

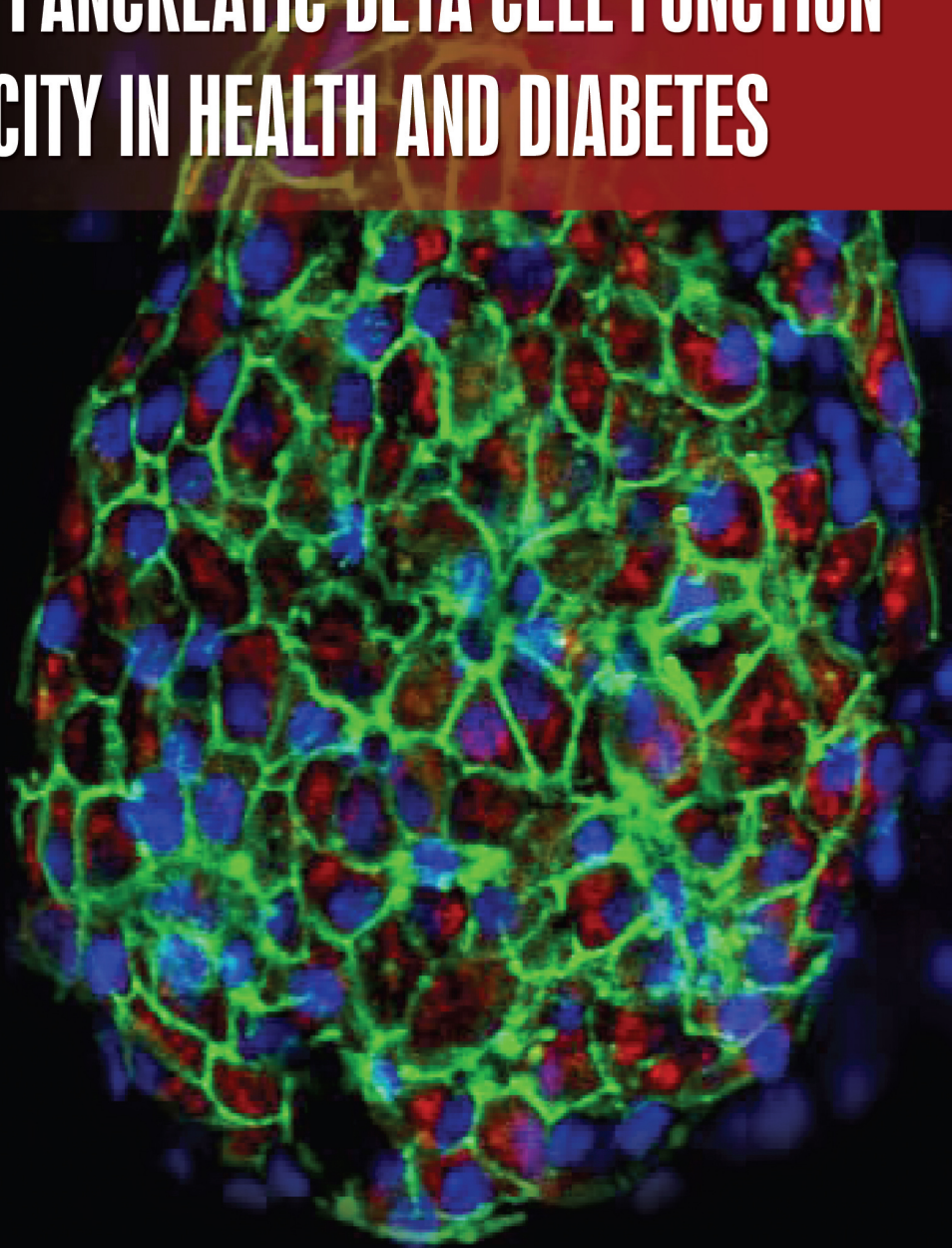
eISBN: 978-1-68108-365-0
ISBN: 978-1-68108-366-7

eISSN: 2468-7545
ISSN: 2468-7537

CURRENT AND FUTURE DEVELOPMENTS IN PHYSIOLOGY

VOLUME 1

CONTROL OF PANCREATIC BETA CELL FUNCTION AND PLASTICITY IN HEALTH AND DIABETES



Editor:
David J. Hill

Bentham  Books

**Current and Future
Developments in Physiology
(Volume 1)**
***Control of Pancreatic Beta Cell
Function and Plasticity in Health
and Diabetes***

Edited by

David J. Hill

*Lawson Health Research Institute
St. Joseph's Health Care,
268 Grosvenor Street, London,
Ontario N6A 4V2,
Canada*

Current and Future Developments in Physiology

Volume # 1

Control of Pancreatic Beta Cell Function and Plasticity in Health and Diabetes

Editor: David J. Hill

ISSN (print): 2468-7537

ISSN (online): 2468-7545

ISBN (online): 978-1-68108-365-0

ISBN (print): 978-1-68108-366-7

©2016, Bentham eBooks imprint.

Published by Bentham Science Publishers – Sharjah, UAE. All Rights Reserved.

BENTHAM SCIENCE PUBLISHERS LTD.

End User License Agreement (for non-institutional, personal use)

This is an agreement between you and Bentham Science Publishers Ltd. Please read this License Agreement carefully before using the ebook/echapter/ejournal (“**Work**”). Your use of the Work constitutes your agreement to the terms and conditions set forth in this License Agreement. If you do not agree to these terms and conditions then you should not use the Work.

Bentham Science Publishers agrees to grant you a non-exclusive, non-transferable limited license to use the Work subject to and in accordance with the following terms and conditions. This License Agreement is for non-library, personal use only. For a library / institutional / multi user license in respect of the Work, please contact: permission@benthamscience.org.

Usage Rules:

1. All rights reserved: The Work is the subject of copyright and Bentham Science Publishers either owns the Work (and the copyright in it) or is licensed to distribute the Work. You shall not copy, reproduce, modify, remove, delete, augment, add to, publish, transmit, sell, resell, create derivative works from, or in any way exploit the Work or make the Work available for others to do any of the same, in any form or by any means, in whole or in part, in each case without the prior written permission of Bentham Science Publishers, unless stated otherwise in this License Agreement.
2. You may download a copy of the Work on one occasion to one personal computer (including tablet, laptop, desktop, or other such devices). You may make one back-up copy of the Work to avoid losing it. The following DRM (Digital Rights Management) policy may also be applicable to the Work at Bentham Science Publishers’ election, acting in its sole discretion:
 - 25 ‘copy’ commands can be executed every 7 days in respect of the Work. The text selected for copying cannot extend to more than a single page. Each time a text ‘copy’ command is executed, irrespective of whether the text selection is made from within one page or from separate pages, it will be considered as a separate / individual ‘copy’ command.
 - 25 pages only from the Work can be printed every 7 days.
3. The unauthorised use or distribution of copyrighted or other proprietary content is illegal and could subject you to liability for substantial money damages. You will be liable for any damage resulting from your misuse of the Work or any violation of this License Agreement, including any infringement by you of copyrights or proprietary rights.

Disclaimer:

Bentham Science Publishers does not guarantee that the information in the Work is error-free, or warrant that it will meet your requirements or that access to the Work will be uninterrupted or error-free. The Work is provided "as is" without warranty of any kind, either express or implied or statutory, including, without limitation, implied warranties of merchantability and fitness for a particular purpose. The entire risk as to the results and performance of the Work is assumed by you. No responsibility is assumed by Bentham Science Publishers, its staff, editors and/or authors for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products instruction,

advertisements or ideas contained in the Work.

Limitation of Liability:

In no event will Bentham Science Publishers, its staff, editors and/or authors, be liable for any damages, including, without limitation, special, incidental and/or consequential damages and/or damages for lost data and/or profits arising out of (whether directly or indirectly) the use or inability to use the Work. The entire liability of Bentham Science Publishers shall be limited to the amount actually paid by you for the Work.

General:

1. Any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims) will be governed by and construed in accordance with the laws of the U.A.E. as applied in the Emirate of Dubai. Each party agrees that the courts of the Emirate of Dubai shall have exclusive jurisdiction to settle any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims).
2. Your rights under this License Agreement will automatically terminate without notice and without the need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.
3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Ltd.

Executive Suite Y - 2

PO Box 7917, Saif Zone

Sharjah, U.A.E.

