IntechOpen

Ultimate Guide to Insulin

Edited by Gaffar Zaman

ULTIMATE GUIDE TO INSULIN

Edited by **Gaffar Zaman**

Ultimate Guide to Insulin
http://dx.doi.org/10.5772/intechopen.71990
Edited by Gaffar Zaman

Contributors

Rafał Skowronek, Nicolás Renna, Rodrigo Damián García, Jesica Ramirez, Roberto M. Miatello, Yoko Shiba, Michiko Saito, Kenneth Strauss, Anders Frid, Laurence Hirsch, Ashish Chauhan, Lucia Agoston-Coldea, Alexandru Zlibut, Lucian Mocan, Teodora Mocan, Ioana Corina Bocsan, Gaffar Sarwar Zaman

Notice

First published in London, United Kingdom, 2019 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Ultimate Guide to Insulin
Edited by Gaffar Zaman

p. cm.

Print ISBN 978-1-78984-921-9

Online ISBN 978-1-78984-922-6

eBook (PDF) ISBN 978-1-83962-001-0

Meet the editor

Dr. Zaman received his Doctor of Medicine degree in Biochemistry (MD) from the Assam Medical College & Hospital, Dibrugarh University, India. He undertook a fellowship in diabetes (FID) from the Royal Liverpool Academy, United Kingdom; a fellowship in applied nutrition (FIAN) from the Medvarsity, Apollo Hospitals, India and a postgraduate diploma in Clinical Research (PGDCR) from the Symbiosis University, India. He has almost 12 years of experience as an assistant professor at the King Khalid Government University and Rajiv Gandhi University of Health Sciences. He is well accustomed in quality development and can help any college to improve its quality. He is also well accustomed in curriculum designing and is trained in e-learning methods. He has almost 40 research publications (most of them original articles) to his credit, in both national and international journals. He has already published a book entitled "Quality Control in Laboratory". Currently, he is also engaged in a book entitled "Clinical Biochemistry: Fundamentals of Medical & Laboratory Science", along with this book.

Contents

Preface

Insulin is one of the most important treatment modalities of any diabetologist. Insulin treatment has undergone a vast change in the last 50 years. From its discovery on the morning of 14 November 1921 and the first injection on 23 January 1922 to Canadian 14-year-old Leonard Thompson, it has become the mainstay for the treatment for diabetes mellitus. Various reports on medical/laboratory fallacy/misconception have increased the necessity to augment insulin safety and resourcefulness. Prominence has relocated from simply diagnosing and treating diabetes to identifying and controlling the disease and risk factors, and maintaining health, for which insulin plays a vital role. This book stresses prominence on five facets: defective insulin exocytosis of diabetic cells; insulin resistance; translocation to the muscle membrane; decreased hepatic clearance of insulin; and obesity related to insulin resistance. In addition to this, the book also focuses on the treatment, immunisation and importance of insurance in diabetes mellitus.

This work would not have been possible without the support of IntechOpen. I am especially indebted to Mr Julian Virag, Author Service Manager, IntechOpen, who has been supportive of my book and who worked actively to provide me with all facilities. I am grateful to all those with whom I have had the pleasure to work during this project. Each of the authors of the different chapters has provided me with extensive professional help and obliged me on this scientific venture wherever possible. I would especially like to thank the authors of the various chapters of the book.

Nobody has been more important to me in the pursuit of this project than the members of my family. I would like to thank my parents, the late Taufiquz Zaman and the late Jaiba Zaman, whose love and guidance are with me in whatever I pursue. Also, I am thankful to my father-in-law, the late Misbahul Hussain, and my mother-in-law, Suraiya Hussain. Most importantly, I wish to thank my loving and supportive wife, Jarin Tanwir Hussain, who is my ultimate role model, and my two wonderful children, Naushad Muntasir Zaman and Umar Sarwar Zaman, who provided me with unending inspiration during the project.

Dr Gaffar S. Zaman
MBBS, MD (Doctor of Medicine in Biochemistry), FID (Fellowship in Diabetes)
FIAN (Fellowship in Applied Nutrition)
PGDCR (Post Graduate Diploma in Clinical Research)
Assistant Professor, Department of Clinical Laboratory Sciences
College of Applied Medical Science
King Khalid University
Kingdom of Saudi Arabia

Chapter 1

Introductory Chapter: Historical Perspective and Brief Overview of Insulin

Gaffar Sarwar Zaman

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.81183

1. Introduction

One of the oldest diseases of mankind is diabetes mellitus. It was only during the later part of the nineteenth and first half of the twentieth centuries that newer advances relating to the pathology, predisposing factors, management, course and complications of diabetes mellitus were discovered. Yet many more demanding solutions relating to the disease are still required. It has been seen that urbanisation and ageing of the population is definitely related to diabetes mellitus. But it is also true that diabetes mellitus affects all ages and all races. It has been estimated that around 400 million people will be affected by diabetes mellitus by 2030 AD. There are three principal forms of diabetes mellitus: type 1, type 2 and gestational diabetes mellitus (GDM). It has been seen that Finland has one of the highest incidence rates of type 1 diabetes mellitus. Type 1 diabetes is most likely a polygenic disease and has a number of potential risk factors. Type 2 diabetes is associated with the interaction of environmental factors and genetic factors. Impaired glucose tolerance (IGT), which has a great potential to be converted to diabetes mellitus, also carries cardiovascular and other risks. It has been seen that the important risk factors for the occurrence of diabetes are (i) changes in lifestyle due to urbanisation, (ii) hereditary, (iii) resistance to insulin, (iv) accumulation of fat around the waist rather than generalised obesity, (v) increasing age and (vi) ethnicity. It has been seen that long-standing diabetes mellitus is associated with an increased prevalence of macrovascular and microvascular diseases. Other chronic complications such as neuropathy and retinopathy are very common in diabetes mellitus.

2. Historical perspective of insulin

Although diabetes mellitus was always recognised as a distinct entity for more than 3000 years, its exact cause was not known until the twentieth century.

Till the early 1920s, many investigators were of the strong suspicion that diabetes was caused by a malfunction in the digestive system related to the pancreas gland. During that time the fatal disease was treated by a diet which was low in sugar and carbohydrates and high in protein and fat.

In those days, the patient usually died shortly after diagnosis, but the above diet allowed them to live for some years.

However, Best and Banting [1], **Figure 1**, two Canadians, provided a great relief to the world when they were able to isolate insulin from canines. They first produced diabetogenic symptoms in dogs and then with the help of insulin injections returned the dogs to normalcy. On the morning of November 14, 1921, they announced their discovery to the world. Then on January 23, 1922, they first injected Canadian 14-year-old Leonard Thompson with insulin and continued the treatment for diabetes mellitus.

Banting [2], **Figure 2**, by virtue of his tedious research, was able to create a pancreatic extract, which enabled him to gather thousands of islet cells. He then prepared extracts of insulin from these islets.

Initially, the insulin was tested on dogs, and it was able to regulate their blood glucose levels. Then in 1922 they tested it on Leonard Thompson, who became the first human being to be

Figure 1. Charles Herbert Best, CC, CH, CBE, FRS, FRSC, FRCP (February 27, 1899–March 31, 1978) and Sir Frederick Grant Banting, KBE, MC, FRS, FRSC, (November 14, 1891–February 21, 1941) (Source: [1]).

http://dx.doi.org/10.5772/intechopen.81183

Figure 2. Sir Frederick Grant Banting, KBE, MC, FRS, FRSC (November 14, 1891–February 21, 1941) (Source: [2]).

given insulin. The first dose was a failure as it was not purified enough, but the second dose which was purified by James B. Collip proved to be successful.

Development of insulin was done further by Banting along with laboratory director John MacLeod, and both of them were awarded the Nobel Prize in Physiology of Medicine in 1923.

The first person who promoted the benefits of a low-carbohydrate diet was Banting [3], **Figure 3**, originally referred to as the 'Banting diet'. After almost a period of 150 years post his publication of the renowned booklet 'Letter on Corpulence', addressed to the public (in the year 1863), the Banting diet has been backed up by several clinical trials as being safe and effective for weight loss, and it is now finally being acknowledged as a beneficial diet for people with diabetes.

It should be noted that Banting was not a scientist, but a highly skilful carpenter. While he was young, Banting became overweight, and he was told by a doctor to exercise more. But it did not help him. He tried a variety of weight loss options but failed in almost all of them.

Then he met Harvey who told him to give up butter, bread, milk, beer, sugar and potatoes—i.e. foods which contained sugar and starch. After 5 months, Banting returned to normal weight and he was more agile.

Best [4], **Figure 4**, also helped Frederick Banting in discovering insulin in 1922 after he became Banting's assistant in the summer of 1921. After being awarded the Nobel Prize in 1923 with

Figure 3. William Banting (c. December 1796–March 16, 1878) (Source: [3]).

Figure 4. Charles Herbert Best, CC, CH, CBE, FRS, FRSC, FRCP (February 27, 1899–March 31, 1978) (Source: [4]).

J.J.R. MacLeod, Banting shared the prize money with Best and the rest of his team that were responsible for insulin being developed. Best was instrumental in doing the chemical tests to measure blood glucose levels while working with the team.

Thompson [5], **Figure 5**, was the first patient having diabetes to receive insulin injections on January 11, 1922. Almost facing death, Leonard survived for another 13 years. Leonard, who was diagnosed some years previously, was admitted in Toronto General Hospital.

He was severely diabetic and was coming in and out of a diabetic coma and was weighing only 65 pounds. It was Leonard's father who gave the consent that his son should be the first person to test insulin, which was never previously been tried on another human being.

Initially the impure form of insulin was unable to make any impact in Leonard's condition; however, a purer version of insulin made him survive and his parameters came back to normal.

One of the most unique approaches to diabetes treatment was provided by Proctor [6], **Figure 6**, in the early 1900s, as he concentrated on patients taking their own responsibility.

Joslin made his mother survive through diabetes for 10 years through a rigorous combination of exercise, meal planning and food management. He was the founder of Joslin Diabetes Center.

The 'starvation diet' was first proposed by Allen [7], **Figure 7**, who proposed it before the discovery of insulin to increase the life span of diabetes patients.

Figure 5. Leonard Thompson (Source: [5]).

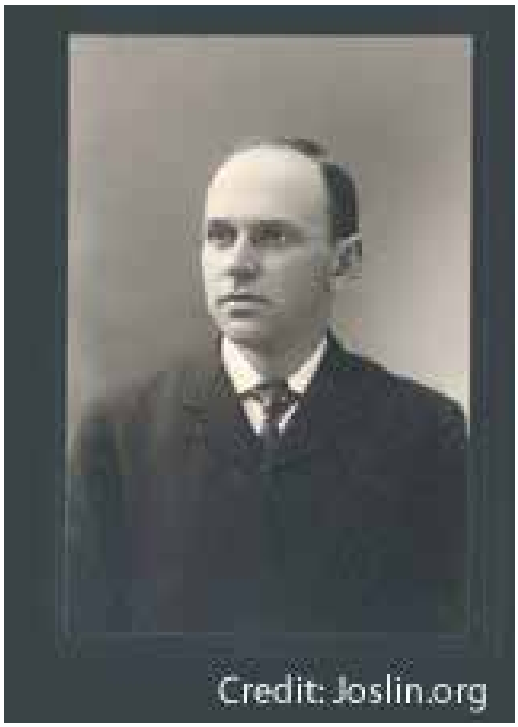

Figure 6. Elliott Proctor Joslin, MD (June 6, 1869–January 28, 1962) (Source: [6]).

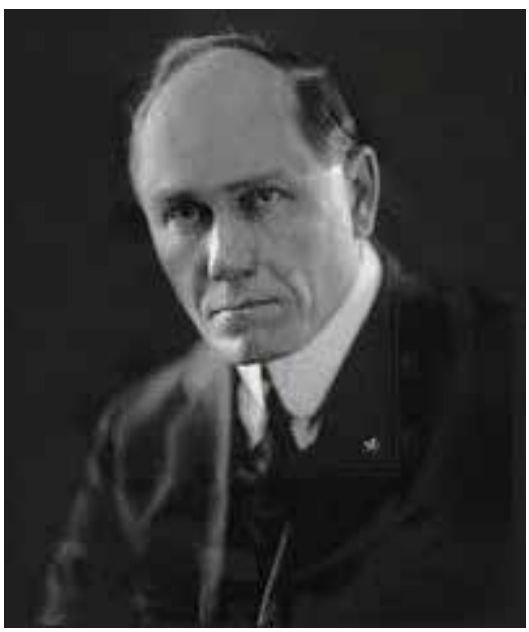

Figure 7. Frederick Madison Allen (1879–1964) (Source: [7]).

It was Allen, a physician, who first proposed that restriction in calorie intake and engaging oneself in regular exercise resulted in the prolongation of the life of insulin-producing beta cells.

3. Early research

Allen, who was born in Iowa, studied medicine at the University of California. He attended Harvard Medical School between 1909 and 1912, and thanks to his father's financing, he also published *Studies on Diabetes and Glycosuria* in 1913.

Insulin receptors were thoroughly studied mostly by Kahn [8], **Figure 8**. He spent most of his career investigating the role of insulin sensitivity in obesity and diabetes.

Kahn currently works as the Chief Academic Officer and Head of Joslin's Section on Integrative Physiology and Metabolism at the Joslin Diabetes Center.

He thoroughly investigated how cells are affected by insulin and the reason behind why only a particular group of cells develops insulin resistance, which is one of the main causes of type 2 diabetes.

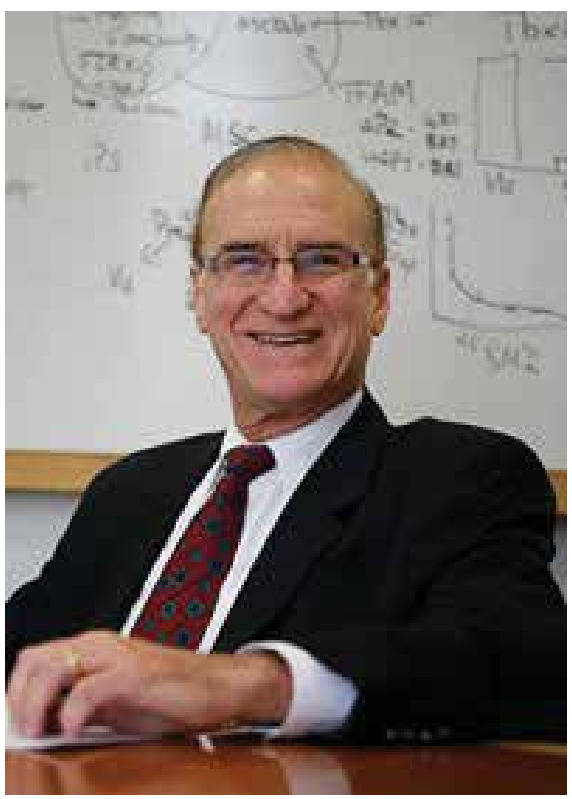

Figure 8. Carl Ronald Kahn (born January 14, 1944) (Source: [8]).

Kahn through his excellence in diabetes study became Research Director of Joslin and Associate Professor of Medicine at Harvard Medical School in 1981 and 1984. He was the first to discover the importance of the role of insulin actions in the brain and the causation of metabolic diseases by fat cells.

Bouchardat [9], **Figure 9**, is considered as the founder of diabetology, who helped in the treatment of diabetic patients before the creation of insulin in 1922. He was the first clinician who educated patients of diabetology to become aware of the disease. He also stressed the importance of exercise and urine glucose self-monitoring in the treatment of diabetes. He was the pioneer of advising against taking of sugars and starchy food to reduce glycosuria. He was also the first to hypothesise the location of diabetes in the pancreas.

Bouchardat wrote and published a number of books on diabetes, amongst them is his most well-known work 'De la Glycosurie ou diabète sucré, son traitement hygénigue'.

It was Collip [10], **Figure 10**, a biochemist, who played an important role in the production of the first insulin dose that was found to be appropriate for injection into humans.

The credit for the discovery of insulin goes to Banting and Best, but their extract was raw and failed to produce beneficial effects after being administered to Leonard Thompson, the first human to receive it.

Figure 9. Apollinaire Bouchardat (July 23, 1809 – April 7, 1886), a French pharmacist and hygienist born in L'Isle-sur-Serein (Source: [9]).

Collip took up the job of purifying the extract within a period of 2 weeks, and it was again administered to Thompson. During the second time, the insulin extract stabilised Thompson's blood glucose levels, which saved his life.

Pancreatic diabetes was discovered by Minkowski [11], **Figure 11**. Minkowski studied at the University of Konigsberg before becoming a professor in Strasburg in 1888. Minkowski was a pioneer in the procedure of pancreatectomy in dogs.

White [12] was the pioneer of research into diabetic woman during pregnancy; it led to the White classification which is being used to assess diabetes during pregnancy (**Figure 12**).

This classification is still used today to differentiate between existing diabetes before pregnancy and gestational diabetes. The White classification established her in the diabetes history.

The secretion of insulin is an energy requiring process which involves the microtubule-microfilament system in beta cells of the islets of Langerhans. Varied numbers of mediators have been implicated in the release of insulin.

The level of glucose in the interstitial fluid regulates the activity of the beta cells. A sharp increase of 8–10 in the secretion of insulin usually occurs in response to an increase in plasma glucose from 70 to 150 mg/dl. During the same phase, a simultaneous decrease in the secretion of glucagon from A cell occurs. There is a greater B-cell response observed following oral as opposed to intravenous glucose administration. This is known as 'incretin' effect.

Of major importance, defects in the below-stated portions of the hormone's properties and journey in the body have been correlated and are most often related to hypertension, insulin resistance and type 2 diabetes [13–19].

Figure 10. James Bertram Collip, CBE, FRS, FRSC, FRCP, FRCPC (November 20, 1892–June 19, 1965) (Source: [10]).

Figure 11. Oskar Minkowski (January 13, 1858–July 18, 1931) (Source: [11]).

Figure 12. Priscilla White (March 17, 1900–December 16, 1989) (Source: [12]).

The journey of insulin can be divided into five stages, which can be related to insulin resistance and type 2 diabetes:

- Diabetic cell having defective insulin exocytosis [20–25].
- Defect in the vasoactive properties of insulin during insulin resistance, which includes capillary recruitment [22, 26–29].
- The GLUT4 translocation to the muscle membrane is diminished in humans [14, 22, 30–36].
- Decreased hepatic clearance of insulin [37] and CEACAM1 expression [38] in obesity.
- There is compromised glomerular function in obese people [22, 39–41].

Most of it is routed to the lysosome for degradation. But most of the degradation of the circulating hormone remaining after second pass through the liver continues in the kidney.

Author details

Gaffar Sarwar Zaman

Address all correspondence to: gffrzaman@gmail.com

King Khalid University, Abha, Kingdom of Saudi Arabia

References

[1] Best CH, Banting FG. https://southcoastherald.co.za/184174/canada-pioneers-diabetes-treatment/

[2] Banting FG. https://www.diabetes.co.uk/pioneers/frederick-banting.html

[3] Banting W. https://www.diabetes.co.uk/pioneers/william-banting.html

[4] Best CH. https://www.diabetes.co.uk/pioneers/charles-herbert-best.html

[5] Thompson L. https://www.diabetes.co.uk/pioneers/leonard-thompson.html

[6] Joslin EP. https://www.diabetes.co.uk/pioneers/dr-elliott-proctor-joslin.html

[7] Allen FM. https://www.diabetes.co.uk/pioneers/frederick-madison-allen.html

[8] Kahn CR. https://www.diabetes.co.uk/pioneers/ronald-kahn.html

[9] Kahn CR. https://www.diabetes.co.uk/pioneers/apollinaire-bouchardat.html

[10] Collip JB. https://www.diabetes.co.uk/pioneers/james-collip.html

[11] Minkowski O. https://www.diabetes.co.uk/pioneers/oskar-minkowski.html

[12] White P. https://www.diabetes.co.uk/pioneers/priscilla-white.html

[13] Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: Insights into insulin action. Nature Reviews. Molecular Cell Biology. 2006;**7**:85-96. DOI: 10.1038/nrm1837

[14] Hoehn KL, Hohnen-Behrens C, Cederberg A, Wu LE, Turner N, Yuasa T, et al. IRS1-independent defects define major nodes of insulin resistance. Cell Metabolism. 2008; **7**:421-433. DOI: 10.1016/j.cmet.2008.04.005

[15] Odegaard JI, Chawla A. Pleiotropic actions of insulin resistance and inflammation in metabolic homeostasis. Science. 2013;**339**:172-177. DOI: 10.1126/science.1230721

[16] Boucher J, Kleinridders A, Kahn CR. Insulin receptor signaling in normal and insulin-resistant states. Cold Spring Harbor Perspectives in Biology. 2014;**6**:a009191. DOI: 10.1101/cshperspect.a009191

[17] DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, et al. Type 2 diabetes mellitus. Nature Reviews Disease Primers. 2015;**1**:15019. DOI: 10.1038/nrdp.2015.19

[18] Samuel VT, Shulman GI. The pathogenesis of insulin resistance: Integrating signaling pathways and substrate flux. The Journal of Clinical Investigation. 2016;**126**:12-22. DOI: 10.1172/JCI77812

[19] Haeusler RA, McGraw TE, Accili D. Biochemical and cellular properties of insulin receptor signalling. Nature Reviews. Molecular Cell Biology. 2018;**19**:31-44. DOI: 10.1038/nrm.2017.89

[20] Ferdaoussi M, MacDonald PE. Toward connecting metabolism to the exocytotic site. Trends in Cell Biology. 2017;**27**:163-171. DOI: 10.1016/j.tcb.2016.10.003

[21] Gandasi NR, Yin P, Riz M, Chibalina MV, Cortese G, Lund P-E, et al. Ca^{2+} channel clustering with insulin-containing granules is disturbed in type 2 diabetes. The Journal of Clinical Investigation. 2017;**127**:2353-2364. DOI: 10.1172/JCI88491

[22] Tokarz VL, MacDonald PE, Klip A. The cell biology of systemic insulin function. The Journal of Cell Biology. 2018. DOI: 10.1083/jcb.201802095. http://jcb.rupress.org/content/early/2018/04/04/jcb.201802095/tab-article-info

[23] Lang DA, Matthews DR, Burnett M, Turner RC. Brief, irregular oscillations of basal plasma insulin and glucose concentrations in diabetic man. Diabetes. 1981;**30**:435-439. DOI: 10.2337/diab.30.5.435

[24] Hollingdal M, Juhl CB, Pincus SM, Sturis J, Veldhuis JD, Polonsky KS, et al. Failure of physiological plasma glucose excursions to entrain high-frequency pulsatile insulin secretion in type 2 diabetes. Diabetes. 2000;**49**:1334-1340. DOI: 10.2337/diabetes.49.8.1334

[25] Laedtke T, Kjems L, Pørksen N, Schmitz O, Veldhuis J, Kao PC, et al. Overnight inhibition of insulin secretion restores pulsatility and proinsulin/insulin ratio in type 2 diabetes.

American Journal of Physiology. Endocrinology and Metabolism. 2000;**279**:E520-E528. DOI: 10.1152/ajpendo.2000.279.3.E52e

[26] de Jongh RT, Serné EH, IJzerman RG, de Vries G, Stehouwer CDA. Impaired microvascular function in obesity: Implications for obesity-associated microangiopathy, hypertension, and insulin resistance. Circulation. 2004;**109**:2529-2535. DOI: 10.1161/01.CIR.0000129772.26647

[27] Clerk LH, Vincent MA, Jahn LA, Liu Z, Lindner JR, Barrett EJ. Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. Diabetes. 2006;**55**:1436-1442. DOI: 10.2337/db05-1373

[28] Keske MA, Clerk LH, Price WJ, Jahn LA, Barrett EJ. Obesity blunts microvascular recruitment in human forearm muscle after a mixed meal. Diabetes Care. 2009;**32**:1672-1677. DOI: 10.2337/dc09-0206

[29] Broussard JL, Castro AVB, Iyer M, Paszkiewicz RL, Bediako IA, Szczepaniak LS, et al. Insulin access to skeletal muscle is impaired during the early stages of diet-induced obesity. Obesity (Silver Spring). 2016;**24**:1922-1928. DOI: 10.1002/oby.21562

[30] Klip A, Ramlal T, Bilan PJ, Cartee GD, Gulve EA, Holloszy JO. Recruitment of GLUT-4 glucose transporters by insulin in diabetic rat skeletal muscle. Biochemical and Biophysical Research Communications. 1990;**172**:728-736. DOI: 10.1016/0006-291X(90)90735-6

[31] Zierath JR, He L, Gumà A, Odegoard Wahlström E, Klip A, Wallberg-Henriksson H. Insulin action on glucose transport and plasma membrane GLUT4 content in skeletal muscle from patients with NIDDM. Diabetologia. 1996;**39**:1180-1189. DOI: 10.1007/BF02658504

[32] Garvey WT, Maianu L, Zhu JH, Brechtel-Hook G, Wallace P, Baron AD. Evidence for defects in the trafficking and translocation of GLUT4 glucose transporters in skeletal muscle as a cause of human insulin resistance. The Journal of Clinical Investigation. 1998;**101**:2377-2386. DOI: 10.1172/JCI1557

[33] Sylow L, Jensen TE, Kleinert M, Højlund K, Kiens B, Wojtaszewski J, et al. Rac1 signaling is required for insulin-stimulated glucose uptake and is dysregulated in insulin-resistant murine and human skeletal muscle. Diabetes. 2013;**62**:1865-1875. DOI: 10.2337/db12-1148

[34] Aslamy A, Thurmond DC. Exocytosis proteins as novel targets for diabetes prevention and/or remediation? American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2017;**312**:R739-R752. DOI: 10.1152/ajpregu.00002.2017

[35] Foley K, Boguslavsky S, Klip A. Endocytosis, recycling, and regulated exocytosis of glucose transporter 4. Biochemistry. 2011;**50**:3048-3061. DOI: 10.1021/bi2000356

[36] Samuel VT, Shulman GI. Mechanisms for insulin resistance: Common threads and missing links. Cell. 2012;**148**:852-871. DOI: 10.1016/j.cell.2012.02.017

[37] Jung S-H, Jung C-H, Reaven GM, Kim SH. Adapting to insulin resistance in obesity: Role of insulin secretion and clearance. Diabetologia. 2018;**61**:681-687

[38] Lee W. The CEACAM1 expression is decreased in the liver of severely obese patients with or without diabetes. Diagnostic Pathology. 2011;**6**:40. DOI: 10.1186/1746-1596-6-40

[39] Kanasaki K, Kitada M, Kanasaki M, Koya D. The biological consequence of obesity on the kidney. Nephrology, Dialysis, Transplantation. 2013;**28**(Suppl. 4):iv1-iv7. DOI: 10.1093/ndt/gft098

[40] Spoto B, Pisano A, Zoccali C. Insulin resistance in chronic kidney disease: A systematic review. American Journal of Physiology. Renal Physiology. 2016;**311**:F1087-F1108. DOI: 10.1152/ajprenal.00340.2016

[41] Robbins DC, Shoelson SE, Tager HS, Mead PM, Gaynor DH. Products of therapeutic insulins in the blood of insulin-dependent (type I) diabetic patients. Diabetes. 1985;**34**:510-519. DOI: 10.2337/diab.34.5.510

Chapter 2

Insulin – Overview, Infections and Benefits of Immunization and Insurance

Ashish Chauhan

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.81346

Abstract

From the time the earliest description of diabetes appeared around 552 BC in the *Ebers Papyrus*, people have been searching for a cure for it. The term 'diabetes' was introduced by Aretaeus of Cappadocia, a Greek physician, (129–199 AD). Before the availability of insulin, any child who had diabetes had a very poor life expectancy, and the prognosis was also poor for any adult having the disease. In 1921, Canadian physician Frederick Banting and medical student Charles H. Best discovered the hormone insulin in the pancreatic extracts of dogs. Earlier, several injections of insulin were required daily; in the 1930s, H.C. Hagedorn, a chemist in Denmark, added protamine to the preparation and found that it prolonged the action of insulin. It was not until 1978 that the first recombinant DNA human insulin was prepared by combining the insulin A- and B-chains. Credit goes to David Goeddel and his colleagues (of Genentech) for this. Some innate (cytokines, complement) humoral immune functions are decreased and some remain the same in patients with diabetes mellitus (DM) compared to those without DM. We all know why diabetics are more prone to infections by this point. The science keeps the mind going but what keeps the heart going? Probably a big size chocolate bar for a diabetic with a blood sugar of. But no! Resistance is a key. If we are not going to resist that candy bar, nor are the bacteria or other organisms causing infection going to. There is not a lot we can do after this point, but there is quite a lot that could be done before—immunisation. Diabetes is an uphill battle for those that suffer from it; but the new health insurance schemes offered by the government should ease a little bit of the pressure. Insulin is not a definitive cure for diabetes but is definitely a form of life support. As of now, there is no complete cure for diabetes, but there may be one to wipe it out in the future due to the advancing technologies in the field of science.

Keywords: diabetes, insulin, immunisation, insurance

1. Introduction

Laughter is the best medicine. Definitely, if someone has diabetes, insulin would probably be needed to be started depending on the clinical scenario.

What is diabetes anyway? Diabetes is the disability of the body to produce or take up insulin, which leads to abnormal carbohydrate anabolism that implies an increase in glucose in the blood. Basically, any food that a person takes in has a certain relative value called 'glycaemic index', which indicates how much food is broken down into sugars and circulated to the rest of the body and utilised by organs for their proper functioning.

However, there is a saying in Chinese, wù jí bì fǎn. In layman terms, this means 'too much of a good thing is bad'; that is where insulin comes into play. Insulin is a protein hormone produced by the beta cells of pancreas and is apparently defective, diminished or even absent in about 415 million people globally, out of which 72.9 million people reside in India according to a 2018 census. It allows body to use sugar (glucose) from carbohydrates in the food that one eats for energy or to store glucose for future use. Insulin helps to keep the blood sugar level from getting too high (hyperglycaemia) or too low (hypoglycaemia).

2. A little bit of history

The term 'diabetes' was first used by the Greeks. It was given by Aretaeus of Cappadocia, a Greek physician, (129–199 AD). It means 'to pass through'; they used it to signify the large amount of water consumed and urine produced by diabetics. The term 'mellitus' was added by the Romans, meaning 'sweet as honey', when they noticed that the urine of diabetics was sweet.

In 1921, Canadian physician Frederick Banting (November 14, 1891 to February 21, 1941) and medical student Charles H. Best discovered the hormone insulin in the pancreatic extracts of dogs. Because the early insulin preparation required several injections daily, scientists worked hard to find ways to prolong its duration of action. H.C. Hagedorn, in the 1930s, who was a chemist in Denmark, prolonged the action of insulin by adding protamine. The first genetically engineered, synthetic 'human insulin' (first recombinant DNA human insulin) was produced in 1978 by David Goeddel and his colleagues (of Genentech) using *E. coli* bacteria.

3. Diabetes and insulin

There are two types of diabetes, type 1 which is hereditary because of variants in the HLA DQA1, HLA DQB1 and HLA DRB1 genes, which are crucial for forming certain proteins in the body, and type 2 which is acquired due to sedentary lifestyle or family predisposition.

Insulin is composed of two peptide chains, chain A which is made up of 21 amino acids and chain B which is made up of 30 amino acids. Both these chains are linked together by two disulphide bonds. Insulin causes the liver to convert more glucose into glycogen by a process

called glycogenesis and primarily forces muscle and fat tissue cells to take up glucose from the blood through the GLUT4 transporter, thus decreasing blood sugar. So, if there is a defect in production of insulin or its uptake, then there will be a rise in blood sugar which can lead to fatal complications if untreated.

These complications are lethal, not going to sugarcoat it because these may worsen it for a diabetic! It starts off with increased risk of developing cardiovascular disease, including atherosclerosis, stroke, peripheral artery disease and kidney disease. Diabetic neuropathy, diabetic nephropathy and stroke are some terminal complications.

Diabetes can be controlled in the form of tablets initially, but progressive stages require subcutaneous administration of insulin. Modern methods include transdermal patches or nasal spray.

Administration of insulin can be tricky if it is by the subcutaneous method. Repeated issuing of insulin into the same area can cause lipohypertrophy, a condition of excess accumulation of fat at the site of injection. The most fatal complication that can arise is hypoglycaemia.

Overdose or improper timing of administration of insulin can lead to dangerously low levels of insulin. Low sugar level initially causes hunger, sweating and shaking, but in the long run, it deprives the brain of its fuel, leading to the patient falling into a coma. Hence, it is always advised for a diabetic to have some food around which is ironic.

One can avoid this menace of a disease by altering the modern-day couch potato lifestyle. Keeping oneself hydrated is a good start, exercise is very important as well. Both these components of a healthy manner of living go hand in hand and can naturally lower blood sugar levels. Decreasing the carbohydrate intake and increasing the protein in an individual's diet may also be of great help. Avoidance of sugary food and drinks is a step in the right direction.

Insulin is not a definitive cure for diabetes but is definitely a form of life support. As of now, there is no complete cure for diabetes, but there may be one to wipe it out in the future due to the advancing technologies in the field of science. Strict adherence to medication and exercise can cause the severity of diabetes to lower down, but a normal lifestyle cannot be guaranteed.

If diabetes is so prevalent around the world, it must mean that the population below poverty line and lower middle class are affected as well up to some extent. Insulin can be costly, as it is not a definitive cure, but more of a life support as mentioned above. This is where health insurance is of maximum necessity, which will be shortly discussed in detail.

Diabetes is not something that should be overlooked. Let us say a 68-year-old, uncontrolled diabetic man came to an ophthalmologist with complaints of clouded vision for a long time, and is required to undergo a cataract surgery as soon as possible. If the surgery takes place without letting the sugar levels normalise, this can lead to a lot of postoperative ocular complications such as endophthalmitis, diabetic retinopathy and other diseases which can terminate in complete loss of vision. Such a simple act of patience like waiting for the uncontrolled sugar levels to subside can be detrimental to both the patient and reputation of the doctor.

The way to prevent major infections is via immunisation.

There is sufficient awareness about diabetes in the community, but not sufficient enough about how pernicious it can be if not controlled. Apprehension of such an ailment should be spread by the health sector as well as the media. Medical camps and general hospitals should ensure the illiterate patients are educated to understand the basic knowledge of health-related issues such as diabetes, and that thorough follow-up with the medication is necessary to sustain a healthy lifestyle.

4. Infection in diabetes

The incidence of infections is increased in patients with diabetes mellitus (DM) [1]. Some of these infections are also more likely to have a complicated course in diabetic than in non-diabetic patients [1]. Diabetic ketoacidosis, for example, is precipitated or complicated by an infection in 75% of the cases. The mortality rate of patients with an infection and ketoacidosis is 43% [1]. In a prospective study of 101,293 adult hospitalised patients, 1640 episodes of bacteraemia were diagnosed. Of 1000 hospitalised patients studied, 2/3 of the bacteraemia were found in patients with DM compared to 1/3 in patients without DM ($P < 0.001$) [2].

5. Defects in innate immunity and relation to humoral innate immunity

The immune system can be divided into innate and adaptive-humoral or cellular immune systems. Concerning the humoral adaptive immunity, serum antibody concentrations in patients with DM are normal and they respond to vaccination with pneumococcal vaccine as well as non-diabetic controls [3, 4]. Furthermore, no differences have been shown in the immune response to intramuscular hepatitis B vaccine between children with DM type 1 and controls [5]. Concerning the adaptive cellular immunity, inhibition of the proliferative response to different stimuli has been observed in the lymphocytes of diabetics with poorly controlled disease [6]. An abnormal delayed-type hypersensitivity reaction (cell-mediated immunity) has also been described in DM type 1 and type 2 patients [7–9].

6. Complement function

In a study of 86 DM type 1 patients, 22 (26%) had a serum complement factor 4 concentration (C4) below the normal range [10]. The low C4 values did not appear to be the result of consumption. Since non-diabetic identical twins also had a C4 concentration below normal, and the genes encoding C4 are linked with the antigens DR3 and DR4 (which are expressed in 95% of the Caucasian diabetic patients in contrast to 40% of the general population [6]), the authors suggest that the reduced C4 level may be an inherited phenomenon [10].

http://dx.doi.org/10.5772/intechopen.81346

7. Cytokines

Studies with whole blood, peripheral blood mononuclear cells (PBMCs), and isolated monocytes of diabetics have to be divided into studies with and without stimulation. Without stimulation, tumour necrosis factor alpha (TNF-α) concentrations in patients with DM type 1 [11], interleukin (IL) 6 concentrations in patients with DM type 2 [12] and IL-8 concentrations in DM type 1 and 2 patients [13] have been studied. Elevated resting values of TNF-α, IL-6 and IL-8 were found in diabetic patients compared to non-diabetic controls.

Studies with PBMCs and isolated monocytes of diabetic patients after stimulation show the following results: in one study [14] the IL-1 secretion of PBMCs in response to lipopolysaccharide (LPS) was reduced in diabetic (type 1 and 2) PBMCs, while the TNF-α response was the same as in the control cells. In another study, monocytes of DM type 1 patients showed a significantly lower production of IL-1 and IL-6, but again no differences in TNF-α concentrations were measured, after stimulation with LPS, compared with monocytes of DM type 2 patients and non-diabetic controls [15]. Possibly most of the TNF-α already disappeared after the incubation period of 24 h [15]. Neither glucose nor insulin showed any effect on the production of IL-1 or IL-6 in isolated monocytes, so the decreased production after stimulation with LPS seemed an intrinsic cellular defect of diabetic cells. It is possible that the elevated resting value of diabetic cells leads to the induction of tolerance to stimulation, which results in lower cytokine secretions after stimulation. This phenomenon has already been described in non-diabetic cells [16].

