial mediating role of hypothalamic agouti-related protein. *Endocrinology*, *143*(2), 558–568. <http://doi.org/10.1210/endo.143.2.8633>
- Tschöp, M., Weyer, C., Tataranni, P. A., Devanarayan, V., Ravussin, E., & Heiman, M. L. (2001). Circulating Ghrelin Levels Are Decreased in Human Obesity. *Diabetes*, *50*(4), 707–709. <http://doi.org/10.2337/diabetes.50.4.707>
- Tsui, H., Razavi, R., Chan, Y., Yantha, J., & Dosch, H.-M. (2007). “Sensing” autoimmunity in type 1 diabetes. *Trends in Molecular Medicine*, *13*(10), 405–413. <http://doi.org/10.1016/j.molmed.2007.07.006>
- Turk, N., Dağistanlı, F. K., Sacan, O., Yanardag, R., & Bolkent, S. (2012). Obestatin and insulin in pancreas of newborn diabetic rats treated with exogenous ghrelin. *Acta Histochemica*, *114*(4), 349–357. <http://doi.org/10.1016/j.acthis.2011.07.003>
- Unniappan, S., & Peter, R. E. (2005). Structure, distribution and physiological functions of ghrelin in fish. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *140*(4), 396–408. <http://doi.org/10.1016/j.cbpb.2005.02.011>
- Veilleux, A., Houde, V. P., Bellmann, K., & Marette, A. (2010). Chronic inhibition of the mTORC1/S6K1 pathway increases insulin-induced PI3K activity but inhibits Akt2 and glucose transport stimulation in 3T3-L1 adipocytes. *Molecular Endocrinology (Baltimore, Md.)*, *24*(4), 766–778. <http://doi.org/10.1210/me.2009-0328>

- Vincent, A., Herman, J., Schulick, R., Hruban, R. H., & Goggins, M. (2011). Pancreatic cancer. *The Lancet*, 378(9791), 607–620.
[http://doi.org/10.1016/S0140-6736\(10\)62307-0](http://doi.org/10.1016/S0140-6736(10)62307-0)
- Volante, M., Allia, E., Gugliotta, P., Funaro, A., Broglio, F., Deghenghi, R., ... Papotti, M. (2002). Expression of Ghrelin and of the GH Secretagogue Receptor by Pancreatic Islet Cells and Related Endocrine Tumors. *The Journal of Clinical Endocrinology & Metabolism*, 87(3), 1300–1308.
<http://doi.org/10.1210/jcem.87.3.8279>
- Wajnrajch, M. P., Ten, I. S., Gertner, J. M., & Leibel, R. L. (2011). Genomic Organization of the Human GHRELIN Gene. *International Journal on Disability and Human Development*, 1(4), 231–234.
<http://doi.org/10.1515/IJDHD.2000.1.4.231>
- Walker, A. K., Gong, Z., Park, W.-M., Zigman, J. M., & Sakata, I. (2013). Expression of Serum Retinol Binding Protein and Transthyretin within Mouse Gastric Ghrelin Cells. *PloS One*, 8(6), e64882.
<http://doi.org/10.1371/journal.pone.0064882>
- Wang, Y., Nishi, M., Doi, A., Shono, T., Furukawa, Y., Shimada, T., ... Nanjo, K. (2010). Ghrelin inhibits insulin secretion through the AMPK–UCP2 pathway in β cells. *FEBS Letters*, 584(8), 1503–1508.
<http://doi.org/10.1016/j.febslet.2010.02.069>
- Warnock, G. L., Kneteman, N. M., Ryan, E., Seelis, R. E. A., Rabinovitch, A., & Rajotte, R. V. (1991). Normoglycaemia after transplantation of freshly isolated and cryopreserved pancreatic islets in Type 1 (insulin-dependent) diabetes mellitus. *Diabetologia*, 34(1), 55–58.
<http://doi.org/10.1007/BF00404026>