Email: subscriptions@benthamscience.org



CONTENTS

FOREWORD	i	
REFERENCES	iv	
PREFACE	v	
REFERENCES	vi	
LIST OF CONTRIBUTORS	vii	
SECTION 1: ORIGINS AND DEVELOPMENTAL BIOLOGY OF β-CELLS		
CHAPTER 1 UNDERSTANDING THE DEVELOPMENTAL BIOLOGY OF β-CELL AS A STRATEGY FOR DIABETES REVERSAL		3
<i>Dgt v cpf 'F w x l n k . 'D g p l c o k p 'D t q e j g . 'C p i g r e 'J g t g p i v 'F c t o q p 'a n d 'R c w i T k e j c t f u</i>		
GENETIC NETWORK CONTROLLING PANCREATIC DEVELOPMENT	4	
The Sequential Implication of Transcription Factors	4	
THE PANCREATIC MESENCHYME AND GROWTH FACTORS	5	
ROLE OF NUTRIENTS IN THE DEVELOPMENT OF β -CELLS	7	
Impact of Protein Levels	7	
The Effects of Glucose	8	
IMPORTANCE OF THE PARTIAL PRESSURE OF OXYGEN IN THE INTRAUTERINE MILIEU	8	
OXIDATIVE STRESS AND B-CELL DEVELOPMENT	9	
FROM DEVELOPMENTAL BIOLOGY STUDIES TO INNOVATIVE THERAPEUTIC STRATEGIES FOR THE TREATMENT OF DIABETES	11	
The Context of Diabetes	11	
Application of Our Knowledge From β -Cell Development to Treating Diabetes	11	
<i>Type 1 Diabetes and Islet Transplantation</i>	11	
THE GENETICS OF PANCREAS EMBRYOGENESIS AND THE OCCURRENCE OF DIABETES ...	13	
CONFLICT OF INTEREST	14	
ACKNOWLEDGEMENTS	14	
REFERENCES	15	
CHAPTER 2 AGING AND β-CELL PROLIFERATION, MOLECULAR AND SIGNALING CHANGES AND WHAT THIS MEANS FOR TARGETED REGENERATION		20
<i>C c t q p 'T O E q z</i>		
AGING AND DIABETES	21	
B-CELL PROLIFERATION AND AGING	21	
AGING AND ADAPTIVE B-CELL PROLIFERATION	23	
MOLECULAR AND SIGNALING CHANGES IN AGING	26	
SIGNIFICANCE FOR TARGETED β -CELL REGENERATION	32	
CONFLICT OF INTEREST	33	
ACKNOWLEDGEMENTS	33	
REFERENCES	33	
CHAPTER 3 HUMAN β-CELL MASS AND DISTRIBUTION IN HEALTH, AGING AND DIABETES		42
<i>L q p c u 'H q y i g t . 'C p c p w 'R q w f g r i a n d 'O c p c o k J c t c</i>		
INTRODUCTION	42	
HUMAN PANCREAS	43	
Anatomy	43	

Regional Differences in β -Cell/Islet Mass	44
The Head of the Pancreas	45
Islet Architecture and Cellular Composition	46
β-CELL MASS IN AGING AND DIABETES	47
Absolute Mass of β -Cells and Islets in Humans	47
β -Cell Mass in Aging and Obesity	47
Loss of β -Cell Mass in Diabetes	47
Physical vs. Functional Loss of β -Cell Mass	48
WHOLE PANCREAS ANALYSIS	49
CONCLUDING REMARKS	51
CONFLICT OF INTEREST	51
ACKNOWLEDGEMENTS	51
REFERENCES	51

SECTION 2: FACTORS CONTROLLING β -CELL MASS AND FUNCTION

CHAPTER 4 GESTATIONAL PROGRAMMING OF β -CELL MASS AND PANCREATIC FUNCTION IN THE NEXT GENERATION

<i>F cxf 'LJ km</i>	58
INTRODUCTION	59
ANIMAL MODELS OF FETAL PROGRAMMING OF THE ENDOCRINE PANCREAS THROUGH NUTRITIONAL DEFICIT	59
ANIMAL MODELS OF FETAL PROGRAMMING OF THE ENDOCRINE PANCREAS THROUGH HYPERGLYCEMIA	62
MECHANISMS OF FETAL PROGRAMMING OF THE PANCREATIC FUNCTION	63
Early Dietary Insult and the mTOR Axis	63
Epigenetic Mechanisms Governing Pancreatic Gene Expression and Their Involvement in Fetal Programming of Metabolic Disease	67
Epigenetic Changes to Pancreatic Gene Expression Caused by Environmental Toxins	70
Prematurity of Cellular Aging	70
REVERSAL STRATEGIES FOR FETAL PROGRAMMING OF THE PANCREAS	72
CONCLUSION	72
CONFLICT OF INTEREST	73
ACKNOWLEDGEMENTS	73
REFERENCES	73

CHAPTER 5 MALPROGRAMMING OF β -CELL FUNCTION BY A DIETARY MODIFICATION IN THE IMMEDIATE POSTNATAL PERIOD

<i>Owzj cpf 'URcvr!and'Utrgj 'Ocj o qqf</i>	84
INTRODUCTION	85
Metabolic Programming After Birth	85
EXPERIMENTAL APPROACHES: 'PUP-IN-A-CUP' RAT MODEL	86
Alterations in Islets Mass	87
Molecular Adaptations	88
MOLECULAR MECHANISM BY WHICH GLUCOSE CAN CONTROL β-CELL MASS	90
Alterations in Nutrient-Mediated Insulin Secretion	91
Alterations in Non-Nutrient-Mediated Insulin Secretion	96
Long-Term Consequences Due to Programming	97
IS IT POSSIBLE TO REVERSE METABOLIC PROGRAMMING IN ADULTHOOD?	98
RELEVANCE TO OBESITY AND TYPE 2 DIABETES	99
CONFLICT OF INTEREST	100
ACKNOWLEDGEMENTS	100
REFERENCES	101

CHAPTER 6 NEW CONCEPTS IN THE INTRA-ISLET CONTROL OF β -CELL FUNCTION AND MASS

.....	107
<i>Dtkcp'VONc{f gp.'Ugr j cplg'Xkrc 'and'Y ktko 'NONqy g</i>	
INTRODUCTION	108
Defining the Importance of β -Cell Function and Mass	108
Known Factors Affecting β -Cell Function and Mass and Their Mode of Action	109
PARACRINE AND AUTOCRINE FACTORS REGULATING β-CELL FUNCTION	110
Anatomic Differences Affect Paracrine and Autocrine Signaling Properties Between Species	110
Emerging Autocrine Factors	112
<i>Insulin</i>	112
<i>Proinsulin C-Peptide</i>	113
<i>Islet Amyloid Polypeptide</i>	113
<i>GABA</i>	114
<i>Serotonin</i>	114
<i>Nicotinic Acid Adenine Dinucleotide Phosphate (NAADP)</i>	115
<i>Cholecystokinin (CCK)</i>	115
<i>Acetate</i>	115
Emerging Paracrine Factors	116
<i>Acetylcholine</i>	116
<i>VEGF-Derived Peptides</i>	116
<i>Approaches to Identify New Autocrine/Paracrine Signaling Factors</i>	117
CONCLUSION	117
CONFLICT OF INTEREST	118
ACKNOWLEDGEMENTS	118
REFERENCES	118

CHAPTER 7 β -CELL ADAPTABILITY DURING PREGNANCY 123

Lgpn'J äk kku'Plgnngp.'Uk pg'J qtp.'Lgcpngw'Mtngi cctf.'Co ctpcfj 'Pcmc 'and'Dki kvg'Uänt wr

INTRODUCTION	124
Morphological Changes	125
Hormonal Changes During Pregnancy	126
Somatotrophic Hormones – Systemic Effects	128
Somatotrophic Hormones – Lessons from <i>h⁺Xktq</i> Studies	129
Somatotrophic Hormones – Lessons from Transgenic Mice	132
Somatotrophic Hormones – Mechanism of Action	133
Somatotrophic Hormones – A Downstream Role of STAT5	135
Role of Glucocorticoid Hormones	137
Role of Progesterone	138
Role of Estrogens	138
Role of Epidermal Growth Factor (EGF)	139
Role of Nerve Growth Factor (NGF)	140
Role of Hepatocyte Growth Factor (HGF)	140
Role of Survivin	140
Role of IGF-Binding Protein 5 (Igfbp5)	141
Role of Glucagon-like Peptide 1 (Glp-1)	141
Role of Glucose and Fatty Acids	142
Role on Neurogenin 3 (Ngn-3)	143
Role of Forkhead Box Protein M1 (FoxM1)	143
Role of Hepatic Nuclear Factor 4 α (Hnf4 α)	143
Role of β -cell Lymphoma 6 Protein Homolog (Bcl6) and Menin (Men1)	144
Role of Betatrophin	144
Role of Osteoprotegerin (OPG)	145

Role of Serotonin	145
Role of Cyclophilin B	146
Role of Stathmins	146
Role of Delta-like 1 (dlk-1)	147
Role of Trefoil Factors (TFF)	147
Other Differentially Expressed Islet Genes	147
Circulating Factors in Pregnancy that may Influence β -Cell Function	148
Diabetes in Pregnancy	148
β -Cell Adaptation Postpartum	149
CONCLUSION	150
CONFLICT OF INTEREST	151
ACKNOWLEDGEMENTS	151
REFERENCES	152

SECTION 3: GENERATION OF β -CELLS AND FUTURE APPLICATIONS

CHAPTER 8 β - CELLS FROM EMBRYONIC AND ADULT STEM CELLS AND PROGENITORS ... 169

Ej thakpg'CODgco kij

INTRODUCTION	169
GENERATION OF β-CELLS FROM STEM CELL SOURCE	171
REGENERATION OF THE ENDOCRINE PANCREAS	174
Generation of Insulin-Expressing Cells <i>h'Xlatq</i>	175
Resident Stem/Progenitor Cells Within the Endocrine Pancreas	177
Promiscuity in Cell Lineage Within the Endocrine Pancreas	179
CONFLICT OF INTEREST	180
ACKNOWLEDGEMENTS	180
REFERENCES	180

CHAPTER 9 INDUCTION OF β -CELL REGENERATION BY HUMAN POSTNATAL STEM CELLS 190

Vlrgt 'VOEqrgrt.'Twj 'OOGri co crl'and'F cxlf'COJ guu

INTRODUCTION	191
Dealing with the Complications and Consequences of Diabetes Mellitus: An Emerging Global Crisis?... 191	
The Advent of Cellular Therapies for Diabetes: The Edmonton Protocol	192
Can Islet Regeneration Occur in the Face of Autoimmunity: The Medalist Study?	193
Bone Marrow Stem Cells Initiate Islet Regeneration: Identifying the Mechanisms?	194
HEMATOPOIETIC STEM AND PROGENITOR CELLS	195
Why Use HPC for Islet Regeneration?	196
Isolation of Human Hematopoietic Progenitor Cells Using High ALDH-Activity	197
Preclinical Xenotransplantation Models to Investigate Islet Regeneration	198
Human HPC with High ALDH-activity Promote Regenerating Islet Cell Proliferation and Vascularization	198
Islet Regenerative Functions by Expanded HPC Subsets with High ALDH-Activity	199
Can HPC Transplantation Abrogate Autoimmunity and Permit Endogenous Islet Regeneration?	200
MULTIPOTENT MESENCHYMAL STROMAL CELLS (MSC)	201
Can MSC Create a Regenerative Niche for New Islet Formation?	203
MSC Immunomodulation and Recruitment of M2 Macrophages to Promote Islet Regeneration	206
Can MSC Reverse Autoimmunity in T1D and Support Endogenous β -Cell Regeneration?	207
ENDOTHELIAL PROGENITOR CELLS (EPC)	208
Can We Utilize EPC to Support Endogenous Islet Regeneration and Revascularization?	210
EPC-induced Vascularization Support the Survival and Function of Islet Allografts?	210
CONCLUSION AND FUTURE DIRECTIONS	211