Studies of cytokine excretion by PBMCs of non-diabetic patients after the addition of different glucose concentrations have shown comparable results as studies with diabetic cells. One study [17] showed that after the addition of different glucose concentrations, unstimulated monocytes of non-diabetics showed an increased TNF-α and IL-6 response. Another study [18] showed that after pokeweed mitogen stimulation, lower IL-2, IL-6 and IL-10 concentrations were found after the addition of glucose (with a dose-response effect). Possibly, the induction of tolerance, described above, can also explain these results. In other words, the presence of glucose leads to a higher resting cytokine production; after stimulation, however, this cytokine production is impaired compared to the situation without glucose. Another substance which may play a role in the increased basal cytokine secretion is the advanced glycation end products (AGEs, which are products of glucose and lysine or arginine residues). An increased formation of AGEs takes place in poorly regulated diabetic patients [19]. Different studies have shown that binding of these AGEs to non-diabetic cells, without stimulation, leads to an increased cytokine production [17, 20, 21]; so, it seemed that the increased formation of these AGEs in diabetics may be responsible for the increased basal cytokine secretion.

8. Hyperglycaemia/glucosuria

Following the 1985 WHO criteria, DM is defined as a fasting glucose concentration of at least 7.8 mmol l^{-1} or a 2-h glucose concentration of 11.1 mmol l^{-1} or higher [22]. As a result of

this, patients with DM (also with medication) very often have hyperglycaemia. This hyperglycaemic environment can enhance the virulence of certain microorganisms. An example is *Candida albicans*, which expresses a surface protein that has great homology with the receptor for complement factor 3b (CR3). Normally, opsonisation of microorganisms takes place by attachment of complement factor 3b (C3b). Receptors on phagocytising cells recognise this bound C3b and attach, thereby initiating ingestion and killing. In a hyperglycaemic environment, the expression of the receptor-like protein of *C. albicans* is increased, which results in competitive binding and inhibition of the complement-mediated phagocytosis [23]. Another example is the presence of glucosuria, as found in poorly regulated patients. We showed [24] that glucosuria enhances bacterial growth of different *Escherichia coli* strains, which probably plays a role in the increased incidence of urinary tract infections in diabetic patients.

9. Other serum factors

In vitro tests analysing the functions of non-diabetic polymorphonuclear cells (PMNs) are carried out by incubating these cells with plasma derived from patients with DM. These defects are not correlated with the amount of glucose present in plasma [6, 25, 26]. An example is the increased adherence of PMNs of non-diabetic patients to bovine aortic endothelium in the presence of diabetic plasma [27]. This increased adherence probably leads to a decrease in diapedesis and exudate formation of PMNs [27]. The question arises which factor in diabetic serum is responsible for the difference mentioned above. It has been suggested [28] that AGEs play a role. Since the formation of AGEs is increased in poorly regulated patients, it seemed that an optimal diabetes regulation possibly can improve the host response.

Another frequently mentioned substance in the pathogenesis of infections in diabetic patients is zinc. Low plasma zinc levels have been reported in DM type 1 and type 2 patients [6]. Nevertheless, in another study, no differences in zinc levels between diabetic and non-diabetic subjects were found [29]. In vitro studies described a disturbed lymphocyte response and depression of chemotaxis in diabetic PMNs when zinc deficiency was present [1, 6, 28]. Other in vitro studies with PBMCs of non-diabetic patients showed an enhanced LPS-induced excretion of pro-inflammatory cytokines after the addition of zinc [30]. Considering the contradictory epidemiological data about zinc deficiency in DM patients, the clinical relevance of the above-mentioned in vitro results in the pathogenesis of infections in diabetic patients remains unclear.

In conclusion, some innate (cytokines, complement) humoral immune functions are decreased and some remain the same in patients with DM compared to those without DM.

10. Cellular innate immunity: PMNs and chemotaxis

A significantly lower chemotaxis has been found in PMNs of diabetic patients (type 1 and type 2) than in those of controls [25, 31, 32]. However, it could not be demonstrated in the study in which (we studied) the PMN function in women with DM having asymptomatic bacteriuria were

compared to non-bacteriuric diabetic women (healthy controls) were studied [33]. All studies used serum from healthy controls. It is possible that the different stimuli (zymosan, complement) of the PMNs and the differences in patient characteristics (duration, regulation and complications of DM, DM type 1 or DM type 2) in the above-mentioned studies may explain these contradictory results. No correlation was found between glucose concentration [25, 32] or haemoglobin A1c (HbA1c, which is a serum marker for the regulation of the DM) level and the chemotactic responses, although one study showed a further reduction in chemotaxis in patients with hyperglycaemia [31]. Interestingly, one of the other studies showed that the chemotactic responses of the PMNs did not alter after the incubation of either glucose or insulin, but returned to normal values after the incubation with glucose and insulin together [32]. Since most PMN functions are energy-dependent processes [34], an adequate energy production is necessary for an optimal PMN function. Glucose needs insulin to enter the PMNs to generate this energy, which may explain the improvement of the chemotactic response after the addition of these two substances.

11. In vitro adherence of diabetic PMNs

Conflicting data have been reported about the in vitro adherence of diabetic PMNs without stimulation [25, 27, 31, 34, 35]. In contrast, no differences have been found between diabetic and control PMNs after stimulation [27, 31]. No correlation was found between plasma glucose or HbA1c and adherence [25, 27, 31]. However, in a small number of DM type 1 and DM type 2 patients with untreated hyperglycaemia, the decreased adherence of PMNs to nylon fibre columns increased after the hyperglycaemia was corrected [34, 35]. Of course, adherence to nylon fibre columns is not the same as to endothelial cells as a first step in the inflammation reaction. However, again a better regulation of the DM seemed to increase the host response.

12. Phagocytosis

PMNs of diabetic patients have shown the same [25, 33] and a lower [31, 36] phagocytotic capacity compared to PMNs of controls. The mean HbA1c concentration was lower (better regulation) in patients without impaired phagocytosis [33] than in those with impaired phagocytosis [31, 36]. One study [36] showed an inverse relationship between the HbA1c levels and the phagocytotic rate. Another study [37] showed that the decreased phagocytosis improved, but did not become normal after 36 h of normoglycaemia. Therefore, it seems that impairment of phagocytosis is found in PMNs isolated from poorly regulated patients and that better regulation of the DM leads to an improved phagocytotic function.

13. Oxidative burst

Chemiluminescence (CL) corresponds to the emission of light directly or indirectly produced in the course of a chemical reaction. This phenomenon is often used to evaluate the oxidative potential of PMNs, a process during which free radicals are synthesised early in the phagocytotic

process [31, 38]. CL correlates well with antimicrobial activity [39] and may be used as a measure of phagocytotic capacity [38]. Compared to controls, CL at baseline was higher [31] or the same [36, 39] in PMNs of diabetic patients. These studies [31, 36, 39] also showed that, after stimulation, the CL of diabetic PMNs was lower than that of control PMNs. It is possible that the reaction of diabetic PMNs to stimuli is quenched as a result of the higher resting CL.

14. Killing

Data about the bactericidal activity of diabetic PMNs have yielded conflicting results [25, 26, 33, 37]. An impaired killing function of diabetic PMNs was found in all studies using *Staphylococcus aureus* as the microorganism [25, 26, 37], but not in the studies in which the killing of *C. albicans* [33] was used as the measure. Killing was impaired in one study that used non-diabetic serum for opsonisation [37], but not in another [33]. Thus, based on these studies, any conclusions about the effect of non-diabetic serum on the killing of diabetic cells cannot be drawn. No correlation was found with glycaemic level [25, 26, 37], although some studies have shown that bactericidal activity improved, but did not normalise after achieving normoglycaemia [6, 37].

15. Cellular innate immunity: monocytes/macrophages

Both impaired chemotaxis and phagocytosis of the monocytes of diabetic patients have been described [1, 40]. Since plasma from healthy controls does not cause any significant change in the phagocytotic capacity of diabetic monocytes [40], it seems that this impaired function is caused by an intrinsic defect in the monocytes themselves.

A lower immune response in children with DM type 1 compared to controls was found after intradermal (instead of intramuscular) administration of the hepatitis B vaccine [5]. It has been suggested that this lower response is probably partly the result of an impaired macrophage function in this patient group [5].

In combination with the earlier mentioned decreased production of pro-inflammatory cytokines after LPS stimulation in DM type 1 patients, it seemed that monocyte/macrophage functions are impaired in DM type 1 patients.

16. Adherence

Adherence of a microorganism to mucosal or epithelial cells is an important step in the pathogenesis of infections. Host-related factors may influence this adherence. For example, women with recurrent urinary tract infections have a greater adherence of *E. coli* to their vaginal and buccal cells compared to controls [41].

C. albicans infection is frequently found in diabetic patients. Since infection mostly is preceded by colonisation, Aly et al. investigated which risk factors increased the risk of *Candida* carriage in diabetic patients [42]. Risk factors for oral *Candida* carriage in patients with DM type 1 were a

lower age and a higher HbA1c level (poor regulation of DM). Continuous wearing of dentures and the presence of glucosuria (also an indication of a poor DM regulation) increased the risk of *Candida* carriage in DM type 2 patients, the mean number of cigarettes smoked per day was correlated with *Candida* carriage in DM type 1 and type 2 grouped together [42]. Cameron et al. extracted lipids from human buccal epithelial cells and found, using chromatogram overlay assays, that some *C. albicans* strains bind to fucose-containing and other *C. albicans* strains to *N*-acetylgalactosamine-containing lipids extracted from human buccal cells. The authors conclude that the existence of several adhesin-receptor systems contributes to the virulence of *C. albicans* [43]. The carbohydrate composition of receptors probably plays an important role in the susceptibility to infections. It has been shown that severely ill patients have a decreased amount of galactose and sialic acid on their buccal cells, compared with minimally ill patients and healthy controls. The investigators mentioned that these receptor changes possibly lead to an increased adherence of microorganisms and play a role in the high prevalence of Gram-negative bacterial colonisation in the respiratory tract of these patients [44]. This mechanism of increased adherence, due to an altered receptor carbohydrate composition, is possibly also present in diabetic patients. Buccal cells from 50 diabetic patients (DM type 1 and type 2) showed an increased in vitro adherence of *C. albicans* compared to buccal cells from controls [45]. A significantly higher incidence of *Candida* infection, but not *Candida* carriage, was also found in this patient group (12% versus 0%) [45]. No relationships, however, were found between the frequency or quantity of *Candida* and age, duration, regulation or type of DM [45]. This increased adherence to diabetic cells might also play a role for other microorganisms, for example the adherence of *E. coli* to uroepithelial cells, which would explain the increased prevalence of infections in patients with DM.

In conclusion, disturbances in cellular innate immunity play a role in the pathogenesis of the increased prevalence of infections in DM patients. In general, a better regulation of the DM leads to an improvement of cellular function. A second important mechanism is the increased adherence of the microorganism to diabetic cells. Furthermore, some microorganisms become more virulent in a high-glucose environment.

17. Types of infections from diabetes

Diabetes could cause the following infections:

- Bladder infections
- Skin infections
- Foot infections
- Oral infections

17.1. Bladder infections

People with diabetes are more likely to get these infections than those without diabetes. It involves the kidney, ureter, bladder and urethra. Major complaint from such infection includes:

- Burning micturition
- Urinary urgency (a sudden compelling urge to urinate)
- Polyuria (producing abnormally large volumes of dilute urine)
- Nocturia (waking up at night for voiding urine)
- Urinary incontinence(uncontrolled leakage of urine)
- Blood in the urine
- Cloudy appearance of urine
- Strong smelly urine

Additionally, if it is an infection in the kidney:

- Upper back and side (flank) pain
- High fever
- Shaking and chills
- Nausea
- Vomiting

If it is an infection in the bladder:

- Pelvic pressure
- Lower abdomen discomfort
- Frequent, painful urination
- Blood in urine

If it is an infection in the urethra:

- Burning on urination
- Discharge

17.2. Skin infections

There is increased risk of bacterial and fungal infections. The best way to avoid this is to take care of skin and look out for early symptoms.

For bacterial infections,

you have to look out for:

- Stye (infection of the glands of the eyelid)
- Boils folliculitis (infections of the hair follicles)

- Carbuncles (deep infections of the skin and the tissue underneath)
- Infections around the nails

For fungal infections it may be:

- Ringworm
- Vaginal yeast infection
- Athlete's foot

Diabetics are more likely to develop overgrowth of yeast-like-fungus, *Candida albicans*. The patient comes with complaints of itchy rashes, moist red areas, surrounded by tiny blisters and scales. Common sights are areas of warm, moist folds of skin. Identifying in the early stages is the key for successful treatment.

17.3. Foot infections

Like already mentioned, foot infections occur due to reduced blood supply to the extremities. Proper care of feet must be done by methods that will be later mentioned.

17.4. Oral infections

Elevated blood sugar leads to elevated sugar levels in the saliva, leading to increased growth of bacteria in the mouth. This predisposes to:

- Plaque on the teeth
- Gum disease
- Breath odour

17.4.1. Gingivitis

Here, the gums are unhealthy, swollen, bleed easily and appear red.

17.4.2. Periodontitis

This is a mild to severe gum disease. Symptoms are:

- Red and swollen bleeding gums
- Gums pulled away from the teeth
- Long-lasting infection between teeth and gums
- Presence of pus between teeth and gums

- Persistent bad breath
- Loose teeth

17.4.3. Thrush

This occurs due to candidiasis, that is, overgrowth of candida yeast as white or red patches in the mouth. This can be treated by fungal medications.

17.4.4. Dry mouth

Here, there is not enough saliva in the mouth. This poses a high risk for gum diseases and cavities. The patient complains of trouble in talking and difficulty in swallowing. The treatment for this is occasionally drinking sips of water and avoiding tobacco, alcohol and caffeine.

17.4.5. Oral burning

This is a sensation of burning in the mouth, dry mouth and bitter taste. Oral care must be followed, which will be mentioned later.

17.5. Prevention

"I may have diabetes, but diabetes does not have me."

The first and foremost way is to control the sugar levels in the blood.

Second most important measure is undergoing immunization which will be taken in detail in the later pages.

Prevention or early diagnosis is mostly done for foot infections and oral infections.

17.5.1. Foot infections

The following should be done, in case of foot infections:

- Wash your feet everyday
- Keep the skin soft and smooth
- Smoothen corns and calluses gently
- Trim your toenails regularly
- Wear shoes and socks at all times
- Ensure that minor cuts do not turn into ulcerated infections which migrate to the bloodstream

http://dx.doi.org/10.5772/intechopen.81346

17.5.2. Oral infections

The following should be done in case of an oral infection:

- Use a soft bristle toothbrush
- Angle the toothbrush towards your gum line
- Use a gentle scrubbing motion
- Brush your tongue and gums
- Replace toothbrush every 3–4 months
- Floss once a day, using a clean section of floss per tooth
- When flossing, scrub up and down, each side of your tooth
- See your dentist regularly for check-ups and cleanings

17.5.3. Urinary infections

This has to be done mostly for women and children. In cases of urinary tract infection (UTI), the following has to be checked for:

- Toilet hygiene
- Regular emptying of bladder
- Prompt urination after sexual intercourse
- Ample fluid intake

17.5.4. Vaginal infections

Yeast infections can be avoided by the following:

- Eating foods with active culture (yoghurt with acidophilus)
- Avoidance of spermicides and douches

17.5.5. Common symptoms of all infections

- Alert for:
  - An increased body temperature
  - Change in blood sugars
  - Foul-smelling vaginal discharge
  - Pain while urination

- Cloudy and bloody appearance of urine
- Painful swallowing
- Irregular bowel movement

18. What are the other consequences of a high sugar level in the blood?

"Sometimes I pretend I'm not diabetic, but that's a dangerous game."
– Unknown

Interestingly, sometimes an infection can lead to diabetes and not the other way round which we encounter usually.

An infection can cause our body to produce higher levels of certain hormones like adrenaline or cortisol. These hormones counter the effect of insulin, thus increasing the levels of sugar in the blood which may trigger an episode of diabetic ketoacidosis.

19. Immunisation

As spoken above about the seriousness of diabetics and insulin and infection, why is not the prevention of that infection not spoken much about? Is it that we always wait for the seriousness of a situation to escalate to maximum to actually take it into consideration and look for cure even after reaching a stage of no cure? Are people only preaching prevention is better than cure but not actually practicing it? Well, fellow humans! We have reached an era of high prevalence in vaccine-preventable diseases and it is time we prevent them for good.

We all know why diabetics are more prone to infections by this point. The science keeps the mind going but what keeps the heart going? Probably a big size chocolate bar for a diabetic with a blood sugar of. But no! Resistance is key. If we are not going to resist that candy bar nor are the bacteria or other organisms causing infection going to. There is not a lot we can do after this point, but there is quite a lot that could be done before—immunisation.

As spoken about already, it is estimated that 415 million people are living with diabetes around the world. That makes it 1 in every 11 people in the world's population. At this rate, it is estimated to reach a dramatic 642 million by the year 2040. Diabetes being non-communicable, hence, increased dramatically over the past few years and according to the statistical staircase imagine how many people will be suffering in the future! If such a non-communicable disease has such an increase, imagine how fast the other infections as spoken above will become more and more common? As if that question did not scare you enough, imagine how many infections will mess with diabetics in the coming future? We have already gone through some very notorious infections that play around with the life of diabetics and surprisingly looking at the bright side, most of them are vaccine preventable.

Influenza, pneumococcal and hepatitis stand as the most important vaccine-preventable infections in diabetics along with many others. Additionally, we need to take care of diphtheria, typhoid, pertussis, tetanus and shingles. These viruses as we have come across already have a high tendency to cause infection in people with weak immune systems, which is important to note among diabetics.

The influenza virus is famous for causing infection in the elderly usually at the age of around 65. People who have weak immune systems with underlying ailments are more prone to such infections. Diabetics are at a risk of more severe form of the disease. Vaccination is available against the flu whose compliance against the disease can be found to be effective seasonally.

Coming to pneumococcal vaccination, there are two types of vaccines for this infection, the pneumococcal conjugate vaccine (PCV13) which protects against 13 types of pneumococcal bacteria and the pneumococcal polysaccharide vaccine (PPSV23) which protects against 23 types of pneumococcal bacteria.

As in the case of typhoid, this vaccine works by exposing you to a small amount of the bacteria, which causes your body to develop immunity to the disease. **Typhoid vaccine will not treat an active infection that has already developed in the body**, and will not prevent any disease caused by bacteria other than *Salmonella typhi*.

As for diphtheria, pertussis and tetanus, the potent Tdap vaccine protects patients against tetanus, diphtheria, and pertussis. Tetanus can lead to tightening of the muscles of the head and neck and kills 1 in 10 people with the infection, while diphtheria can cause breathing problems, heart failure, paralysis and death, according to the centers for disease control and prevention (CDC). Pertussis causes serious coughing fits that can strain breathing, cause vomiting and disturb sleep.

As for shingles, the zoster vaccine protects individuals against shingles, which is the reactivation of the chicken pox virus. Shingles presents as a painful rash, with pain that can persist even after the rash clears up.

We all know by this point that people with diabetes are more prone to infection. So, it is not surprising to note that people with diabetes are prone to hepatitis B infection more than nondiabetics. Hence, hepatitis B vaccination is recommended for everyone. It is a series of three vaccine shots. The second shot is taken 1 month after the first and the third dose is taken 6 months after the first.

The centre for disease control and prevention had recommended several other vaccines for diabetic patients. Various vaccines are provided against measles, mumps, rubella, chicken pox, herpes, diphtheria, tetanus and many others.

It is always recommended to be aware of the vaccinations taken in the past and to enquire with a doctor to know more information on your status and keep a record hence forth.

No matter how much we keep speaking about these great vaccines which have always given us a second chance, it is important to seek its help before it is too late. That is where the importance of early immunisation comes in. Immunisation is a simple way of protecting against so many harmful diseases. But many times, it is neglected and it is not surprising to know that it is not hard for diabetics to neglect vaccination either. Many infectious diseases are rare or eradicated

now as a result of our immunisation programmes that have been ongoing from the past, but new infectious diseases are appearing around the world which increases the need to promote the importance of early immunisation. Immunising yourself not only means protection to yourself but also protection to the future generations against these deadly diseases. It is a crucial step in eradication of some of the most deadly diseases. It protects and continues to protect you and everyone around you from easily preventable disease. There are people in the community who take this matter into utmost importance and take their vaccines when required and make sure people around them do so too. But quite a few do not do so. Some may not be able to take vaccine if they might be too weak or sick as they might be vulnerable to the infection if vaccine is taken: these people are usually an acceptation but those who do not get vaccinated just because of mere carelessness or unawareness are something that can be changed. On a positive note, the importance of early immunisation can be spread around the world only when those taking it show a difference than those who do not. But, why wait for that difference? You never know when you might get infected by a needle prick. For all we know, people doing their daily duties for a living are getting infected even when they take all measures to prevent it. Except one: the person taking vaccination. It may be a simple step, but it makes all the difference.

Every country must do its part to make this world a safer and better place for us and our future generations to live in. Immunisation stands as priority not only in diabetics but also for everyone whether or not they are suffering from any diseases, because, remember, prevention is better than cure. That being said, WHO has recommended every country to have a national immunisation programme based on the country statistics on prevalent infectious diseases that are vaccine preventable. India has put to action the Universal Immunisation Programme (UIP) which was launched in 1985. The programme now consists of vaccination for 12 diseases: tuberculosis, diphtheria, pertussis (whooping cough), tetanus, polio, measles, hepatitis B, diarrhoea, Japanese encephalitis, rubella, pneumonia (Haemophilus influenza type B) and pneumococcal diseases (pneumococcal pneumonia and meningitis). Our country has come a long way in planning for the health and well-being for us and generations to come. The plan has been put to use but it is not going to be 100% effective unless everyone does their part in creating awareness and spreading the importance for early immunisation.

As we slowly approach the end of the discussion on immunisation, let us discuss the other modes of preventing infection in diabetics other than immunisation.

The most important measure to protect themselves from infection that most diabetics neglect is to maintain clean foot hygiene and always wear footwear or socks to protect the foot from minor injuries. Every now and then, the foot must be checked for any scratches or cuts or other skin problem which could give way for an infection. If any such things are present, they should be maintained clean so that the infection is not given a chance to enter the bloodstream and aggravate. Good urinary hygiene should also be maintained. In women, clean vaginal hygiene is of utmost importance as well. Eating food rich in active cultures are helpful in preventing yeast infections.

All these measures can help people suffering with diabetics protect themselves from further infections. If you are a diabetic and have not followed the above prevention methods, then, it is time to make change because it is better late than never. But if you do not have diabetes and are happy, well my friend, get yourself immunised! You do not have to be a diabetic to be susceptible

to infection. Anyone can get infected anywhere, any time. So, do not laze around or ignore the seriousness of the situation because prevention is better than cure. Spend a few rupees now getting vaccinated. It saves much more than when you have to spend to treat the disease later on.

20. Insurance

20.1. Introduction to insurance

India has an expenditure of 1.5 lakh crore on availability of insulin preparations to the general public, and yet many people are unable to afford the medication required.

Since majority of India's population is not in the upper or upper middle class, a major hurdle is faced by the health sector in order to keep the physical quality of life index (PQLI) of the population in check. To ensure that the money spent on health necessities by the country is balanced out with the rise in the demand of medication by people who cannot afford it, the government introduced health insurance into the country. Ironically, only a small portion of individuals are making use of this advantage and a majority of the population have probably never even heard of the term insurance!

Based on the age, type of diabetes and various other factors, the government targeted certain groups of people to make sure that the insurance scheme is most favourable to them.

Diabetes is an uphill battle for those that suffer from it, and their providers. Medical expenses and the complications that may occur for diabetics are far more than those for nondiabetics. It is a major struggle in the present world, but the new health insurance schemes offered by the government should ease a little bit of the pressure.

As mentioned before, awareness must be spread by the government and those employed in the health sector via medical camps. In this modern era, everyone is hooked onto at least one type of social media platform.

Therefore, spreading the word on various social media is a great step in the right direction and must be implemented more extensively.

The amount of awareness and utilisation of these insurance schemes among the population have a direct correlation to diminished expenditure from the diabetics.

This way, many lives that are taken due to the mere absence of medication can be avoided and even diabetics among the economically unstable can lead to a better life.

20.2. Insurance schemes

A few government-implemented health insurance schemes in India are:

- Central government heath scheme
- Universal health insurance scheme
- Aam Aadmi Bima Yojana, Rashtiya Swasthiya Bima Yojana

There are also several private sector insurance companies offering health insurance, such as:

- Star health insurance
- ICICI prudential
- Apollo Munich Health insurance
- National insurance
- Religare Health Insurance, etc.

which are all diabetic safe. They all offer health care insurance services to the people of our country.

21. I-I-I-I as a full unit

The above all, that is, **insulin**, **infection**, **immunisation** and **insurance,** are interrelated.

A decrease or lack of **insulin** causes diabetes, which is a great predisposing factor to make the individual more susceptible to various **infections.** The various infections can be prevented mostly by using **immunisation**. If immunisation is not done for the individual at an early age, he will be under the financial burden of the medicines, hospital visits and doctor consultations related to diabetes, in the later stages of his life, for which the government has come up with various **insurance** schemes.

Therefore, as we have seen above, **insulin is just the tip of the iceberg.** So, when we treat a case of diabetes, it is not sufficient to look at just the amount of insulin administered to the patient, but also taking care that the patient does not get any infections. Additionally, from a preventive stand point, it is important to make sure that the entire population is immunised at a very early age against the various infections that diabetes get. Lastly, it is also important to look at the various insurance schemes because diabetes as a disease is a great financial burden, not only to the family but also to the country as a whole.

As already mentioned, India is the diabetic capital of the world and hence all these issues must be taken care of as soon as possible. This is not only for the good of the people, but for the good of the country as a whole. Lastly, I would like to say **'better late than never'**.

Author details

Ashish Chauhan

Address all correspondence to: martin_ashish@yahoo.com

Apollo, Hyderabad, Andhra Pradesh, India

References

[1] Deresinski S. Infections in the diabetic patient: Strategies for the clinician. Infectious Disease Reports. 1995;**1**:1-12

[2] Carton JA, Maradona JA, Nuno FJ, Fernandez-Alvarez R, Perez-Gonzalez F, Asensi V. Diabetes mellitus and bacteraemia: A comparative study between diabetic and non-diabetic patients. The European Journal of Medicine. 1992;**1**:281-287

[3] Lederman MM, Schiffman GA, Rodman HM. Pneumococcal immunization in adults. Diabetes. 1981;**30**:119-121

[4] Beam TRJ, Crigler ED, Goldman JR, Schifmann G. Antibody response to polyvalent pneumococcal polysaccharide vaccine in diabetics. Journal of the American Medical Association. 1980;**244**:2641-2644

[5] LiVolti S, Caruso Nicoletti M, Biazzo F, Sciacca A, Mandara G, Mancuso M, et al. Hypo-responsiveness to intradermal administration of hepatitis B vaccine in insulin dependent diabetes mellitus. Archives of Disease in Childhood. 1998;**78**:54-57

[6] Moutschen MP, Scheen AJ, Lefebvre PJ. Impaired immune responses in diabetes mellitus: Analysis of the factors and mechanisms involved in relevance to the increased susceptibility of diabetic patients to specific infections. Diabetes & Metabolism. 1992;**18**: 187-201

[7] Casey JI, Heeter BJ, Klyshevich KA. Impaired response of lymphocytes of diabetic subjects to antigen of *Staphylococcus aureus*. The Journal of Infectious Diseases. 1977;**136**:495-501

[8] Maccuish AC, Urbaniak SJ, Campbell CJ, Duncan LJP, Irvine W. Phytohemagglutinin transformation and circulating lymphocyte subpopulations in insulin-dependent diabetics. Diabetes. 1974;**23**:708-712

[9] Plouffe JF, Silva J, Fekety FRJ, Allen JL. Cell-mediated immunity in diabetes mellitus. Infection and Immunity. 1978;**21**:425-429

[10] Vergani D, Johnston C, B-Abdullah N, Barnett AH. Low serum C4 concentrations: An inherited predisposition to insulin dependent diabetes. British Medical Journal. 1983;**286**:926-928

[11] Mysliwska J, Zorena K, Bakowska A, Skuratowicz-Kubica A, Mysliwski A. Significance of tumor necrosis factor-α in patients with long-standing type-1 diabetes mellitus. Hormone and Metabolic Research. 1998;**30**:158-161

[12] Pickup JC, Crook MA. Is type II diabetes mellitus a disease of the innate immune system. Diabetologia. 1998;**41**:1241-1248

[13] Zozulinska D, Majchrzak A, Sobieska M, Wiktorowicz K, Wierusz-Wysocka B. Serum interleukin-8 level is increased in diabetic patients. Diabetologia. 1999;**42**:117-118

[14] Mooradian AD, Reed RL, Meredith KE, Scuderi P. Serum levels of tumor necrosis factor and IL1α and IL-1β in diabetic patients. Diabetes Care. 1991;**14**:63-65

[15] Ohno Y, Aoki N, Nishimura A. In vitro production of interleukin-1, interleukin-6, and tumor necrosis factor-α in insulin-dependent diabetes mellitus. The Journal of Clinical Endocrinology and Metabolism. 1993;**77**:1072-1077

[16] Ziegler Heitbrock HWL, Wedel A, Schraut W, Strobel M, Wendelgass P, Sternsdorf T, et al. Tolerance to lipopolysaccharide involves mobilization of nuclear factor kB with predominance of p50 homodimers. The Journal of Biological Chemistry. 1994;**269**:17001-17004

[17] Morohoshi M, Fujisawa K, Uchimura I, Numano F. The effect of glucose and advanced glycosylation end products on IL-6 production by human monocytes. Annals of the New York Academy of Sciences. 1995;**748**:562-570

[18] Reinhold D, Ansorge S, Schleicher ED. Elevated glucose levels stimulate transforming growth factor-b1 (TGF-B1), suppress interleukin IL-2, IL-6 and IL-10 production and DNA synthesis in peripheral blood mononuclear cells. Hormone and Metabolic Research. 1996;**28**:267-270

[19] Stehouwer C, Lambert J, Donker A, van Hinsbergh VWM. Endothelial dysfunction and pathogenesis of diabetic angiopathy. Cardiovascular Research. 1997;**34**:55-68

[20] Vlassara H, Brownlee M, Manogue KRD, Inarello CA, Pasagian A. Cachectin/TNF and IL-1 induced by glucose-modified proteins: Role in normal tissue remodeling. Science. 1988;**240**:1546-1548

[21] Imani F, Horii Y, Suthanthiran M, Skolnik EY, Makita Z, Sharma V, et al. Advanced glycosylation endproduct-specific receptors on human and rat T-lymphocytes mediate synthesis of interferon gamma: Role in tissue remodeling. The Journal of Experimental Medicine. 1993;**178**:2165-2172

[22] Wahl PW, Savage PJ, Psaty BM, Orchard TJ, Robbins JA, Tracy RP. Diabetes in older adults: Comparison of 1997 American Diabetes Association classification of diabetes mellitus with 1985 WHO classification. Lancet. 1998;**352**:1012-1015

[23] Hostetter MK. Perspectives in diabetes. Handicaps to host defense. Effects of hyperglycemia on C3 and *Candida albicans*. Diabetes. 1990;**39**:271-275

[24] Geerlings SE, Brouwer EC, Gaastra W, Verhoef J, Hoepelman AIM. The effect of glucose and pH on uropathogenic and non-uropathogenic *Escherichia coli*: Studies using urine from diabetics and non-diabetics. Journal of Medical Microbiology. 1999;**48**:535-539

[25] Tater D, Tepaut B, Bercovici JP, Youinou P. Polymorphonuclear cell derangements in type I diabetes. Hormone and Metabolic Research. 1987;**19**:642-647

[26] Tan JS, Anderson JL, Watanakunakorn C, Phair JP. Neutrophil dysfunction in diabetes mellitus. The Journal of Laboratory and Clinical Medicine. 1975;**85**:26-33

[27] Andersen B, Goldsmith GH, Spagnuolo PJ. Neutrophil adhesive dysfunction in diabetes mellitus; the role of cellular and plasma factors. The Journal of Laboratory and Clinical Medicine. 1988;**111**:275-285

[28] Bloomgarden ZT. Endothelial dysfunction, neuropathy and the diabetic foot, diabetic mastopathy, and erectile dysfunction. Diabetes Care. 1998;**21**:183-189

[29] Zargar AH, Shah NA, Masoodi SR, Laway BA, Dar FA, Khan AR, et al. Copper, zinc, and magnesium levels in non-insulin dependent diabetes mellitus. Postgraduate Medical Journal. 1998;**74**:665-668

[30] Wellinghausen N, Schromm AB, Seydel U, Brandenburg K, Luhm J, Kirchner H, et al. Zinc enhances lipopolysaccharide-induced monkine secretion by alteration of fluidity state of lipopolysaccharide. Journal of Immunology. 1996;**157**:3139-3145

[31] Delamaire M, Maugendre D, Moreno M, Le Goff M, Allannic H, Genetet B. Impaired leucocyte functions in diabetic patients. Diabetic Medicine. 1997;**14**:29-34

[32] Mowat AG, Baum J. Chemotaxis of polymorphonuclear leukocytes from patients with diabetes mellitus. The New England Journal of Medicine. 1971;**284**:621-627

[33] Balasoiu D, van Kessel KC, van Kats-Renaud HJ, Collet TJ, Hoepelman AI. Granulocyte function in women with diabetes and asymptomatic bacteriuria. Diabetes Care. 1997; **20**:392-395

[34] Bagdade JD, Stewart M, Walters E. Impaired granulocyte adherence. A reversible defect in host defense in patients with poorly controlleddiabetes. Diabetes. 1978;**27**:67781

[35] Bagdade JD, Walters E. Impaired granulocyte adherence in mildly diabetic patients. Effects of tolazamide treatment. Diabetes. 1980;**29**:309-311

[36] Marhoffer W, Stein M, Maeser E, Federlin K. Impairment of polymorphonuclear leukocyte function and metabolic control of diabetes. Diabetes Care. 1992;**15**:256-260

[37] Gin H, Brottier E, Aubertin J. Influence of glycaemic normalisation by an artificial pancreas on phagocytic and bactericidal functions of granulocytes in insulin dependent diabetic patients. Journal of Clinical Pathology. 1984;**37**:1029-1031

[38] Saeed F, Castle GE. Neutrophil chemiluminescence during phagocytosis is inhibited by abnormally elevated levels of acetoacetate: Implications for diabetic susceptibility to infections. Clinical and Diagnostic Laboratory Immunology. 1998;**5**:740-743

[39] Shah SV, Wallin JD, Eilen SD. Chemiluminescence and superoxide anion production by leukocytes from diabetic patients. The Journal of Clinical Endocrinology and Metabolism. 1983;**57**:402-409

[40] Katz S, Klein B, Elian I, Fishman P, Djaldetti M. Phagocytotic activity of monocytes from diabetic patients. Diabetes Care. 1983;**6**:479-482

[41] Schaeffer AJ, Jones JM, Dunn JK. Association of in vitro *Escherichia coli* adherence to vaginal and buccal epithelial cells with susceptibility of women to recurrent urinary-tract infections. The New England Journal of Medicine. 1981;**304**:1062-1066

[42] Aly FZ, Blackwell CC, Mackenzie DAC, Weir DM, Clarke BF. Factors influencing oral carriage of yeasts among individuals with diabetes mellitus. Epidemiology and Infection. 1992;**109**:507-518

[43] Cameron BJ, Douglas LJ. Blood group glycolipids as epithelial cell receptors for *Candida albicans*. Infection and Immunity. 1996;**64**:891-896

[44] Weinmeister KD, Dal Nogare AR. Buccal cell carbohydrates are altered during critical illness. American Journal of Respiratory and Critical Care Medicine. 1994;**150**:131-134

[45] Darwazeh AMG, Lamey PJ, Samaranayake LP, Mac Farlane TW, Fisher BM, Mac Rury SM, et al. The relationship between colonisation, secretor status and in vitro adhesion of *Candida albicans* to buccal epithelial cells from diabetics. Journal of Medical Microbiology. 1990;**33**:43-49

Chapter 3

ER Stress, Secretory Granule Biogenesis, and Insulin

Michiko Saito and Yoko Shiba

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76131

Abstract

Insulin is secreted from pancreatic β-cells, and the high demand of insulin biosynthesis is known to cause β-cell dysfunction in patients with type 2 diabetes mellitus. The insulin biosynthetic pathway has been extensively studied and is still an exciting area for future studies. In this chapter, first, we focus on proinsulin biosynthetic pathway in the endoplasmic reticulum (ER) and recent progress of our knowledge about ER stress. We discuss about how ER stress is involved in the development of diabetes. Second, we focus on the formation of insulin secretory granules. The biogenesis of secretory granules has been explored for several decades; however, it still has been debated and has yet to be understood. We review the current knowledge about the secretory granules and discuss about the problems for future studies.

Keywords: insulin, islets of Langerhans, ER stress, Golgi, secretory granules, biosynthetic pathway

1. Introduction

Pancreatic β-cells synthesize insulin and secrete it in response to the increase of blood glucose levels. Insulin is synthesized as proinsulin in the endoplasmic reticulum (ER) and transported to the Golgi apparatus. In the trans-Golgi network (TGN), insulin becomes hexamer, and then packaged into secretory granules (**Figure 1**). In secretory granules, proinsulin is processed to form mature insulin and C-peptide. By the stimulation of high glucose concentration in blood, insulin granule is exocytosed and insulin and C-peptide are secreted into blood.