- Watanabe, M., Hayasaki, H., Tamayama, T., & Shimada, M. (1998). Histologic distribution of insulin and glucagon receptors. *Brazilian Journal of Medical and Biological Research = Revista Brasileira De Pesquisas Médicas E Biológicas / Sociedade Brasileira De Biofísica ... [et Al.]*, 31(2), 243–256.
- Weerakiet, S., Srisombut, C., Rojanasakul, A., Panburana, P., Thakkinstian, A., & Herabutya, Y. (2004). Prevalence of gestational diabetes mellitus and pregnancy outcomes in Asian women with polycystic ovary syndrome. *Gynecological Endocrinology: The Official Journal of the International Society of Gynecological Endocrinology*, 19(3), 134–140.
- Wells, B., DiPiro, J., Schwinghammer, T., Matzke, G., Yee, G., Posey, L. M., & Talbert, R. L. (2008). *Pharmacotherapy Handbook EBOOK*. McGraw Hill Professional.
- White, R., & Sherman, C. (1999). Exercise in Diabetes Management *Maximizing Benefits, Controlling Risks. The Physician and Sportsmedicine*, 27(4), 0. <http://doi.org/10.3810/psm.1999.04.787>
- WHO | Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. (n.d.). Retrieved July 31, 2014, from http://www.who.int/diabetes/publications/diagnosis_diabetes2006/en/
- Wierup, N., Yang, S., McEvelly, R. J., Mulder, H., & Sundler, F. (2004). Ghrelin is expressed in a novel endocrine cell type in developing rat islets and inhibits insulin secretion from INS-1 (832/13) cells. *The Journal of Histochemistry and Cytochemistry: Official Journal of the Histochemistry Society*, 52(3), 301–310.

- Wild, S., Roglic, G., Green, A., Sicree, R., & King, H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*, 27(5), 1047–1053.
- Wu, J. T., & Kral, J. G. (2004). Ghrelin: integrative neuroendocrine peptide in health and disease. *Annals of Surgery*, 239(4), 464–474.
- Xu, G., Stoffers, D. A., Habener, J. F., & Bonner-Weir, S. (1999). Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes*, 48(12), 2270–2276.
- Yada, T., Damdindorj, B., Rita, R. S., Kurashina, T., Ando, A., Taguchi, M., ... Dezaki, K. (2014). Ghrelin signalling in β -cells regulates insulin secretion and blood glucose. *Diabetes, Obesity & Metabolism*, 16 Suppl 1, 111–117. <http://doi.org/10.1111/dom.12344>
- Yang, C., Liu, Z., Liu, K., & Yang, P. (2014). Mechanisms of Ghrelin anti-heart failure: inhibition of Ang II-induced cardiomyocyte apoptosis by down-regulating AT1R expression. *PloS One*, 9(1), e85785. <http://doi.org/10.1371/journal.pone.0085785>
- Yildirim, S., Sundler, F., & Bolkent, S. (2007). Ghrelin and insulin gene expression changes in streptozotocin-induced diabetic rats after rosiglitazone pretreatment. *European Journal of Histochemistry: EJH*, 51(1), 11–18.
- Zander, M., Madsbad, S., Madsen, J. L., & Holst, J. J. (2002). Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β -cell function in type 2 diabetes: a parallel-group study. *The Lancet*, 359(9309), 824–830. [http://doi.org/10.1016/S0140-6736\(02\)07952-7](http://doi.org/10.1016/S0140-6736(02)07952-7)

- Zhang, S., Zhang, Q., Zhang, L., Li, C., & Jiang, H. (2013). Expression of ghrelin and leptin during the development of type 2 diabetes mellitus in a rat model. *Molecular Medicine Reports*, 7(1), 223–228.
<http://doi.org/10.3892/mmr.2012.1154>
- Zhao, Z., Liu, H., Xiao, K., Yu, M., Cui, L., Zhu, Q., ... Zhou, Y. (2014). Ghrelin administration enhances neurogenesis but impairs spatial learning and memory in adult mice. *Neuroscience*, 257, 175–185.
<http://doi.org/10.1016/j.neuroscience.2013.10.063>
- Zhu, Q., Xiao, K., Yu, M., Niu, M., Li, C., Gao, Y., ... Zhou, Y. (2013). Ghrelin but not nesfatin-1 affects certain forms of learning and memory in both rats and mice. *Brain Research*, 1541, 42–51.
<http://doi.org/10.1016/j.brainres.2013.10.022>
- Zigman, J. M., Jones, J. E., Lee, C. E., Saper, C. B., & Elmquist, J. K. (2006). Expression of ghrelin receptor mRNA in the rat and the mouse brain. *The Journal of Comparative Neurology*, 494(3), 528–548.
<http://doi.org/10.1002/cne.20823>
- Zigman, J. M., Nakano, Y., Coppari, R., Balthasar, N., Marcus, J. N., Lee, C. E., ... Elmquist, J. K. (2005). Mice lacking ghrelin receptors resist the development of diet-induced obesity. *The Journal of Clinical Investigation*, 115(12), 3564–3572. <http://doi.org/10.1172/JCI26002>

List of Publications

- Conferences:
 - Graduate Students Research Conference, March 22 – 24, 2015