CONFLICT OF INTEREST	213
ACKNOWLEDGEMENTS	213
REFERENCES	213
SUBJECT INDEX	224

FOREWORD

The incidence of both type 1 (T1D) and type 2 diabetes (T2D) has increased over the past few decades and is frequently described as an epidemic. It is now recognized that loss of β -cell mass occurs in both forms of diabetes, through autoimmune destruction in the case of T1D and by exhaustion in the case of T2D. Despite current treatments, blood glucose levels are not restored to normal and this leads to serious injury in several organ systems. Ideally it should be possible to treat or reverse both forms of diabetes by re-establishing sufficient functioning β -cells to maintain normoglycemia. There is general agreement that the cells in the adult pancreas can regenerate, but the routes by which this occurs remain controversial. New β -cells are produced early in life by replication of existing cells, but it is less clear how β -cells could be produced naturally or induced in adults through post-injury ductal neogenesis, activation of resident progenitors/stem cells or transdifferentiation of other non- β -cells (e.g., α -cells or acinar cells). A further complication is understanding to what extent these processes are influenced by environmental conditions.

It has recently been appreciated that terminally differentiated and stem/progenitor cells can differentiate into other cells in the pancreas, consistent with the idea of plasticity in cell fate that depends in part on modification of developmental programs by environmental factors [1]. The fate of cells depends on the type of stress encountered, for example pregnancy, metabolic demand due to hyperglycemia and insulin resistance (glucotoxicity), or ectopic expression or deletion of lineage specific factors.

The importance of restoring lost β -cell mass in T1D has been demonstrated by transplanting islets from cadaveric donors. Unfortunately, this solution is only temporary, requires the recipient to be on powerful immunosuppressive drugs and has side effects. Further constraints are the requirement for 2-3 pancreata/recipient and the limited number of pancreata available for transplantation. Hence, a strong research effort is underway to find other sources of β -cells. These include transplanting pig islets, promoting transdifferentiation of non- β -cells into functioning, insulin-producing β -like cells or enhancing proliferation, maintenance and function of remaining β -cells. Several attempts have been made to develop functioning β -cells from embryonic or adult pluripotent stem cells. However, most reports described the production of immature β -like cells from human pluripotent stem cells that did not respond normally to glucose. A recent report from the Melton group described the *in vitro* production from human embryonic stem cells of glucose-sensitive, insulin-producing β -cells that resemble mature β -cells [2]. It has been pointed out that the basis of this (and other) attempts to find new sources of β -cells comes from a vastly improved and expanded knowledge of pancreas developmental biology [3] and the conditions required to change cell fate, areas

which are addressed in this e-book. As with any discovery, there is good news and bad news. The use of embryonic stem cells still raises ethical concerns and there are technical issues to overcome before such a therapy can be considered for use in the clinic. Others must replicate these findings. It must also be remembered that these cells do not have the exact genetic profile and they are not identical copies of β -cells *in situ* [3]. It is unclear what will be the long term effects of transplanting such cells in patients. As with any “replacement” β -cells, they must be protected from the host’s immune system. To do this, they will likely be placed in protective capsules that are themselves the target of a fibrotic process in the body that blocks the exchange of nutrients and insulin across the membrane. In short, it will be a while before these cells are ready for prime time.

Many questions remain unanswered regarding β -cell plasticity. Is it only a subset of cells of a certain lineage (*e.g.*, acinar) that respond to external stress and express transcription factors that promote self renewal or multi-potency directly or through de-differentiation? An ever present danger is the potential for these processes to go unchecked if fate constraints are lost, leading to cancer. It has been pointed out that it is difficult to evaluate transdifferentiation *per se* because the characteristics that reflect the extent of the new cell’s maturity and stability of the phenotype are unclear [1]. Does the phenotype of these new cells differ from that of the cells that occur during development?

The editor, Dr. David Hill, has brought together experts in the field who provide extensive and sometimes provocative state-of-the-art discussions of various key aspects of β -cell function and plasticity in health and diabetes. The book is divided into three sections. In the first section entitled: “Origins and developmental biology of β -cells”, Dr. Bertrand Duvill   and colleagues (Paris) review advances in understanding the hierarchy of transcription factors and discuss the influence of growth factors, partial oxygen pressure and nutrients in the intrauterine environment on β -cell maturation. This is followed by Aaron Cox’s (Houston) discussion of the role of aging on β -cell development and how the decline in β -cell proliferation is affected by age-related impairment in signal transduction, altered cell cycle progression and epigenetic regulation of genes is likely to affect attempts to regenerate β -cells. Part 1 ends with a chapter on β -cell mass across the spectrum of aging, health and diabetes by Dr. Manami Hara and colleagues (Chicago). They discuss the heterogeneous distribution and function of β -cells among and within individuals and the need to identify markers of dysfunctional β -cells.

The second section addresses “Factors controlling β -cell mass and function”. Beginning with Dr. David Hill’s (London, ON) discussion of gestational programming of β -cell mass and function in which he emphasizes that the period of gestation affects cell fate and β -cell function in the offspring. Using fetal growth retardation as a model, he notes several systems are adversely affected leading to decreased β -cell mass, proliferation, increased apoptosis and

later impaired glucose regulation. The mechanisms are discussed and the point is made that giving micronutrient supplements to the dam and β -cell trophic peptide hormones to the neonate decreases disease risk. The second article by Drs. Mulchand S. Patel and Saleh Mahmood (Buffalo) addresses modification of β -cell function by diet in neonates. They suggest that the feeding of carbohydrate rich food adversely affects islet structure and β cell function in suckling neonates leading to hyperinsulinemia attributable to increased β cell plasticity. The changes noted in developmental gene expression and β -cell function lead to obesity in the adult which could promote development of T2D. The third chapter in this section is by Dr. Brian T. Layden and colleagues (Chicago) in which they provide a discussion of intra-islet control of β -cell function and mass. They highlight the role of under-appreciated autocrine and paracrine factors that contribute to β -cell mass and function. The last chapter in this section by Dr. Jens HøiriisNielsen and colleagues (Copenhagen) deals with beta cell adaptability during pregnancy. Although it is well known that β -cell mass expands during pregnancy, they describe in detail the many growth factors, transcriptional modifications and changes in gene expression that have recently been characterized. The mechanism involved in β -cell mass expansion during pregnancy remains unclear, in particular the degree to which neogenesis is involved in rodents and humans.

The third and last part of the book deals with “Generation of β -cells and future applications”. Chapter 8 by Dr. Christine A. Beamish (London, ON) addresses the production of β -cells from embryonic and adult stem cells and progenitors as a source of replacement β -cells in diabetes. The plasticity of cell fate in the endocrine pancreas is discussed. The last chapter is by Dr. Tyler T. Cooper and colleagues (London, ON) in which they describe the latest thinking on the role of bone marrow-derived stem cells for β -cell regeneration.

It is now clear that T1D is far more complex than previously appreciated [4]. In particular, the long history of mostly immune-based therapies has not resulted in new treatments or cures. Despite more than 20 clinical trials, most focused on immune suppression, there has been a remarkable lack of success with respect to prevention or reversal of T1D [5]. The picture continues to evolve with respect to T2D as well, as a role for adipose tissue inflammation is now being investigated. To address this level of complexity, both forms of diabetes must be thought of in terms of their integrative biology. That is, how do genetic predisposition, inappropriate immune reactivity, inappropriate β -cell mass/function/regenerative capacity and environmental exposures come together to result in diabetes? We are entering a new era of big data obtained through readily available high throughput analyses and higher computer power. This has raised the possibility of personalized or so-called “precision medicine” to address the heterogeneity in the human population and reveal the many pathways by which diabetes likely occurs. A further new approach involves the acknowledgement that there is unlikely to be a single infectious agent akin to *helicobacter pylori* and ulcers in the

environment that causes diabetes. To address the multiplicity of environmental factors that undoubtedly influence diabetes and other chronic diseases, a new “omics” designation was coined, termed the exposome [6]. And finally, it must be said that timing is indeed everything. Thus, the timing of these interactions and exposures is crucial in determining the success of the early developmental program for appropriate β -cell mass/function or its potential recapitulation following injury, inflammation or disease. The articles in this e-book provide up-to-date summaries of key elements affecting natural and induced forms of β -cell plasticity and its potential as a therapy for diabetes. The reader will no doubt enjoy this timely collection.