Pancreatic β-cells are located in islets of Langerhans. In pancreas, there are islet-like cell clusters that are stained differently from other parts of pancreatic tissues (**Figure 2**). It was named as islets of Langerhans, from the name of the person who found this structure. Paul Langerhans found this structure in his doctoral thesis. The cell clusters appeared to be different

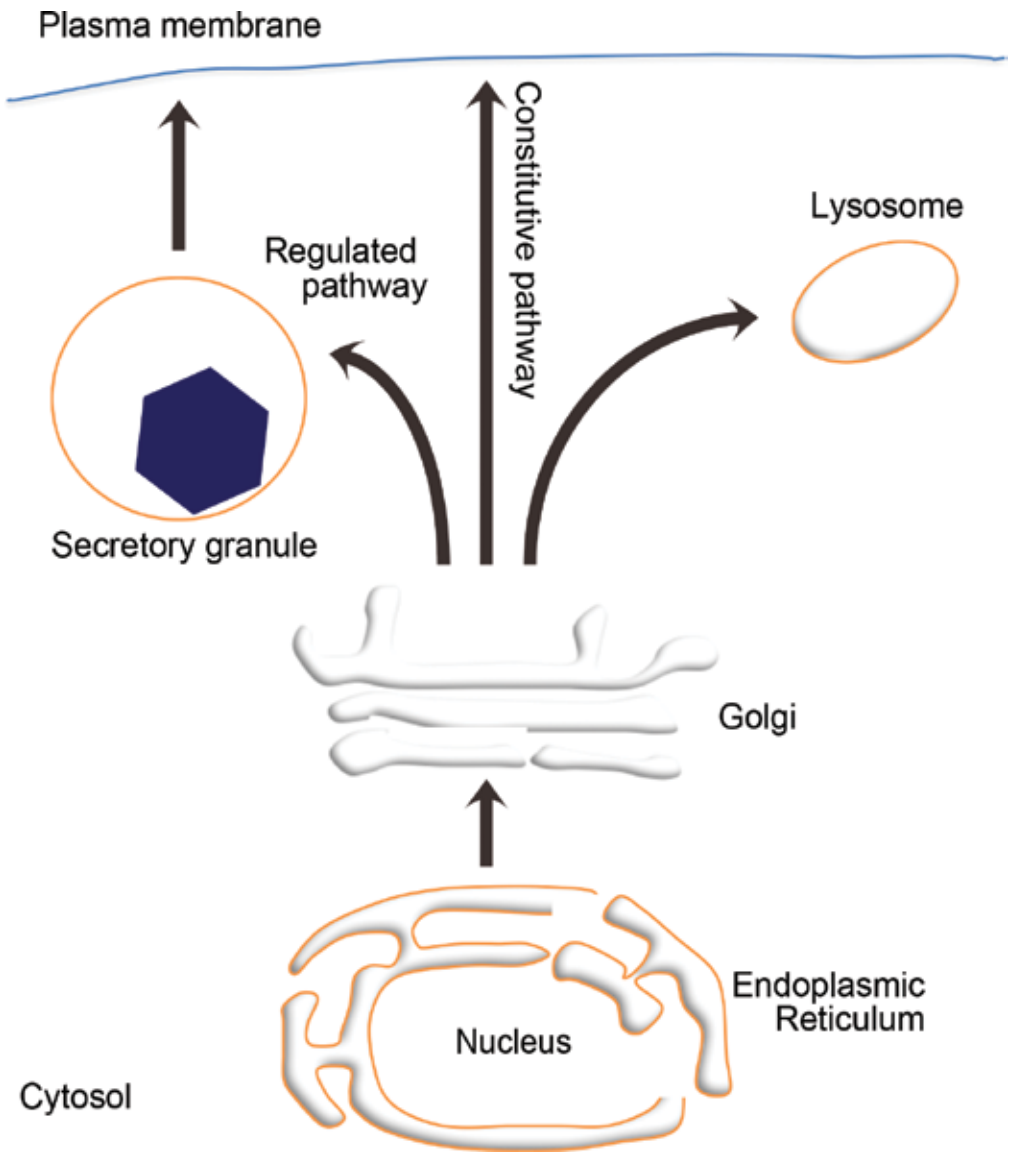

Figure 1. Biosynthetic pathway of secretory proteins. Intracellular transport in mammalian cells. Insulin is synthesized in the endoplasmic reticulum (ER), transported to the Golgi apparatus, and then packaged into secretory granules (SGs). Upon stimulation, SGs fuse with the plasma membrane (PM) and insulin is secreted. Insulin follows regulated secretory pathway in β-cells.

from the cells that secrete pancreatic enzymes, but he did not know what the function of this structure was. As he also found the cells that have dendrites in skin, his name is used for these cells as Langerhans cells, the dendritic cells [1].

Pancreas contains exocrine cells that secrete digestive enzymes including amylase and trypsin, and endocrine cells that secrete hormones including insulin and glucagon. The ratio of exocrine

http://dx.doi.org/10.5772/intechopen.76131

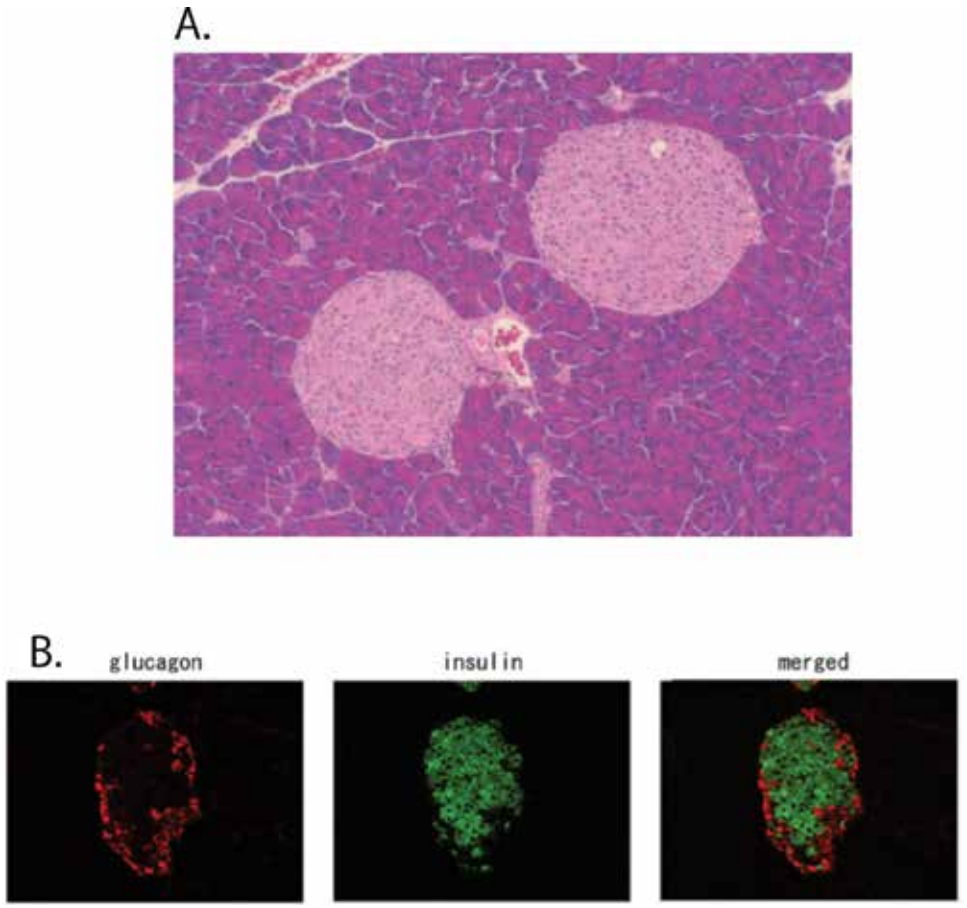

Figure 2. Islets of Langerhans in mouse. (A) Pancreatic segments from adult male mouse were stained by Hematoxylin-Eosin (HE). The lightly stained regions are islets of Langerhans. The densely stained regions that surround islets contain exocrine cells. (B) Islet of Langerhans captured by Immunofluorescence Pancreatic segments from adult male mouse were fixed and stained by anti-insulin antibody (green) and anti-glucagon antibody (red). Pancreatic β-cells that secrete insulin accumulate in the center, whereas α-cells that secrete glucagon are located in periphery.

and endocrine cells is approximately 9:1, meaning that less than 10% of cells are endocrine cells located in islets of Langerhans scattered in pancreas. Islets of Langerhans contain α, β, δ, ε, and PP cells. There are a million of islets of Langerhans in pancreas in human and 200–300 in adult mouse. In islets of Langerhans, 15–20% of cells are α-cells, 75–80% are β-cells, 5% are δ-cells, 1% are ε-cells, and 4% are PP cells. α-cells secrete glucagon that raises the blood glucose levels, β-cells secrete insulin that is the only hormone to decrease the blood glucose levels, δ-cells secrete somatostatin that inhibits the secretion of insulin and glucagon, ε-cells promote appetite and secrete ghrelin that inhibits insulin secretion, and PP cells secrete pancreatic peptide whose function is yet to be understood. In rodents, islets of Langerhans have a clear mantle core structure in which β-cells are located in the center of islets of Langerhans and surrounded by α, δ, ε, and PP cells (**Figure 1**). When rat islets are trypsinized and maintained in culture medium, the cells reassembled into aggregates that have a similar organization of intact islets; β-cells are

located in the center and the other cells are in the periphery [2]. Human islets of Langerhans do not have such clear structures. β-cells are mixed with the other cells. In avian pancreas, α-cells are in the center of islets. In zebrafish, their pancreas shares the basic structure with mammalian pancreas [3]. Recent studies show that zebrafish is a good model to study pancreatic development and diabetes mellitus [4–7].

2. ER stress and insulin

2.1. Proinsulin translation and folding in the ER

Human has INS gene as only insulin gene, whereas rodents have INS1 and INS2 genes for insulin. Human insulin gene encodes preproinsulin that has 110 amino acids containing N-terminal signal peptide following B chain, C-peptide, and A chain. Preproinsulin mRNA translation begins in the cytosol in pancreatic β-cells, and the signal peptide is recognized by signal recognition particle (SRP) to translate proinsulin across the membrane of the ER. In the ER, signal peptide is cleaved by signal peptidase to produce proinsulin that has 86 amino acids consisting of B chain, C-peptide, and A chain (**Figure 3**). Proinsulin is folded in the ER by chaperones including protein disulfide isomerase (PDI) family and BiP. Molecular chaperons and PDIs bind to the hydrophobic regions of proteins to promote folding and inhibit the aggregation of proteins [8]. Proinsulin has three disulfide bonds in A6–A11, A7–B7, and A20–B19 (**Figure 3**) [9]. *N*-glycosylation is often used as a marker for proper folding of newly synthesized proteins in the ER; however, proinsulin does not have *N*-glycosylation site.

2.2. ER stress

The high demand of insulin synthesis under a high plasma glucose condition causes ER stress that could cause β-cell dysfunction. Generally, the secretory proteins and transmembrane proteins are folded and acquire a variety of modification in the ER. Environmental and genetic factors affect protein folding in the ER. If protein folding is inhibited, unfolded proteins accumulate in the ER, leading to ER stress. Cells that sense ER stress cause unfolded protein response (UPR) that includes the inhibition of general protein translation, the induction of expression of ER chaperons, and ER-associated degradation (ERAD). UPR is a cellular response to recover ER homeostasis. In mammalian cells, there are three ER stress sensors, PERK, IRE1α, and ATF6 (**Figure 4**).

Protein kinase RNA-like ER kinase (PERK) is type-I transmembrane protein that localizes in the ER. Under ER stress, PERK undergoes autophosphorylation to be activated and oligomerized. Oligomerized PERK phosphorylates translation initiation factor, eIF2 α-subunit to inhibit protein translation. The inhibition of protein translation attenuates the accumulation of unfolded ER proteins as well as ER stress [10]. On the other hand, this inhibition of protein translation promotes the translation of ATF4, a transcription factor that induces genes related to apoptosis, amino acid metabolism, and antioxidants. Still, when cells cannot deal with ER stress even by these measures and ER stress is continued, ATF4 induces the transcription of C/EBP homologous

http://dx.doi.org/10.5772/intechopen.76131

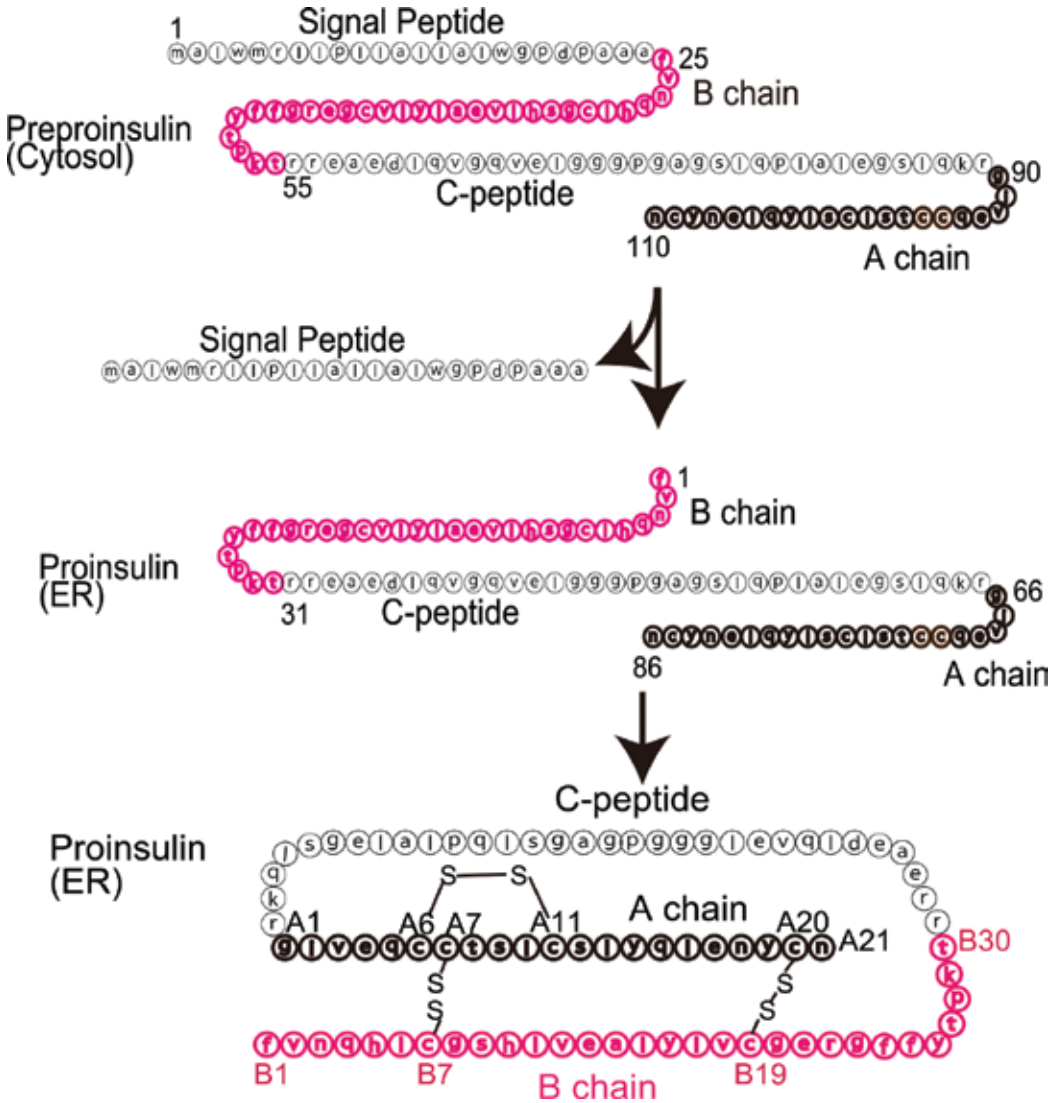

Figure 3. Insulin biosynthesis in the ER. The schematic structure of human insulin is shown. Insulin has signal peptide in its N-terminus followed by B chain, C-peptide, and A chain. Insulin mRNA translation is initiated in the cytosol as preproinsulin and cotranslationally inserted to the ER. Signal peptide is cleaved by endopeptidase during insertion into the ER and proinsulin is generated. In the ER, proinsulin is folded by three disulfide bonds of A and B chain.

proteins (CHOP/GADD153). CHOP and ATF4 form a heterodimer that induces the transcription of each downstream genes and promotes apoptosis [11, 12].

Inositol-requiring enzyme 1 (IRE1) has two isoforms: IRE1α that is expressed ubiquitously [13] and IRE1β that is expressed in goblet cells that secrete mucin in the digestive tract and lung [14, 15]. IRE1α is the type-I transmembrane protein localized in the ER. IRE1α has kinase and ribonuclease domains in its cytoplasmic region, and its lumenal domain has the binding site

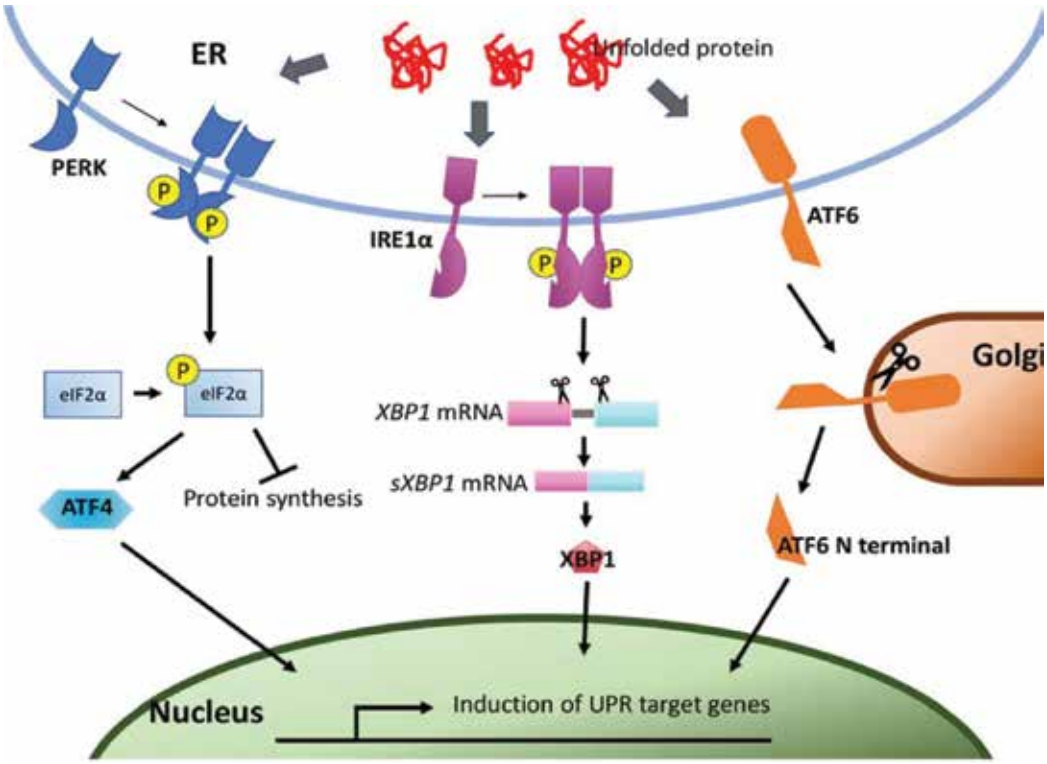

Figure 4. ER stress and activation of the unfolded protein response (UPR) pathways in mammalian cells. Accumulation of unfolded protein in the ER can be recognized by ER stress sensors, IRE1α, PERK and ATF6. These proteins are activated and mediate ER stress response, including inhibition of translation and induction of transcription of ER chaperones.

for BiP. In normal condition, IRE1α is in inactive form and binds to BiP, as well as other ER-stress sensors PERK and ATF6. In ER stress condition, BiP is released from IRE1α and IRE1α forms oligomer to be in an active form [16–21]. Autophosphorylation of the kinase domain in cytoplasmic region of IRE1α activates the ribonuclease (RNase) domain in the C-terminal region of cytoplasmic domain of IRE1α [22–25]. The activated RNase domain cleaves the precursor form of XBP1 (XBP1 unspliced; XBP1u mRNA) in specific two sites to produce mature XBP1 mRNA (XBP1 spliced; XBP1s mRNA) that produces functional transcription factor to induce the transcription of genes related to ER chaperones, ERAD, and lipid metabolism to recover ER homeostasis [26, 27].

Activating transcription factor 6 (ATF6) is a type-II transmembrane protein functioning as a transcription factor. ATF6 localizes in the ER but has the Golgi-localizing signal in its lumenal region that is inhibited in normal condition by binding to BiP. Under ER-stress condition, BiP is released from the lumenal region of ATF6, and its Golgi-localizing signal is exposed to transport ATF6 to the Golgi apparatus [28, 29]. In the Golgi, site 1 protease (S1P) and site 2 protease (S2P) cleave the transmembrane region of ATF6 and produce ATF6 that has only cytoplasmic region containing DNA-binding site [30, 31]. The cleaved ATF6 is translocated

into the nucleus to function as a transcription factor that induces the transcription of genes related to ER chaperones [10, 32]. Prolonged ER stress promotes ATF6 to bind to ATF4 to induce the transcription of CHOP, resulting in apoptosis [33–35].

2.3. ER stress and insulin

Pancreatic β-cells are specialized cells to synthesize and secrete a large amount of insulin. Insulin biosynthesis in pancreatic β-cells accounts for 10–50% of total protein synthesis [36, 37]. Therefore, the burden to the ER (ER stress) in pancreatic β-cells is constitutively high even in physiological condition. It is also known that pancreatic β-cells are sensitive against oxidative stress and hypoxia [38] as well as ER stress. The expression level of glutathione peroxidase, an antioxidant, is very low in pancreatic β-cells; therefore, pancreatic β-cells are sensitive to oxidative stress [39]. The islets of Langerhans are surrounded by blood vessels and supplied with nutrients and oxygen. Hypoxia affects insulin secretion of pancreatic islets and the survival rate of grafted islets [40, 41].

In type-II diabetes, it was reported that pancreatic β-cell mass is decreased [42, 43]. Huang et al. reported that the rat model of type-II diabetes expressing human islet amyloid polypeptide (hIAPP) showed the decrease of β-cell mass due to β-cell apoptosis, and the proteins related to ER stress including CHOP is highly expressed in β-cells [44]. The relationship between ER stress and diabetes has been studied by a variety of animal models and human genetic diseases. Akita mouse, another mouse model of diabetes named by Akio Koizumi in Akita University, has a single mutation in insulin 2 gene. Although there are no gross defects in the transcription of the wild-type insulin 2 allele and the two alleles of insulin 1, the phenotype of a single mutation of insulin 2 is dominant. Insulin 2 gene in Akita mouse has tyrosine instead of cysteine 96 (C96Y), and the mutated proinsulin does not form the disulfide bond between A chain and B chain (A7–B7). The mutated proinsulin cannot be transported to the Golgi apparatus and its secretion is inhibited [45]. The mutated proinsulin is accumulated in the ER that causes UPR to result in the induction of the expression of GRP78, XBP1, and CHOP. Eventually, pancreatic β-cells die by apoptosis. The necessity of ER stress for β-cell death was demonstrated by the delay of the development of diabetes in mouse produced by crossing Akita mouse with CHOP–knock-out mouse [46]. It was reported that human also has the same mutation [47].

Wolcott-Rallison syndrome (WRS) is caused by the malfunction of Eif2ak3 gene that encodes PERK [48]. WRS is an autosomal-recessive disorder that has neonatal diabetes, epiphyseal dysplasia, osteoporosis, and growth retardation. Patients with WRS have the point mutation in the kinase domain of PERK or the mutation that causes the deletion mutant of PERK. The mutation causing kinase dead of PERK develops diabetes after several months of birth, whereas the mutation that still maintains kinase activity of PERK delays the development of diabetes after 30 months. As well as WRS, PERK knock-out mice showed the secretory defects in many tissues causing diabetes and growth defects [49–51]. Furthermore, the knock-in mice having the mutation of phosphorylation site of eIF2α, the downstream molecule of PERK signaling, are unable to inhibit translation leading to over-synthesis of insulin and resulting in the dysfunction of pancreatic β-cells and β-cell death [52].

ATF6 knock-out mice do not show gross defects in normal diet, whereas high-fat diet causes the dysfunction of pancreatic β-cells [53, 54]. Furthermore, strong ER stress promotes the death of pancreatic β-cells [55, 56].

The knock-out mice of IRE1α specifically deleted in pancreatic β-cells cause diabetic phenotype [57, 58]. The mRNA levels of preproinsulin are not impaired; however, the protein level of proinsulin and mature insulin decreases, and protein and mRNA levels of five PDI protein families, PDI, PDIR, P5, ERp44, and ERp46, also decrease. These results indicate that these five PDI families are involved in proinsulin folding downstream of IRE1α, and upregulation of these PDI families could be the next approach for the treatment of diabetes.

3. Biogenesis of insulin secretory granules

After reaching the Golgi from the ER, secretory proteins are sorted in the *trans*-Golgi network (TGN) (**Figure 1**). One of the secretory pathways is the constitutive pathway in which proteins are constitutively secreted. When there is no sorting signal, proteins are thought to follow this pathway in mammalian cells. By contrast, another secretory pathway is the regulated pathway in which secretory proteins are packaged into the immature secretory granules (ISGs) (**Figure 5**). ISGs mature into mature secretory granules (MSGs), then MSGs are fused with the plasma membrane (PM) upon the stimulation of secretagogues to secrete the contents of MSGs. Proinsulin follows the regulated secretory pathway after the Golgi apparatus.

Secretory proteins destined for regulated pathway are segregated from other proteins and packaged into ISGs. This is termed as sorting by entry. On the other hand, in the process of formation and maturation of ISGs, other proteins are eliminated from ISGs. It is termed as sorting by exit, or sorting by retention [59–61].

3.1. Proinsulin transport to immature secretory granules

The molecular mechanisms of proinsulin sorting in the TGN are yet to be understood. It is thought that the selective aggregation of proinsulin occurs in the TGN [62, 63]. Insulin secretory granules contain a clear electron-dense core structure suggesting that insulin is crystallized in the granules. In pituitary AtT-20 cells, insulin granules can be formed by transfecting insulin gene, and hemagglutinin that flows in a constitutive pathway is segregated from the dense-core structure. Hemagglutinin is distributed evenly through the Golgi stacks as well as proinsulin; however, they are segregated after the TGN [62]. Therefore, the proinsulin sorting from constitutive pathway could occur in the TGN.

The sorting receptor that recognizes proinsulin and transport proinsulin into ISGs remains unidentified [59]. Carboxypeptidase E (CPE), an enzyme involved in insulin processing, was proposed to play a role as the sorting receptor [64]; however, the islets from mice that lost CPE by its mutation showed that insulin is efficiently secreted by secretagogues as well as in control islets, whereas the constitutive secretion of insulin remains as low as 1% similar to that in control islets [65]. Therefore, the possibility that CPE plays a role as a sorting receptor in

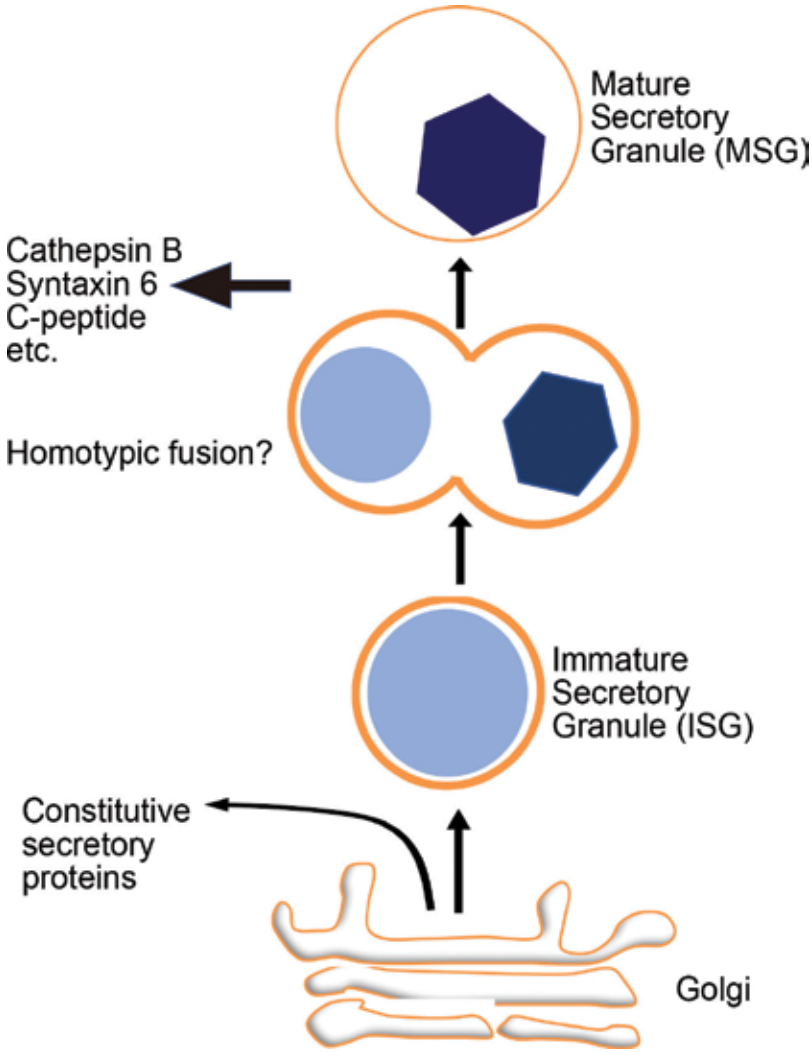

Figure 5. Insulin Secretory Granule (SG) formation. After folded in the ER, proinsulin is transported to the Golgi apparatus, then packaged into immature secretory granules (ISG) from the *trans*-Golgi network (TGN). ISGs mature into mature secretory granules (MSGs). There are several steps to make SGs; segregation from constitutive secretory proteins, possibly homotypic fusion and removal of proteins not required for MSGs.

pancreatic β-cells is questionable [59]. While research to find out the sorting receptor has been going on, another possibility was proposed; cargo aggregation/oligomerization is the sorting signal for ISGs [60, 61, 66].

It is proposed that aggregated proteins directly bind to lipid micro-domain in the TGN membranes and these micro-domains could be recognized by cytosolic machineries [59, 67]. Secretory granules (SGs) contain a high amount of cholesterol, and the depletion or addition of cholesterol affects glucose-stimulated insulin secretion (GSIS) [68]. Secretogranin III is one of

the components of SGs and known to bind to cholesterol-rich membranes [69]. Although the role of secretogranin III in SG biogenesis in mice is not clear [70], it could be important to investigate the role of cholesterol.

There is an interesting phenomenon using SEGFP that is the green fluorescent protein (GFP) having a signal peptide in its N-terminus. In cultured insulin-secreting cells (INS-1 cells), SEGFP is sorted to secretory granules and secreted by secretagogues similar to insulin, whereas secreted alkaline phosphatase (SEAP), a model protein of constitutive pathway, is constitutively secreted [71]. SEGFP forms oligomer by a disulfide bond, and its oligomerization could unexpectedly function as a sorting signal to ISGs. The results may support the idea that cargo oligomerization itself, rather than specific sequences on cargo, is required for sorting. The involvement of lipids or other sorting proteins in this case is unclear.

Zinc and calcium ions play important roles in insulin oligomerization. Structural studies showed that insulin forms a dimer, and in the presence of zinc and calcium ions, it forms a hexamer [72, 73]. It is thought that the concentration of zinc and calcium ions rises in the TGN [9], and these ions are enriched in SGs [74, 75]. The oligomerization regions of insulin and proinsulin are essentially the same with or without C-peptide [72]. The cleavage of C-peptide from proinsulin hexamer decreases the solubility of insulin hexamer leading to crystallization of insulin in mature secretory granules (MSGs). Insulin crystals are thought to be stable and can be stored in MSGs for a long time without being degraded [73]. ZnT8 zinc transporter, the product of *SLC30A8* gene, is highly expressed in pancreatic β-cells, and the combined deletion of ZnT8 and ZnT7 inhibits GSIS [76]. However, ZnT8 mutation is protective against type 2 diabetes [77]. The precise function of ZnT8 in insulin biosynthetic pathway and its relationship with the development of diabetes remains unclarified.

Although the molecular mechanisms of sorting are yet to be understood, recent studies revealed the molecules to be involved in the fission process of SGs from the TGN. Arfaptin 1 has a lipid-binding domain termed Bin/Amphiphysin/Rvs (BAR) domain that binds to a curved membrane structure [78, 79] and implicated in a regulating membrane fission [80]. Arfaptin-1 binds to small GTP-binding proteins, Arf1- and Arf-like protein 1 (Arl1), and recruited to the Golgi membrane by a GTP-bound form of Arf1 and Arl1 [81, 82]. Arfaptin 1 is phosphorylated by Protein Kinase D (PKD) that is activated by diacylglycerol (DAG) enriched in the neck of budding vesicles [83]. Non-phosphorylated mutant of Arfaptin 1 (S132A) or PKD inhibitor blocks insulin SG fission from the TGN [84]. The expression of Arfaptin 1 (S132A) or Arfaptin 1 depletion inhibits GSIS. As Arfaptin-1 was reported to be involved in other transport pathways [82, 85, 86], the specificity of Arfaptin 1 in SG biogenesis needs to be carefully addressed. Although Arfaptin 1 is proposed to play a role in membrane fission [84, 87], it could be interesting to investigate the upstream molecules of Arfaptin 1 to look for the sorting machinery for SG biogenesis.

3.2. Maturation of secretory granules

3.2.1. Insulin processing

The excursion of C-peptide decreases the solubility of insulin hexamer and causes insulin crystallization within SGs [73]. Proinsulin is processed into mature insulin by prohormone

convertases (PC1/3 and PC2) and carboxypeptidase E (CPE) [88] (**Figure 6**). PC1 (also known as PC3) cleaves 32–33 junction between B chain and C-peptide, and then CPE removes 31, 32 arginine residues. The intermediate form of proinsulin that is cleaved in B–C junction but is yet to be cleaved in A–C junction is termed as des-31, 32 split proinsulin. PC2 cleaves 65–66 junction between A chain and C peptide, and CPE removes 64, 65 lysine and arginine residues to form another intermediate termed des-64, 65 split proinsulin [9]. The cleavage of B–C junction tends to occur first before the cleavage of A–C junction [89]. PC1/3 and PC2 are Ca^{2+}- and pH-dependent endopeptidases. The optimal pH of both enzymes is pH 5.5 [90, 91]. The pH at the TGN is reported to be ~6.0 [92]. The pH of ISGs varies from 5.5 to 7.0 and the pH of

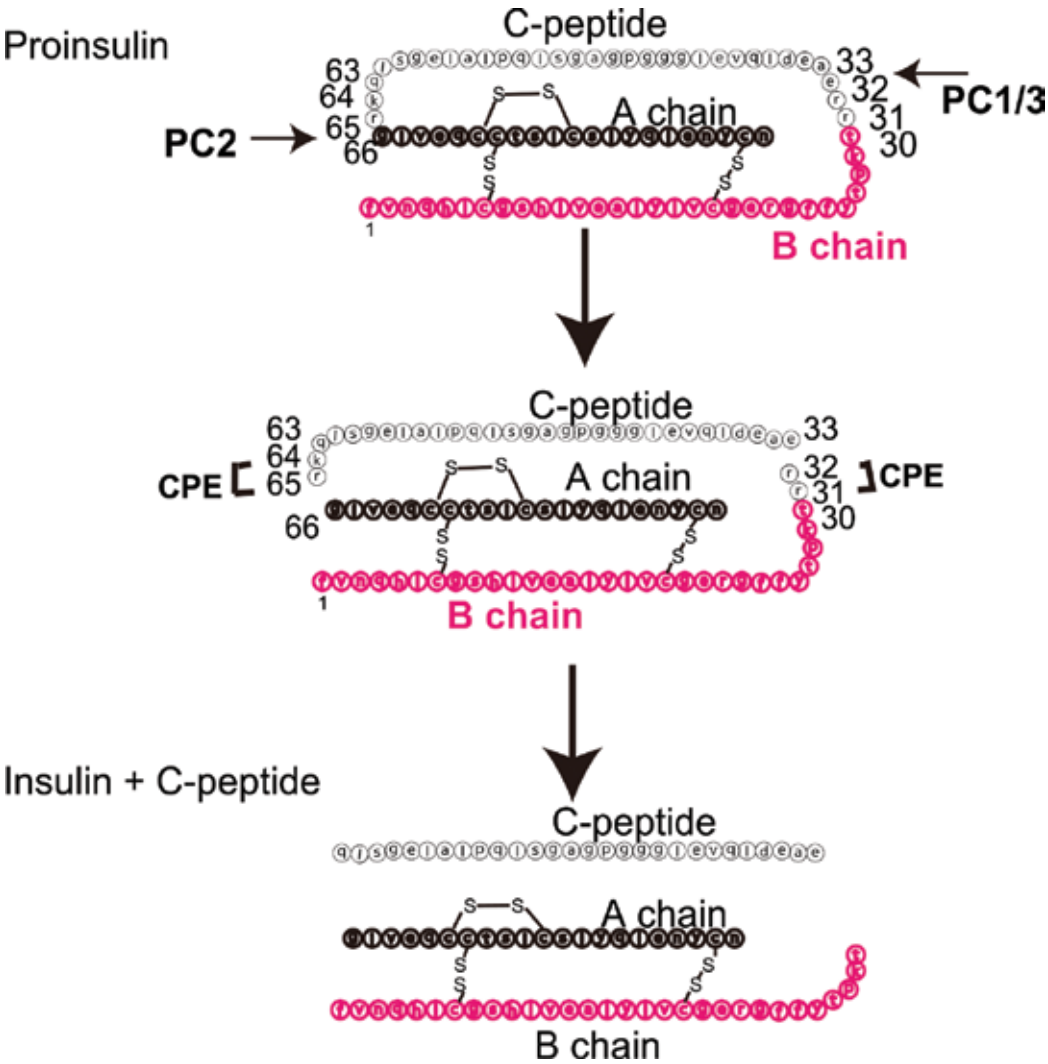

Figure 6. Proinsulin processing. Proinsulin processing is thought to be initiated in the TGN and continue to undergo in ISGs. Prohormone convertases PC1/3 and PC2 cleave C-peptide from proinsulin then Carboxypeptidase E (CPE) removes di-basic residues (Arg-Arg or Arg-Lys) to produce mature insulin and C-peptide.

MSGs is ~5.0 [89]. PC1/3 could be active in the TGN as well as in ISGs [93], whereas PC2 is thought to be active in ISGs and MSGs [91, 94].

3.2.2. Sorting by retention

As ISGs mature, ISGs produce vesicles to remove proteins that are not required for MSGs, whereas insulin is retained in MSGs. For example, ISGs are known to produce constitutive-like vesicles that contain excess C-peptide than insulin to be secreted [95, 96]. Also, in contrast to constitutive secretory proteins, lysosomal enzymes are thought to be segregated from ISGs in pancreatic β-cells [97, 98]. Generally, lysosomal enzymes are synthesized in the ER as well as secretory proteins and transported to the Golgi apparatus (**Figure 1**). In the TGN and endosomes, lysosomal enzymes are recognized by mannose 6-phosphate receptors (MPRs) and then packaged into clathrin-coated vesicle (CCVs). Clathrin is a coat protein that forms a cage-like structure to produce CCVs in the post-Golgi compartment [99]. AP-1 is a clathrin adaptor that binds to MPRs and clathrin and mediates to form clathrin/AP-1-coated vesicles [100, 101]. Proinsulin ISGs have clathrin and AP-1 on their surfaces as well as MPRs [98]. Also, MSGs lose the signal of cathepsin B, a lysosomal protease, whereas ISGs still have a strong cathepsin B signal. These results suggest that lysosomal enzymes recognized by MPRs are removed from ISGs [98].

3.2.3. Homotypic fusion

Syntaxin 6, a SNARE protein that is reported to be important for the homotypic fusion of ISGs in neuroendocrine cells [102], is also removed from insulin ISGs [98]. In neuroendocrine cells, it is thought that homotypic fusion plays an important role in SG maturation, and the fusion machineries required for homotypic fusion are different than that required for MSGs fusion to the PM [67, 102]. It is proposed that membrane fusion machinery is remodeled in the end of ISG maturation. The role of Syntaxin 6 and homotypic fusion in insulin granule maturation is not clear [103]. However, recent study showed that homotypic fusion could also be important in insulin granule maturation [104]. In islets from HID-1 KO mice, Vamp-4, another SNARE protein that is proposed in ISG-derived vesicle fusion to the PM in neuroendocrine cells [67], is mislocalized. Proinsulin processing and acidification are delayed, and by 3D electron microscopy, there are less homotypic fusion events [104]. Although the role of HID-1 and Vamp-4 in homotypic fusion in pancreatic β-cells should be addressed in the future, it is possible that homotypic fusion might also be important for insulin granule formation.

The MSGs are fused with the PM, and insulin and C-peptide are secreted upon stimulation. For details about the exocytosis of insulin granules, see the review articles [59, 105, 106].

4. Conclusion

Because of the importance of insulin in diabetes mellitus, insulin secretory pathway has been extensively studied. Recent advance in the understanding of biosynthetic pathway reveals the importance of ER stress in β-cell dysfunction and novel machineries of secretory granule biogenesis. However, still many questions remain. What are the mechanisms by which ER-stress

sensors regulate proinsulin translation and folding? Is it relevant to prevent β-cell death by preventing UPR? The inhibition of CHOP has been studied to prevent β-cell death for the treatment of diabetes [46, 107–109]; however, it should be addressed carefully that even if β-cells survive by preventing CHOP, and too much accumulation of unfolded proteins in the ER may prevent normal proinsulin folding and would not support the function of islets of Langerhans. Decreasing the continuous high demand of insulin synthesis is anyway the primary importance for diabetes; then thinking about how to support proinsulin folding, packaging proinsulin into secretory granules, and elimination of unfolded proteins from β-cells would help for developing new treatments of diabetes.

Author details

Michiko Saito[1] and Yoko Shiba[2]*

*Address all correspondence to: shibay@iwate-u.ac.jp

1 Bio-Science Research Center, Kyoto Pharmaceutical University, Kyoto, Japan

2 Faculty of Science and Engineering, Iwate University, Morioka, Japan

References

[1] Ronald Kahn C, Weir G, King G, Jacobson A, Smith R, Moses A. Joslin's Diabetes Mellitus. 14th ed. Lippincott Williams & Wilkins; 2004

[2] Halban PA et al. Spontaneous reassociation of dispersed adult rat pancreatic islet cells into aggregates with three-dimensional architecture typical of native islets. Diabetes. 1987;**36**(7):783-790

[3] Kinkel MD, Prince VE. On the diabetic menu: Zebrafish as a model for pancreas development and function. BioEssays. 2009;**31**(2):139-152

[4] Zang L, Shimada Y, Nishimura N. Development of a novel zebrafish model for type 2 diabetes mellitus. Scientific Reports. 2017;**7**(1):1461

[5] Tehrani Z, Lin S. Antagonistic interactions of hedgehog, Bmp and retinoic acid signals control zebrafish endocrine pancreas development. Development. 2011;**138**(4):631-640

[6] Kimmel RA, Meyer D. Zebrafish pancreas as a model for development and disease. Methods in Cell Biology. 2016;**134**:431-461

[7] Prince VE, Anderson RM, Dalgin G. Zebrafish pancreas development and regeneration: Fishing for diabetes therapies. Current Topics in Developmental Biology. 2017;**124**:235-276

[8] Otero JH, Lizak B, Hendershot LM. Life and death of a BiP substrate. Seminars in Cell & Developmental Biology. 2010;**21**(5):472-478

[9] Liu M et al. Proinsulin entry and transit through the endoplasmic reticulum in pancreatic beta cells. Vitamins and Hormones. 2014;**95**:35-62

[10] Yoshida H et al. A time-dependent phase shift in the mammalian unfolded protein response. Developmental Cell. 2003;**4**(2):265-271

[11] Han J et al. ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. Nature Cell Biology. 2013;**15**(5):481-490

[12] Harding HP et al. Regulated translation initiation controls stress-induced gene expression in mammalian cells. Molecular Cell. 2000;**6**(5):1099-1108

[13] Tirasophon W, Welihinda AA, Kaufman RJ. A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. Genes & Development. 1998;**12**(12):1812-1824

[14] Martino MB et al. The ER stress transducer IRE1beta is required for airway epithelial mucin production. Mucosal Immunology. 2013;**6**(3):639-654

[15] Tsuru A et al. Negative feedback by IRE1beta optimizes mucin production in goblet cells. Proceedings of the National Academy of Sciences of the United States of America. 2013; **110**(8):2864-2869

[16] Bertolotti A et al. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nature Cell Biology. 2000;**2**(6):326-332

[17] Kimata Y et al. Genetic evidence for a role of BiP/Kar2 that regulates Ire1 in response to accumulation of unfolded proteins. Molecular Biology of the Cell. 2003;**14**(6):2559-2569

[18] Kimata Y et al. A role for BiP as an adjustor for the endoplasmic reticulum stress-sensing protein Ire1. The Journal of Cell Biology. 2004;**167**(3):445-456

[19] Kimata Y et al. Two regulatory steps of ER-stress sensor Ire1 involving its cluster formation and interaction with unfolded proteins. The Journal of Cell Biology. 2007;**179**(1):75-86

[20] Korennykh AV et al. The unfolded protein response signals through high-order assembly of Ire1. Nature. 2009;**457**(7230):687-693

[21] Li H et al. Mammalian endoplasmic reticulum stress sensor IRE1 signals by dynamic clustering. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**(37):16113-16118

[22] Welihinda AA et al. Protein serine/threonine phosphatase Ptc2p negatively regulates the unfolded-protein response by dephosphorylating Ire1p kinase. Molecular and Cellular Biology. 1998;**18**(4):1967-1977

[23] Papa FR et al. Bypassing a kinase activity with an ATP-competitive drug. Science. 2003; **302**(5650):1533-1537

[24] Han D et al. IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. Cell. 2009;**138**(3):562-575

[25] Ali MM et al. Structure of the Ire1 autophosphorylation complex and implications for the unfolded protein response. The EMBO Journal. 2011;**30**(5):894-905

[26] Sidrauski C, Walter P. The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. Cell. 1997;**90**(6):1031-1039

[27] Yoshida H et al. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell. 2001;**107**(7):881-891

[28] Shen J et al. ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. Developmental Cell. 2002;**3**(1):99-111

[29] Shen J et al. Stable binding of ATF6 to BiP in the endoplasmic reticulum stress response. Molecular and Cellular Biology. 2005;**25**(3):921-932

[30] Wang Y et al. Activation of ATF6 and an ATF6 DNA binding site by the endoplasmic reticulum stress response. The Journal of Biological Chemistry. 2000;**275**(35):27013-27020

[31] Chen X, Shen J, Prywes R. The luminal domain of ATF6 senses endoplasmic reticulum (ER) stress and causes translocation of ATF6 from the ER to the Golgi. The Journal of Biological Chemistry. 2002;**277**(15):13045-13052

[32] Ye J et al. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Molecular Cell. 2000;**6**(6):1355-1364

[33] Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nature Reviews. Molecular Cell Biology. 2007;**8**(7):519-529

[34] Mori K. Signalling pathways in the unfolded protein response: Development from yeast to mammals. Journal of Biochemistry. 2009;**146**(6):743-750

[35] Kimata Y, Kohno K. Endoplasmic reticulum stress-sensing mechanisms in yeast and mammalian cells. Current Opinion in Cell Biology. 2011;**23**(2):135-142

[36] Schuit FC, In't Veld PA, Pipeleers DG. Glucose stimulates proinsulin biosynthesis by a dose-dependent recruitment of pancreatic beta cells. Proceedings of the National Academy of Sciences of the United States of America. 1988;**85**(11):3865-3869

[37] Van Lommel L et al. Probe-independent and direct quantification of insulin mRNA and growth hormone mRNA in enriched cell preparations. Diabetes. 2006;**55**(12):3214-3220

[38] Eizirik DL et al. Major species differences between humans and rodents in the susceptibility to pancreatic beta-cell injury. Proceedings of the National Academy of Sciences of the United States of America. 1994;**91**(20):9253-9256

[39] Robertson RP, Harmon JS. Pancreatic islet beta-cell and oxidative stress: The importance of glutathione peroxidase. FEBS Letters. 2007;**581**(19):3743-3748

[40] Dionne KE, Colton CK, Yarmush ML. Effect of oxygen on isolated pancreatic tissue. ASAIO Transactions. 1989;**35**(3):739-741

[41] Carlsson PO et al. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. Diabetes. 2001;**50**(3):489-495

[42] Butler AE et al. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes. 2003;**52**(1):102-110

[43] Rahier J et al. Pancreatic beta-cell mass in European subjects with type 2 diabetes. Diabetes, Obesity & Metabolism. 2008;**10**(Suppl 4):32-42

[44] Huang CJ et al. High expression rates of human islet amyloid polypeptide induce endoplasmic reticulum stress mediated beta-cell apoptosis, a characteristic of humans with type 2 but not type 1 diabetes. Diabetes. 2007;**56**(8):2016-2027

[45] Wang J et al. A mutation in the insulin 2 gene induces diabetes with severe pancreatic beta-cell dysfunction in the Mody mouse. The Journal of Clinical Investigation. 1999; **103**(1):27-37

[46] Oyadomari S et al. Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. The Journal of Clinical Investigation. 2002;**109**(4):525-532

[47] Stoy J et al. Insulin gene mutations as a cause of permanent neonatal diabetes. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(38): 15040-15044

[48] Delepine M et al. EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. Nature Genetics. 2000;**25**(4):406-409

[49] Harding HP et al. Diabetes mellitus and exocrine pancreatic dysfunction in perk-/-mice reveals a role for translational control in secretory cell survival. Molecular Cell. 2001;**7**(6): 1153-1163

[50] Zhang W et al. PERK EIF2AK3 control of pancreatic beta cell differentiation and proliferation is required for postnatal glucose homeostasis. Cell Metabolism. 2006;**4**(6):491-497

[51] Gao Y et al. PERK is required in the adult pancreas and is essential for maintenance of glucose homeostasis. Molecular and Cellular Biology. 2012;**32**(24):5129-5139

[52] Scheuner D et al. Control of mRNA translation preserves endoplasmic reticulum function in beta cells and maintains glucose homeostasis. Nature Medicine. 2005;**11**(7):757-764

[53] Yamamoto K et al. Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. Developmental Cell. 2007;**13**(3):365-376

[54] Usui M et al. Atf6alpha-null mice are glucose intolerant due to pancreatic beta-cell failure on a high-fat diet but partially resistant to diet-induced insulin resistance. Metabolism. 2012;**61**(8):1118-1128

[55] Song B et al. Chop deletion reduces oxidative stress, improves beta cell function, and promotes cell survival in multiple mouse models of diabetes. The Journal of Clinical Investigation. 2008;**118**(10):3378-3389

[56] Fonseca SG et al. Wolfram syndrome 1 gene negatively regulates ER stress signaling in rodent and human cells. The Journal of Clinical Investigation. 2010;**120**(3):744-755

[57] Hassler JR et al. The IRE1alpha/XBP1s pathway is essential for the glucose response and protection of beta cells. PLoS Biology. 2015;**13**(10):e1002277

[58] Tsuchiya Y et al. IRE1-XBP1 pathway regulates oxidative proinsulin folding in pancreatic β cells. The Journal of Cell Bioligy. Published Online: 5 March; 2018

[59] Hou JC, Min L, Pessin JE. Insulin granule biogenesis, trafficking and exocytosis. Vitamins and Hormones. 2009;**80**:473-506

[60] Molinete M et al. Trafficking/sorting and granule biogenesis in the beta-cell. Seminars in Cell & Developmental Biology. 2000;**11**(4):243-251

[61] Tooze SA. Biogenesis of secretory granules in the trans-Golgi network of neuroendocrine and endocrine cells. Biochimica et Biophysica Acta. 1998;**1404**(1–2):231-244

[62] Orci L et al. The trans-most cisternae of the Golgi complex: A compartment for sorting of secretory and plasma membrane proteins. Cell. 1987;**51**(6):1039-1051

[63] Chanat E, Huttner WB. Milieu-induced, selective aggregation of regulated secretory proteins in the trans-Golgi network. The Journal of Cell Biology. 1991;**115**(6):1505-1519

[64] Cool DR et al. Carboxypeptidase E is a regulated secretory pathway sorting receptor: Genetic obliteration leads to endocrine disorders in Cpe(fat) mice. Cell. 1997;**88**(1):73-83

[65] Irminger JC et al. Proinsulin targeting to the regulated pathway is not impaired in carboxypeptidase E-deficient Cpefat/Cpefat mice. The Journal of Biological Chemistry. 1997;**272**(44):27532-27534

[66] Tooze SA, Martens GJ, Huttner WB. Secretory granule biogenesis: Rafting to the SNARE. Trends in Cell Biology. 2001;**11**(3):116-122

[67] Kogel T, Gerdes HH. Maturation of secretory granules. Results and Problems in Cell Differentiation. 2010;**50**:1-20

[68] Tsuchiya M et al. Cholesterol biosynthesis pathway intermediates and inhibitors regulate glucose-stimulated insulin secretion and secretory granule formation in pancreatic beta-cells. Endocrinology. 2010;**151**(10):4705-1476

[69] Hosaka M, Watanabe T. Secretogranin III: A bridge between core hormone aggregates and the secretory granule membrane. Endocrine Journal. 2010;**57**(4):275-286

[70] Maeda Y et al. Impaired processing of prohormones in Secretogranin III-null mice causes maladaptation to an inadequate diet and stress. Endocrinology. 2018;**159**(2):1213-1227

[71] Molinete M et al. Trafficking of non-regulated secretory proteins in insulin secreting (INS-1) cells. Diabetologia. 2000;**43**(9):1157-1164

[72] Dodson G, Steiner D. The role of assembly in insulin's biosynthesis. Current Opinion in Structural Biology. 1998;**8**(2):189-194

[73] Dunn MF. Zinc-ligand interactions modulate assembly and stability of the insulin hexamer – A review. Biometals. 2005;**18**(4):295-303

[74] Howell SL, Montague W, Tyhurst M. Calcium distribution in islets of Langerhans: A study of calcium concentrations and of calcium accumulation in B cell organelles. Journal of Cell Science. 1975;**19**(2):395-409

[75] Zalewski PD et al. Video image analysis of labile zinc in viable pancreatic islet cells using a specific fluorescent probe for zinc. The Journal of Histochemistry and Cytochemistry. 1994;**42**(7):877-884

[76] Syring KE et al. Combined deletion of Slc30a7 and Slc30a8 unmasks a critical role for ZnT8 in glucose-stimulated insulin secretion. Endocrinology. 2016;**157**(12):4534-4541

[77] Flannick J et al. Loss-of-function mutations in SLC30A8 protect against type 2 diabetes. Nature Genetics. 2014;**46**(4):357-363

[78] Peter BJ et al. BAR domains as sensors of membrane curvature: The amphiphysin BAR structure. Science. 2004;**303**(5657):495-499

[79] Gehart H, Ricci R. Saving the neck from scission. Communicative & Integrative Biology. 2013;**6**(2):e23098

[80] Boucrot E et al. Membrane fission is promoted by insertion of amphipathic helices and is restricted by crescent BAR domains. Cell. 2012;**149**(1):124-136

[81] Kanoh H, Williger BT, Exton JH. Arfaptin 1, a putative cytosolic target protein of ADP-ribosylation factor, is recruited to Golgi membranes. The Journal of Biological Chemistry. 1997;**272**(9):5421-5429

[82] Man Z et al. Arfaptins are localized to the trans-Golgi by interaction with Arl1, but not Arfs. The Journal of Biological Chemistry. 2011;**286**(13):11569-11578

[83] Szule JA, Fuller NL, Rand RP. The effects of acyl chain length and saturation of diacylglycerols and phosphatidylcholines on membrane monolayer curvature. Biophysical Journal. 2002;**83**(2):977-984

[84] Gehart H et al. The BAR domain protein Arfaptin-1 controls secretory granule biogenesis at the trans-Golgi network. Developmental Cell. 2012;**23**(4):756-768

[85] Williger BT, Ostermann J, Exton JH. Arfaptin 1, an ARF-binding protein, inhibits phospholipase D and endoplasmic reticulum/Golgi protein transport. FEBS Letters. 1999; **443**(2):197-200

[86] Huang LH et al. Arfaptin-1 negatively regulates Arl1-mediated retrograde transport. PLoS One. 2015;**10**(3):e0118743

[87] Anitei M et al. Spatiotemporal control of lipid conversion, actin-based mechanical forces, and curvature sensors during clathrin/AP-1-coated vesicle biogenesis. Cell Reports. 2017; **20**(9):2087-2099

[88] Steiner DF. The proprotein convertases. Current Opinion in Chemical Biology. 1998;**1**:31-39

[89] Orci L et al. pH-independent and -dependent cleavage of proinsulin in the same secretory vesicle. The Journal of Cell Biology. 1994;**126**(5):1149-1156

[90] Hutton JC. Insulin secretory granule biogenesis and the proinsulin-processing endopeptidases. Diabetologia. 1994;**37**(Suppl 2):S48-S56

[91] Bailyes EM et al. Differences between the catalytic properties of recombinant human PC2 and endogenous rat PC2. The Biochemical Journal. 1995;**309**(Pt 2):587-594

[92] Demaurex N et al. Mechanism of acidification of the trans-Golgi network (TGN). In situ measurements of pH using retrieval of TGN38 and furin from the cell surface. The Journal of Biological Chemistry. 1998;**273**(4):2044-2051

[93] Bailyes EM et al. A member of the eukaryotic subtilisin family (PC3) has the enzymic properties of the type 1 proinsulin-converting endopeptidase. The Biochemical Journal. 1992;**285**(Pt 2):391-394

[94] Lamango NS et al. The proteolytic maturation of prohormone convertase 2 (PC2) is a pH-driven process. Archives of Biochemistry and Biophysics. 1999;**362**(2):275-282

[95] Arvan PKR, Prabakaran D, Zavacki AM, Elahi D, Wang S, Pilkey D. Protein discharge from immature secretory granules displays both regulated and constitutive characteristics. The Journal of Biological Chemistry. 1991:14171-14174

[96] Kuliawat RAP. Protein targeting via the "constitutive-like" secretory pathway in isolated pancreatic islets: Passive sorting in the immature granule compartment. The Journal of Cell Biology. 1992;**3**:521-529

[97] Kuliawat RAP. Distinct molecular mechanisms for protein sorting within immature secretory granules of pancreatic beta-cells. The Journal of Cell Biology. 1994;**126**(1):77-86

[98] Klumperman J et al. Mannose 6-phosphate receptors are sorted from immature secretory granules via adaptor protein AP-1, clathrin, and syntaxin 6-positive vesicles. The Journal of Cell Biology. 1998;**141**(2):359-371

[99] Robinson MS. Forty years of Clathrin-coated vesicles. Traffic. 2015;**16**(12):1210-1238

[100] Le Borgne R, Hoflack B. Protein transport from the secretory to the endocytic pathway in mammalian cells. Biochimica et Biophysica Acta. 1998;**1404**(1–2):195-209

[101] Ghosh P, Dahms NM, Kornfeld S. Mannose 6-phosphate receptors: New twists in the tale. Nature Reviews. Molecular Cell Biology. 2003;**4**(3):202-212

[102] Wendler FPL, Urbé S, Tooze SA. Homotypic fusion of immature secretory granules during maturation requires syntaxin 6. Molecular Biology of the Cell. 2001;**12**(6):1699-1709

[103] Kuliawat R et al. Syntaxin-6 SNARE involvement in secretory and endocytic pathways of cultured pancreatic beta-cells. Molecular Biology of the Cell. 2004;**15**(4):1690-1701

[104] Du W et al. HID-1 is required for homotypic fusion of immature secretory granules during maturation. eLife. 2016:**5**

[105] Rorsman P, Braun M. Regulation of insulin secretion in human pancreatic islets. Annual Review of Physiology. 2013;**75**:155-179

[106] Izumi T, Kasai K, Gomi H. Secretory vesicle docking to the plasma membrane: Molecular mechanism and functional significance. Diabetes, Obesity & Metabolism. 2007;**9**(Suppl 2): 109-117

[107] Gurlo T et al. CHOP contributes to, but is not the only mediator of, IAPP induced beta-cell apoptosis. Molecular Endocrinology. 2016;**30**(4):446-454

[108] Yang Y et al. Transcription factor C/EBP homologous protein in health and diseases. Frontiers in Immunology. 2017;**8**:1612

[109] Nam DH et al. CHOP deficiency ameliorates ERK5 inhibition-mediated exacerbation of Streptozotocin-induced hyperglycemia and pancreatic beta-cell apoptosis. Molecules and Cells. 2017;**40**(7):457-465

Chapter 4

Optimal Insulin Delivery

Anders Frid, Laurence Hirsch and Kenneth Strauss

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76232

Abstract

Insulin therapy is only effective if it is delivered into the right tissue in the right way. Exogenous insulin is intended for the subcutaneous (SC) tissue, not the muscle or skin. If delivered into the latter, its absorption (pharmacokinetics (PK)) and action (pharmacodynamics (PD)) are unpredictable, which often leads to poor glucose control. Correct insulin therapy begins with matching the insulin to the site used. Typically, four sites are used for insulin injection or infusion: the abdomen lateral to the umbilicus all the way to the flanks, the anterior lateral upper half of the thigh, the deltoid region of the arm, and the upper outer quadrant of the buttocks. Regular insulin and neutral protamine Hagedorn (NPH) are both absorbed more rapidly from the arm and abdominal sites and more slowly from the thigh and buttocks. The newer insulin analogs, both rapid- and slow-acting, do not appear to be influenced by the site used for injection. In order to avoid intramuscular (IM) injections, patients should use the shortest needles currently available (the 4-mm pen needle and the 6-mm syringe needle). Very young children should raise a skin fold and inject into it even when using the 4-mm needle. Giving injections with the 6-mm needle at a 45° angle converts this needle into the equivalent of the 4 mm. Injection sites should be rigorously rotated, with the new injection being approximately 1 cm from previous injections. This measure helps prevent the most common complication of injection therapy, lipohypertrophy (LH). Injecting into LH leads to unstable PK and PD and deregulated glucose control, manifested as unexpected hypoglycemia, glycemic variability, and elevated HbA1c values. Comprehensive insulin deliver recommendations have recently been published.

Keywords: insulin, injection needles, subcutaneous, lipodystrophy, lipohypertrophy

1. Introduction

Current insulin therapy requires delivery into the subcutaneous (SC) tissue either by injection or by infusion. Optimal insulin delivery requires that accidental intramuscular (IM) or

intradermal (ID) delivery be avoided since the pharmacokinetics (PK) and pharmacodynamics (PD) of insulin are significantly altered in these tissue spaces. Optimal delivery also requires that sites of injection or infusion be rotated systematically in order to avoid the most common complication of insulin therapy, lipohypertrophy (LH). Insulin delivered into LH also has significantly altered PK and PD, which can lead to unexpected hypoglycemic episodes and glycemic variability. The latter are associated with worsened overall glucose control, increased short- and long-term complications, and higher costs.

Recently, new recommendations have been published as a consensus document from international diabetes experts [1]. This publication was the collective output of 183 experts from 54 countries who wrote and vetted a practical, evidence-based roadmap for optimal insulin delivery at the FITTER (Forum for Injection Technique and Therapy: Expert Recommendations) workshop from October 23 to 24, 2015, in Rome. FITTER was the fourth in a sequence of workshops on optimal insulin delivery [2–4]. The FITTER recommendations were also based on the results of the fourth Injection Technique Questionnaire (ITQ) survey conducted from 2014 to 2015. In total, 13,289 insulin-injecting patients from 42 countries participated [5].

Each recommendation is followed by a grade (e.g., A2). The letter indicates the strength of each recommendation: A. Strongly recommended; B. Recommended; C. Unresolved issue. The number indicates the degree of scientific support for each recommendation: 1. At least one rigorously performed study which is peer-reviewed and published; 2. At least one observational, epidemiologic, or population-based study which is peer-reviewed and published; 3. Consensus expert opinion based on patient experience. Since FITTER, many diabetes groups from countries around the world have adapted and adopted these recommendations as local guidelines. We draw on certain of these recommendations in the review that follows as well as summarize studies that have been performed since FITTER and will follow a thematic format, beginning with the anatomy of injection sites.

2. Current insulin delivery practice

A large survey of current insulin delivery has shown that there are many aspects of injection practice which are suboptimal [5, 6]. For decades, professionals had been advising patients to use insulin needles which, we now know, were too long for them, with no scientific rationale. However, after the shortest pen needles (4 mm) became available and studies on injection site anatomy and needle performance began to be published, starting in 2010 [7, 8], showing the safety and efficacy of these needles, the recommendations of experts changed. It was recognized that 4-mm pen needles were the optimal choice for nearly all injecting patients, whether adults or children: thin, normal-weight or obese, male or female, and of all ethnicities. These needles were felt to be a key step toward reducing the risk of IM injections. As a result, the use of the 8-mm needle, the dominant size in 2010, has decreased dramatically since then, with a corresponding increase in the use of the 4-mm needle.

However, the latest survey revealed that the longer lengths (8 mm and higher) are still being used by approximately 30% of patients worldwide and that the 5- and 6-mm needles are still used by approximately 20% each. This means that only 30% of patients worldwide currently use the recommended 4-mm needles. Longer needles are being used in sites where IM injection risk is very high (thighs and arms) and by patients who are at an increased risk because they have thin SC layers (slim and normal-weight adults as well as all adolescents and children).

The same survey has shown that lipohypertrophy (LH) is very common at injection sites. LH was found in almost a third of patients worldwide, many of them having LH at multiple injection sites. Injecting into LH has serious consequences for glucose control as well as possibly adverse effects on long-term outcomes and costs. Patients with LH consume a mean of over 10 IU more insulin per day than those without LH, and their HbA1c is on average 0.55% higher. LH is associated with increased rates of unexplained hypoglycemia, glucose variability, and more frequent diabetic ketoacidosis (DKA).

The survey showed that LH is most frequently associated with an incorrect rotation of injection sites and reusing pen needles. Rotating injection sites carefully appears to be the best method of avoiding LH. HbA1c values are lower in patients who rotate their injections over larger injection areas and who get their sites inspected regularly. Checking of injection sites routinely by health-care givers is associated with less LH and lower HbA1c levels, yet nearly 40% of patients reported that they could not remember their injection sites ever being examined. Patients are also more likely to rotate correctly if they have obtained injection instruction from their carer in the last 6 months. However, less than two out of five claim to have obtained such instructions on injecting in that time period. Ten percent of total injectors claim that they have never obtained injection training at all. The survey also shows that incorrect disposal of sharps after use is rampant. The majority of used sharps end up in public trash and constitute a major risk factor for accidental needle sticks.

3. Skin thickness

The skin is the main obstacle the needle must overcome. Needles must be at least long enough to traverse the skin and reach the SC tissue. Adult skin, according to a number of studies using imaging techniques ranging from ultrasound (US) to computer tomography (CT), has yielded remarkably similar results across genders, ethnicities, age groups, and body mass index (BMI) categories. The skin averages approximately 2–2.5-mm thick and varies in its 95% confidence interval (CI) between 1.25 and 3.25 mm. These studies included patients with type 2 diabetes (T2DM) from the Philippines [9], Korea [10], China [11], and India [12]; both type 1 diabetes (T1DM) and T2DM adults from the USA (including four different ethnic groups) [7]; and children from South Africa [13] and Italy [14].

The skin in children is slightly thinner than in adults, but these differences are largely irrelevant for insulin infusions and injections. Skin thickness increases during adolescence and reaches adult size in the late teens.

4. SC thickness

The SC tissue is the target for insulin. Injections must reach the SC tissue, but not go deeper into the muscle fascia or the muscle itself. Therefore, the thickness of the SC is critical in determining the desired length of the needle as well as the injection technique (e.g., lifting a skin fold or not). SC tissue thickness varies widely depending on gender, site of injection, and BMI. Women, on average, have approximately 5-mm thicker SC fat than men, when one controls for BMI and body site. Truncal sites (abdomen and buttocks) have more SC fat than limbs (arm and thigh), in the same patient. The higher the BMI, the thicker the SC fat. Studies within the last decade have used precision US to determine the SC tissue thickness in a diverse group of adults [7, 9, 15, 16], adolescents, and children [13, 14].

Needle length	Combined	Thigh	Arm	Abdomen	Buttock
4 mm	0.4%	1.6%	1.0%	0.3%	0.1%
5 mm	1.8%	4.7%	3.1%	1.1%	0.5%
6 mm	5.7%	10.0%	7.0%	2.8%	1.3%
8 mm	15.3%	25.0%	19.5%	9.7%	5.5%
12.7 mm	45.0%	63.0%	55.0%	38.0%	26.9%

*Assumes injection straight at 90° without pinch-up (the table adapted from Hirsch [16]).

With kind permission from Hirsch L et al. [16]. Intramuscular risk at insulin injection sites—measurement of the distance from the skin to the muscle and rationale for shorter-length needles for subcutaneous insulin therapy.

Table 1. Estimated IM injection risk in adults, by body site*.

	Marran, 2014 [13]				Lo Presti, 2014 (pooled) [14]		
	Arm	Thigh	Abdomen	Buttock	Ages 2–6	Ages 7–13	Ages 14–17
4 mm	27.5%	12.5%	12.5%	0%	20.2%	4.6%	2.4%
5 mm	47.5%	22.5%	30.0%	0%	46.0%	18.4%	16.1%
6 mm	62.5%	30.0%	37.5%	5.0%	66.5%	38.0%	34.5%
8 mm	87.5%	62.5%	50.0%	15.0%	83.9%	65.3%	66.1%
12.7 mm	100%	90.0%	85.0%	35.0%	97.2%	93.9%	96.4%

*Assumes that injections are into flat skin and not into lifted skin fold.

Table 2. Calculated risk of IM injection in children and adolescents as a function of injection site, age, and needle length*.

Babies have more SC tissue than preschool children. Children from 2 to 6 years have very little SC tissue regardless of gender. Children from 7 to 13 years gain SC tissue slowly but SC tissue thickness is almost the same in both genders until puberty. At puberty, girls increase their SC tissue more rapidly than boys as a result of hormonal differences.

SC tissue thickness when combined with the currently available needle lengths yields a relatively clear indication of the risk of IM injection. **Tables 1** and **2** show the risks for adult and pediatric persons with diabetes, respectively. It is clear from these data that the shorter the needle, the lower the risk of IM injection.

5. IM insulin

IM-injected insulins have a much greater variability in absorption and effect (PK and PD) compared to SC-injected. This variability is influenced by both exercise and the properties of the individual insulins. Human insulins and the new analogs also differ as to their PK when injected IM. In general, IM insulin is often associated with a more rapid absorption and unexplained hypoglycemia [17–19]. Because of the difficulty of predicting the impact of IM injections on PK and PD, various measures can be taken to avoid injecting IM: using of shorter needles, lifting of a skin fold into which one injects the insulin, or choosing injection sites with thicker layers of SC fat. A combination of the above techniques can also be used [20].

6. Needle length

In the last decade, insulin needle lengths have decreased dramatically. Previously, adults were given needles that were ≥ 8 mm long and children ≥6 mm. As shown in **Tables 1** and **2**, these lengths are now universally recognized as too long. They make IM injections more likely, and on the whole, the length of the needle has little or nothing to do with glucose control, according to a multitude of studies [8, 21–28]. Longer needles also tend to have larger diameters (smaller G or gauge), which correlates with a greater injection pain.

Hirsch [8] compared the 4-mm pen needle to 5- and 8-mm needles and showed the former to be safe and efficacious in adults (i.e., comparable glucose control); leakage from the skin was equivalent and both pain scores and overall preference were better with the 4 mm. In Japan, Miwa [29] compared the 4-mm needle with 6 mm and showed equivalent results, as did Nagai [30] when comparing 4-to 5-mm pen needles. Hirose [31] found equivalent modeled PK/PD results for the 4 mm compared to the 6- and the 8-mm needles, in young non-diabetics. Birkebaek [32] found a reduced IM risk with 4 versus 6-mm PNs in children and lean adults. Lo Presti [14] measured the skin and SC in children and adolescents with diabetes (ages 2–17) and concluded that the safest needle length for all ages is the 4 mm.

In obese adults, Bergenstal [33] recently showed that the 4-mm pen needles deliver equivalent glycemic control (HbA1c) to both 8- and 12.7-mm pen needles. These obese patients were taking

relatively high doses of glargine (>40 IU), with total daily dose (TDD) insulin up to 300 U daily. No differences between 4- and both 8- and 12.7-mm PNs in hypo- or hyperglycemic events or insulin leakage were found in obese subjects with BMI up to nearly 60 kg/m^2. The 4-mm needle was found to be less painful, easier to use, easier to insert, and less anxiety-provoking than 8 or 12.7 mm (all at $p < 0.05$).

Based on these studies, FITTER recommended the following:

- The 4-mm needle inserted perpendicularly is long enough to penetrate the skin and enter the subcutaneous tissue, with little risk of intramuscular (or intradermal) injection. Therefore, it should be considered the safest pen needles for adults and children regardless of age, gender, ethnicity, and BMI. **A1**
- The 4-mm needle should be inserted perpendicular to the skin (at 90° to the skin surface), not at an angle, regardless of whether a skin fold is raised. A1.
- Very young children (6 years old and under) and very thin adults should use the 4-mm needle by lifting a skin fold and inserting the needle perpendicularly into it. Others may inject using the 4-mm needle without lifting a skin fold. A1
- Patients with tremors or other disorders, which make them unable to hold a 4-mm pen needle in place, may need longer needles. B3

7. Injection site care

Recommended injection sites include the abdomen, lateral thigh, arms, and buttocks [34–38]. In the abdomen, injections or infusions in adults may be given within the following boundaries: 1 cm above symphysis pubis, 1 cm below the lowest rib, 1 cm away from the umbilicus, and laterally at the flanks. In the lateral thighs, patients should use the upper third anteriorly. The posterior lateral aspect of the upper buttocks and flanks may be used. In the arm, one may use the mid-third posterior aspect. In children, the abdominal boundaries are similar to adults, but 2 cm is used instead of 1 cm for all distances. A degree of clinical judgment must be used in all cases, adult and pediatric.