Dr. Fraser W. Scott

The Ottawa Hospital Research Institute

Department of Medicine

Department of Biochemistry, Microbiology and Immunology

University of Ottawa

Ottawa, Ontario, Canada

REFERENCES

- [1] Puri S, Folias AE, Hebrok M. Plasticity and dedifferentiation within the pancreas: development, homeostasis, and disease. *Cell Stem Cell* 2015; 16(1): 18-31.
[<http://dx.doi.org/10.1016/j.stem.2014.11.001>] [PMID: 25465113]
- [2] Pagliuca FW, Millman JR, Gürtler M, *et al.* Generation of functional human pancreatic β cells *in vitro*. *Cell* 2014; 159(2): 428-39.
[<http://dx.doi.org/10.1016/j.cell.2014.09.040>] [PMID: 25303535]
- [3] Mfopou JK, Bouwens L. Diabetes: β cells at last. *Nat Rev Endocrinol* 2015; 11(1): 5-6.
[<http://dx.doi.org/10.1038/nrendo.2014.200>] [PMID: 25385037]
- [4] Atkinson MA, von Herrath M, Powers AC, Clare-Salzler M. Current concepts on the pathogenesis of type 1 diabetes: considerations for attempts to prevent and reverse the disease. *Diabetes Care* 2015; 38(6): 979-88.
[<http://dx.doi.org/10.2337/dc15-0144>] [PMID: 25998290]
- [5] Lord S, Greenbaum CJ. Disease modifying therapies in type 1 diabetes: Where have we been, and where are we going? *Pharmacol Res* 2015; 98: 3-8.
[<http://dx.doi.org/10.1016/j.phrs.2015.02.002>] [PMID: 25771310]
- [6] Wild CP. Complementing the genome with an exposome: the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol Biomarkers Prev* 2005; 14(8): 1847-50.
[<http://dx.doi.org/10.1158/1055-9965.EPI-05-0456>] [PMID: 16103423]

PREFACE

An adaptive metabolic axis has been a major evolutionary advantage in allowing humans to colonize every part of the globe from arid deserts to permanent ice fields. Prior to an effective food supply chain, metabolic plasticity evolved to deal with seasonal famines balanced by times of plenty, and a greater diversity of diets than perhaps any other mammalian species. In the developed world there are new challenges to metabolic plasticity including food overabundance, unbalanced diets, child and adult obesity, and an increasing rate of type 1 and 2 diabetes. A plasticity of pancreatic β -cell mass and function are key to metabolic adaptation. The β -cell mass normally increases proportionally to fetal and child growth, in response to the added metabolic stress of pregnancy, and in response to the nutritional stress of an obesogenic diet. Yet, in the face of the autoimmune challenge of type 1 diabetes or the glucotoxicity of type 2 diabetes there is a net loss of β -cells with limited potential for endogenous regeneration. Thus lies the paradox. How can a highly physiologically-adaptive β -cell mass prove so difficult to manipulate following the pathological loss that accompanies diabetes?

Key to creating and testing strategies for the therapeutic manipulation of β -cell number is to know their developmental origins and normal ontogeny. The first section of this volume addresses current knowledge around the developmental origins of pancreatic β -cells, and how β -cell mass and proliferation change throughout the human lifespan. The second section explores the mechanisms responsible for β -cell plasticity, drawing from animal models and clinical studies revealing environmental, epigenetic, endocrine and paracrine regulators that contribute to the normal homeostatic processes, and the delicate balance of proliferation vs. apoptotic loss that optimizes β -cell mass during normal metabolic homeostasis. The final section examines the presence and potential of resident stem cells within the pancreas or bone marrow, β -cell progenitors, and the potential for pancreatic endocrine cell differentiation or trans-differentiation.

Underlying each of these chapters is the assumption that β -cells can potentially be replaced endogenously, but only through a thorough understanding of normal development and the exploitation of existing, but perhaps sub-optimal adaptive physiology. There is great reason for confidence. In humans there is reproducible histological evidence of β -cell turnover involving mitogenesis and apoptosis throughout life, including both children and adults with type 1 or type 2 diabetes [1 - 5]. The regenerative potential of human β -cells may normally be age-limited, since new cells were not generated in the short-term in patients aged over 50 following surgical reduction of pancreatic mass [6]. However, insulin release had improved between 2-4 years post-surgery, suggesting that even in older individuals a slower adaptive replacement of β -cells can occur [7]. Overcoming such physiological limitations to optimize

β -cell mass and function to match metabolic demand will likely be a major focus for diabetes research in the coming decade.

Dr. David J. Hill

Lawson Health Research Institute
St. Joseph's Health Care, 268 Grosvenor Street
London, Ontario N6A 4V2
Canada
Tel: 519 6466100 Ext. 64716
E-mail: david.hill@lhrionhealth.ca

REFERENCES

- [1] Gepts W, De Mey J. Islet cell survival determined by morphology. An immunocytochemical study of the islets of Langerhans in juvenile diabetes mellitus. *Diabetes* 1978; 27 (Suppl. 1): 251-61. [<http://dx.doi.org/10.2337/diab.27.1.S251>] [PMID: 75815]
- [2] Yoneda S, Uno S, Iwahashi H, *et al.* Predominance of β -cell neogenesis rather than replication in humans with an impaired glucose tolerance and newly diagnosed diabetes. *J Clin Endocrinol Metab* 2013; 98(5): 2053-61. [<http://dx.doi.org/10.1210/jc.2012-3832>] [PMID: 23539729]
- [3] Keenan HA, Sun JK, Levine J, *et al.* Residual insulin production and pancreatic β -cell turnover after 50 years of diabetes: Joslin Medalist Study. *Diabetes* 2010; 59(11): 2846-53. [<http://dx.doi.org/10.2337/db10-0676>] [PMID: 20699420]
- [4] Meier JJ, Bhushan A, Butler AE, Rizza RA, Butler PC. Sustained beta cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? *Diabetologia* 2005; 48(11): 2221-8. [<http://dx.doi.org/10.1007/s00125-005-1949-2>] [PMID: 16205882]
- [5] Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG. Evidence of increased islet cell proliferation in patients with recent-onset type 1 diabetes. *Diabetologia* 2010; 53(9): 2020-8. [<http://dx.doi.org/10.1007/s00125-010-1817-6>] [PMID: 20532863]
- [6] Menge BA, Tannapfel A, Belyaev O, *et al.* Partial pancreatectomy in adult humans does not provoke β -cell regeneration. *Diabetes* 2008; 57(1): 142-9. [<http://dx.doi.org/10.2337/db07-1294>] [PMID: 17959931]
- [7] Menge BA, Breuer TG, Ritter PR, Uhl W, Schmidt WE, Meier JJ. Long-term recovery of β -cell function after partial pancreatectomy in humans. *Metabolism* 2012; 61(5): 620-4. [<http://dx.doi.org/10.1016/j.metabol.2011.09.019>] [PMID: 22079939]

List of Contributors

Aaron R. Cox	McNair Medical Institute, Baylor College of Medicine, Houston, Texas
Amarnadh Nalla	Department of Biomedical Sciences, University of Copenhagen, Denmark Centre for Fetal Programming, Copenhagen, Denmark
Ananta Poudel	Department of Medicine, The University of Chicago, Chicago, USA
Angela H. Darmon	INSERM, U1016, Institut Cochin, Paris, France Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France
Benjamin Broche	INSERM, U1016, Institut Cochin, Paris, France Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France
Bertrand Duvillié	INSERM, U1016, Institut Cochin, Paris, France Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France
Birgitte Søstrup	Department of Biomedical Sciences, University of Copenhagen, Denmark Centre for Fetal Programming, Copenhagen, Denmark
Brian T. Layden	Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois, USA
Christine A. Beamish	Department of Surgery, Islet Transplantation Laboratory, The Methodist Hospital Research Institute, Houston Texas, USA
David A. Hess	Molecular Medicine Research Group, Krembil Centre for Stem Cell Biology, Robarts Research Institute; Department of Physiology & Pharmacology, Schulich School of Medicine & Dentistry, Western University, London, Ontario, Canada
David J. Hill	Lawson Health Research Institute, St. Joseph's Health Care, 268 Grosvenor Street, London, Ontario N6A 4V2, Canada
Jeanette Kirkegaard	Department of Biomedical Sciences, University of Copenhagen, Denmark Novo Nordisk A/S, Måløv, Denmark
Jens H. Nielsen	Department of Biomedical Sciences, University of Copenhagen, Denmark Centre for Fetal Programming, Copenhagen, Denmark
Jonas Fowler	Department of Medicine, The University of Chicago, Chicago, USA
Manami Hara	Department of Medicine, The University of Chicago, Chicago, USA
Mulchand S. Patel	Department of Biochemistry, School of Medicine and Biomedical Sciences, University at Buffalo, The State University of New York, Buffalo, New York 14214, USA

- Paul Richards** INSERM, U1016, Institut Cochin, Paris, France
Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France
- Ruth M. Elgamal** Molecular Medicine Research Group, Krembil Centre for Stem Cell Biology, Robarts Research Institute; Department of Physiology & Pharmacology, Schulich School of Medicine & Dentistry, Western University, London, Ontario, Canada
- Saleh Mahmood** Department of Biochemistry, School of Medicine and Biomedical Sciences, University at Buffalo, The State University of New York, Buffalo, New York 14214, USA
- Signe Horn** Department of Biomedical Sciences, University of Copenhagen, Denmark
Novo Nordisk A/S, Måløv, Denmark
- Stephanie Villa** Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA
- Tyler T. Cooper** Molecular Medicine Research Group, Krembil Centre for Stem Cell Biology, Robarts Research Institute; Department of Physiology & Pharmacology, Schulich School of Medicine & Dentistry, Western University, London, Ontario, Canada
- William L. Lowe** Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

Section 1: Origins and Developmental Biology of β -Cells

CHAPTER 1**Understanding the Developmental Biology of β -Cells as a Strategy for Diabetes Reversal****Bertrand Duvillié^{1,2,*}, Benjamin Broche^{1,2}, Angela Herengt Darmon^{1,2} and Paul Richards^{1,2}**¹ INSERM, U1016, Institut Cochin, Paris, France² Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France

Abstract: In recent decades, intense efforts have been made to understand the cellular and molecular mechanisms controlling β -cell development. This process is well coordinated and consists of multiple steps. Many studies have tried to identify (i) molecular signals governing the proliferation of progenitors and (ii) their differentiation into mature pancreatic β -cells. A number of laboratories have focused on the role of transcription factors, and well constructed experiments have contributed to defining a hierarchy, highlighting the importance of each transcription factor in the interconnected network. Moreover, studies over the last 10 years have shown that the pancreatic mesenchymal cells, which are in contact with progenitors, influence pancreas organogenesis. Recent work has also indicated that the intra-uterine milieu influences gene expression and endocrine development. Indeed, nutrients, locally expressed growth factors and even the partial pressure of oxygen also control pancreas development. In a more applied setting, these understandings may improve our knowledge on the different forms of diabetes and, importantly, allow us to mimic a similar developmental process *in vitro*. This is because the precise understanding of each step *in vivo* seems to be necessary for designing protocols to generate β -cells from embryonic stem (ES) cells or induced pluripotent stem cells (iPS). These stem cell-derived β -cells should, in theory, provide new sources of insulin-secreting cells for transplantation into diabetic patients. A description of the recent advances in the field will be presented and illustrated in this chapter.