8. Human insulin

Soluble human insulin (e.g., regular insulin) has a slower absorption profile than the rapid-acting analogs (lispro, aspart, and glulisine). The PK and PD of regular insulin, as well as those of neutral protamine Hagedorn (NPH), are highly dependent on the body site injected and the technique used. FITTER recommendations state that:

- IM injections of NPH and long-acting insulin analogs must be strictly avoided due to the risk of hypoglycemia [17, 39–41]. **A2**

- The abdomen is the preferred site for soluble human insulin (regular), since absorption of this insulin is fastest there [35, 42–46]. **A1**
- The regular/NPH mix should be given in the abdomen to increase the speed of absorption of the short-acting insulin in order to cover postprandial glycemic excursions [18]. **A1**
- If there is risk of nocturnal hypoglycemia, NPH- and NPH-containing mixes given in the evening should be injected into the thigh or the buttock as these sites have slower absorption of NPH [38, 47, 48]. **A1**

9. Insulin analogs

There are fewer studies of optimal delivery methods for the newer insulin analogs and GLP-1 s. However, insulin analogs are *not* as dependent on injection sites as are human insulins or NPH. From the existing studies, FITTER recommended the following:

- Rapid-acting insulin analogs may be given at any of the injection sites, as absorption rates do not appear to be site-specific [49–53]. **A2**
- Rapid-acting analogs should not be given IM although studies have shown that absorption rates are similar from fat tissue and resting muscle. Absorption from working muscle has not, however, been studied [50, 51, 54]. **A2**
- Pending further studies, patients may inject long-acting insulin analogs in any of the usual injecting sites, with appropriate technique to prevent IM injection which can lead to profound hypoglycemia [55]. **B2**

10. GLP-1 agents

- Pending further studies, patients using non-insulin injectable therapies should follow the recommendations already established for insulin injections with regards to needle length, site selection, and site rotation [56–58]. **A2**

11. Lipohypertrophy

LH is the most common complication of insulin injection [59–62] or infusion [63, 64], with prevalence rates of 50% or higher. Risk factors for LH appear to be longer time on insulin, more daily injections, failure to carefully rotate injection sites, and extensive reuse of needles [59, 65–68]. The latter two risk factors are modifiable. Insulin injected into LH has been reported to have delayed or erratic absorption which may worsen glucose control, although these trials are older with less rigor, less precise insulin assays, or very small sample sizes

which in one case led to a conclusion that injecting into LH did not worsen inherent variability of insulin uptake [69–72]. A crossover glucose clamp study [73] showed that both insulin absorption and action when injected into LH are blunted and are 3–5X more variable than when the same insulin dose is injected into non-LH areas. A controlled mixed-meal tolerance test in the same study also showed a reduced insulin absorption, and prolonged postprandial hyperglycemia when the insulin was injected into the LH area. When patients change from delivering insulin into LH and move to normal tissue, they are at risk of hypoglycemia and must lower their doses. Gentile [74, 75] has shown convincingly that HCPs trained to detect LH can do so with extremely high efficiency using the physical examination alone, achieving up to 97% consistency levels. FITTER issued the following recommendations:

- Switching injections from lipohypertrophy to normal tissue often requires a decrease in the dose of insulin injected. The amount of change varies from one individual to another and should be guided by frequent blood glucose measurements. Reductions often exceed 20% of their original dose [66, 76]. **A1**
- Injections should be systematically rotated in such a way that they are spaced at least 1 cm (or approximate width of an adult finger) from each other in order to avoid repeat tissue trauma. **A2**
- One scheme with proven effectiveness involves dividing the injection site into quadrants (or halves when using the thighs or the buttocks), using one quadrant per week and moving quadrant to quadrant in a consistent direction (e.g., clockwise) [77]. **A3**

A multicenter interventional study in the UK [78] showed that education focused on these recommendations resulted in significantly reduced clinically detectable LH after 6 months, with LH either disappearing completely or decreasing by approximately 50% from its original size. The mean HbA1c fell by more than 4 mmol/L, and there were significantly reduced levels of unexpected hypoglycemia and glycemic variability. The mean TDD of insulin in the study population fell by an average of 5.6 IU by study close.

In a controlled, prospective, multicenter study in French patients [79], all of whom had LH, the intervention consisted of instructions to move injections to non-LH areas, to correctly rotate within injection sites, to forego needle reuse, and to switch to 4-mm needles in order to facilitate correct rotation without increased IM injections. These patients were also given intensive education on the injection recommendations as summarized in this chapter. Control patients were informed of the presence of LH and were told that injections should not be given into LH. They received usual and standard education. In the intervention group, there was a significant decrease of TDD of insulin of approximately 5 IU versus baseline ($P = 0.035$). There were significant decreases in HbA1c (up to 0.5%) in both intervention and control groups, with no significant differences between groups. A significant number of intervention patients improved their IT habits. The authors concluded that the intervention was effective in both groups, but that intensive education in LH management yielded more rapid and superior outcomes.

An interventional study in Moscow [80] followed three groups of T1DM and T2DM patients for 6 months. Two groups received structured injection training (with one group receiving 4-mm needles for each injection while the other did not) and a control group which did not get training or needles. Both training groups had HbA1C reductions of approximately 1% but the non-training group saw no change. Needle reuse and LH declined in the training groups and injection technique improved but none of these changes were seen in the non-training group.

The available data from intervention trials in patients with insulin-related LH show consistently positive outcomes. However, there are limitations to each trial—some were not randomized; in another, the control group received meaningful parts of the educational intervention [79]. Results of one or more ongoing randomized clinical trials should provide more definitive answers to the impact of injection technique training in the near future.

12. Needle reuse

Reusing needles is a common practice of injecting patients, mainly for reasons of convenience and cost-saving. However, a number of studies have linked needle reuse to LH [59, 65, 66, 81–83], especially when the reuse is excessive (≥5 times/needle). Injection pain was associated with reuse in one study [84] although another one disputed these results [85]. Another study found bacterial growth on reused needles and inflammatory changes (skin redness) at injection sites of patients who reused needles [86, 87]. Although local infections or abscesses have not been reported with needle reuse, FITTER recommends against reusing needles, which are labeled by regulatory agencies for single use.

13. Safety

Patients should never share insulin pens, whether in the hospital or at home setting. Blood can be aspirated back into the pen cartridge even after one injection, and this could possibly transmit a blood-borne disease such as HIV or hepatitis to the next user. Sonoki [88] found hemoglobin in a number of cartridges which patients had used only once. Le Floch [89] also studied the contamination of cartridges after one use and found similar findings. A recent US study corroborated these findings [90]. The rule with insulin injections is clear: one patient/one insulin pen.

Insulin needles are the most commonly used sharp worldwide. If not disposed of properly, needle-stick injuries with used insulin needles could transmit hepatitis, HIV, or other blood-borne pathogens. This is a major public health issue. Technologies exist to minimize this risk. FITTER recommended the following to minimize the risk of needle-stick injuries, particularly in a hospital or other inpatient setting:

- Safety-engineered devices play a critical role in protecting injectors, pump users, and downstream workers [91]. **A1**
- Needle recapping should not be done. **A2**
- Sharp containers must be easily accessible at the point of care beside the patient, prior to the injection or infusion. **A2**
- Safe disposal should be taught to patients, caregivers, and all others who may come in contact with the sharp device from the beginning of injection or infusion therapy and reinforced throughout [92] **A2**
- Under no circumstance should sharps material be disposed of into the public trash or rubbish system. **A3**

14. Insulin infusion

Continuous subcutaneous insulin infusion (CSII) has been used for 40 years [93, 94]. Insulin infusion sets (IISs) deliver insulin into the SC, and they have been associated with numerous adverse side effects [95]. It is generally agreed that if a patient has otherwise unexplained hyperglycemia, they should administer a correction bolus via their pump. If the blood glucose does not decline at least 50 mg/dL by 90 min, they should (1) remove the set, (2) give a correction with a pen or a syringe, and (3) insert a new set. FITTER recommended the following additional recommendations for CSII and IIS users:

- CSII cannula should be changed every 48–72 h in order to minimize infusion site adverse events and potential metabolic deterioration. However, these times are very patient-dependent and should be adjusted accordingly. **A1**
- All CSII patients should be taught to rotate infusion sites along the same principles that injecting patients are taught to rotate injection sites. **A1**
- Any CSII patients with unexplained glucose variability including frequent hypoglycemia/hyperglycemia should have infusion sites checked for lipohypertrophy, nodules, scarring, inflammation, or other skin and SC conditions that could affect insulin flow or absorption. **A1**

15. Conclusion

Insulin has a very low therapeutic index. The margin between its greatest therapeutic benefit and its unacceptable side effects is low. Without careful attention to optimal insulin delivery, patients can find themselves on either side of a slippery slope: either suboptimal therapeutic benefit or high toxicity. Optimal insulin delivery is complex and involves choices that

patients and professionals may not have previously considered: the choice of injection sites as a function of the insulin delivered, the choice of needle length as a function of SC thickness, the injection or infusion technique which ensure consistently effective SC delivery, the precise and systematic rotation of delivery sites, reduced or non-reuse of sharps, and safe disposal of used sharps which reduces needle-stick injury risk to family members or the community at large [96]. We have provided both evidence-based recommendations and proof that these work in practice and deliver insulin with an improved therapeutic index and better outcomes—both clinical- and patient-reported. The challenge now is to scale these recommendations so that all insulin-using patients and insulin-prescribing professional know and follow them.

Conflict of interest

Authors KS and LH are employees of BD, a manufacturer of injecting devices. All other authors declare that they have no conflict of interest.

Authorship

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval to the version to be published.

Abbreviations

BMI	body mass index
CSII	continuous subcutaneous insulin infusion
DKA	diabetic ketoacidosis
FIT	Forum for Injection Technique
FITTER	Forum for Injection Technique and Therapy: Expert Recommendations
G	gauge (of needle).
GCP	good clinical practice.
GLP-1	glucagon-like peptide-1 receptor agonists

HbA1c	glycated hemoglobin
HBV	hepatitis B virus
HCP	health care professional
HCV	hepatitis C virus
ID	intradermal
IM	intramuscular
ITQ	injection technique questionnaire
IU	international unit (of insulin)
LH−	patient without lipohypertrophy
LH	lipohypertrophy
LH+	patient with lipohypertrophy
NPH	neutral protamine hagedorn (also known as insulin N)
NSI	needle-stick injury
PD	pharmacodynamics
PK	pharmacokinetics.
SC	subcutaneous
T1DM	type 1 diabetes
T2DM	type 2 diabetes.
TDD	total daily dose (of insulin)

Author details

Anders Frid[1], Laurence Hirsch[2] and Kenneth Strauss[3]*

*Address all correspondence to: kenneth.strauss@bd.com

1 Department of Endocrinology, Skane University Hospital, Malmö, Sweden

2 BD Diabetes Care, Franklin Lakes, NJ, USA

3 BD Diabetes Care, Erembodegem, Belgium

References

[1] Frid AH et al. New insulin delivery recommendations. Mayo Clinic Proceedings; **91**(9): 1231-1255. Open Access at: http://www.mayoclinicproceedings.org/article/S0025-6196(16)30321-4/fulltext

[2] Strauss K. Insulin injection techniques: Report from the 1st international insulin injection technique workshop, Strasbourg, France—June 1997. Practical Diabetes International. 1998;**15**(6):16-20

[3] Strauss K, De Gols H, Letondeur C, Matyjaszczyk M, Frid A. The second injection technique event (SITE), May 2000, Barcelona. Practical Diabetes International. 2002;**19**(1):17-21

[4] Frid A, Hirsch L, Gaspar R, Hicks D, et al. New injection recommendations for patients with diabetes. Diabetes & Metabolism. 2010;**36**(2):S3-S18

[5] Frid AH et al. Worldwide injection technique questionnaire study: Population parameters and injection practices. Mayo Clinic Proceedings. 2016;**91**:1212-1223. Open Access at: http://www.mayoclinicproceedings.org/article/S0025-6196(16)30322-6/fulltext

[6] Frid AH et al. Worldwide injection technique questionnaire study: Injecting complications and role of the professional. Mayo Clinic Proceedings. 2016;**91**:1224-1230. Open Access at: http://www.mayoclinicproceedings.org/article/S0025-6196(16)30326-3/fulltext

[7] Gibney MA, Arce CH, Byron KJ, Hirsch LJ. Skin and subcutaneous adipose layer thickness in adults with diabetes at sites used for insulin injections: Implications for needle length recommendations. Current Medical Research and Opinion. 2010;**26**(6):1519-1530

[8] Hirsch L, Klaff L, Bailey T, Gibney M, Albanese J, Qu S, Kassler-Taub K. Comparative glycemic control, safety and patient ratings for a new 4 mm\32G insulin pen needle in adults with diabetes. Current Medical Research and Opinion. 2010;**26**:1531-1541

[9] Catambing I, Villa M. Ultrasonographic measurement of skin and subcutaneous thickness at insulin injection sites among adult Filipinos with diabetes. Journal of the ASEAN Federation of Endocrine Societies. 2014;**29**(1):24-32

[10] Sim KH, Hwang MS, Kim SY, Lee HM, Chang JY, Lee MK. The appropriateness of the length of insulin needles based on determination of skin and subcutaneous fat thickness in the abdomen and upper arm in patients with type 2 diabetes. Diabetes and Metabolism Journal. 2014;**38**(2):120-133

[11] Wang W, et al. Skin and subcutaneous layer thickness at insulin injection sites in Chinese diabetic patients. Diabetes Technology & Therapeutics. 2014 Dec;**16**(12):867-873. DOI: 10.1089/dia.2014.0111. Epub 2014 Oct 20

[12] Jain SM, Pandey K, Lahoti A, Rao PK. Evaluation of skin and subcutaneous tissue thickness at insulin injection sites in Indian, insulin naïve, type-2 diabetic adult population. Indian Journal of Endocrinology and Metabolism. 2013;**17**(5):864-870

[13] Marran K, Segal D. SKINNY – Skin thickness and needles in the young. South African Journal of Chemistry. 2014;**8**(3):92-95. DOI: 10.7196/SAJCH.687

[14] Lo Presti D, Ingegnosi C, Strauss K. Skin and subcutaneous thickness at injecting sites in children with diabetes: Ultrasound findings and recommendations for giving injection. Pediatric Diabetes. 2012;**13**(7):525-533

[15] Wang W et al. Skin and subcutaneous layer thickness at insulin injection sites in Chinese diabetic patients. Diabetes Technology & Therapeutics. 2015, submitted

[16] Hirsch L, Byron K, Gibney M. Intramuscular risk at insulin injection sites-measurement of the distance from skin to muscle and rationale for shorter-length needles for subcutaneous insulin therapy. Diabetes Technology & Therapeutics. 2014;**16**(12):867-873

[17] Karges B, Boehm BO, Karges W. Early hypoglycaemia after accidental intramuscular injection of insulin glargine. Diabetes Medicine. 2005;**22**(10):1444-1445

[18] Frid A, Gunnarson R, Guntner P, Linde P. Effects of accidental intramuscular injection on insulin absorption in IDDM. Diabetes Care. 1988;**11**(1):41-45

[19] Spraul M, Chantelau E, Koumoulidou J, Berger M. Subcutaneous or nonsubcutaneous injection of insulin. Diabetes Care. 1988;**11**(9):733-736

[20] Gentile S, Agrusta M, Guarino G, Carbone L, Cavallaro V, et al. Metabolic consequence of incorrect insulin administration techniques in aging subjects with diabetes. Acta Diabetologica. 2011;**48**:121-125

[21] Kreugel G, Keers JC, Jongbloed A, Verweij-Gjaltema AH, Wolffenbuttel BHR. The influence of needle length on glycemic control and patient preference in obese diabetic patients. Diabetes. 2009;**58**:A117

[22] Schwartz S, Hassman D, Shelmet J, et al. A multicenter, open-label, randomized, two-period crossover trial comparing glycemic control, satisfaction, and preference achieved with a 31 gauge × 6 mm needle versus a 29 gauge × 12.7 mm needle in obese patients with diabetes mellitus. Clinical Therapeutics. 2004;**26**(10):1663-1678

[23] Ross SA, Jamal R, Leiter LA, et al. Evaluation of 8 mm insulin pen needles in people with type 1 and type 2 diabetes. Practical Diabetes International. 1999;**16**(5):145-148

[24] Tubiana-Rufi N, Belarbi N, Du Pasquier-Fediaevsky L, et al. Short needles (8 mm) reduce the risk of intramuscular injections in children with type 1 diabetes. Diabetes Care. 1999;**22**(10):1621-1625

[25] Strauss K, Hannet I, McGonigle J, et al. Ultra-short (5mm) insulin needles: Trial results and clinical recommendations. Practical Diabetes International. 1999;**16**(7):218-221

[26] Kreugel G, Keers JC, Kerstens MN, Wolffenbuttel BH. Randomized trial on the influence of the length of two insulin pen needles on glycemic control and patient preference in obese patients with diabetes. Diabetes Technology & Therapeutics. 2011;**13**(7):737-741

[27] Iwanaga M, Kamoi K. Patient perceptions of injection pain and anxiety: A comparison of Novo Fine 32-gauge tip 6 mm and micro fine plus 31-gauge 5 mm needles. Diabetes Technology & Therapeutics. 2009;**11**(2):81-86

[28] McKay M, Compion G, Lytzen L. A comparison of insulin injection needles on patients' perceptions of pain, handling, and acceptability: A randomized, open-label, cross-over study in subjects with diabetes. Diabetes Technology & Therapeutics. 2009;**11**(3): 195-201

[29] Miwa T, Itoh R, Kobayashi T, Tanabe T, Shikuma J, Takahashi T, Odawara M. Comparison of the effects of a new 32-gauge × 4-mm pen needle and a 32-gauge × 6-mm pen needle on glycemic control, safety, and patient ratings in Japanese adults with diabetes. Diabetes Technology & Therapeutics. 2012 Dec;**14**(12):1084-1090

[30] Nagai Y, Ohshige T, Arai K, Kobayashi H, Sada Y, Ohmori S. Comparison between shorter straight and thinner microtapered insulin injection needles. Diabetes Technology & Therapeutics. 2013;**15**(7):550-555

[31] Hirose T, Ogihara T, Tozaka S, Kanderian S, Watada H. Identification and comparison of insulin pharmacokinetics injected with a new 4-mm needle vs 6- and 8-mm needles accounting for endogenous insulin and C-peptide secretion kinetics in non-diabetic adult males. Journal of Diabetes Investigation. 2013;**4**(3):287-296

[32] Birkebaek NH, Solvig J, Hansen B, Jorgensen C, Smedegaard J, Christiansen JS. A 4-mm needle reduces the risk of intramuscular injections without increasing backflow to skin surface in lean diabetic children and adults. Diabetes Care. 2008;**31**(9):e65

[33] Bergenstal RM et al. Safety and efficacy of insulin therapy delivered via a 4mm pen needle in obese patients with diabetes. Mayo Clinic Proceedings. 2015;**90**(3):329-338

[34] Koivisto VA, Felig P. Alterations in insulin absorption and in blood glucose control associated with varying insulin injection sites in diabetic patients. Annals of Internal Medicine. 1980;**92**(1):59-61

[35] Annersten M, Willman A. Performing subcutaneous injections: A literature review. Worldviews on Evidence-Based Nursing. 2005;**2**(3):122-130

[36] Vidal M, Colungo C, Jansà M. Actualización sobre técnicas y sistemas de administración de la insulina (I). Avances en Diabetologia. 2008;**24**(3):175-190

[37] Fleming D, Jacober SJ, Vanderberg M, Fitzgerald JT, Grunberger G. The safety of injecting insulin through clothing. Diabetes Care. 1997;**20**(3):244-247

[38] Bantle JP, Neal L, Frankamp LM. Effects of the anatomical region used for insulin injections on glycaemia in type 1 diabetes subjects. Diabetes Care. 1993;**16**(12):1592-1597

[39] Personal Communication: Anders Frid. Data on file: Novo Nordisk

[40] Frid A, Östman J, Linde B. Hypoglycemia risk during exercise after intramuscular injection of insulin in thigh in IDDM. Diabetes Care. 1990;**13**(5):473-477

[41] Vaag A, Handberg A, Laritzen M, et al. Variation in absorption of NPH insulin due to intramuscular injection. Diabetes Care. 1990;**13**(1):74-76

[42] Frid A, Linde B. Intraregional differences in the absorption of unmodified insulin from the abdominal wall. Diabetic Medicine. 1992;**9**(3):236-239

[43] Frid A, Linde B. Clinically important differences in insulin absorption from the abdomen in IDDM. Diabetes Research and Clinical Practice. 1993;**21**(2):137-141

[44] Zehrer C, Hansen R, Bantle J. Reducing blood glucose variability by use of abdominal insulin injection sites. The Diabetes Educator. 1985;**16**(6):474-477

[45] Henriksen JE, Djurhuus MS, Vaag A, et al. Impact of injection sites for soluble insulin on glycaemic control in type 1 (insulin-dependent) diabetic patients treated with a multiple insulin injection regimen. Diabetologia. 1993;**36**(8):752-758

[46] Sindelka G, Heinemann L, Berger M, Frenck W, Chantelau E. Effect of insulin concentration, subcutaneous fat thickness and skin temperature on subcutaneous insulin absorption in healthy subjects. Diabetologia. 1994;**37**(4):377-340

[47] Henriksen JE, Vaag A, Hansen IR, Lauritzen M, Djurhuus MS, Beck-Nielsen H. Absorption of NPH (isophane) insulin in resting diabetic patients; evidence for subcutaneous injection in the thigh as preferred site. Diabetic Medicine. 1991;**8**(5):453-457

[48] Kølendorf K, Bojsen J, Deckert T. Clinical factors influencing the absorption of 125 I-NPH insulin in diabetic patients. Hormone and Metabolic Research. 1983;**15**:274-278

[49] Mudaliar SR, Lindberg FA, Joyce M, et al. Insulin aspart (B28 asp-insulin): A fast-acting analog of human insulin: Absorption kinetics and action profile compared with regular human insulin in healthy nondiabetic subjects. Diabetes Care. 1999;**22**(9):1501-1506

[50] Rave K, Heise T, Weyer C, et al. Intramuscular versus subcutaneous injection of soluble and lispro insulin: Comparison of metabolic effects in healthy subjects. Diabetic Medicine. 1998;**15**(9):747-751

[51] Frid A. Fat thickness and insulin administration, what do we know? Infusystems Internationl. 2006;**5**(3):17-19

[52] Guerci B, Sauvanet JP. Subcutaneous insulin: Pharmacokinetic variability and glycemic variability. Diabetes & Metabolism. 2005;**31**(4):4S7-4S24

[53] Ter Braak EW, Woodworth JR, Bianchi R, et al. Injection site effects on the pharmacokinetics and glucodynamics of insulin lispro and regular insulin. Diabetes Care. 1996;**19**(12): 1437-1440

[54] Lippert WC, Wall EJ. Optimal intramuscular needle-penetration depth. Pediatrics. 2008;**122**(3):e556-e563

[55] Owens DR, Coates PA, Luzio SD, Tinbergen JP, Kurzhals R. Pharmacokinetics of 125I-labeled insulin glargine (HOE 901) in healthy men: Comparison with NPH insulin and the influence of different subcutaneous injection sites. Diabetes Care. 2000;**23**(6):813-819

[56] Byetta Pen User Manual. Eli Lilly and Company; 2007

[57] Calara F, Taylor K, Han J, et al. A randomized, open-label, crossover study examining the effect of injection site on bioavailability of exenatide (synthetic exendin-4). Clinical Therapeutics. 2005;**27**(2):210-215

[58] Gentile S, Strollo F. Subcutaneous nodules during treatment with an exenatide long-actin once-weekly formulation: An ultrasound evaluation. Diversity and Equality in Health and Care. 2016;**13**(4):313-318

[59] Blanco M, Hernández MT, Strauss KW, Amaya M. Prevalence and risk factors of lipohypertrophy in insulin-injecting patients with diabetes. Diabetes & Metabolism. 2013 Oct;**39**(5):445-453

[60] Grassi G, Scuntero P, Trepiccioni R, et al. Optimizing insulin injection technique and its effect on blood glucose control. Journal of Clinical & Translational Endocrinology. 2014;**1**(4):145-150. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/)

[61] Ji L, Li Q, Wei G. Lipohypertrophy - prevalence, risk factors and clinical characteristics of insulin-requiring patients in China. Abstract, EASD Vienna. 2014. Tracking Number: A-14-747

[62] Gentile S, Ceriello A, Strollo F. Insulin Shot Dependent Lipodystrophy: Evidence, Uncertainties and Current Terminology Overlaps. Journal of Diabetes, Metabolic Disorders & Control. 2016;**3**(3):00067. DOI: 10.15406/jdmdc.2016.03.00067

[63] Conwell LS, Pope E, Artiles AM, Mohanta A, Daneman A, Daneman D. Dermatological complications of continuous subcutaneous insulin infusion in children and adolescents. The Journal of Pediatrics. 2008;**152**:622-628

[64] Pickup J, Yemane N, Brackenridge A, Pender S. Nonmetabolic complications of continuous subcutaneous insulin infusion: A patient survey. Diabetes Technology & Therapeutics. 2014;**16**:145-149

[65] Vardar B, Kizilci S. Incidence of lipohypertrophy in diabetic patients and a study of influencing factors. Diabetes Research and Clinical Practice. 2007;**77**:231-236

[66] Saez-de Ibarra L, Gallego F. Factors related to lipohypertrophy in insulin-treated diabetic patients; role of educational intervention. Practical Diabetes International. 1998;**15**:9-11

[67] Raile K, Noelle V, Landgraf R, Schwarz HP. Insulin antibodies are associated with lipoatrophy but also with lipohypertrophy in children and adolescents with type 1 diabetes. Experimental and Clinical Endocrinology & Diabetes. 2001;**109**(8):393-396

[68] Sandro Gentile S, Strollo F, Ceriello A. Lipodistrophy and associated risk factors in insulin-treated people with diabetes. International Journal of Endocrinology Metabolism. 2016;**14**(2):e33997. DOI: 10.5812/ijem.33997 Published online 2016 Apr 26

[69] Young RJ, Hannan WJ, Frier BM, Steel JM, Duncan LJ. Diabetic lipohypertrophy delays insulin absorption. Diabetes Care. 1984;**7**:479-480

[70] Chowdhury TA, Escudier V. Poor glycaemic control caused by insulin induced lipohypertrophy. British Medical Journal. 2003;**327**:383-384

[71] Johansson UB. Impaired absorption of insulin aspart from lipohypertrophic injection sites. Diabetes Care. 2005;**28**:2025-2027

[72] Frid A, Linden B. Computed Tomography of Injection Sites in Patients with Diabetes Mellitus. Injection and Absorption of Insulin. Stockholm: Thesis. 1992

[73] Famulla S, Hövelmann U, Fische A, et al. Insulin injection into lipohypertrophic tissue: Blunted and more variable insulin absorption and action and impaired postprandial glucose control. Diabetes Care. 2016;**39**:1486-1492. DOI: 10.2337/dc16-0610

[74] Gentile S, Guarino G, Giancaterini A, Guida P, Strollo F, AMD-OSDI Italian Injection Technique Study Group. A suitable palpation technique allows to identify skin lipohypertrophic lesions in insulin-treated people with diabetes. Springerplus. 2016;**5**:563. DOI: 10.1186/s40064-016-1978-y eCollection 2016

[75] Gentile S, Strollo F, Guarino G, Giancaterini A, Ames PRJ, Speese K, Guida P, Strauss K, On behalf of the AMDOSDI Italian Injection Technique Study Group. Factors hindering correct identification of unapparent lipohypertrophy. Journal of Diabetes and Metabolic Disorders Control. 2016;**3**:00065. DOI: 10.15406/jdmdc.2016.03.00065

[76] Jansà M, Colungo C, Vidal M. Actualización sobre técnicas y sistemas de administración de la insulina (II). Avances en Diabetologia. 2008;**24**(4):255-269

[77] Diagrams courtesy of Lourdes Saez-de Ibarra and Ruth Gaspar, Diabetes Nurses and Specialist Educators from La Paz Hospital, Madrid, Spain

[78] Smith M, Clapham L, Strauss K. UK LIpohypertrophy intervention study. Diabetes Research and Clinical Practice. 2017;**126**:248-253

[79] Campinos C et al. An effective intervention for diabetic lipohypertrophy: Results of a randomised, controlled, prospective, multicentre study in France. Diabetes Technology & Therapeutics. 2017;**19**:623-632. DOI: 10.1089/dia.2017.0165 Epub 2017 Oct 23

[80] Misnikova I, Gubkina V, Lakeeva T, Dreval A. A randomized controlled trial to assess the impact of proper insulin injection technique training on glycemic control. Diabetes Therapy. 2017;**8**(6):1309-1318. DOI: 10.1007/s13300-017-0315-y Epub 2017 Oct 13

[81] De Coninck C, Frid A, Gaspar R, et al. Results and analysis of the 2008-2009 insulin injection technique questionnaire survey. Journal of Diabetes. 2010;**2**(3):168-179

[82] Strauss K, De Gols H, Hannet I, Partanen TM, Frid A. A pan-European epidemiologic study of insulin injection technique in patients with diabetes. Practical Diabetes International. 2002;**19**:71-76

[83] Hirsch L, Ji L, Sun Z, Li Q, et al. Lipohypertrophy – Prevalence, risk factors and clinical characteristics of insulin-requiring patients in China. DTT. 2015;**17**(Suppl 1):A57-A58

[84] Misnikova I, Dreval A, Gubkina V, Rusanova E. The risk of repeated use of insulin pen needles in patients with diabetes mellitus. Journal of Diabetology. 2011;**1**:1-5

[85] Puder J, Atar M, Muller B, Pavan M, Keller U. Using insulin pen needles up to five times does not affect needle tip shape nor increase pain intensity. Diabetes Research and Clinical Practice. 2005;**67**:119-123

[86] Schuler G, Pelz K, Kerp L. Is the reuse of needles for insulin injection systems associated with a higher risk of cutaneous complications? Diabetes Research and Clinical Practice. 1992;**16**:209-212

[87] Thomas DR, Fischer RG, Nicholas WC, Beghe C, Hatten KW, Thomas JN. Disposable insulin syringe reuse and aseptic practices in diabetic patients. Journal of General Internal Medicine. 1989;**4**:97-100

[88] Sonoki K, Yoshinari M, Iwase M, Tashiro K, Iino K, Wakisaka M, Fujishima M. Regurgitation of blood into insulin cartridges in the pen-like injectors. Diabetes Care. 2001;**24**(3): 603-604

[89] Floch JPL, Lange F, Herbreteau C, Perlemuter L. Biologic material in needles and cartridges after insulin injection with a pen in diabetic patients. Diabetes Care. 1998;**21**: 1502-1504

[90] Herdman M, Larck C, Hoppe Schliesser S, Jelic T. Biological contamination of insulin pens in a hospital setting. American Journal of Health-System Pharmacy. 2013;**70**: 1244-1248

[91] Jagger J et al. The impact of U.S. policies to protect healthcare workers from bloodborne pathogens: The critical role of safety-engineered devices. Journal of Infection and Public Health. 2008;**1**(2):62-71

[92] Bain A, Graham A. How do patients dispose of syringes? Practical Diabetes International. 1998;**15**(1):19-21

[93] Pickup JC et al. Continuous subcutaneous insulin infusion: An approach to achieving normoglycaemia. British Medical Journal. 1978;**1**(6107):204-207

[94] Dean Kamen, son of Mad Magazine cartoonist Jack Kamen, patented the Auto Syringe AS6C in 1977 (personal communication, Anders Frid, Dec. 12, 2017)

[95] Heinemann L, Krinelke L. Insulin infusion set: The Achilles heel of continuous subcutaneous insulin infusion. Journal of Diabetes Science and Technology. 2012;**6**(4):954-964

[96] Spollett G, Edelman SV, Mehner P, Walter C, Penfornis A. Improvement of insulin injection technique: Examination of current issues and recommendations. The Diabetes Educator. 2016;**42**(4):379-394. DOI: 10.1177/0145721716648017 Epub 2016 May 23

Chapter 5

Role of Insulin Resistance in Vascular Inflammation

Nicolás Federico Renna, Jésica Magalí Ramirez,
Rodrigo Damián Garcia and
Roberto Miguel Miatello

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77287

Abstract

Cardiovascular diseases (CVDs) such as ischemic heart disease (IHD), stroke, and peripheral artery disease (PAS) are the leading causes of mortality and morbidity around the world; about 30% of global deaths and 10% of global disease burden a year are due to CVDs. In this chapter, we will analyze the classic concepts of vascular remodeling, to later expand the concepts and physiopathological mechanisms of vascular inflammation. The role of immunomodulation from IL-6R alpha and the JAK/STAT3 intracellular cascade, will be proposed as an activator of vascular remodeling mechanisms. In addition, the role of new drugs such as LCZ696 and immunomodulators involved in the local inflammatory response will also be analyzed. The concept of remodeling and vascular inflammation, which a decade ago was only important at the level of basic research, step-by-step has proven crucial in the appearance of atherosclerosis, called subclinical atherosclerosis. Even though much progress has been made in the treatment and discovery of pathophysiological mechanisms, it has not been possible to reduce of cardiovascular risk, this is perhaps, it the decade in which we can advance in this.

Keywords: vascular remodeling, vascular inflammation, LCZ696, insulin resistance, IL-6R alpha

1. Introduction

Cardiovascular diseases (CVDs) such as ischemic heart disease (IHD), stroke, and peripheral artery disease (PAS) are the leading causes of mortality and morbidity around the world; about 30% of global deaths and 10% of global disease burden a year are due to CVDs [1, 2]. In the past three decades, these diseases have been increasing in underdeveloped and developing

countries. Although deaths from CVDs have declined in some developed countries with better healthcare interventions and systems and primary prevention, population growth and aging will drive up global CVDs in the coming decades [1, 2].

Vascular diseases, including atherosclerosis, media calcification, macrovascular expression of diabetes vascular disease, and microangiopathy, are very prevalent in these patients and are primary causes of death and disability in these individuals (see **Figure 1**) [3].

The nexus between microangiopathy and macroangiopathy is not yet fully explained, but it is much more important than a simple time line, although it could be said that microangiopathic pathology precedes macroangiopathy in 5–10 years in the natural history of the disease. Atherosclerosis occurs earlier in patients with diabetes, frequently with greater severity and a more diffuse distribution. Diabetes and metabolic syndrome are associated with vascular function abnormalities and ensuing morphological changes associated with vascular remodeling and atherosclerosis [4, 5].

One way to study vascular disease in diabetes mellitus is through experimental models in animals. One of them consists of feeding with carbohydrate-enriched diets to normal rats for induced metabolic syndrome [6, 7]. Fructose-fed rats (FFR) have been used to assess the pathophysiological mechanisms involved in the development of this syndrome [8]. This model has proven to be very interesting, since it allows the study of vascular changes, associated with metabolic syndrome, without the effects produced by hypercholesterolemia.

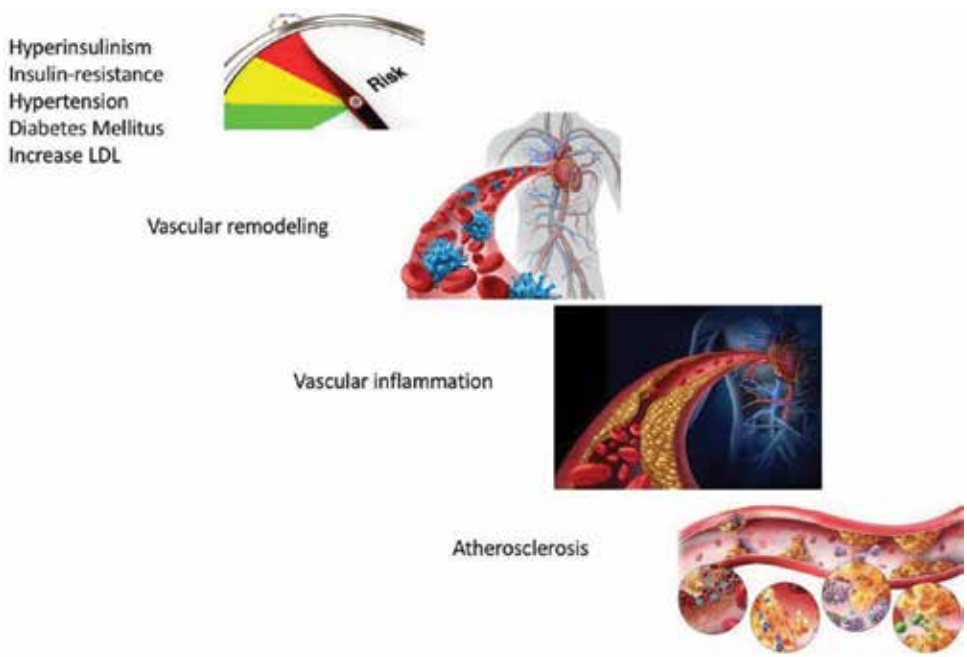

Figure 1. The evolution of vascular disease, with atherosclerosis being the final stage and vascular remodeling of the vascular disease manifested as vascular insufficiency.

http://dx.doi.org/10.5772/intechopen.77287

2. Physiopathological changes

2.1. Role of insulin

Recent studies have suggested that insulin and Ang II share a cross talk at multiple levels (**Figure 2**). Insulin signaling is initiated by binding to its receptor. The insulin receptor is a heterotetrameric tyrosine kinase that after binding insulin undergoes a rapid tyrosine autophosphorylation that activates the receptor kinase and allows transient interaction with IRS-1. Interaction of tyrosine-phosphorylated IRS-1 with PI3K results in PI3K activation and Akt phosphorylation, which stimulates translocation of Glut-4 to the sarcolemma to facilitate glucose uptake and NO production in the endothelium to induce vasorelaxation [9].