* **Corresponding author Bertrand Duvillié:** Institut Curie, Centre de Recherche, Laboratoire Eychene, INSERM U1021, CNRS UMR3347, Bât. 110, Centre Universitaire, 91405 ORSAY Cedex, France; Tel: (33) 1 69 86 30 74, Fax: (33) 1 69 86 30 51; E-mail: bertrand.duvillie@curie.fr

Keywords: Beta cell, Development, Differentiation, FGF, HIF, Insulin, Oxygen, Pancreas, Proliferation, Reactive oxygen species.

GENETIC NETWORK CONTROLLING PANCREATIC DEVELOPMENT

The pancreas originates from the dorsal and ventral regions of the foregut endoderm, located directly behind the stomach. Signals from adjacent mesodermal structures, notochord, dorsal aorta and cardiac mesoderm are important for the emergence and early development of the pancreas [1 - 5]. During these early stages, *i.e.* E11.5 in the mouse, the pancreas is composed of an undifferentiated epithelium surrounded by mesenchyme. The epithelial fraction contains all the precursor cells that develop into the exocrine and endocrine compartments. A sequential expression of transcription factors is required to determine the commitment of these early progenitors to the different endocrine and exocrine cell types.

The Sequential Implication of Transcription Factors

These precursor cells express the gene *Pancreatic and Duodenal Factor 1 (Pdx1)*. This is a master gene as its deletion leads to the complete agenesis of the pancreas in the mouse. In humans, the paralogue of Pdx1 is called insulin promoting factor 1 (IPF1). Interestingly, a pancreatic agenesis was also described in a patient with a homozygous single nucleotide deletion in the codon 63 of IPF1. The role of Pdx1 is thus conserved between rodents and humans. Following Pdx1 expression, the transcription factor Neurogenin 3 (NGN3) is transiently expressed in endocrine progenitors. The disruption of *Ngn3* results in the absence of α -, β -, δ - and PP-cells [6]. Other transcription factors are involved more specifically in exocrine cell development, for example MIST1 [7]. The genetic network controlling pancreas development is represented in Fig. (1).

In addition to these complex genetic mechanisms, it has been shown that the local environment releases signals that determine not only proliferation of the precursor cells but also their differentiation. A detailed review of these signals will be presented in the next section.

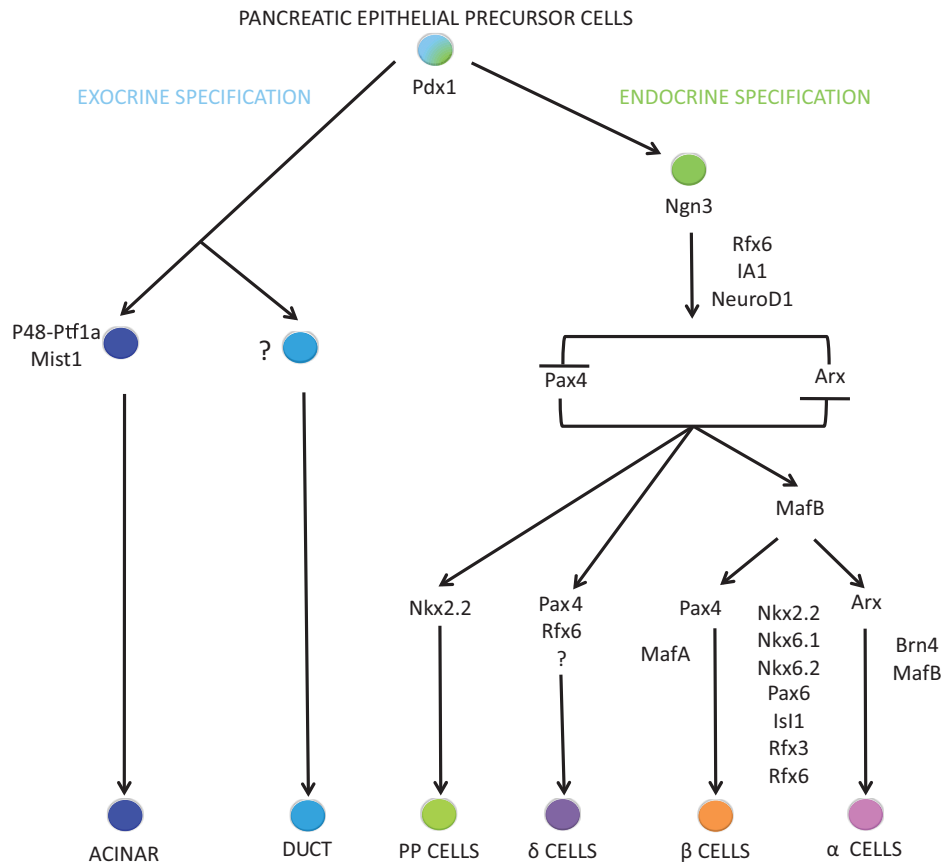


Fig. (1). The genetics of pancreas development [8].

THE PANCREATIC MESENCHYME AND GROWTH FACTORS

For many years the exact role of the mesenchymal cells that surround the epithelial precursors remained elusive and was the subject of much debate. In the laboratory of R Scharfmann, an *in vitro* model allowing the development of the embryonic pancreas in the presence or absence of the mesenchyme was designed [9]. In these conditions, more acinar cells developed in the presence rather than in the absence of the mesenchyme. To investigate the mechanism by which the mesenchyme exerts its effects, the expression of tyrosine kinase receptors was researched. A special interest was paid to the Fibroblast Growth Factors family (FGFs). There are four FGF receptors, called FGF1 to FGF4, with several isoforms. Of importance, the FGFR2IIIb isoform expression is specific to the

Aging and β -Cell Proliferation, Molecular and Signaling Changes and What This Means for Targeted Regeneration

Aaron R. Cox*

McNair Medical Institute, Baylor College of Medicine, Houston, Texas, U.S.A

Abstract: Increased age confers a greater risk for the development of type 2 diabetes (T2D), and also has significant consequences for β -cell growth and regeneration. Pancreatic insulin-producing β -cells are long-lived, and exhibit very little turnover in adult life. The severe decline in β -cell proliferation contributes to a decreased capacity for β -cell regeneration with age. β -cell regeneration is dependent on mitogenic signals, receptor and downstream signal transduction, cell cycle progression, and epigenetic regulation of gene expression, all of which are significantly affected by increasing age. Studies suggest that circulating growth factors and their receptors are decreased with age, along with important intracellular signaling molecules, such as Pdx-1 and FoxM1. Cell cycle progression is inhibited by an increased expression of cell cycle inhibitors and a reduction in cell cycle kinase complexes (Cyclin/Cdks). Moreover, decreased expression of epigenetic silencers, such as polycomb group proteins, results in de-repression of the cell cycle inhibitor p16, and a significant reduction in β -cell proliferation. Collectively, these age-induced changes present obstacles for the design of β -cell regenerative therapies for diabetes; however, some reports suggest that even very old β -cells can re-enter cell cycle. Future studies will further define the effects of aging on β -cell proliferation and elucidate new drug targets for diabetes therapy.

Keywords: Aging, β -cell regeneration, Cell cycle, Diabetes, Epigenetics, Molecular signals, Proliferation.

* Corresponding author Aaron R. Cox: McNair Medical Institute, Baylor College of Medicine, Houston, Texas, U.S.A; Tel/Fax: 832-824-0711; E-mail: racox@bcm.edu

AGING AND DIABETES

Aging is associated with an increased risk for several diseases including metabolic syndrome and type 2 diabetes (T2D). The incidence of T2D increases with age from ~8% in middle age (40-59 years) to ~33% in older adults (>60 years) [1]. These rates increase substantially when accounting for individuals with prediabetes, defined as impaired fasting glucose or glucose tolerance. There are many factors which may contribute to the age associated increased risk of diabetes. Peripheral insulin sensitivity declines with age, and can be attributed to increased obesity, reduced physical activity, and decreased lean muscle mass [2 - 4]. These changes are moderately compensated by increased β -cell function and hyperinsulinemia [5]. However, aging is also associated with a progressive decrease in β -cell function [6 - 8], which may be impacted by decreased incretin levels [9, 10], Sirt1-mediated glucose stimulate insulin release [11], mitochondrial function and ATP content [12, 13], as well as increased oxidative stress [12] and glucolipotoxicity [14]. Moreover, isolated islets demonstrate greater sensitivity to glucose-induced β -cell apoptosis with increasing age [8]. Thus, chronic hyperglycemia and insulin resistance in the setting of aging and declining β -cell function collectively contribute to drive β -cell loss. Human post-mortem samples demonstrate increased rates of apoptosis and reduced β -cell mass in patients with T2D [15]. The inability of β -cells to adequately compensate through increased β -cell mass in the presence of insulin resistance leads to β -cell apoptosis and T2D. Elucidation of the mechanisms of aging that contribute to failed β -cell compensation will be critical for diabetes therapies. Aging is also an important factor in type 1 diabetes (T1D) given that over 50% of T1D cases occur in adults [16], and moreover, with insulin therapy and quality health care, the life expectancy of juveniles with T1D extends well into adulthood [17]. Considering the large adult population with T1D, age associated changes in β -cell function and mass may influenced the design of successful diabetes therapies.