Ang II has been shown to inhibit the insulin-PI3K signaling pathway in both vascular and skeletal muscle cells. Ang II inhibits downstream signaling, including Akt phosphorylation, Glut-4 translocation to the sarcolemma, and NO production in the endothelium [9].

In the vasculature, insulin stimulates two major signaling transduction cascades: PI3K and MAPK. Insulin stimulation of NO production through activation of the PI3K pathway leads to vasodilation and increased blood flow and subsequent augmentation of glucose disposal in skeletal muscle. Insulin also stimulates the MAPK pathway, which mediates cellular growth and migration as well as production of prothrombotic and profibrotic factors [10].

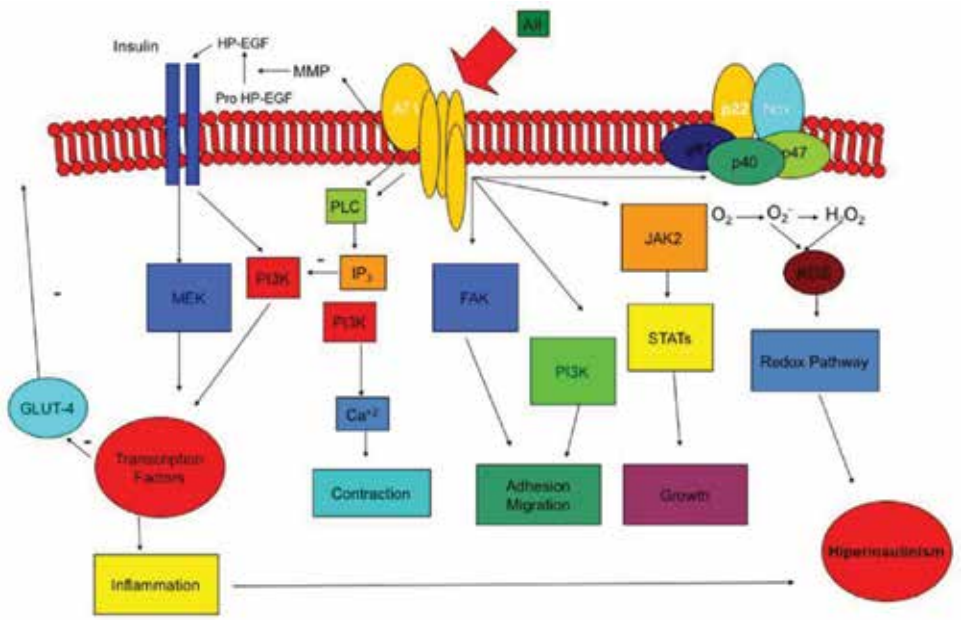

Figure 2. Insulin-Ang II relationship. Insulin signaling is initiated by binding to its receptor. The insulin receptor is a heterotetrameric tyrosine kinase that after binding insulin undergoes a rapid tyrosine autophosphorylation that activates the receptor kinase and allows transient interaction with IRS-1. Interaction of tyrosine-phosphorylated IRS-1 with PI3K results in PI3K activation and Akt phosphorylation, which stimulates translocation of Glut-4 to the sarcolemma to facilitate glucose uptake and NO production in the endothelium to induce vasorelaxation.

Fasting plasma insulin levels in a normal insulin-sensitive individual are usually in the low-picomolar range (50–150 pM). At this range, insulin constitutively stimulates the PI3K pathway, which participates in the regulation of the metabolic effects of insulin and maintenance of vascular tone [11].

In insulin-resistant states, such as obesity and diabetes, fasting insulin levels may reach the nanomolar range and are often associated with activation of RAAS. In addition, insulin stimulation of the PI3K pathway is selectively impaired.

2.2. Vascular changes

The spectrum of clinical and morphological changes that can be displayed has changed over time. One of the classifications we find is that proposed by author Gibbons [12]. These changes are shown predominantly in the relationship of light/medium vessel by changing the ratio of wall thickness by an increase in muscle mass or reorganization of the cellular and non-cellular components. These changes increase vascular reactivity, which promotes increased peripheral resistance in diseases such as hypertension. Another form of vascular remodeling involves primarily changes in the dimensions of the light. In this example, the restructuring of the active components of cellular and noncellular vascular wall results in significant changes in the dimensions of the vascular lumen, with relatively small changes in wall thickness. The clinical examples of this type include remodeling associated with vascular dilation of blood flow which is consistently high, for example, an arteriovenous fistula or loss in cellularity and proteolysis of extracellular matrix, resulting in the formation of an aneurysm. By contrast, a mass reduction vascular caliber results from a long-term reduction in blood flow. In fact, rarefaction of the microcirculation is another form of vascular remodeling. The architecture of the vascular wall is also markedly changed in response to vascular injury. Neointima is formed as part of a repair response to injury involving thrombosis, migration, and proliferation of vascular cells, production of the matrix, and infiltration of inflammatory cells.

The term “remodeling” is limited to situations in which there is a change in the lumen of a vessel relaxed, measured under a standard intravascular pressure, and where changes in the characteristics of the wall material (i.e., the wall stiffness) do not consider the change in the vascular lumen [13].

Chronic changes in hemodynamic forces produce structural alterations in the vascular wall, as stated above. Furthermore, hemodynamic changes are not the only production mechanisms of vascular remodeling [14], and the role of the inflammatory response and changes in matrix components have been suggested as mediators in this process of vascular adaptation [15].

To complete the above concept, the vascular wall remodeling is the result of changes in cellular and noncellular components, depending on the disease process causing the changes. Changes in growth and migration of VSMC, endothelial dysfunction, the inflammatory process, synthesis, or degradation of extracellular matrix components may be present in this process.

2.3. Vascular remodeling and inflammation

The traditional view of atherosclerosis as a lipid storage disease crumbles in front of the large and growing evidence that inflammation contributes to the center at all stages of the

disease, from initial injury until the final stage of thrombotic complications that compromise the bloodstream. Researchers now appreciate that the mere narrowing of the arterial lumen does not necessarily presage myocardial infarction and that simply treating narrowed blood vessels does not prolong life. Although invasive procedures such as angioplasty and coronary bypass will remain necessary in some cases, we now understand that medical treatment and lifestyle modification (diet and physical activity) produce benefits that may result from reductions in the processes inflammatory [16].

2.4. Initiation of atherosclerosis

It has been shown that atherosclerosis is not only a disease of lipid deposition but also a complex interaction between resident cells, inflammatory cells, and extracellular matrix, associated with a characteristic phenotypic change of macrophages to foam cells.

A key part of this interaction between the endothelium and the leukocytes is the vascular cell adhesion molecule-1 (VCAM-1). VCAM-1 binds to monocytes and T lymphocytes; these leukocytes are found in early atherosclerotic plaques. The most important stimuli for the membrane docking of this molecule are the nuclear factor kB (NF-kB) as well as the interleukin-1β (IL-1β) and the tumor necrosis factor (TNF-α) [10].

Cell adhesion molecules (CAM) are essential in the mediation of adhesion and transendothelial migration of leukocytes. In several murine models, the absence of CAM reduces atherogenesis [17]. We have demonstrated the presence of VCAM-1 in the endothelium in an experimental model of metabolic syndrome, in which the expression of this protein is familiar with the AT1 receptor (AT1R) and the local inflammatory process. High levels of ICAM-1 are predictive of cardiac events and are also independent cardiovascular risk factors [18]. This relationship was examined by Pradhan et al. [19], who showed that men with and without prior ischemic heart disease, accelerated atherogenesis, are associated with elevated levels of ICAM-1.

VCAM-1 is expressed in endothelial cells at sites predisposed to plaque formation [20]. By contrast, ICAM-1 is expressed throughout the plate; VCAM-1 is detected only in areas of rupture. In addition, VCAM-1 levels have a consistent association with atherosclerosis; high levels of VCAM-1 in the transcardiac gradient correlate with endothelial dysfunction and the progression of coronary atherosclerosis [21].

3. Pharmacology on vascular remodeling

3.1. New drugs

LCZ696 is the first of a new class of drugs that simultaneously block angiotensin 1 receptor blocker (ARB) and neprilysin or neutral endopeptidase protein (NEP); hence, they are referred to with the acronym ARNI [22].

This complex system results in multiple effects on the cardiovascular system. In the first instance, according to different experiments, LCZ696 can increase the half-life of BNP through the initiation of NEP, managing to increase natriuresis and vasodilation through activation

of the NPRA receptor. On the other hand, the blockade of AT1R can decrease fibrosis, induce vasodilation, reduce the retention of sodium and water, lower blood pressure, and other effects [23].

The most important clinical study that demonstrated the reduction of cardiovascular morbidity and mortality in patients with heart failure was the PARADIGM-HF study, which showed a clear benefit in patients who were in the branch receiving LCZ696. However, studies in experimental animals and pathophysiological analyses are scarce. Thus, the precise mechanism by which LCZ696 reduces cardiovascular mortality remains unclear. Some authors have proposed different hypotheses: (1) a sustained increase in natriuretic peptides by inhibition of NEP, (2) a direct hemodynamic effect that reduces stress on the left ventricular wall, (3) a reduction in arrhythmias and by a reduction in fibrosis or myocardial hypertrophy, and (4) an improvement of regional myocardial perfusion [4].

Previously, drug-intervention studies in this model have shown that some antihypertensive treatments, with candesartan, telmisartan, and losartan, not only lower blood pressure but also cause an improvement in redox balance and regression of structural changes in resistance arteries, although not a regeneration of the endothelium. In other words, reversing adverse effects associated with hypertension does not improve the normal structure and function of endothelial cells [8, 9].

Within the inflammatory cascades activated in this experimental model, we found that the determinant of the previously evidenced changes is that of the IL-6 receptor. Two different forms of IL-6 cell receptors have been described: an 80 kDa ligand-binding chain, known as IL-6R (IL-6Ra, CD126), and a 130 kDa signal transduction chain, gp130 (IL-6Rb, CD130). Gp130 is present in many places and situations; in contrast, IL-6R shows a more limited expression pattern [24].

In a recent publication of our group, it can be concluded that LCZ696 can reverse the changes associated with vascular remodeling, even more important than just blocking AT1R. The proposed pathway to demonstrate this finding was through the inhibition of the intracellular cascade of STAT3/JAK in intimate relation with IL-6R alpha, thus demonstrating an intrinsic anti-inflammatory effect. In addition, from the inhibition of STAT3, LCZ696 managed to significantly increase the amount of EPC at the vascular level, thus mediating endothelial repair [25].

The physiopathological mechanisms proposed in our work are summarized in **Figure 3**. The dual blocking of NEP/AT1R by LCZ696 could reduce the expression and phosphorylation of STAT3 through JAK, either by blocking AT1R, reducing oxidative stress, or controlling systolic blood pressure.

The reduction of STAT3 produced a decrease in the inflammatory transcription factors in the nucleus and a release of hsCRP in the blood circulation, which produces an increasing docking of the alpha subunit of IL-6R toward the membrane. Through this pathway, vascular remodeling and LVH were reduced because part of the growth factors and migration of muscle cells depend on the activation of the inflammatory cascade.

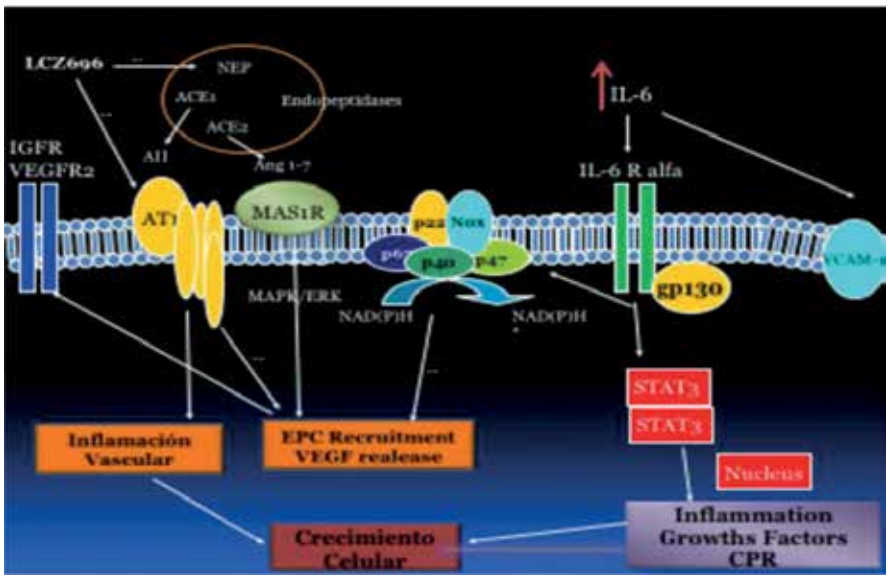

Figure 3. The phytopathological mechanisms proposed in our work are summarized in this image. The dual blocking of NEP/AT1R by LCZ696 could reduce the expression and phosphorylation of STAT3 through JAK, either by blocking AT1R, reducing oxidative stress, or controlling systolic blood pressure. The reduction of STAT3 produced a decrease in the inflammatory transcription factors in the nucleus and a release of hsCRP in the blood circulation, which produces an increasing docking of the alpha subunit of IL-6R toward the membrane. On the other hand, NEP and ACE2 probably induce the conversion of angiotensin II in different intermediate metabolites, such as angiotensin 1-7 (Ang 1-7), which produces antagonistic effects to angiotensin II by MAS1R. The intracellular cascade of MAS1R, by MAPK/ERK, produces a fundamental effect, namely, the production of VEGF and its two receptors: VEGFR1 and 2. From this mechanism, the endothelium could be repaired and/or replaced, favoring the maturation of circulating EPCs on resident EPCs at the endothelial level. MAS1R could be counter-regulated by IL-1ß.

On the other hand, NEP and ACE2 probably induce the conversion of angiotensin II in different intermediate metabolites, such as angiotensin 1-7 (Ang 1-7), which produces antagonistic effects to angiotensin II by MAS1R. The intracellular cascade of MAS1R, by MAPK/ERK, produces a fundamental effect, namely, the production of VEGF and its two receptors, VEGFR1 and VEGFR2. From this mechanism, the endothelium could be repaired and/or replaced, favoring the maturation of circulating EPCs on resident EPCs at the endothelial level. MAS1R could be counter-regulated by IL-1ß [26–28].

In conclusion, we postulate that LCZ696, by MAS1R activation, is not only able to improve endothelial function but also able to repair the endothelium, and this probably allows for improved functionality of the entire cardiovascular system. In addition, LCZ696 could reduce the expression of hsCRP through reduction in the expression of STAT3, a sign also demonstrated in different clinical studies such as JUPITER and CANTOS, which have allowed a great reduction in morbidity and mortality, revolutionizing modern cardiology. The anti-inflammatory and angio-repairing effect of LCZ696 is probably reflected in an improvement in the survival of patients who receive a treatment regimen with this drug in studies such as PARADIGM-HF [29–31].

3.2. Gliptin on vascular inflammation

Renna et al. suggest that incretin system dysfunction, as happens in patients with diabetes mellitus or metabolic syndrome, allows activation of inflammatory response in different levels. The consequence is the creation of a vascular microenvironment that is conducive to the creation, perpetuation, progression, and destabilization of vascular injury, with either a simple eutrophic mechanism of vascular remodeling or the generation of an atherosclerotic lesion [32].

Several mechanisms may underlie these results: (1) increase the circulating levels of GLP-1 [33]. The cardiovascular actions of GLP-1 may occur either directly through the GLP-1 receptor or through a GLP-1 receptor-independent effect of the degradation product of GLP-1 [38]; (2) DPP-IV also degrades GIP and potentially cytokines and certain chemokines (including stromal-derived factor 1-α). Thus, other substrates of DPP-IV may be responsible for the improvement in endothelial function. Alternatively, DDP-IV inhibition might improve endothelial function by influencing insulin and glucose levels. Insulin causes vasodilation by increasing endothelial production of NO [34].

The improvement in endothelial function and oxidative stress could result in a decrease in activation of the inflammatory process.

Other authors have suggested that the DDP-IV inhibitors may have anti-inflammatory effects, such as reduced activation of TNF-alpha during macrophage activation [33, 35].

4. Conclusion

There is sufficient evidence to show that insulin resistance and hyperinsulinism produce significant changes at the vascular level [7, 25, 32, 36, 37]. The proposed mechanisms are (1) the IGF-1 receptor, (2) through the coactivation between IG-1 and AT1R, (3) by activating nuclear transcription factors such as NF-KB or AP-1, (4) by dimerization of IL-6R, and (5) from the activation of oxidative cascades such as NADP (H) oxidase, peroxynitrites, or superoxide dismutase (SOD). However, the effects of hyperglycemia are more erratic: moderate hyperglycemia is sufficient to induce adverse structural changes in the mesenteric vasculature, but more severe hyperglycemia is essential to cause endothelial dysfunction.

It is more interesting that the blocking of these pathways has significant effects on the activation/deactivation of vascular remodeling, independent of the correction or not, of hyperinsulinism or insulin resistance. This shows that intracellular cascades, in most of these mechanisms, have no feedback from insulin or glucose receptors.

On the other hand, it is likely that the vascular remodeling associated with insulin resistance, due to the stimulation of growth factors, from the pathways, is due to changes in vascular hemodynamics or to the increase in peripheral resistances, as in the case of arterial hypertension.

The new drugs, which modify the inflammatory modulating response, such as tocilizumab (anti-IL-6R alpha) and canakinumab (anti-IL-1b), will be drugs that could further modify the cardiovascular risk of these patients in the future, since it could modify the vascular inflammatory

microenvironment, preventing vascular modeling and the subsequent formation of atheromatous plaques. Another pharmacological group that has gained importance in recent years is LCZ696, which, as several clinical studies have shown, modifies the cardiovascular morbidity and mortality of one of the most frequent pathologies of clinical practice, heart failure. However, new studies show that it is capable of producing effects in vascular repair, increasing the CPE at the vascular level, and avoiding vascular remodeling, even in experimental models with insulin resistance.

The concept of remodeling and vascular inflammation, which a decade ago was only important at the level of basic research, step-by-step has proven crucial in the appearance of atherosclerosis, called subclinical atherosclerosis. Much progress has been made in the treatment and discovery of pathophysiological mechanisms, rest improve the studies of deterrence, and its correlation with the reduction of cardiovascular risk; this is, perhaps, the decade in which we can advance in this.

Conflict of interest

The authors have no "conflict of interest" to declare.

Author details

Nicolás Federico Renna[1,2]*, Jésica Magalí Ramirez[3], Rodrigo Damián Garcia[1,2] and Roberto Miguel Miatello[1,2]

*Address all correspondence to: nicolasfede@gmail.com

1 Department of Pathology, School of Medicine, National University of Cuyo, Argentina

2 National Council of Scientific and Technical Research (IMBECU-CONICET), Argentina

3 Department of Cardiology, Spanish Hospital of Mendoza, Argentina

References

[1] Shanthi M, Pekka P, Bo N. Global Atlas on Cardiovascular Disease Prevention and Control. Geneva: World Health Organization; 2011

[2] Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. Heart disease and stroke statistics-2017 update: A report from the American Heart Association. Circulation. 2017;**135**:e146-e603

[3] Kaplan N. The deadly quartet hyperbody obesity, glucose intolerance, hypertriglyceridemia, and hypertension. Archives of Internal Medicine. 1989;**149**:1514-1520

[4] Reaven G, Laws A. Insulin resistance, compensatory hyperinsulinemia and coronary heart disease. Diabetologia. 1994;**37**:948-952

[5] de Champlain J, Wu R, Girouard H, Karas M, El Midaoui A, Laplante MA, Wu L. Oxidative stress in hypertension. Clinical and Experimental Hypertension. 2004;**26**:593-601

[6] Hwang I, Ho H, Hoffman B, Reaven G. Fructose-induced insulin resistance and hypertension in rats. Hypertension. 1987;**10**:512-516

[7] Miatello R, Risler N, Castro C, Gonzalez S, Rüttler M, Cruzado M. Aortic smooth muscle cell proliferation and endothelial nitric oxide synthase activity in fructose-fed rats. American Journal of Hypertension. 2001;**14**:1135-1141

[8] Bell R, Ryan E, Finegood D. Consequences of high dietary fructose in the islet-transplanted rat with suboptimal beta-cell mass. The American Journal of Physiology. 1996;**270**: E292-E298

[9] Andreozzi F, Laratta E, Sciacqua A, Perticone F, Sesti G. Angiotensin II impairs the insulin signaling pathway promoting production of nitric oxide by inducing phosphorylation of insulin receptor substrate-1 on Ser(312) and Ser(616) in human umbilical vein endothelial cells. Circulation Research. 2004;**94**:1211-1218

[10] Nigro J, Osman N, Dart AM, Little PJ. Insulin resistance and atherosclerosis. Endocrine Reviews. 2006;**27**:242-225

[11] Zhou MS, Schulman IH, Zeng Q. Link between the renin–angiotensin system and insulin resistance: Implications for cardiovascular disease. Vascular Medicine. Oct 2012;**17**(5): 330-341

[12] Gibbons GH, Dzau VJ. The emerging concept of vascular Remodeling. The New England Journal of Medicine. 1994;**330**(20):1431-1438

[13] Norrelund H, Christensen KL, Samani NJ, Kimber P, Mulvany MJ, Korsgaard N. Early narrowed afferent arteriole is a contributor to the development of hypertension. Hypertension. 1994;**24**:301-308

[14] Hacking WJ, VanBavel E, Spaan JA. Shear stress is not sufficient to control growth of vascular networks: A model study. American Journal of Physiology. Heart and Circulatory Physiology. 1996;**270**(1):H364-H375

[15] Pasterkamp G, Galis ZS, de Kleijn DPV. Expansive arterial remodeling: Location, location, location. Arteriosclerosis, Thrombosis, and Vascular Biology. 2004;**24**(4):650-657

[16] Libby P. Inflammation and cardiovascular disease mechanisms. The American Journal of Clinical Nutrition. 2006;**83**(2):456S-4460S

[17] Miller MA et al. Cellular adhesion molecules and blood pressure: Interaction with sex in a multi-ethnic population. Journal of Hypertension. 2004;**22**(4):705-711

[18] Ridker P, Hennekens C, Roitman-Johnson B. Plasma concentrations of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet. 1998;**351**:88-92

[19] Pradhan AD, Rifai N, Ridker PM. Soluble intercellular adhesion molecule-1, soluble vascular adhesion molecule-1, and the development of symptomatic peripheral arterial disease in men. Circulation. 2002;**106**(7):820-825

[20] Cook-Mills JM. VCAM-1 signals during lymphocyte migration: Role of reactive oxygen species. Molecular Immunology. 2002;**39**(9):499-508

[21] Ridker PM, Buring JE, Rifai N. Soluble P-selectin and the risk of future cardiovascular events. Circulation. 2001;**103**(4):491-495

[22] Braunwald E. The path to an angiotensin receptor antagonist-Neprilysin inhibitor in the treatment of heart failure. Journal of the American College of Cardiology. 2015;**65**(10): 1029-1041

[23] Desai AS, McMurray JJ, Packer M, et al. Effect of the angiotensin-receptor-neprilysin inhibitor LCZ696 compared with enalapril on mode of death in heart failure patients. European Heart Journal. 2015;**36**:1990-1997

[24] Kishimoto T. IL-6: From its discovery to clinical applications. International Immunology. 2010;**22**:347-352

[25] Garcia R, Ramirez J, Peral de Bruno M, Miatello R, Renna NF. Dual ARB/NEP Inhibition with LCZ696 improved endothelial regeneration in an experimental model of metabolic syndrome. Journal of Hypertensio. 2018 (in press)

[26] Hoffmann BR, Stodola TJ, Wagner JR, Didier DN, Exner EC, Lombard JH, Greene AS. Mechanisms of Mas1 receptor-mediated Signaling in the vascular endothelium. Arteriosclerosis, Thrombosis, and Vascular Biology. 2017;**37**:433-445

[27] Fraga-Silva RA, Da Silva DG, Montecucco F, Mach F, Stergiopulos N, da Silva RF, Santos RA. The angiotensin-converting enzyme 2/angiotensin-(1-7)/mas receptor axis: A potential target for treating thrombotic diseases. Thrombosis and Haemostasis. 2012;**108**:1089-1096

[28] Ridker PM. Closing the loop on inflammation and atherothrombosis: why perform the CIRT and CANTOS trials? Transactions of the American Clinical and Climatological Association. 2013;**124**:174-190

[29] Vidt DG, Ridker PM, Monyak JT, Schreiber MJ, Cressman MD. Longitudinal assessment of estimated glomerular filtration rate in apparently healthy adults: A post hoc analysis from the JUPITER study (justification for the use of statins in prevention: an intervention trial evaluating rosuvastatin). Clinical Therapeutics. Jun 2011;**33**(6):717-725

[30] Ridker P, Everett B, Thuren T, MacFadyen J, Chang W, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker S, et al, CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. New England Journal of Medicine. Sep 21, 2017; **377**:1119-1131

[31] Simpson J, Jhund PS, Silva Cardoso J, Martinez F, Mosterd A, Ramires F, Rizkala AR, Senni M, Squire I, Gong J, Lefkowitz MP, Shi VC, Desai AS, Rouleau JL, Swedberg K, Zile MR, McMurray JJV, Packer M, Solomon SD, PARADIGM-HF Investigators and Committees. Comparing LCZ696 with enalapril according to baseline risk using the MAGGIC and EMPHASIS-HF risk scores: An analysis of mortality and morbidity in PARADIGM-HF. Journal of the American College of Cardiology. Nov 10, 2015; **66**(19):2059-2071

[32] Renna NF, Diez E, Miatelo RM. Effects of dipeptidyl-peptidase 4 inhibitor about vascular inflammation in a metabolic syndrome model. PLoS One. Sep 3, 2014;**9**(9):e106563

[33] Kahlberg N, Qin CX, Anthonisz J, Jap E, Ng HH, Jelinic M, Parry LJ, Kemp-Harper BK, Ritchie RH, Leo CH. Adverse vascular remodeling is more sensitive than endothelial dysfunction to hyperglycemia in diabetic rat mesenteric arteries. Pharmacological Research. Sep 2016;**111**:325-335

[34] Shah Z, Kampfrath T, Deiuliis JA, Zhong J, Pineda C, et al. Long-term dipeptidyl-peptidase 4 inhibition reduces atherosclerosis and inflammation via effects on monocyte recruitment and chemotaxis. Circulation. 2011;**124**:2338-2349

[35] Ussher JR, Drucker DJ. Cardiovascular biology of the incretin system. Endocrine Reviews. 2012;**33**:187-215

[36] Renna NF, Lembo C, Diez E, Miatello RM. Role of renin-angiotensin system and oxidative stress on vascular inflammation in insulin resistance model. International Journal of Hypertension. 2013;**2013**:420979

[37] Renna NF. Oxidative stress, vascular remodeling, and vascular inflammation in hypertension. International Journal of Hypertension. 2013;**2013**:710136

[38] Nauck M, Meier J, Cavender M, Abd El Aziz M, Drucker D. Cardiovascular Actions and Clinical Outcomes With Glucagon-Like Peptide-1 Receptor Agonists and Dipeptidyl Peptidase-4 Inhibitors. Circulation. 2017;**136**:849-870. DOI: 10.1161/CIRCULATIONAHA.117.028136

Chapter 6

Biomarkers in Metabolic Syndrome

Alexandru Zlibut, Lucia Agoston-Coldea, Teodora Mocan, Ioana Corina Bocsan and Lucian Mocan

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79427

Abstract

Nowadays, biomarkers are useful in the early detection and risk stratification of metabolic syndrome (MetS) patients. Studies confirmed the implication of adipokines, neuropeptides, inflammatory cytokines, prothrombotic factors, and others in MetS pathogenesis. Leptin:adiponectin ratio is useful in predicting insulin resistance and MetS severity; leptin is correlated with obesity and waist size and adiponectin is inversely related with MetS components. Ghrelin is inversely correlated with MetS components, and studies confirmed its role in MetS prediction. Regarding the pro-inflammatory cytokines, studies confirmed that interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha are positively correlated with hypertriglyceridemia, hypertension, fasting glucose levels, insulin resistance, and in postmenopausal women with central obesity. Oxidized low-density lipoprotein (LDL) levels could be implicated in insulin resistance. Recent studies also confirmed that novel biomarkers such as pentraxin-3 are positively correlated with MetS severity and the presence of vascular lesions, and it could bring new data on the MetS mechanism. Within this chapter, we review data on the contribution of biomarkers as well as on the stratification of MetS patients, discussing their key contribution for creating a risk assessment algorithm.

Keywords: metabolic syndrome, biomarkers, cytokines, obesity, insulin resistance, leptin, adiponectin, ghrelin, pentraxin-3, paraoxonase, interleukins

1. Introduction

Metabolic syndrome (MetS) is a cluster of cardiovascular risk factors with a reported prevalence of 20–25% in general population [1] and also with an increased two-fold risk to develop

cardiovascular disease [2]. Recent studies have shown that, being involved in MetS pathogenesis, some adipokines, neuropeptides, inflammatory cytokines, prothrombotic factors, and others could be used in diagnosing and monitoring these patients.

Various studies confirmed that the leptin:adiponectin ratio (LAR) could have a superior predictive power in determining insulin resistance and MetS severity than the use of leptin or adiponectin alone [3]. Leptin is positively correlated with obesity and waist size [4–8]. Adiponectin has important physiological functions in maintaining metabolic balance and is inversely related with MetS components independently of body mass index (BMI) [7, 9].

Ghrelin is inversely correlated with MetS components, and studies confirmed its role in MetS prediction. Also, a positive correlation of ghrelin levels with hypertension, insulin resistance, and obesity has been found [10–16].

Regarding the pro-inflammatory cytokines, studies confirmed that interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) are positively correlated with hypertriglyceridemia, hypertension, fasting glucose levels, insulin resistance, and in postmenopausal women with central obesity [17, 18, 19–25]. Oxidized low-density lipoprotein (LDL) levels have been found to be correlated with insulin resistance, hyperinsulinemia, impaired glycemic control, and excessive adipose tissue and could predict the occurrence of MetS [26–28].

Recent studies also confirmed that novel biomarkers such as pentraxin-3 are positively correlated with MetS severity and the presence of vascular lesions, and it could bring new data on the MetS mechanism. Also, pentraxin-3 (PTX3) was found to be correlated with low high-density lipoprotein (HDL) cholesterol levels and high triglycerides [29–31].

Paraoxonase-1 (PON-1) was inversely correlated with the presence of MetS, more precisely with central obesity, hypertriglyceridemia, and hypertension [32–35]. Interleukin-10 (IL-10) is an anti-inflammatory cytokine, and decreased levels of IL-10 are associated with insulin resistance and the presence of MetS [36–39].

2. Leptin, adiponectin, and leptin:adiponectin ratio

2.1. Leptin

Leptin is a hormone produced mainly by white adipose tissue, but also by non-adipose ones (placenta, stomach, mammary gland, and immune system) [40, 41]. Its regulation is achieved through various factors dependable on the metabolic status (**Figure 1**). Thus, the implications of leptin in pathogenic mechanisms comprise energy homeostasis, obesity syndromes, metabolic dysfunctionalities, neuroendocrine function, and bone metabolism. The pathogenic pathways of leptin follow similar targets through different mechanisms [40]. Leptin binds to its functional receptor and activates several transduction pathways, such as Janus kinase (JAK)/signal transducers and activators of transcription (determines autophosphorylation of JAK1 and JAK2 with STAT3 activation), mitogen-activated protein kinase (activates this MAPK pathway in central and peripheral tissues), phosphatidylinositol-4,5-bisphosphate

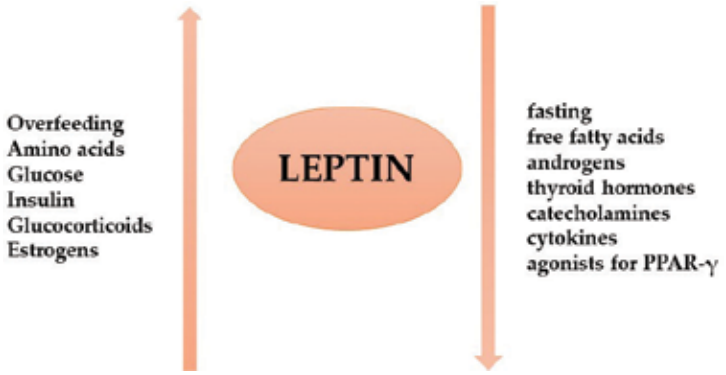

Figure 1. Factors that regulate leptin plasmatic levels.

3-kinase/protein kinase B (leptin activates directly PI3K in peripheral tissue), and AMP-activated protein kinase [42, 43].

Since its discovery, many studies have focused on the role of leptin in the evaluation of cardiovascular risk. High levels of leptin lead to a global and/or selective leptin resistance. MetS is a condition that favors leptin resistance through systemic inflammation, insulin resistance, hyperlipidemia, hypertension, atherosclerosis, and obesity [44]. Leptin levels correlate mainly with obesity and waist circumference, as it has been confirmed in numerous studies, the aspects of which are detailed in **Table 1** [4–8].

2.2. Adiponectin

Adiponectin is a protein hormone produced exclusively by adipocytes. Its high-molecular weight form is proved to have the most intense metabolic activity. Circulating levels of adiponectin are higher in females than in males due to the stimulating activity of testosterone on adiponectin secretion [45]. It plays an important role in metabolic balance, and its lower levels are correlated with an increased cardiac, vascular, and metabolic risk.

Study	Year	Subjects	Leptin and MetS
García-Jiménez et al.	2014	204	Leptin is strongly correlated with BMI; plasma leptin concentration is proportional to the degree of central obesity causing leptin resistance
Yoshinaga et al.	2008	321	Leptin was the most sensitive marker for predicting MetS in elementary school children
Lee et al.	2012	153	Elevated leptin in MetS women in postmenopausal
Gannage-Yared et al.	2006	153	Leptin was strongly correlated with waist size in Lebanese population
Yun et al.	2010	9995	Serum leptin levels increased as the components of MetS, thus reduction of leptin levels may be protective

Table 1. Leptin correlations with metabolic syndrome.

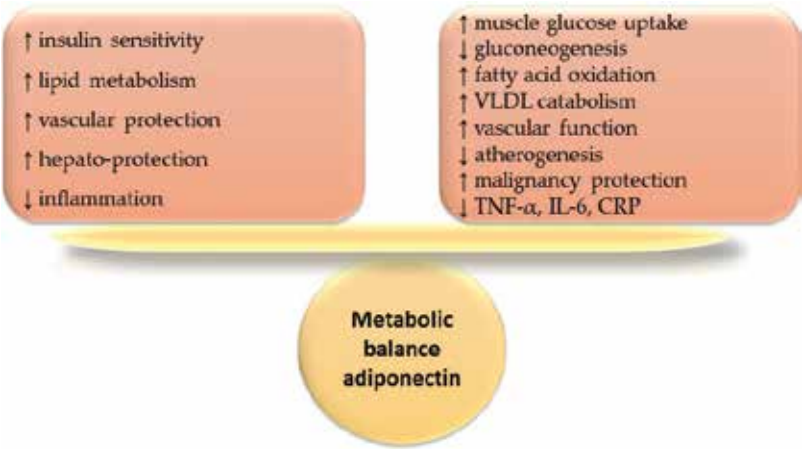

Figure 2. Metabolic balance mediated by adiponectin.

In normal subjects, adiponectin has important physiological functions in maintaining the metabolic balance (**Figure 2**); therefore, in patients with MetS, adiponectin levels are decreased [43]. Numerous studies have demonstrated its positive effect on metabolic protection, mainly based on its potentially inhibitory activity on the atherogenic process [46]. Recent studies have shown that adiponectin is inversely correlated with MetS components and that it has benefic effects on metabolic disorders [47]. Hypoadiponectinemia induced by visceral obesity determines vascular changes and insulin resistance. Likewise, two clinical studies conducted by Gannage-Yared et al. and by Santaneimi et al. have demonstrated the correlation of adiponectin with MetS independent of BMI [7, 9].

2.3. Leptin:adiponectin ratio

Various studies recommend using the leptin:adiponectin ratio (LAR) due to its increased predictive power, despite determining leptin and/or adiponectin alone. Recent data suggest the fact that leptin and adiponectin are two molecules that possess antagonistic effects. In addition, the study by Thorand et al. has been suggested that leptin and adiponectin interact with each other in order to modulate the risk of diabetes [3]. Therefore, Finucane et al. have demonstrated that LAR is a useful marker of insulin resistance in non-diabetic adults [48]. Lopez-Jaramillo et al. have emphasized the use of LAR in the evaluation of insulin resistance, and Kotani et al. have confirmed the predictive value of LAR in Japanese patients with MetS; other studies have also shown the correlation between LAR with all five MetS components [49–51].

3. Ghrelin

3.1. Generalities

Ghrelin is a peptide hormone produced in the gastrointestinal tract, and it has an important role in regulating the use of energy in human organism. Ghrelin undergoes posttranslational changes resulting in two circulating forms: unacylated ghrelin (UAG) and acylated ghrelin (AG) [51].

This hormone acts directly on hypothalamus and indirectly by increasing the expression of orexigenic peptides such as neuropeptide Y, Agouti-related protein, proopiomelanocortin, and corticotropin-releasing hormone [52].

In addition to its effect on hunger, ghrelin has important effects on glucose homeostasis, energy homeostasis, heart, muscular atrophy, bone metabolism, and tumors [53]. Recent studies emphasize that AG excess is correlated with insulin resistance and metabolic alterations; thereby, the AG/UAG ratio could play a role in the development of MetS [54].