β -CELL PROLIFERATION AND AGING

Pancreatic β -cell mass rapidly increases in young rodents and slowly expands with advancing age [18, 19]. Maintenance and expansion of β -cell mass is dependent on a fine balance between cell birth (self-replication and neogenesis)

and cell death (apoptosis). Postnatal β -cell neogenesis is extremely controversial, and while many suggest that β -cells arise from exocrine duct cell differentiation [20 - 22], there is strong evidence to suggest that postnatal β -cell growth exclusively occurs by self-duplication [23 - 27]. Thus, it is critically important to understand the underlying biology governing β -cell proliferation for developing diabetes therapies.

β -cell proliferation in the early postnatal period is $\sim 20\%$ in rodents [18, 28], declining dramatically in adolescence and into young adulthood [18, 19, 28, 29]. This decline stabilizes within the first 100 days of life to $\sim 1-4\%$ per day [18]. Based on this data, it was estimated that the lifespan of a β -cell was between 1 and 3 months [18]. Limited data at older ages prevented conclusive determination of the β -cell replication rate in older rats. Subsequently, Montanya *et al.* [19] measured intra-islet proliferation in rats up to 20 months of age. The authors determined that intra-islet cell proliferation stabilized at $\sim 0.1\%$ using a 6-h BrdU pulse ($\sim 0.4\%$ per day) at 7 months of age. Mouse β -cell proliferation was extremely low at 1 year of age, $\sim 0.04\%$ per day, and remained constant at 19-months [28, 30]. Similarly, β -cell apoptosis in metabolically normal rodents is rare and decreases with age [28, 31, 32]. β -cell turnover is governed by the replication refractory period, which prevents cell cycle entry immediately following cell division, thus limiting the frequency a β -cell can divide [24]. This replication refractory period of β -cell turnover is lengthened with age [24, 33]. Collectively, these studies suggest that β -cells are largely post-mitotic and long-lived, contributing to a very slowly expanding adult tissue through self-renewal. Therefore, rodent β -cell turnover is minimal with increasing age.

Human β -cell proliferation also decreases with age [15, 34 - 37]. Meier *et al.* [34] measured β -cell proliferation by Ki67 in 46 tissue samples from children and young adults from 2 weeks to 21 years. In some infants, β -cell proliferation was quite high ($\sim 2.5\%$), and quickly declined with age. Surprisingly, pancreata from several individuals indicated no β -cell proliferation even at an early age (<10 years). Low replication rates were also observed in middle age [35]. By elderly age (~ 78 years), data from 17 non-diabetic individuals indicated that β -cell proliferation rates had fallen below 0.1% Ki67⁺ β -cells [15]. While Ki67⁺ expression is a static measurement at the time of pancreas collection, thymidine

Human β -Cell Mass and Distribution in Health, Aging and Diabetes

Jonas L. Fowler, Ananta Poudel and Manami Hara*

Department of Medicine, The University of Chicago, Chicago, USA

Abstract: Regulation of pancreatic β -cell mass is an essential matter to understand pathophysiology of diabetes. Physiological and pathological changes of β -cell mass associated with aging, obesity and diabetes have been reported for over a century. However, the degree of compensation or alteration significantly varies among literature. The difficulty in studying the human pancreas is its large size and uneven distribution of β -cells/islets. Whole pancreas analysis has revealed intra-individual (regional) and inter-individual heterogeneity in β -cell mass, which hampers accurate quantification. Furthermore, physical β -cell loss is not the only contributing factor, but “dysfunctional” β -cells may be involved in insulin deficiency as well. Development of a practical stereological approach to quantify β -cell mass to overcome intra-individual and inter-individual heterogeneity would provide a standardized methodology in the field. Identification of marker(s) for quantifying dysfunctional β -cells that synthesize insulin but are deficient in insulin secretion should lead to a better understanding of β -cell pathophysiology.

Keywords: Aging, β -cell mass, Diabetes, Islets.

INTRODUCTION

Accurate quantification of β -cell mass in the human pancreas is challenging due to its large size. Furthermore, there is marked variability in the β -cell/islet distribution in the different regions of the pancreas. The head region is anatomically and developmentally distinct, and it exclusively contains a pancreatic polypeptide

* **Corresponding author Manami Hara:** Department of Medicine, The University of Chicago, Chicago, USA; Tel/Fax: 773-702-3727/773-834-0486; E-mail: mhara@uchicago.edu

(PP) cell rich area, which can confound analysis of β -cell mass if not analyzed separately. Besides these regional differences, there is also marked variability among individuals that show no direct association with sex, age, BMI or type 2 diabetes (T2D). Starting with the basics of the human pancreas from its anatomy to intrinsic heterogeneity in β -cell mass, this chapter will review recent studies on β -cell mass and distribution in health, aging and diabetes and current difficulties of accurately assessing β -cell mass will be discussed.

HUMAN PANCREAS

Anatomy

The human pancreas is commonly divided into three main regions based on the anatomy, which are referred to the head, body, and tail. The head is localized closely to the duodenum on the right side of the abdomen. The superior mesenteric vessels beneath the neck are inferior and to the left of the head of the pancreas, while the uncinate process lies within the head posterior and to the right of the superior mesenteric vessels. The body region extends laterally to the left of the head, lying along the floor of the lesser sac, which is covered by peritoneum derived from the superior leaf of the transverse mesocolon. The tail region extends from the body laterally into the left side of the abdomen towards the spleen, where there is no coverage from transverse mesocolon derived peritoneum.

The head region is distinct from the rest of the pancreas both developmentally and anatomically [1] (Fig. 1). The pancreas has two main sources of blood supply. The superior pancreaticoduodenal artery supplies the head region, while the splenic artery supplies both the body and tail. Hepatic ducts from the liver and cystic duct from the gall bladder merge into the common bile duct, which flows down to the head region of the pancreas and at the ampulla of Vater connects with the pancreatic duct. The ductal network is controlled by innervations from the celiac plexus and vagus. The sphincter of Oddi, a circular muscle band, functions as a valve to regulate the flow of pancreatic enzymes and bile into the duodenum, separating the pancreas from the intestinal environment.

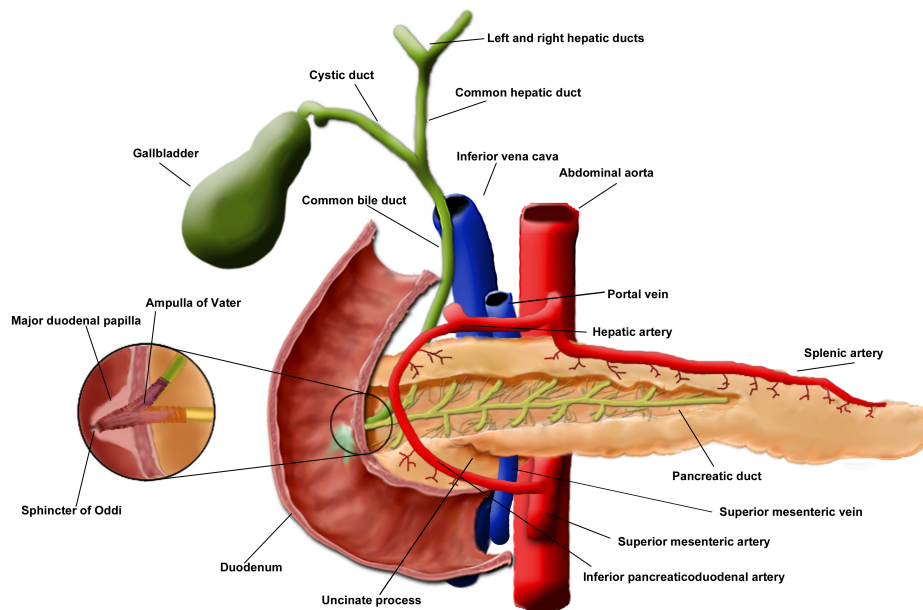


Fig. (1). Head region of the human pancreas (Reproduced from [1]).

Regional Differences in β -Cell/Islet Mass

The pancreatic islet is a highly vascularized micro-organ that is composed of multiple cell types including relatively large populations of β -, α - and δ -cells and small populations of PP- and ϵ -cells. There is an intrinsic regional difference in β -cell mass and correspondingly islet mass [2, 3]. While it is comparable between the head and body region, there is a gradual increase in β -cell and islet mass toward the tail region resulting in a ~ 2 -fold difference. This regional difference in β -cell/islet density has important implications for the assessment of β -cell mass. Random inter-specimen comparisons between different regions may confound study accuracy. For example, a study of the tail region alone where the measured values are normalized using the pancreas volume or weight could result in overestimation of β -cell/islet mass, whereas using only the head or body regions could result in underestimation.