3.2. Ghrelin and metabolic syndrome

Ghrelin is inversely associated with MetS components, and progressively lower ghrelin levels are being correlated with its severity. Ukkola O et al. emphasized the correlation of low ghrelin levels in obese patients with metabolic syndrome [55]. Also, the positive correlation of ghrelin levels with hypertension, insulin resistance, and obesity has been confirmed by numerous studies. McLaughlin et al. have concluded that ghrelin correlates with MetS mainly based on obesity as well as they identified lower ghrelin levels in patients with MetS and obesity than in non-obese MetS patients [10]. Likewise, many studies confirm the relation between MetS and ghrelin [11–16].

4. Interleukin-6

4.1. Interleukin-6 and inflammation response

IL-6 is a human cytokine that plays important roles in acute and chronic inflammation, immune cell development, and the pathogenesis of autoimmune disease. It is known that the increased activity of IL-6 gene is associated with an elevated risk of developing diabetes mellitus [56]. Likewise, IL-6 is linked with all the components of the inner immunity and yields a pro-inflammatory effects explained by different pathways **(Figure 3)**. Nevertheless,

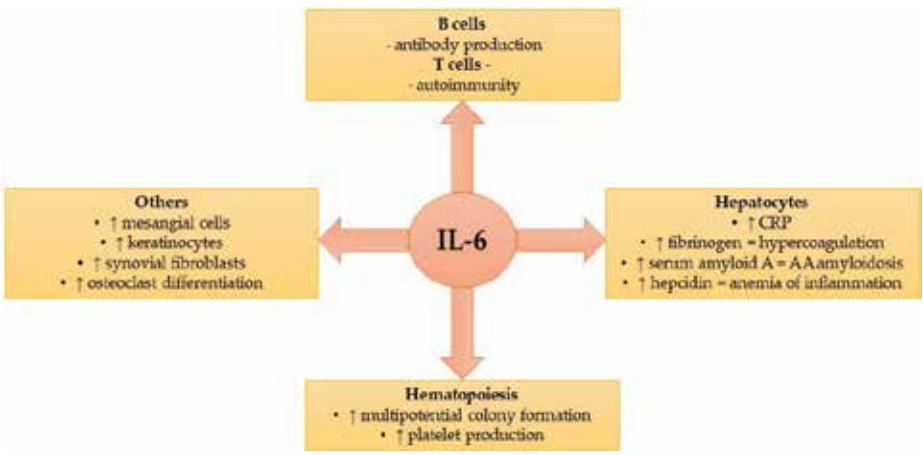

Figure 3. Inflammation pathways that involve IL-6.

studies confirmed that IL-6 also controls processes involved in the resolution of inflammation, emphasizing its anti-inflammatory function [57].

4.2. Interleukin-6 in metabolic syndrome

Studies confirmed that IL-6 is correlated with all five of MetS components. The main explanation relies on the fact that the dysfunctional adipose tissue induces macrophagic proliferation with increased IL-6 production [58]. Weiss et al. have found that IL-6 is associated with hypertriglyceridemia, fasting plasma glucose, and hypertension [59]. The same results are confirmed by Sarbijani et al. [17]. They also reported that increasing levels of IL-6 are correlated with MetS severity [17, 59]. Also, Chedraui et al. found increased levels of IL-6 in women with abdominal obesity, lower levels of HDL-C, and hypertriglyceridemia [18]. Another study demonstrated that high IL-6 levels within hepatocytes in a state of chronic inflammation could be a determining cause of MetS development [60].

5. Tumoral necrosis factor-alpha

5.1. Tumoral necrosis factor-alpha in human metabolism

TNF-α is an inflammatory cytokine mainly produced by macrophage cells, but also by other type of inflammatory cells. Among its many roles, TNF-α is an acute inflammatory response protein, which increases C-reactive protein levels and also determines insulin resistance by interacting with insulin receptor [18]. TNF-α plays important roles in regulating lipid metabolism **(Figure 4),** cholesterol metabolism, and adipokine synthesis [61].

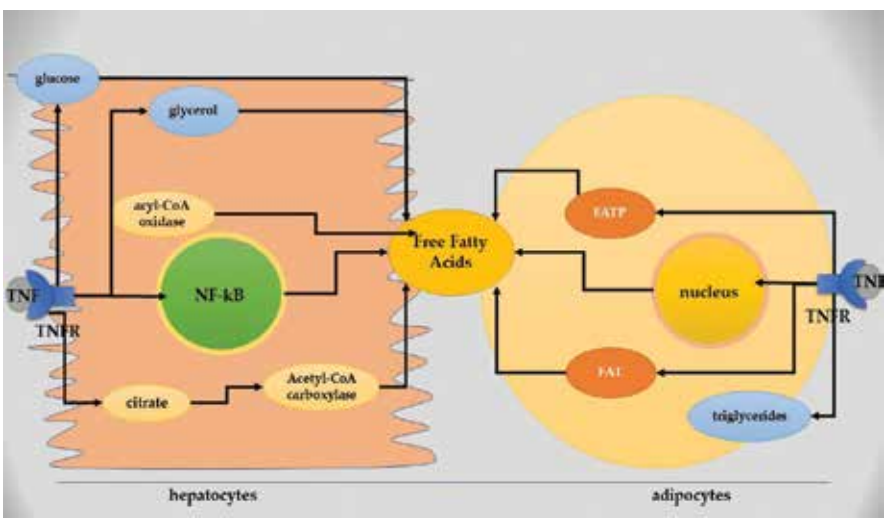

Figure 4. Effects of TNF-α production of free fatty acids in hepatocytes and adipocytes.

http://dx.doi.org/10.5772/intechopen.79427

5.2. Tumoral necrosis factor-alpha and metabolic syndrome

TNF-α can be produced by inflammatory cells from the dysfunctional adipose tissue, similar to IL-6. TNF-α is involved in numerous MetS pathways and alterations, in insulin resistance through similar mechanism of mTOR and protein C kinase activation and systemic inflammation [62]. As many studies have shown, TNF-α is being associated with all MetS components.

In the study by Moon et al. on obese adolescents, it was confirmed that TNF-α had higher levels in obese patients, even higher in male subjects, also, TNF-α positively correlated with BMI and waist circumference. Initially, TNF-α correlated positively with triglyceride levels and diastolic blood pressure, and inversely with HDL cholesterol, but after adjustment for BMI and waist circumference, only the association with triglyceride levels persisted [19].

In the meta-analysis of Sookoian et al. conducted on 16 homogeneous studies, it has been shown that obesity, systolic blood pressure, and serum insulin levels positively correlate with TNF-α -308A gene (genetic polymorphism that influences the plasmatic level of cytokine) variant and determine a 23% increased risk to develop MetS [20].

Obesity induces a systemic inflammatory status that determines dysfunctions of the macrophages and adipocytes and inappropriate cytokine production [21]. As a result, higher levels of TNF-α determine insulin resistance through various mechanisms and promote disease progression in patients with MetS **(Figure 5).** Studies emphasize that insulin resistance caused by TNF-α is based on abnormal insulin signaling, overexpression of tissular and plasmatic levels of TNF-α in subjects with insulin resistance, and administration of TNF-α determines and TNF-α neutralization improves insulin resistance [22–25]. Therefore, TNF-α is involved in MetS pathogenesis and progression and could be used in determining patients with MetS.

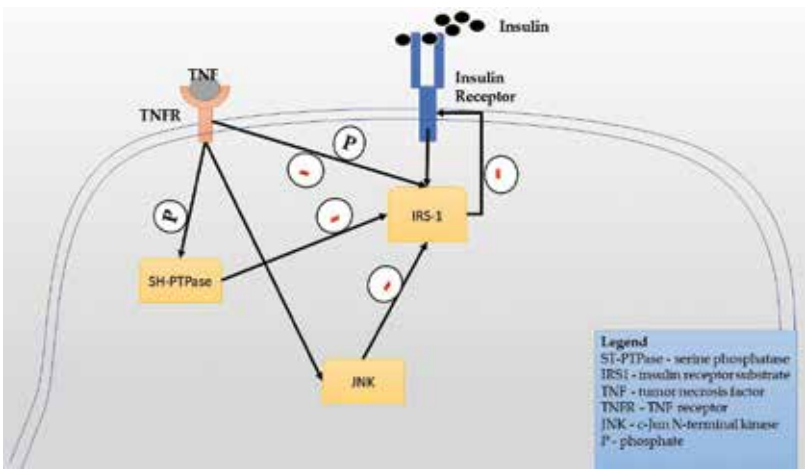

Figure 5. TNF-α and insulin resistance.

6. Oxidized low-density lipoproteins

6.1. Pathogenesis of oxidized LDL

In human organism, LDL particles undergo a series of oxidation processes, resulting in reactive oxygen species (ROS) and oxidized LDL (Ox-LDL) particles. These products create negative electric charges that will cause macrophagic stimulation and inflammation.

During LDL oxidation process, a series of products are generated: fatty acid oxidation products, lipid-derived products, protein oxidation products **(Figure 5)** [63].

Lara-Guzman et al. have shown that THP-1 human macrophage exposure to Ox-LDL caused a series of changes, such as an increased intake of Ox-LDL, overexpression of its receptors, and ROS production. Likewise, in the same study, it has been demonstrated that Ox-LDL determines the synthesis of isoprostanes as oxidation markers and of prostaglandines and prostaglandine metabolites as inflammation markers. Therefore, this study emphasizes that Ox-LDL links oxidative stress with inflammation via macrophages, resulting in systemic and local consequences [64]. Besides that, Schwarz et al. demonstrated that Ox-LDL increases Jun activation domain-binding protein-1 and stimulates inflammatory signaling in macrophages [65].

6.2. Oxidized LDL and endothelial dysfunction

Atherosclerosis represents one of the main alterations caused by MetS, and endothelial dysfunction is the earliest event within it. As mentioned earlier, Ox-LDL triggers inflammation and oxidation process that determines macrophagic activation and ROS production with cytotoxic effect on vascular endothelium [66].

Ox-LDL interacts with lectin-type oxidized LDL receptor 1 (LOX-1) from the surface of endothelial cells and determines their activation [67]. Withal, Ox-LDL causes endothelial

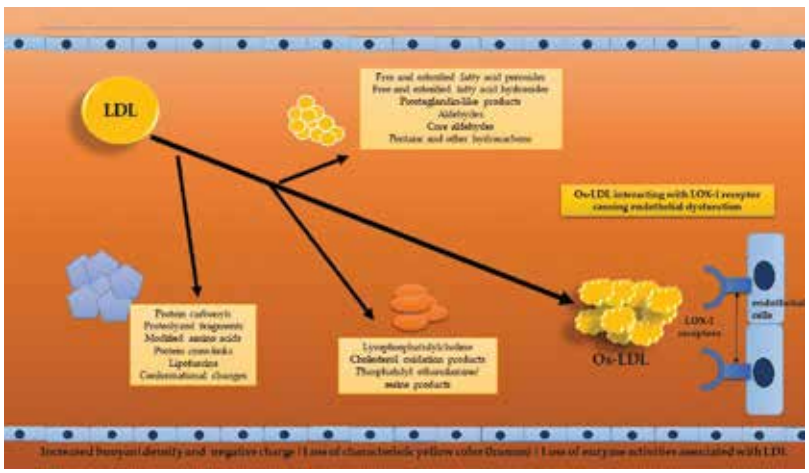

Figure 6. LDL oxidation products.

dysfunction by increasing endothelial adhesivity, by recruiting inflammatory cells into the endothelial wall, and by reducing nitric oxide production **(Figure 6)** [68, 69].

6.3. Oxidized LDL and metabolic syndrome

Various studies have shown that Ox-LDL levels are associated with MetS. Holvoet et al. demonstrated that patients with MetS had higher Ox-LDL values. They also reported that hyperinsulinemia and impaired glycemic control were associated with increased Ox-LDL levels, independent from lipid levels. The same research found that elevated Ox-LDL levels could predict the development of MetS in future [26].

Hurtado-Roca et al. in a study conducted on 3987 subjects demonstrated that Ox-LDL levels are positively correlated with MetS and its components even after adjustments for central obesity and insulin resistance. The strongest association was with triglyceride levels [27]. Another study conducted on overweighted/obese children showed that Ox-LDL positively correlated with BMI, percent body fat, waist circumference, percent trunk fat, abdominal visceral fat, abdominal subcutaneous fat (all p-values <0.0001), and with insulin resistance [28].

7. Pentraxin-3

7.1. The role of pentraxins in human organism

Pentraxins are a cluster of seric proteins with similar structures and calcium-dependent ligands that play important roles in body protection and in inflammatory mediation. The main mechanism is based on complement activation and interaction with Fc receptors [70].

PTX3 is being produced by immune cells as a response to bacterial substances, endotoxins, IL-1, and TNF-alpha. PTX3 is an acute phase protein with very low serum levels. PTX3 levels rise rapidly as a response to diverse inflammation stimuli. Therefore, PTX3 is considered to be a marker of local and general inflammatory and immune response [71–73].

7.2. Pentraxin-3 and metabolic syndrome

Recently, it has been shown that increased PTX3 levels are associated with MetS development and progression. In a study conducted on adolescent subjects with obesity, Kardas et al. have shown that subjects with obesity and MetS had higher values of PTX3 than the subjects without MetS. They also observed that low HDL cholesterol and high triglyceride levels were associated with increased PTX3 levels [29]. Also, Zanetti et al. demonstrated that PTX3 was higher in patients with MetS and subclinical atherosclerosis and that PTX3 was independently correlated with low HDL cholesterol levels [30]. Furthermore, a recent study found that PTX3 correlates with the severity of MetS, more precisely, after multivariate analysis PTX3 correlation persisted for glucose level ($\beta = 0.23$, $p < 0.001$), waist circumference ($\beta = 0.37$, $p < 0.001$), and HDL cholesterol ($\beta = -0.31$, $p < 001$) [31]. In conclusion, PTX3 could be a valuable biomarker in the prediction of MetS, but further studies should be conducted.

8. Paraoxonase

8.1. Paraoxonase-1

PON-1 is an enzyme produced mostly by the liver that protects against lipid oxidation and exogenous toxics. PON-1 extends the lag phase of the oxidation process and reduces the aldehyde concentration, resulting in protective effects on LDL and HDL molecules [74]. Aharoni et al. in a murinic study demonstrated that PON-1 interacts with macrophages scavenger receptor class B type I, thus inhibiting IL-6 and TNF-α production and promoting PON-1 anti-inflammatory effects [75].

The anti-inflammatory role of PON-1 is mainly validated by its anti-atherogenic effect [32]. Likewise, in the study of Ikhlef et al., it has been found that PON-1 could regulate cholesterol homeostasis by stimulating cholesterol efflux via HDL and by potentiating inverse cholesterol transport [33]. On the contrary, in subjects with diabetes, it is assumed that PON-1 becomes malfunctional by excessive glycation, thus it lowers its protective effects and potentiates the atherosclerotic lesion [34].

8.2. Paraoxonase 1 and metabolic syndrome

PON-1 has scientifically confirmed to be connected with MetS. A cross-sectional study conducted on 354 Caucasian subjects with MetS has shown that PON-1 activity was significantly lower among patients who met all five MetS criteria ($p < 0.05$). The same study revealed that lower levels of HDL cholesterol and ApoA1 decrease the PON-1 activity [35]. A like, in a study conducted on 2404 subjects with MetS criteria, it has been demonstrated that PON-1 activity followed a downward trend with increasing MetS components and increasing lipid peroxides [76]. In conclusion, it is assumed that PON-1 through its antioxidant and anti-inflammatory effects could have important roles in lowering of the progression of MetS.

9. Interleukin-10

9.1. Interleukin-10 and metabolic syndrome

IL-10 is a potent anti-inflammatory cytokine that modulates the immune response in order to prevent excessive activation and auto-damage [36]. Based on its properties, IL-10 plays important roles in modulating insulin resistance and atherosclerotic development and, in a cross-sectional study conducted on children and young adolescents, it has been found that plasmatic IL-10 levels were lower in overweight/obese children, and they concluded that IL-10 could be a marker of metabolic risk [37]. On the contrary, Esposito et al. found that IL-10 levels were lower in obese compared with normal weight women, but were lower in both groups that had MetS criteria [38]. Likewise, van Exel et al. found reduced plasmatic levels of IL-10 in patients with MetS and diabetes mellitus [39].

9.2. Interleukin-10 and adiponectin

MetS is characterized by low levels of both adiponectin and IL-10, and recent studies have been evaluating if there is any link between the two molecules. In a study conducted on 117 men, it has been found that IL-10 levels significantly correlated with adiponectin levels especially in patients with MetS, but the correlation was stronger in MetS patients who presented abdominal obesity [77]. Also, Wolf et al. demonstrated that adiponectin modulates human monocytes and macrophages in producing anti-inflammatory cytokines such as IL-10 and IL-1RA [78].

10. Conclusions

The combined use of biomarkers of MetS could increase the rate of an early diagnosis and could prevent the complications of this disease. Associated usage of these biomarkers would increase their predictive value. However, to be able to create a diagnosis algorithm, their cutoff value for the presence of MetS and the causes that would yield false results should be determined. Last but not least, the usefulness of these biomarkers could be extended into guiding pharmacological and non-pharmacological therapeutic interventions. Also, treatment efficiency could be monitored by determining these biomarkers dynamically.

Author details

Alexandru Zlibut[1], Lucia Agoston-Coldea[1]*, Teodora Mocan[2], Ioana Corina Bocsan[3] and Lucian Mocan[4]

*Address all correspondence to: luciacoldea@yahoo.com

1 2nd Department of Internal Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2 Department of Physiology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3 Department of Pharmacology, Toxicology and Clinical Pharmacology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

4 3rd Department of General Surgery, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

References

[1] Alberti KG, Zimmet P, Shaw J. Metabolic syndrome—A new world-wide definition. A consensus statement from the international diabetes federation. Diabetic Medicine. 2006;**23**:469-480

[2] Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;**120**: 1640-1645. DOI: 10.1161/ CIRCULATIONAHA.109.192644

[3] Thorand B, Zierer A, Baumert J, Meisinger C, Herder C, Koenig W. Associations between leptin and the leptin/adiponectin ratio and incident Type 2 diabetes in middle-aged men and women: Results from the MONICA/KORA Augsburg study 1984-2002. Diabetic Medicine. 2010;**27**:1004-1011. DOI: 10.1111/j.1464-5491.2010.03043.x

[4] García-Jiménez S, Bernal FG, Martínez MF, Monroy NA, Toledano JC, Meneses AA, et al. Serum leptin is associated with metabolic syndrome in obese Mexican subjects. Journal of Clinical Laboratory Analysis. 2015;**29**:5-9. DOI: 10.1002/jcla.21718

[5] Yoshinaga M, Sameshima K, Tanaka Y, Wada A, Hashiguchi J, Tahara H, et al. Adipokines and the prediction of the accumulation of cardiovascular risk factors or the presence of metabolic syndrome in elementary school children. Circulation Journal. 2008;**72**:1874-1878. DOI: 10.1253/circj.CJ-08-0180

[6] Lee SW, Jo HH, Kim MR, You YO, Kim JH. Association between metabolic syndrome and serum leptin levels in postmenopausal women. Journal of Obstetrics and Gynaecology. 2012;**32**:73-77. DOI: 10.3109/01443615.2011.618893

[7] Gannage-Yared MH, Khalife S, Semaan M, Fares F, Jambart S, Halaby G. Serum adiponectin and leptin levels in relation to the metabolic syndrome, androgenic profile and somatotropic axis in healthy non-diabetic elderly men. European Journal of Endocrinology. 2006;**155**:167-176. DOI: 10.1530/eje.1.02175

[8] Yun JE, Kimm H, Jo J, Jee SH. Serum leptin is associated with metabolic syndrome in obese and nonobese Korean populations. Metabolism. 2010;**59**:424-429. DOI: 10.1016/j.metabol.2009.08.012

[9] Santaniemi M, Kesaniemi YA, Ukkola O. Low plasma adiponectin concentration is an indicator of the metabolic syndrome. European Journal of Endocrinology. 2006;**155**:745-750. DOI: 10.1530/eje.1.02287

[10] McLaughlin T, Abbasi F, Lamendola C, Frayo RS, Cummings DE. Plasma ghrelin concentrations are decreased in insulin-resistant obese adults relative to equally obese insulin-sensitive controls. The Journal of Clinical Endocrinology and Metabolism. 2004;**89**:1630-1635. DOI: 10.1210/jc.2003-031572

[11] Chedraui P, Perez-Lopez FR, Escobar GS, Pallac G, Montt-Guevara M, Cecchi E, et al. Circulating leptin, resistin, adiponectin, visfatin, adipsin and ghrelin levels and insulin resistance in postmenopausal women with and without the metabolic syndrome. Maturitas. 2014;**79**:86-90. DOI: 10.1016/j.maturitas.2014.06.008

[12] Mora M, Adam V, Palomera E, Blesa S, Díaz G, Buquet X. Ghrelin gene variants influence on metabolic syndrome components in aged Spanish population. PLoS One. 2015;**10**:e0136931. DOI: 10.1371/journal.pone.0136931

[13] Tabak O, Gelişgen R, Cicekçi H, Senateş E, Erdenen F, Müderrisoğlu C. Circulating levels of adiponectin, orexin-A, ghrelin and the antioxidant paraoxonase-1 in metabolic syndrome. Minerva Medica. 2012;**103**:323-329

[14] Ahmed MB, Ismail MI1, Meki AR. Relation of osteoprotegerin, visfatin and ghrelin to metabolic syndrome in type 2 diabetic patients. International Journal of Health Sciences. 2015;**9**:127-139

[15] Cho HY, Lee SY, Jeong DW, Cho AR, Jeon JS, KIM YJ, et al. Metabolic syndrome is associated with lower plasma levels of desacyl ghrelin and total ghrelin in asymptomatic middle-aged Korean men. Journal of Obesity & Metabolic Syndrome. 2017;**26**:114-121. DOI: 10.7570/jomes.2017.26.2.114

[16] Langenberg C, Bergstrom J, Laughlin GA, Barrett-Connor E. Ghrelin, adiponectin, and leptin do not predict long-term changes in weight and body mass index in older adults: Longitudinal analysis of the Rancho Bernardo cohort. American Journal of Epidemiology. 2005;**162**:1189-1197. DOI: 10.1093/aje/kwi338

[17] Sarbijani HM, Khoshnia M, Marjani. The association between metabolic syndrome and serum levels of lipid peroxidation and interleukin-6 in Gorgan. Diabetes and Metabolic Syndrome: Clinical Research and Reviews. 2016;**10**:86-89. DOI: 10.1016/j.dsx.2015.09.024

[18] Chedraui P, Escobar GS, Pérez-López FR, Palla G, Montt-Guevara M, Cecchi E. Angiogenesis, inflammation and endothelial function in postmenopausal women screened for the metabolic syndrome. Maturitas. 2014;**77**:370-374. DOI: 10.1016/j.maturitas.2014.01.014

[19] Moon YS, Kim DH, Song DK. Serum tumor necrosis factor-alpha levels and components of the metabolic syndrome in obese adolescents. Metabolism. 2004;**53**:863-867. DOI: 10.1016/j.metabol.2004.02.007

[20] Sookoian SC, Gonzalez C, Pirola CJ. Meta-analysis on the G-308A tumor necrosis factor α gene variant and phenotypes associated with the metabolic syndrome. Obesity Research. 2005;**13**:2122-2131. DOI: 10.1038/oby.2005.263

[21] Wang B, Trayhurn P. Acute and prolonged effects of TNF-alpha on the expression and secretion of inflammation-related adipokines by human adipocytes differentiated in culture. Pflügers Archiv. 2006;**452**:418-427. DOI: 10.1007/s00424-006-0055-8

[22] Borst SE. The role of TNF-alpha in insulin resistance. Endocrine. 2004;**23**:177-182. DOI: 10.1385/ENDO:23:2-3:177

[23] Hossain M, Faruque MO, Kabir G, Hassan N, Sikdar D, Nahar Q, Ali L. Association of serum TNF-α and IL-6 with insulin secretion and insulin resistance in IFG and

IGT subjects in a Bangladeshi population. International Journal of Diabetes Mellitus. 2010;**2**:165-168. DOI: 10.1016/j.ijdm.2010.08.004

[24] Nieto-Vazquez I, Fernández-Veledo S, Krämer DK, Vila-Bedmar R, Garcia-Guerra L, Lorenzo M. Insulin resistance associated to obesity: The link TNF-alpha. Archives of Physiology and Biochemistry. 2008;**114**:183-194. DOI: 10.1080/13813450802181047

[25] Swaroop JJ, Rajarajeswari D, Naidu JN. Association of TNF-α with insulin resistance in type 2 diabetes mellitus. The Indian Journal of Medical Research. 2012;**135**:127-130. DOI: 10.4103/0971-5916.93435

[26] Holvoet P, De Keyzer D, Jacobs DR. Oxidized LDL and the metabolic syndrome. Future Lipidology. 2008;**3**:637-649. DOI: 10.2217/17460875.3.6.637

[27] Hurtado-Roca Y, Bueno H, Fernandez-Ortiz A, Ordovas JM, Ibanez B, Fuster V, et al. Oxidized LDL is associated with metabolic syndrome traits independently of central obesity and insulin resistance. Diabetes. 2017;**66**:474-482. DOI: 10.2337/db16-0933

[28] Kelly AS, Jacobs DR, Sinaiko AR, Moran A, Steffen LM, Steinberger J. Relation of circulating oxidized LDL to obesity and insulin resistance in children. Pediatric Diabetes. 2010;**11**:552-555. DOI: 10.1111/j.1399-5448.2009.00640.x

[29] Kardas F, Akın L, Kurtoglu S, Kendirci M, Kardas Z. Plasma Pentraxin 3 as a biomarker of metabolic syndrome. Indian Journal of Pediatrics. 2015;**82**:35-38. DOI: 10.1007/s12098-014-1542-0

[30] Zanetti M, Bosutti A, Ferreira C, Vinci P, Biolo G, Fonda M. Circulating pentraxin 3 levels are higher in metabolic syndrome with subclinical atherosclerosis: Evidence for association with atherogenic lipid profile. Clinical and Experimental Medicine. 2009;**9**:243-248

[31] Karakas MF, Buyukkaya E, Kurt M, Motor S, Akcay AB, Karakas E, et al. Serum Pentraxin-3 levels are associated with the severity of metabolic syndrome. Medical Principles and Practice. 2013;**22**:274-279. DOI: 10.1159/000343904

[32] Litvinov D, Mahini H, Garelnabi M. Antioxidant and anti-inflammatory role of Paraoxonase 1: Implication in arteriosclerosis diseases. North American Journal of Medical Sciences. 2012;**4**:523-532. DOI: 10.4103/1947-2714.103310

[33] Ikhlef S, Berrougui H, Kamtchueng Simo O, Zerif E, Khalil A. Human paraoxonase 1 overexpression in mice stimulates HDL cholesterol efflux and reverse cholesterol transport. PLoS One. 2017;**12**:e0173385. DOI: 10.1371/journal.pone.0173385

[34] Mackness B, Mackness M. Anti-inflammatory properties of paraoxonase-1 in atherosclerosis. Advances in Experimental Medicine and Biology. 2010;**660**:143-151. DOI: 10.1007/978-1-60761-350-3_13

[35] Staňková B, Vávrová L, Rychlíková J, Žák A. Changes in Paraoxonase 1 activity and concentration of conjugated dienes in connection with number of metabolic syndrome components. Klinical Biochemical Metabolism. 2016;**24**:88-93

[36] Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Critical Reviews in Immunology. 2012;**32**:23-63

[37] Chang JS, Bai CH, Huang ZC, Owaga E, Chao KC, Chang CC, et al. Interleukin 10 and clustering of metabolic syndrome components in pediatrics. European Journal of Clinical Investigation. 2014;**44**:384-394. DOI: 10.1111/eci.12247

[38] Esposito K, Pontillo A, Giugliano F, Giugliano G, Marfella R, Nicoletti G, et al. Association of low interleukin-10 levels with the metabolic syndrome in obese women. The Journal of Clinical Endocrinology and Metabolism. 2003;**88**:1055-1058. DOI: 10.1210/jc.2002-021437

[39] van Exel E, Gussekloo J, de Craen AJ, Frölich M, Bootsma-Van Der Wiel A, Westendorp RG. Low production capacity of interleukin-10 associates with the metabolic syndrome and type 2 diabetes: The Leiden 85-plus study. Diabetes. 2002;**51**:1088-1092. DOI: 10.2337/diabetes.51.4.1088

[40] Mantzoros CS. The role of leptin in human obesity and disease: A review of current evidence. Annals of Internal Medicine. 1990;**130**:671-680. DOI: 10.7326/0003-4819-130-8-199904200-00014

[41] Matarese G, Moschos S, Mantzoros CS. Leptin in immunology. Journal of Immunology. 2005;**174**:3137-3142. DOI: 10.4049/jimmunol.174.6.3137

[42] Hegyi K, Fülöp K, Kovács K, Tóth S, Falus A. Leptin-induced signal transduction pathways. Cell Biology International. 2004;**28**:159-169. DOI: 10.1016/j.cellbi.2003.12.003

[43] Maroni P, Bendinelli P, Piccoletti R. Intracellular signal transduction pathways induced by leptin in C2C12 cells. Cell Biology International. 2005;**29**:542-550. DOI: 10.1016/j.cellbi.2005.03.008

[44] Dong R, Ren J. What fans the fire: Insights into mechanisms of leptin in metabolic syndrome-associated heart diseases. Current Pharmaceutical Design. 2014;**20**:652-658. DOI: 10.2174/13816128200414021316093O

[45] Robinson K, Prins J, Venkatesh B. Clinical review: Adiponectin biology and its role in inflammation and critical illness. Critical Care. 2011;**15**:221. DOI: 10.1186/cc10021

[46] Matsuzawa Y, Funahashi T, Kihara S, Shimomura I. Adiponectin and metabolic syndrome. Arteriosclerosis, Thrombosis, and Vascular Biology. 2004;**24**:29-33. DOI: 10.1161/01.ATV.0000099786.99623.EF

[47] Fu Y. Adiponectin signaling and metabolic syndrome. Progress in Molecular Biology and Translational Science. 2014;**121**:293-319. DOI: 10.1016/B978-0-12-800101-1.00009-0

[48] Finucane FM, Luan J, Wareham NJ, Sharp SJ, O'Rahilly S, Balkau B, et al. Correlation of the leptin:adiponectin ratio with measures of insulin resistance in non-diabetic individuals. Diabetologia. 2009;**52**:2345-2349. DOI: 10.1007/s00125-009-1508-3

[49] López-Jaramillo P, Gómez-Arbeláez D, López-López J, López-López C, Martínez-Ortega J, Gómez-Rodríguez A. The role of leptin/adiponectin ratio in metabolic syndrome and

diabetes. Hormone Molecular Biology and Clinical Investigation. 2014;**18**:37-45. DOI: 10.1515/hmbci-2013-0053

[50] Kotani K, Sakane N. Leptin:adiponectin ratio and metabolic syndrome in the general Japanese population. The Korean Journal of Laboratory Medicine. 2011;**31**:162-166. DOI: 10.3343/kjlm.2011.31.3.162

[51] Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing-acylated peptide from stomach. Nature. 1999;**402**:656-660. DOI: 10.1038/45230

[52] Cowley MA, Smith RG, Diano S, Tschöp M, Pronchuk N, Grove KL. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron. 2003;**37**:649-661. DOI: 10.1016/S0896-6273(03)00063-1

[53] Pradhan G, Samson SL, Sun Y. Ghrelin: Much more than a hunger hormone. Current Opinion in Clinical Nutrition and Metabolic Care. 2014;**16**:619-624. DOI: 10.1097/MCO.0b013e328365b9be

[54] Barazzoni R, Zanetti M, Ferreira C, Vinci P, Pirulli A, Mucci M. Relationships between desacylated and acylated ghrelin and insulin sensitivity in the metabolic syndrome. The Journal of Clinical Endocrinology and Metabolism. 2007;**92**:3935-3940. DOI: 10.1210/jc.2006-2527

[55] Ukkola O. Ghrelin and metabolic disorders. Current Protein & Peptide Science. 2009;**10**:2-7. DOI: 10.2174/138920309787315220

[56] Qu D, Liu J, Lau CW, Huang Y. IL-6 in diabetes and cardiovascular complications. British Journal of Pharmacology. 2014;**171**:3595-3603. DOI: 10.1111/bph.12713

[57] Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. Nature Immunology. 2015;**16**:448-457. DOI: 10.1038/ni.3153

[58] Aroor AR, McKarns S, Demarco VG, Jia G, Sowers JR. Maladaptive immune and inflammatory pathways lead to cardiovascular insulin resistance. Metabolism. 2013;**62**:1543-1552

[59] Weiss TW, Arnesen H, Seljeflot I. Components of the interleukin-6 transsignalling system are associated with the metabolic syndrome, endothelial dysfunction and arterial stiffness. Metabolism. 2013;**62**:1008-1013. DOI: 10.1016/j.metabol.2013.07.001

[60] Kim JH, Bachmann RA, Chen J. Interleukin-6 and insulin resistance. In: Begley TP, Means AR, O'Malley BW, Riddiford L, Tashjian AH, editors. Vitamins and Hormones. 80th Volume. Amsterdam: Elsevier; 2009. pp. 613-633. DOI: 10.1016/S0083-6729(08)00621-3

[61] Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proceedings of the National Academy of Sciences of the United States of America. 1994;**91**:4854-4858. DOI: 10.1073/pnas.91.11.4854

[62] Chen X, Xun K, Chen L, Wang Y. TNF-alpha, a potent lipid metabolism regulator. Cell Biochemistry and Function. 2009;**27**:407-416. DOI: 10.1002/cbf.1596

[63] Parthasarathy S, Raghavamenon A, Garelnabi MO, Santanam N. Oxidized low-density lipoprotein. Methods in Molecular Biology. 2010;**610**:403-417. DOI: 10.1007/978-1-60327-029-8_24

[64] Lara-Guzmán OJ, Gil-Izquierdo A, Medina S, Osorio E, Álvarez-Quintero R, Zuluaga N, et al. Oxidized LDL triggers changes in oxidative stress and inflammatory biomarkers in human macrophages. Redox Biology. 2018;**15**:1-11. DOI: 10.1016/j.redox.2017.11.017

[65] Schwarz A, Bonaterra GA, Schwarzbach H, Kinscherf R. Oxidized LDL-induced JAB1 influences NF-κB independent inflammatory signaling in human macrophages during foam cell formation. Journal of Biomedical Science. 2017;**24**(12). DOI: 10.1186/s12929-017-0320-5

[66] Sawamura T, Kume N, Aoyama T, Moriwaki H, Hoshikawa H, Ariba Y, et al. An endothelial receptor for oxidized low-density lipoprotein. Nature. 1997;**386**:73-77. DOI: 10.1038/386073a0

[67] Frostegard J, Haegerstrand A, Gidlund M, Nilsson J. Biologically modified LDL increases the adhesive properties of endothelial cells. Atherosclerosis. 1991;**90**:119-126. DOI: 10.1016/0021-9150(91)90106-D

[68] Quinn MT, Parthasarathy S, Fong LG, Steinberg D. Oxidatively modified low density lipoproteins: A potential role in recruitment and retention of monocyte/macrophages during atherogenesis. Proceedings of the National Academy of Sciences of the United States of America. 1987;**84**:2995-2998

[69] Blair A, Shaul PW, Yuhanna IS, Conrad PA, Smart EJ. Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmalemmal caveolae and impairs eNOS activation. The Journal of Biological Chemistry. 1999;**274**:32512-32519. DOI: 10.1074/jbc.274.45.32512

[70] Martinez de la Torre Y, Fabbri M, Jaillon S, Bastone A, Nebuloni M, Vecchi A, et al. Evolution of the pentraxin family: The new entry PTX4. Journal of Immunology. 2010;**184**:5055-5064. DOI: 10.4049/jimmunol.0901672

[71] Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. Annual Review of Immunology. 2005;**23**:337-366. DOI: 10.1146/annurev.immunol.23.021704.115756

[72] Muller B, Peri G, Doni A, Torri V, Landmann R, Bottazzi B, Mantovani A. Circulating levels of the long pentraxin PTX3 correlate with severity of infection in critically ill patients. Critical Care Medicine. 2001;**29**:1404-1407

[73] Ohbayashi H, Miyazawa C, Miyamoto K, Sagara M, Yamashita T, Onda R. Pitavastatin improves plasma pentraxin 3 and arterial stiffness in atherosclerotic patients with hypercholesterolemia. Journal of Atherosclerosis and Thrombosis. 2009;**16**:490-500

[74] Mackness MI, Arrol S, Abbott C, Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. Atherosclerosis. 1993;**104**:129-135. DOI: 10.1016/0021-9150(93)90183-U

[75] Aharoni S, Aviram M, Fuhrman B. Paraoxonase 1 (PON1) reduces macrophage inflammatory responses. Atherosclerosis. 2013;**228**:353-361. DOI: 10.1016/j.atherosclerosis.2013.03.005

[76] Senti M, Tomas M, Fito M, Weinbrenner T, Covas MI, Sala J, et al. Antioxidant Paraoxonase 1 activity in the metabolic syndrome. The Journal of Clinical Endocrinology and Metabolism. 2003;**88**:5422-5426. DOI: 10.1210/jc.2003-030648

[77] Nishida M, Moriyama T, Sugita Y, Yamauchi-Takihara K. Interleukin-10 associates with adiponectin predominantly in subjects with metabolic syndrome. Circulation Journal. 2007;**71**:1234-1238. DOI: 10.1253/circj.71.1234

[78] Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. Biochemical and Biophysical Research Communications. 2004;**323**:630-635. DOI: 10.1016/j.bbrc.2004.08.145

Chapter 7

Insulin in Forensic Medicine and Toxicology

Rafał Skowronek

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76691

Abstract

Today insulin is used not only in medicine in the treatment of diabetes but also in sport as a doping agent and for criminal purposes. Suicides and homicides with insulin maybe are not so common, but are seen in the routine medicolegal and toxicological-clinical practice. Despite the often quite clear circumstances of death and a well-established mechanism of action of insulin and its analogs, it is difficult to analytically confirm its excessive exogenous administration in postmortem biological material. There are no uniform international standards of conduct in such cases, both at the stage of the material sampling during autopsy and forensic laboratory analysis and the final interpretation of the obtained results. The aim of the study is to present the current state of basic knowledge about nonmedical use of insulin, with particular emphasis on the possibility of postmortem diagnosis. The study also highlighted the little known clinical problem of insulin abuse for recreational purposes.

Keywords: overdose, homicide-suicide, postmortem diagnostics, thanatobiochemistry, medicolegal autopsy, forensic histopathology

1. Introduction

Insulin is a potent anabolic polypeptide hormone which stimulates the uptake and storage of carbohydrates, fatty acids, and amino acids into glycogen, fat, and protein, respectively [1]. The typical physiological effect of its action is hypoglycemia (reduction in blood glucose). The result of a casual or intentional overdose of insulin is a hypoglycemic coma and in extreme cases even death of the user. Today it is used not only in medicine in the treatment of diabetic patients but also in competitive sport as a common doping agent for body building [2, 3] and for different criminal purposes [4–8]. Suicides and homicides with insulin maybe are not so common, but are seen in the routine medicolegal and toxicological-clinical practice. The first

documented case of murder by insulin is dated to the year 1957 (Kenneth Barlow case) [9]. Vincent Marks in his review has analyzed case histories of 66 people alleged or proven to have been poisoned by insulin (murders, manslaughters, attempted murders, Munchausen-by-proxy cases) [7].

Of course, some insulin overdoses are accidental and associated with incorrect dosage of the drug by the patient [10, 11]. Most of these cases are not clinically serious. It seems, however, that the risk of intentional (suicidal) insulin overdose in patients with diabetes of both types (1 and 2) is underestimated. The population-based study of suicide victims in Northern Finland performed by Löfman et al. revealed that 3.1% of all suicide victims had diabetes (34.6% type 1 and 65.4% type 2) [12]. In victims with type 1 diabetes, insulin as a suicide method covered half of the self-poisoning cases, while the proportion in type 2 diabetes was 13%. It is known that the risk of depression and attempted suicide is higher in patients with chronic diseases, including diabetes, so physicians who treat diabetic patients should evaluate co-occurring depression and substance abuse, both of which are major risk factors of suicide [13, 14].

The aim of the author is to present the current state of basic knowledge about the nonmedical use of insulin, with particular emphasis on the possibility of postmortem diagnosis. The study also highlighted the little known, rare clinical problem of insulin abuse for recreational purposes.

2. Case reports

In order to illustrate the abovementioned problems, I present two typical cases from routine medicolegal practice of the Department of Forensic Medicine and Forensic Toxicology in Katowice, School of Medicine in Katowice, Medical University of Silesia, Poland [15, 16].

2.1. Suicide

A 44-year-old nondiabetic man was found dead lying on the bed in his flat. Near the body, an ampoule and almost empty syringe were found and taken for further analysis. Two days earlier, the man had called his wife and said that he is going to commit suicide. The forensic autopsy did not reveal the cause of death. The initial stage of putrefaction, blood fluidity, acute blood stagnation (hyperemia) in the internal organs, and two supravital point wounds on the right thigh, which might have been injection sites, were found. Histopathological findings in the main internal organs were the following: brain, hyperemia with numerous petechiae, and edema; heart, adipositas, medium grade of atherosclerosis of the coronary arteries, and local fragmentation of muscle fibers; and lungs, hyperemia with local hemorrhages into alveoli and edema. Additional histochemical staining (Periodic Acid-Schiff, PAS) disclosed low amounts of glycogen in the liver (**Figure 1**). The standard toxicological analysis disclosed no evidence of drug abuse or alcohol, so due to the suspicion of suicide by insulin injection, a directed analysis with immunoradiometric assay (IRMA Kit Immunotech), routinely used for the in vitro determination of insulin in human serum and plasma, was conducted. It revealed a high insulin concentration level—24.42 μIU/ml in the vitreous humor (measuring range, 0.5–300 μIU/ml; the norm for serum, <22 μIU/ml)—and the presence of insulin in the material

http://dx.doi.org/10.5772/intechopen.76691

secured at the crime scene (in the syringe, 1853.91 μIU/ml). All these results (information from the prosecutor about the crime scene, results of medicolegal autopsy, results of histopathological and toxicological studies) clinched the thesis of insulin overdose.

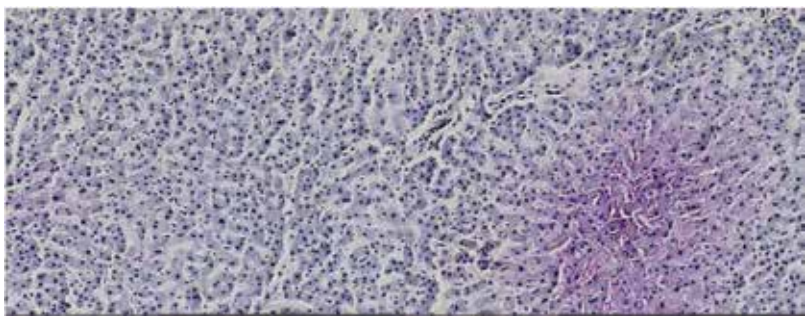

Figure 1. Low amounts of glycogen in the liver—50% of control sections taken during autopsy of sudden traumatic death victims.

Figure 2. Empty packages after insulin, NovoMix 30 Penfill (a mixture of fast and long-acting insulin analogue), revealed in the apartment of victim. Needles can be analyzed by forensic geneticists for the presence of DNA mixture of the victim and murderer in the case of homicide-suicide death.

2.2. Homicide-suicide

According to the information of the Prosecutor's Office, a 63-year-old man was supposed to kill his wife and dog and then commit suicide. Such situation in forensic medicine is called homicide-suicide or dyadic death. In the apartment a farewell letter and empty packages after insulin, NovoMix 30 Penfill (a mixture of fast and long-acting insulin analogue), were revealed (**Figure 2**). External medicolegal examination and forensic autopsies carried out at the Department of Forensic Medicine and Forensic Toxicology of the Medical University of Silesia in Katowice did not explain the cause of death. However, potential injection sites on the thighs and the shoulder of woman were revealed (**Figure 3**). Different biological materials for additional tests—biochemical, chemical-toxicological, and histopathological and for forensic genetics—were taken. Due to the inability to quickly determine insulin level in body fluids and the site of injection using the reference chromatographic methods [17–20], the determination of this hormone was ordered to two clinical diagnostic laboratories (by chemiluminometric and immunoradiometric methods). In addition, C-peptide (short 31-amino-acid

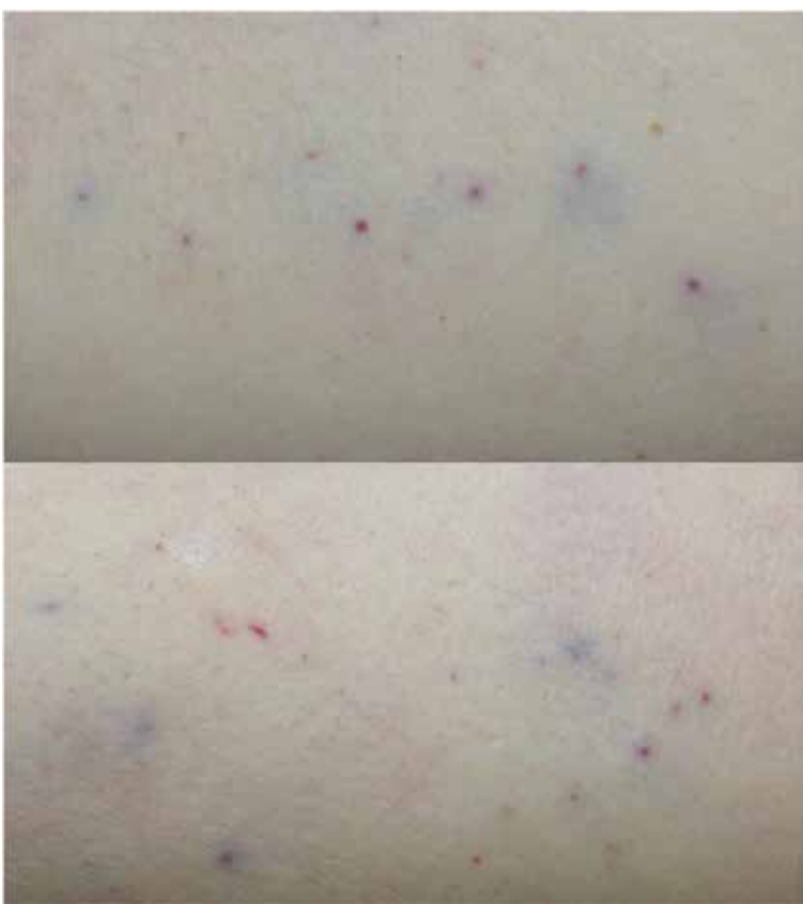

Figure 3. Numerous supravital point wounds and surrounding bruises on the thighs—potential insulin injection sites.

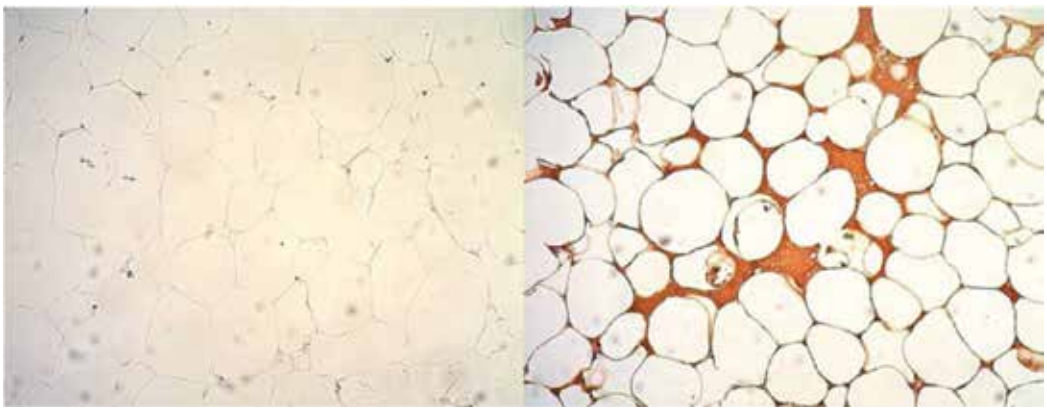

Figure 4. Positive immunohistochemical (IHC) detection of insulin in the subcutaneous tissue around needle tracts between adipocytes (right) and control section from distant area of the skin with no reaction (left).

polypeptide that connects insulin's A-chain to its B-chain in the endogenous proinsulin molecule), glycated hemoglobin (HbA1c, a form of hemoglobin that is measured primarily to identify the 2- to 3-month average plasma glucose concentration), glucose, and lactate (it is known that one mole of glucose during the process of glycolysis produces two moles of lactate) were ordered. Incomplete, difficult-to-interpret results were obtained. In addition, a successful attempt of immunohistochemical (IHC) detection of insulin in samples taken from the injection sites was made (**Figure 4**) [21, 22]. The results of the tests carried out in the abovementioned clinical laboratories confirmed our previous experience with the low usefulness of insulin determinations in the autopsy hemolyzed blood specimens (article in press).

3. Postmortem diagnostics of fatal insulin poisoning

3.1. Medicolegal autopsy

A classic postmortem macroscopic examination of the corpses (forensic autopsy) usually does not explain the cause and mechanism of death [23, 24]. Typically a feature of acute cardiorespiratory failure and nonspecific lesions related to the age of victim (e.g., atherosclerotic changes in vessels) can be found. For this reason, additional laboratory tests are necessary in each case. In addition to routinely collected sections from internal organs and body fluids (blood and urine), it is worth to take at least the sample of vitreous humor (VH) and the samples from potential injection sites for both histopathological and directed toxicological analyses.

3.2. Forensic histopathology and immunohistochemistry

A detailed histological examination of all internal organs, especially of the pancreas and liver, aiming at detection of insulinoma (tumor of the pancreas that is derived from β cells and secretes insulin) and morphological symptoms of hypoglycemia, respectively, should

be always performed by experienced pathologist. The content of glycogen (multibranched polysaccharide of glucose that serves as a form of main energy storage) in the liver may be evaluated by the Periodic Acid-Schiff (PAS) or Best's Carmine staining. Its low amounts can indirectly confirm insulin overdose, as it was presented in the first case [15]. Another useful option is to perform IHC staining for the presence of insulin at the injection site. It is not necessary to buy special antibodies. These routinely used in clinical histopathology can be successfully used for this purpose, as we demonstrated in the second case [16].

3.3. Forensic toxicology and thanatobiochemistry (postmortem biochemistry)

In routine forensic practice, usually antemortem blood samples of the victim, who sometimes is hospitalized before the death, are unavailable for forensic toxicologists, so they can analyze only postmortem biological material taken during autopsy and nonbiological specimens revealed at the crime scene, like syringes, ampoules, vials, or remnants of the infusion solution and tubings [24–26].

What is important from the medicolegal point of view is that the interpretation of insulin levels in the postmortem biological material is difficult and still in doubt [27–31]. The number of published papers dealing with this problem is relatively low. The time of survival after insulin injection depends on many different factors: type of insulin (differentiated onset of action and insulin half-life), method of administration (injection or insulin infusion pump), anatomical localization of injection sites on the body (different rate of absorption), etc. [32]. It certainly influences the insulin levels detected in the postmortem biological material. Unfortunately, in the forensic practice, investigators usually do not know that time, because the cadavers not infrequently are found after a long time since death at an advanced stage of late postmortem changes, for example, when the victim lived alone or the killer committed suicide [21].

Additionally, insulin determination in postmortem blood has a low diagnostic and testimonial value, mainly because of ongoing thanatochemical processes of autolysis and putrefaction [8, 24]. The main barrier that prevents receiving correct and trustworthy results of insulin determinations in postmortem blood with radioimmunological methods is the blood hemolysis (rupturing of red blood cells and the release of their contents into surrounding plasma). This fact was confirmed in the literature and by our own studies performed in the Department of Forensic Medicine and Forensic Toxicology in Katowice [32, 33].

Fortunately, insulin crosses the blood-retinal barrier and may be identified in the VH, which is generally very valuable alternative material for many different chemical-toxicological analyses [34, 35]. The advantage of this material is that it is easy to obtain during typical forensic autopsy. Another advantage is anatomical isolation, useful especially in the case of advanced autolytic and putrefactive changes *in corpore*. It has also a very low cell count, so there is a small postmortem metabolism of glucose and other substances by surviving cells.

In 2011 Thevis et al. have published the first successful mass spectrometry-based analysis of postmortem material (VH) related to an insulin poisoning case [17]. The natural levels of insulin in vitreous humor determined by the authors were below the liquid chromatography–tandem mass spectrometry (LC-MS/MS) limit of detection. LC-MS/MS is modern advanced

instrumental method widely used in analytical chemistry. This was a significant advance in postmortem biochemistry. Our own experience shows that in cases where suicide by insulin poisoning is suspected, determination of its concentration in the vitreous humor and nonbiological material using the immunoradiometric assay (IRMA) gives the opportunity, similarly as the LC-MS/MS analysis, of objective confirmation of the poisoning, so both methods can be used in forensic practice [32].

In our department, we have analyzed material consisted of 93 samples of vitreous humor taken during forensic autopsies. Analysis revealed that in 86 vitreous humor samples (92.5%), the concentration of insulin, determined with IRMA, was below the limit of detection of this method (below 0.5 μIU/ml). The concentration of insulin in vitreous humor was determined only in seven cases (range of results, 1.42–24.42 μIU/ml). We have described above one of these cases, where insulin was used to commit suicide [15].

The IRMA method is known as sensitive, specific, and relatively cheap in comparison to modern methods, but it requires adequate apparatus for the measurement of radioactivity and some experience in its interpretation. It is worth knowing that the studies on insulin determination using antibody-radiolabeled antigen reaction in the late 1950s were the beginning of a new medical discipline—radioimmunology [36]. Until the introduction of radioimmunoassay (RIA), death caused by insulin overdose was extremely difficult to prove [4]. In turn, the huge advantage of modern chromatographic methods (LC-MS/MS) is the possibility of differentiation between different types of insulins (human or animal insulin and their synthetic derivatives/analogues). In 2015, Palmiere et al. have presented preliminary results of postmortem determination of insulin using chemiluminescence enzyme immunoassay (CLEIA). Their conclusion was that the analysis of vitreous humor with CLEIA may provide suitable data, similar to analysis with LC-MS/MS and immunoradiometric assay, to support the hypothesis of insulin overdose [37].

Regardless of the method used in toxicological investigation, an analytically confirmed higher level of insulin in the vitreous humor plays an important and even a decisive role in structuring the final medicolegal opinion about the cause of death. This is the reason why the vitreous humor should be routinely collected and analyzed during forensic autopsy in every case with an "insulin" background [29, 37].

3.4. Forensic molecular biology

An interesting observation, so far unused in the forensic practice, is an increase in the expression of certain genes stimulated by insulin, especially in hyperinsulinemic conditions. This is a potentially promising area for further research. An example might be the changes of neuropeptide Y (NPY) gene expression and its release during hypoglycemic stress. Han et al. found that subcutaneous insulin injection produced an immediate increase in plasma NPY immunoreactivity and delayed increases in adrenal and neuronal NPY mRNA and adrenal NPY immunoreactivity in rats [38]. They have concluded that these results suggest that NPY may play a role in insulin-induced hypertension. Another example can be increased vascular resistance in the equine digit and overexpression of endothelin-1 (ET-1) in the laminar tissue due to the short-term hyperinsulinemia [39].

4. Clinical toxicology of insulin poisoning

An overdose of insulin is a potential life-threatening condition and requires urgent medical attention [40–46]. The clinical manifestations of hypoglycemia occur when the blood glucose level is less than 2.2–2.8 mmol/l (40–50 mg%). Symptomatology includes two groups of symptoms. The first one is caused by stimulation of the autonomic nervous system and includes profuse sweating, anxiety, tremor, and hunger. The second one is caused by progressive dysfunction of the central nervous system (CNS) due to neuroglycopenia and includes nausea, headache, dizziness, blurred vision, abnormal intellectual processes, behavioral disturbances, and finally loss of consciousness, convulsions, and death.

The most optimal place of the treatment is clinical toxicology ward, but patients who are overdosed with insulin can be also treated in typical intensive care units or in less serious cases—in general internal wards. To differentiate endogenous and exogenous insulin overdose, usually insulin/C-peptide [mol/mol] ratio is used, both in clinical and forensic settings [47]. Physiologically for every molecule of insulin formed, a corresponding molecule of C-peptide is formed. If the above-described ratio is >1, it indicates exogenous origin of insulin (as a result of accident, suicide, or homicide). However, it should be remembered that C-peptide is very unstable in postmortem blood [4].

Treatment of hypoglycemia is initially based on the securing of basic vital functions (breathing and circulation). Subsequently, infusions of glucose solution adjusted to the current blood glucose levels are used. Depending on the clinical situation, other drugs are administered s.c. or i.v. (e.g., glucagon which is a glycogenolysis stimulator), as it was presented in above-cited clinical emergency case reports. In the past such specific methods of treatment and management have been reported as excision of insulin injection site or the use of artificial pancreas [48–50]. Assessment of patient prognosis relies on clinical findings. According to the results of prospective study of Mégarbane et al., the observed plasma insulin EC50 (the concentration which induces a response halfway between the baseline and maximum after a specified exposure time) is 46 mIU/l [51].

Tsujimoto et al. have described rare case of rapid onset reversible glycogen storage hepatomegaly caused by suicidal administration of a massive dose of long-acting insulin glargine and subsequent supplementation with large doses of glucose in a 41-year-old type-2 diabetic patient [52]. Supravital liver biopsy revealed hepatocytic glycogen deposition with edematous degeneration. PAS staining revealed many PAS-positive granules containing glycogen. The hepatic computed tomography (CT) attenuation was 83.7 Hounsfield units (HU), being markedly higher than the splenic attenuation (49.5 HU), which indicated pathology of the liver. Such situation (initially higher level of hepatocytic glycogen deposition) must be considered not only by clinicians but also by forensic histopathologist during examination of the insulin fatal poisoning victim's liver.

5. Insulin abuse

An interesting, still not fully explained phenomenon, connecting toxicology, diabetology, and psychiatry, is abuse of insulin as a psychoactive substance. Intentional abuse of insulin is quite rare, but not exceptional. Pudlo et al. described an insulin abuser—the 58-year-old patient who

injected himself with overdoses of insulin or consumed considerable amounts of pure sugar to increase its dose [53]. No other reason for his insulin abuse was found than pleasure seeking. According to the patient, he felt "pleasure" after insulin. Sheehy has counted 55 cases of patients developing hypoglycemic episodes by intentional insulin injecting [54]. Sometimes people suffering from Munchausen syndrome can also apply themselves an excessive dose of the drug to cause factitious hypoglycemia and get to the hospital [7, 55]. In the case of Munchausen syndrome by proxy (per procuram), the victims may be the relatives, most often children [56].

6. Conclusions

Despite the often quite clear circumstances of death and a well-established mechanism of action of insulin and its analogues, it is difficult to analytically confirm its excessive exogenous administration in postmortem biological material [8]. There are no uniform standards of conduct in this type of cases, both at the stage of the material sampling and laboratory analysis and in the interpretation of the obtained results.

If insulin overdose is suspected, it is necessary to take the different biological material during autopsy for further testing and to cautiously interpret its results [7]. It seems necessary to immediately develop a unified international standards/algorithm of conduct, similar to those used in clinical medicine, including the determination of insulin level and other parameters of carbohydrate metabolism in the postmortem biological material, taking into account all above-described possibilities and limitations of laboratory analysis [57–59].

Acknowledgements

The author would like to thank Marcin Tomsia, MSc, PhD, for his review of the manuscript; Joanna Nowicka, MSc, PhD; and Ewa Czech, MSc, PhD, for their valuable comments during the preparation of previous articles and presentations and Czesław Chowaniec, MD, PhD, and Christian Jabłoński, MD, PhD, for creating optimal conditions for scientific work.

Conflict of interest

The author has no conflict of interest to declare.

Author details

Rafał Skowronek

Address all correspondence to: rafal-skowronek@wp.pl

Department of Forensic Medicine and Forensic Toxicology, School of Medicine in Katowice, Medical University of Silesia in Katowice, Katowice, Poland

References

[1] Thevis M, Thomas A, Schänzer W. Insulin. In: Thieme D, Hemmersbach P, editors. Doping in Sports, Handbook of Experimental Pharmacology 195. Springer-Verlag: Berlin Heidelberg; 2010. pp. 209-226. DOI: 10.1007/978-3-540-79088-4_10

[2] Holt RI, Sönksen PH. Growth hormone, IGF-I and insulin and their abuse in sport. British Journal of Pharmacology. 2008;**154**(3):542-556. DOI: 10.1038/bjp.2008.99

[3] Thomas A, Brinkkötter P, Schänzer W, Thevis M. Metabolism of human insulin after subcutaneous administration: A possible means to uncover insulin misuse. Analytica Chimica Acta. 2015;**897**:53-61. DOI: 10.1016/j.aca.2015.09.036

[4] DiMaio D, DiMaio VJM. Forensic Pathology. 2nd ed. CRC Press; 2001. 592 p

[5] Marks V. Hypoglycaemia: Accidents, violence and murder. Part 1. Practical Diabetes International. 2005;**22**(8):303-306. DOI: 10.1002/pdi.854

[6] Marks V. Hypoglycaemia: Accidents, violence and murder. Part 2. Practical Diabetes International. 2005;**22**(9):352-358-54. DOI: 10.1002/pdi.875

[7] Marks V. Murder by insulin: Suspected, purported and proven—A review. Drug Testing and Analysis. 2009;**1**(4):162-176. DOI: 10.1002/dta.38

[8] Marks V, Wark G. Forensic aspects of insulin. Diabetes Research and Clinical Practice. 2013;**101**(3):248-254. DOI: 10.1016/j.diabres.2013.05.002

[9] Marks V, Richmond C. Kenneth Barlow: The first documented case of murder by insulin. Journal of the Royal Society of Medicine. 2008;**101**(1):19-21. DOI: 10.1258/jrsm.2007.071002

[10] Batalis NI, Prahlow JA. Accidental insulin overdose. Journal of Forensic Sciences. 2004;**49**(5):1117-1120

[11] von Mach MA, Meyer S, Omogbehin B, Kann PH, Weilemann LS. Epidemiological assessment of 160 cases of insulin overdose recorded in a regional poisons unit. International Journal of Clinical Pharmacology and Therapeutics. 2004;**42**(5):277-280

[12] Löfman S, Hakko H, Mainio A, Timonen M, Räsänen P. Characteristics of suicide among diabetes patients: A population based study of suicide victims in Northern Finland. Journal of Psychosomatic Research. 2012;**73**(4):268-271. DOI: 10.1016/j.jpsychores.2012.08.002

[13] Sarkar S, Balhara YS. Diabetes mellitus and suicide. Indian Journal of Endocrinology and Metabolism. 2014;**18**(4):468-474. DOI: 10.4103/2230-8210.137487

[14] Russell KS, Stevens JR, Stern TA. Insulin overdose among patients with diabetes: A readily available means of suicide. Primary Care Companion to the Journal of Clinical Psychiatry. 2009;**11**(5):258-262. DOI: 10.4088/PCC.09r00802

[15] Skowronek R, Nowicka J, Czech E, Chowaniec M, Rygol K. A case of suicidal insulin overdose—Significance of directed toxicological and histopathological studies for medico-legal opinion. In: Abstracts of the 22nd International Meeting of Forensic Medicine Alpe-Adria-Pannonia; 5-8 June 2013; Kraków, Poland; Archiwum Medycyny Sadowej I Kryminologii. 2013;1:44-45

[16] Skowronek R, Nowicka J, Czech E, Kulikowska J, Pieprzyca E, Rygol K, Kabiesz-Nenic zka S. Analysis of another case of extended suicide with insulin—Dilemmas and controversies in forensic-medical opinioning. In: Abstracts of the XXI Annually Conference of Polish Forensic Toxicologists; 7-9 May 2014; Ciechocinek, Poland; 2013. pp. 63-64

[17] Thevis M, Thomas A, Schänzer W, Ostman P, Ojanperä I. Measuring insulin in human vitreous humour using LC-MS/MS. Drug Testing and Analysis. 2012;**4**(1):53-56. DOI: 10.1002/dta.368

[18] Ojanpera I, Sajantila A, Vinogradova L, Thomas A, Schanzer W, Thevis M. Post-mortem vitreous humour as potential specimen for detection of insulin analogues by LC-MS/MS. Forensic Science International. 2013;(1-3):328-332. DOI: 10.1016/j.forsciint.2013.10.009

[19] Hess C, Madea B, Daldrup T, Musshoff F. Determination of hypoglycaemia induced by insulin or its synthetic analogues post mortem. Drug Testing and Analysis. 2013;**5**: 802-807. DOI: 10.1002/dta.1500

[20] Hess C, Thomas A, Thevis M, Stratmann B, Quester W, Tschoepe D, Madea B, Musshoff F. Simultaneous determination and validated quantification of human insulin and its synthetic analogues in human blood serum by immunoaffinity purification and liquid chromatography-mass spectrometry. Analytical and Bio analytical Chemistry. 2012;**404**(6-7):1813-1822. DOI: 10.1007/s00216-012-6271-5

[21] Lutz R, Pedal I, Wetzel C, Mattern R. Insulin injection sites: Morphology and immunohistochemistry. Forensic Science International. 1997;**90**(1-2):93-101

[22] Wehner F, Mittmeyer HJ, Wehner HD, Schieffer MC. Insulin- or morphine-injection? Immunohistochemical contribution to the elucidation of a case. Forensic Science International. 1998;**95**(3):241-246. DOI: 10.1016/S0379-0738(98)00099-1

[23] Kernbach-Wighton G, Püschel K. On the phenomenology of lethal applications of insulin. Forensic Science International. 1998;**93**(1):61-73. DOI: 10.1016/S0379-0738(98)00032-2

[24] Labay LM, Bitting CP, Legg KM, Logan BK. The determination of insulin overdose in postmortem investigations. Academic Forensic Pathology. 2016;**6**(2):174-183. DOI: 10.23907/2016.019

[25] Koskinen PJ, Nuutinen HM, Laaksonen H, Klossner JA, Irjala KM, Kalimo H, Viikari JS. Importance of storing emergency serum samples for uncovering murder with insulin. Forensic Science International. 1999;**105**(1):61-66. DOI: 10.1016/S0379-0738(99)00111-5

[26] Junge M, Tsokos M, Püschel K. Suicide by insulin injection in combination with beta-blocker application. Forensic Science International. 2000;**113**(1-3):457-460. DOI: 10.1016/S0379-0738(00)00283-8

[27] Hess C, Musshoff F, Madea B. Disorders of glucose metabolism-post mortem analyses in forensic cases: Part I. International Journal of Legal Medicine. 2011;**125**(2):163-170. DOI: 10.1007/s00414-010-0509-6

[28] Musshoff F, Hess C, Madea B. Disorders of glucose metabolism: Post mortem analyses in forensic cases: Part II. International Journal of Legal Medicine. 2011;**125**(2):171-180. DOI: 10.1007/s00414-010-0510-0

[29] Hess C, Madea B. Insulin and hypoglycemia. Forensic considerations. Rechtsmedizin. 2014;**24**(5):429-443. DOI: 10.1007/s00194-014-0965-2

[30] Palmiere C, Mangin P. Postmortem chemistry update part I. International Journal of Legal Medicine. 2012;**126**(2):187-198. DOI: 10.1007/s00414-011-0625-y

[31] Belsey SL, Flanagan RJ. Postmortem biochemistry: Current applications. Journal of Forensic and Legal Medicine. 2016;**41**:49-57. DOI: 10.1016/j.jflm.2016.04.011

[32] Nowicka J, Skowronek R, Czech E, Kulikowska J, Olszowy Z. Comments on 'Measuring insulin in human vitreous humour using LC-MS/MS' by Thevis et al. Drug Testing and Analysis. 2013;**5**:1-17. DOI: 10.1002/dta.1392

[33] Chevenne D, Letailleur A, Trivin F, Porquet D. Effect of hemolysis on the concentration of insulin in serum determined by RIA and IRMA. Clinical Chemistry. 1998;**44**(2):354-356

[34] Kubo Y, Hosoya K. Inner blood-retinal barrier transporters: Relevance to diabetic retinopathy. In: Shamsul Ola M, editor. Diabetic Retinopathy. InTech; 2012. pp. 91-108. DOI: 10.5772/33992

[35] Bévalot F, Cartiser N, Bottinelli C, Fanton L, Guitton J. Vitreous humor analysis for the detection of xenobiotics in forensic toxicology: A review. Forensic Toxicology. 2016;**34**:12-40. DOI: 10.1007/s11419-015-0294-5

[36] Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. The Journal of Clinical Investigation. 1960;**39**:1157-1175. DOI: 10.1172/JCI104130

[37] Palmiere C, Sabatasso S, Torrent C, Rey F, Werner D, Bardy D. Post-mortem determination of insulin using chemiluminescence enzyme immunoassay: Preliminary results. Drug Testing and Analysis. 2015;**7**(9):797-803. DOI: 10.1002/dta.1775

[38] Han S, Chen X, Wu YM, Naes L, Westfall T. Elevated neuropeptide Y gene expression and release during hypoglycemic stress. Peptides. 1997;**18**(9):1335-1340. DOI: 10.1016/S0196-9781(97)00212-X

[39] Gauff F, Patan-Zugaj B, Licka TF. Hyperinsulinaemia increases vascular resistance and endothelin-1 expression in the equine digit. Equine Veterinary Journal. 2013;**45**(5):613-618. DOI: 10.1111/evj.12040

[40] Fuller ET, Miller MA, Kaylor DW, Janke C. Lantus overdose: Case presentation and management options. The Journal of Emergency Medicine. 2009;**36**(1):26-29. DOI: 10.1016/j.jemermed.2007.02.038

[41] Lu M, Inboriboon PC. Lantus insulin overdose: A case report. The Journal of Emergency Medicine. 2011;**41**(4):374-377. DOI: 10.1016/j.jemermed.2010.04.007

[42] Matsumura M, Nakashima A, Tofuku Y. Electrolyte disorders following massive insulin overdose in a patient with type 2 diabetes. Internal Medicine. 2000;**39**(1):55-57. DOI: 10.2169/internalmedicine.39.55

[43] Shibutani Y, Ogawa C. Suicidal insulin overdose in a type 1 diabetic patient: Relation of serum insulin concentrations to the duration of hypoglycemia. Journal of Diabetes and its Complications. 2000;**14**(1):60-62. DOI: 10.1016/S1056-8727(00)00057-X

[44] Thewjitcharoen Y, Lekpittaya N, Himathongkam T. Attempted suicide by massive insulin injection: A case report and review of the literature. Journal of the Medical Association of Thailand. 2008;**91**(12):1920-1924

[45] Tofade TS, Liles EA. Intentional overdose with insulin glargine and insulin aspart. Pharmacotherapy. 2004;**24**(10):1412-1418. DOI: 10.1592/phco.24.14.1412.43147

[46] Wong OF, Tsui KL, Kam CK. A case of acute insulin poisoning. Hong Kong Journal of Emergency Medicine. 2006;**13**(4):232-234

[47] Iwase H, Kobayashi M, Nakajima M, Takatori T. The ratio of insulin to C-peptide can be used to make a forensic diagnosis of exogenous insulin overdosage. Forensic Science International. 2001;**115**(1-2):123-127. DOI: 10.1016/S0379-0738(00)00298-X

[48] Campbell IW, Ratcliffe JG. Suicidal insulin overdose managed by excision of insulin injection site. British Medical Journal (Clinical Research Ed.). 1982;**285**(6339):408-409

[49] Levine DF, Bulstrode C. Managing suicidal insulin overdose. British Medical Journal (Clinical Research Ed.). 1982;**285**(6346):974-975

[50] Gin H, Larnaudie B, Aubertin J. Attempted suicide by insulin injection treated with artificial pancreas. British Medical Journal (Clinical Research Ed.). 1983;**287**(6387):249-250

[51] Mégarbane B, Deye N, Bloch V, Sonneville R, Collet C, Launay JM, Baud FJ. Intentional overdose with insulin: Prognostic factors and toxicokinetic/toxicodynamic profiles. Critical Care. 2007;**11**:R115. DOI: 10.1186/cc6168

[52] Tsujimoto T, Takano M, Nishiofuku M, Yoshiji H, Matsumura Y, Kuriyama S, Uemura M, Okamoto S, Fukui H. Rapid onset of glycogen storage hepatomegaly in a type-2 diabetic patient after a massive dose of long-acting insulin and large doses of glucose. Internal Medicine. 2006;**45**(7):469-473. DOI: 10.2169/internalmedicine.45.1548

[53] Pudlo R, Pudlo M, Matysiakiewicz JA. Abuse of insulin as psychoactive substance. Postępy Psychiatrii i Neurologii. 2000;**9**(suppl. 3):47-50

[54] Sheehy TW. Case report: Factitious hypoglycemia in diabetic patients. The American Journal of the Medical Sciences. 1992;**304**(5):298-302

[55] Jermendy G. Hypoglycemia factitia: Munchhausen syndrome in diabetes mellitus. Journal of Diabetes and its Complications. 1996;**10**(4):223-225

[56] Kucuker H, Demir T, Oral R. Pediatric condition falsification (Munchausen syndrome by proxy) as a continuum of maternal factitious disorder (Munchausen syndrome). Pediatric Diabetes. 2010;**11**(8):572-578. DOI: 10.1111/j.1399-5448.2009.00631.x

[57] Wunder C, Kauert GF, Toennes SW. Factors leading to the degradation/loss of insulin in postmortem blood samples. Forensic Science International. 2014;**241**:173-177. DOI: 10.1016/j.forsciint.2014.06.003

[58] Madea B, Musshoff F. Postmortem biochemistry. Forensic Science International. 2007;**165**(2-3):165-1671. DOI: 10.1016/j.forsciint.2006.05.023

[59] Harmonisation of Medico-Legal Autopsy Rules. ECLM update of the principles and rules relating to medico-legal autopsy procedures. European Council of Legal Medicine. London, Fall/Winter 1994-1995. Updated, Dubai, January 2014. Available from: http://eclm.info/docs/Documents/ECLM_Harmonisation_of_Autopsy_Rules_2014.pdf

Edited by Gaffar Zaman

The book presents a qualitative and quantitative approach to understand, manage and enforce the integration of insulin into diabetes mellitus. Utilizing a sound theoretical and practical foundation and illustrating procedural techniques through scientific examples, the book bridges the gap between insulin and diabetes mellitus management. Detailed procedures have been omitted because of the variety of equipment and commercial kits used in today's clinical laboratories.

Published in London, UK

IntechOpen

ISBN 978-1-83962-001-0