Section 2: Factors Controlling β -Cell Mass and Function

Gestational Programming of β -Cell Mass and Pancreatic Function in the Next Generation

David J. Hill*

Lawson Health Research Institute, St. Joseph's Health Care, 268 Grosvenor Street, London, Ontario N6A 4V2, Canada

Abstract: The gestational environment can have profound effects on the future health of the offspring, including a greater risk of type 2 diabetes and of cardiovascular diseases. Whilst the function of numerous tissues that can impact on future metabolism are altered by an adverse fetal environment, including the hypothalamic control of appetite and the release of glucocorticoids, hepatic function, and the insulin sensitive tissues such as skeletal muscle and adipose, some of the most definitive data concerns changes in the phenotype and function of the pancreatic β -cells. A number of animal models of intrauterine growth restriction (IUGR) have been utilized to study the long-term effects on the offspring, such as a reduced maternal calorie intake, a reduced protein content of the diet, uterine vessel occlusion, and nicotine administration. Changes to the pancreatic β -cells are remarkably similar and include a reduced tissue mass, lower rate of proliferation, increased developmental apoptosis, less plasticity following damage postnatally, higher sensitivity to cytotoxic cytokines, and reduced glucose-stimulated insulin release. These changes persist into adulthood and result in impaired glucose tolerance. Similar changes are also seen in offspring from pregnancies complicated by maternal diabetes. The mechanisms responsible for the altered β -cells function include changes to the mTOR signaling pathway, epigenetic changes altering the expression of key genes involved with β -cell growth and insulin synthesis, and changes in the rate of telomere shortening resulting in premature cellular aging. These pathways may also be influenced by environmental toxins during pregnancy. Nutritional intervention by micronutrient supplementation of the mother, or treatment of the newborn with peptide hormones trophic for the β -cells can reverse the pancreatic phenotype and reduce the risk of adult metabolic disease.

* **Corresponding author David J. Hill:** Lawson Health Research Institute, St. Joseph's Health Care, 268 Grosvenor Street, London, Ontario N6A 4V2, Canada; Tel/Fax: 519 6466100 Ext. 64716; E-mail: david.hill@lhrionhealth.ca

David J. Hill (Ed.)

All rights reserved-© 2016 Bentham Science Publishers

Keywords: β -cell mass, Epigenetics, Fetal programming, Gestational diabetes, Intra-uterine growth restriction, MTOR, Pregnancy, Type 2 diabetes.

INTRODUCTION

The pre-conceptual and gestational environment can have profound effects on the future health of the offspring, including a greater risk for type 2 diabetes, cardiovascular diseases and other chronic diseases [1]. This was convincingly shown in cohort studies of individuals exposed to the Dutch hunger winter of 1944/45 where severe maternal calorie restriction during first trimester resulted in a reduced birth weight and a higher rate of offspring obesity by 19 years of age, associated with diabetes and vascular disorders in later life [2]. Surprisingly, similar disease risks are observed in the offspring of women who were obese or had diabetes prior to conception, or who developed hyperglycemia, with or without gestational diabetes [3 - 6]. The impact of relative under- or over-nutrition of the fetus on future health has been shown to involve programmed changes to a number of key tissues involved with energy homeostasis, including adipose, liver, and muscle [7], as well as changes to the neuronal architecture of the hypothalamus resulting in altered production of peptides that determine appetite, such as NPY [8], responsiveness to leptin [9] and the basal levels of cortisol and tissue glucocorticoid receptors [10]. Fetal programming of future metabolic diseases is therefore likely to represent an accumulated effect of developmental changes across multiple tissues. However, some of the most profound and well-studied determinants involve changes to the phenotype and function of the pancreatic β -cells. This chapter will review some of the animal models used and the mechanisms proposed for environmental programming of the developing endocrine pancreas prior to birth, and some of the corrective strategies so far investigated.

ANIMAL MODELS OF FETAL PROGRAMMING OF THE ENDOCRINE PANCREAS THROUGH NUTRITIONAL DEFICIT

A number of animal models that disrupt maternal nutritional availability or uteroplacental function have been used to represent the human small-for-gestational age term infant, as reviewed previously [11]. These include reduced

calorie availability to the pregnant rat or mouse [12, 13], uterine vessel occlusion to reduce utero-placental blood flow [14] and nicotine administration to the mother [15]. In each case the resulting changes to endocrine pancreas morphology and function are remarkably similar. We and others have utilized a maternal low protein diet (LP) made isocaloric by the addition of carbohydrate from gestation to either parturition, or continued until weaning. If LP diet was given to rats or mice throughout pregnancy it resulted in a lower β -cell mass and mean islet size in the offspring at birth, and this was further exacerbated at weaning [16 - 19]. This was due to less β -cell proliferation together with an increased rate of developmental apoptosis. Further analysis showed that the cell cycle kinetics of β -cell replication had been altered with an extended G1 phase [16]. Whilst some recovery of β -cell mass was possible if the LP dietary insult was removed at parturition, severe deficits remained throughout life if the insult was extended to weaning [17]. Glucose-stimulated insulin release was reduced and β -cells were more susceptible to cytokine-induced cell death *in vitro* [16, 20]. These deficits were transmitted to the F2 generation through females, even when the F1 offspring received a normal diet through gestation. Once adult at 130 days of age the offspring of the LP-fed rats were glucose intolerant with peripheral glucose resistance [19]. A direct human correlate of the maternal LP diet model in rodents is the reduced birth weight associated with restricted maternal protein intake experienced by vegetarian women in rural India, as characterized by the Pune Maternal Nutrition Study [21, 22].

Exposure to LP diet during gestation also had phenotypic effects on the anatomy of the microvasculature of the pancreas. This is important as paracrine signaling occurs across the basement membrane juxtaposing the β -cell and the capillary endothelium, mediated by integrins and by various peptide growth factors, including vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) and [23 - 25]. The presence of VEGF-A is necessary to maintain a fenestrated islet endothelium that allows for rapid glucose sensing from the circulation and the export of secreted insulin [24]. We reported that intra-islet vascular volume and the abundance of the VEGF receptor were both lower in the offspring of LP-fed animals at birth [26]. Islet microvascular density remained compromised in the pancreas of the offspring until adulthood. Similarly, the

Malprogramming of β -Cell Function by a Dietary Modification in the Immediate Postnatal Period

Mulchand S. Patel* and Saleh Mahmood

Department of Biochemistry, School of Medicine and Biomedical Sciences, University at Buffalo, The State University of New York, Buffalo, New York 14214, USA

Abstract: The development of the structure and function of the endocrine pancreas is known to be influenced by altered nutritional experience during the fetal period. Nutritional modifications in the suckling period are also recognized as contributing factors to developmental programming of the endocrine pancreas. In this chapter we describe the malprogramming of rat pancreatic islet structure and β cell functions in response to an increased intake of carbohydrate-derived calories in a milk formula (HC) during the suckling period. Alterations in β cell function of HC rat pups result in the development of hyperinsulinemia due to β cell plasticity in the immediate postnatal period. These modifications include: altered islet architecture and increased insulin-producing mass, increased insulin secretion capacity with a leftward shift in glucose-stimulated insulin secretion, insulin secretion in the absence of glucose and/or Ca^{2+} , increased gene transcription of several genes crucial for β cell development and function, and increased parasympathetic input, as well as malprogramming of orexigenic circuitry in the hypothalamus. Interestingly, these alterations in β cell function are maintained even after weaning of HC rats on a standard rodent chow, resulting in adult-onset obesity due to development of hyperphagia. It is possible that early introduction of carbohydrate-rich infant supplemental foods could contribute to modified β cell functions in infants which could, in turn, over a longer period predispose to the development of childhood obesity and/or adult-onset obesity and its associated metabolic complications including type 2 diabetes.

* **Corresponding author Mulchand S. Patel:** Department of Biochemistry, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY 14214, USA; Tel: 716-829-3074, Fax: 716-829-2725; E-mail: mspatel@buffalo.edu

Keywords: Artificial rearing of rat pups, β cell neogenesis, β cell proliferation, Hyperinsulinemia, Hypothalamic programming, Insulin secretion, Metabolic programming, Nutritional modification, Obesity, *Pdx-1* gene expression.

INTRODUCTION

Metabolic Programming After Birth

Numerous lines of evidence from animal models, epidemiological studies and clinical investigations indicate that altered nutritional exposures during critical periods of early development (fetal and immediate postnatal periods) can have permanent effects at the cellular, molecular, and biochemical levels in several tissues [1 - 7]. During this rapid growth period, the endocrine pancreas in rodents undergoes both normal structural and biochemical maturation of the endocrine functions [8, 9]. Altered nutrition during these early phases of life can induce permanent adaptations in these processes which continue to be expressed in adulthood [6, 10 - 14]. This phenomenon was initially proposed by Barker [15] as 'Fetal Origins of Adult Disease' followed by other similar terms and more recently referred to as 'Developmental Origins of Health and Disease'. Altered nutritional experiences during the fetal period can be exerted due to maternal malnutrition (such as caloric restriction, protein deficiency, calorie plus protein deficiency, vitamin deficiency), maternal obesity and maternal diabetes.

Accumulated evidence indicates that nutritional alterations (undernutrition, overnutrition and altered milk composition) in the immediate postnatal period (referred to as the suckling period) alone can malprogram metabolic capacities of tissues (*e.g.* pancreatic β cells and hypothalamus) with increased risk for the development of metabolic disorders in adulthood. Human milk is the natural choice for infants because it not only provides nutrients for optimal growth but is also the source of bioactive compounds and immunoglobulins for immunity development during the immediate postnatal period [16]. Commercially available infant formulas are devoid of the latter components. The health benefits of breastfeeding over infant formula feeding are widely recognized, including optimal growth during childhood and longer term benefits such as lower rates of cardiovascular risk and obesity in adulthood [16, 17]. During the suckling period,

over-nourishment may be caused by infant milk-formula feeding (bottle feeding with unrestricted supply of milk formula) with or without early introduction of carbohydrate-enriched infant foods. Although exclusive breast-feeding of babies for the first six months is recommended by the American Dietetic Association [18], formula feeding (aka bottle feeding) and early introduction of complementary infant foods are widely practiced in Westernized societies. It is quite possible that feeding formula alone may result in over-feeding causing increased weight gain and possible metabolic programming of infants. Rodent models of overnutrition by intake of excess maternal milk during the suckling period (*e.g.* by reducing the litter size) are well characterized for metabolic programming of the hypothalamus and pancreatic β cells, resulting in excess weight gain during the immediate postnatal period and predisposition for the development of adult on-set obesity [19 - 22].

Unlike human milk and infant milk formula with a higher level of calories derived from fat, supplementary infant foods (fruits, juices, cereals) are highly enriched with carbohydrate-derived calories but have few calories from fats. Hence, early introduction of infant foods prior to 6 months of age can increase carbohydrate-derived calorie ingestion. The focus of our research has been on metabolic programming effects in β cells due to increased intake of carbohydrate *via* a milk-substitute formula by rat pups during the suckling period [23 - 25]. To investigate this phenomenon we have employed a rat model (referred to as Pup-in-a-Cup model) in which rat pups are artificially reared by intra-gastric feeding of a milk formula high in carbohydrate-derived calories during the suckling period. Based on the observations on the HC rat model, as presented in this chapter, it is possible that early introduction of sugar-dense supplementary foods for human infants could be a contributing factor to metabolic programming of β cells and hence in the etiology of obesity in childhood and adulthood.

EXPERIMENTAL APPROACHES: ‘PUP-IN-A-CUP’ RAT MODEL

To feed a modified rat milk-substitute formula enriched in carbohydrate-derived calories to neonates during the suckling period, we took advantage of an artificial rearing technique employing intragastric feeding of rats [26 - 28]. On postnatal day 4, pups born to normal dams consuming standard laboratory chow *ad libitum*

New Concepts in the Intra-Islet Control of β -Cell Function and Mass

Brian T. Layden^{1,2,*}, Stephanie Villa¹ and William L. Lowe¹

¹ Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

² Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois, USA

Abstract: The regulation of pancreatic β -cell function and mass is critical to the maintenance of euglycemia. β -cells integrate numerous signals from the host to secrete appropriate amounts of insulin and maintain tight control of blood glucose levels. Together with glucose; nutrients, amino acids, hormones, and metabolic by-products contribute to this physiologic response. Within the islet microenvironment, where β -cells reside, there exists a network of interacting pathways that contribute to insulin secretion and regulation of β -cell mass. While factors within these pathways are often sourced from digestive processes and peripheral tissues, intra-islet-derived factors are also important components in the ability of β -cells to accurately integrate metabolic demands with β -cell function. In recent years, many biologic factors have been found to have previously unappreciated autocrine and paracrine roles within the islet. Moreover, differences have been described between signaling within rodent and human islets that are important for informing our understanding of autocrine/paracrine signaling between species. In this review, we highlight these new findings and future directions for this field of study.

Keywords: Autocrine, β -cells, β -cell function, β -cell mass, Diabetes, Glucose, Insulin, Islets, Nutrient-sensing, Paracrine.

* **Corresponding author Brian T. Layden:** Division of Endocrinology, Metabolism, and Molecular Medicine, Department of Medicine, Northwestern University, Chicago, IL, USA; Tel/Fax: 312-503-0006/312-908-9032; E-mail: b-layden@northwestern.edu

David J. Hill (Ed.)

All rights reserved-© 2016 Bentham Science Publishers

INTRODUCTION

Defining the Importance of β -Cell Function and Mass

The Islets of Langerhans are composed of multiple cell types. β -cells, the most abundant cell type within islets, produce and secrete insulin in response to glucose. The metabolism of glucose initiates biochemical signaling cascades that ultimately result in β -cell depolarization, insulin granule fusion, and insulin secretion. The secretion of insulin, which is synthesized primarily in pancreatic islets, is largely dependent on extracellular glucose levels and tightly regulated to maintain euglycemia. While glucose is the primary mediator of insulin secretion, other factors (such as nutrients, cytokines, and hormones) fine-tune insulin secretion based on the physiologic needs of the organism. Deficient insulin secretion is central to the pathogenesis of diabetes mellitus (DM) and results in diminished uptake of glucose by peripheral tissues, which leads to hyperglycemia. Understanding the additional pathways involved in the regulation of insulin secretion and maintenance of glucose homeostasis has provided novel mechanistic insight into DM and pathways to target for its treatment.

While blood glucose levels are the primary driver of insulin secretion, changes in insulin sensitivity that accompany physiologic conditions such as pregnancy [1] and obesity [2] require the pancreas to increase its capacity for insulin secretion. To support this demand, β -cells have the capacity to expand their overall mass through the regulation of β -cell proliferation and apoptosis, β -cell hypertrophy, and/or β -cell neogenesis [3]. However, the pathways and mechanisms (proliferation/apoptosis, hypertrophy or neogenesis) that predominate are still debated. Regardless, the end result of β -cell mass expansion is to provide increased insulin secretory capacity under conditions of increased insulin demand. Because of this, identification of endogenous and exogenous factors that promote β -cell mass expansion provides insight into mechanisms used to compensate for deficient insulin secretion and its alteration in states such as type 2 diabetes (T2D) and gestational diabetes mellitus (GDM).

Known Factors Affecting β -Cell Function and Mass and Their Mode of Action

As noted above, glucose is the primary insulin secretagogue, and elevated glucose has been shown to increase β -cell mass by inducing β -cell proliferation in both mouse and human islets [4, 5]. Along with glucose, other nutrients (fatty acids and amino acids) and hormones such as glucagon-like peptide-1 (GLP-1) have a documented role in insulin secretion and β -cell mass regulation (Table 1). In general, these nutrients and hormones are derived either from dietary sources or by secretion from a peripheral tissue and delivered to islets through the systemic circulation. Thus, these factors act as endocrine hormones. However, hormones can also be secreted and signal locally through a paracrine (action on neighboring cells) or autocrine (action on cells the factor was secreted by) effect.

Table 1. Partial listing of extracellular signaling factors affecting insulin secretion and β -cell proliferation.

	Signal type	Mode of action at β -cell	β -cell effect
Insulin	Autocrine	Receptor tyrosine kinase	Potentiate GSIS?
GLP-1 [6]	Endocrine/Paracrine	GPCR	Potentiate GSIS Stimulate β -cell proliferation Protect against β -cell death
Glucose-dependent insulinotropic polypeptide (GIP) [6]	Endocrine	GPCR	Potentiate GSIS Stimulate β -cell proliferation Protect against β -cell death
Somatostatin [7]	Paracrine	GPCR	Inhibit GSIS
Amino acids [8]	Endocrine	GPCR/ Intracellular metabolism	Inhibit or potentiate GSIS (depending on amino acid type)
Ghrelin [7]	Endocrine/Paracrine/Autocrine	GPCR	Inhibit GSIS
Vasoactive intestinal polypeptide (VIP) [7]	Neuropeptide	GPCR	Potentiate GSIS

β -Cell Adaptability During Pregnancy

Jens Høiriis Nielsen^{1,2,*}, Signe Horn^{1,3}, Jeannette Kirkegaard^{1,3}, Amarnadh Nalla^{1,2} and Birgitte Søstrup^{1,2}

¹ Department of Biomedical Sciences, University of Copenhagen, Denmark

² Centre for Fetal Programming, Copenhagen, Denmark

³ Novo Nordisk A/S, Måløv, Denmark

Abstract: Pregnancy is a physiological condition associated with β -cell mass expansion occurring in response to increased insulin demand. If the insulin resistance is not compensated by proper augmented insulin production gestational diabetes will occur. As reviewed herein, pregnancy induced hormonal changes have occupied scientists since the beginning of the last century where important discoveries of the hormonal regulation of metabolism during pregnancy have been accomplished. Of the multiple hormonal and metabolic changes the somatolactogenic hormones, placental lactogens (PL) and placental growth hormone (GH-V) are the most described and are found to have dual roles by induction of insulin resistance and promotion of β -cell function and expansion. More recently, the direct effects on isolated pancreatic islets and the influence of signaling pathways involved in the adaptation of β -cell growth and function during pregnancy have been elucidated. This has identified contributions of a number of known peptide hormones and growth factors (EGF, NGF, HGF, IGFs, GLP-1) and steroid hormones (progesterone, estrogens, glucocorticoids). In addition, glucokinase has been found to be essential for the both proliferation and glucose stimulated insulin secretion during pregnancy. Some transcriptional activators and repressors (FoxM1, HNF4 α , Myc, Bcl6, Men1) have been implicated in β -cell growth and survival, but also systemic factors like betatrophin, serotonin and osteoprotegerin have been reported to stimulate β -cell proliferation during pregnancy. Gene expression studies and proteomics of islets from pregnant rodent have furthermore revealed upregulation of a number of genes (*e.g.* cyclophilin B, stathmins, dlk-1, trefoil factor-3 and several others) that may influence β -cell growth and function during pregnancy although the mechanisms driving these changes are not yet known. Similarly,

* **Corresponding author Jens Høiriis Nielsen:** Department of Biomedical Sciences, University of Copenhagen, Denmark; Tel: +45 28757721; E-mail: jenshn@sund.ku.dk

David J. Hill (Ed.)

All rights reserved-© 2016 Bentham Science Publishers

circulating factors in serum from pregnant women have been identified. Among the stimulating factors are peptide fragments of alpha-1 antitrypsin, kininogen-1, apolipoprotein-1, fibrinogen alpha chain and angiotensinogen. An intriguing question remains about the origin of the increased β -cell mass in pregnancy. In humans, studies have primarily reported an increase in the number of small islets, suggesting that neogenesis as the primary driver of β -cell mass expansion in human. In rodents, however, β -cell replication is believed to be the primary mechanism, although increased expression the neogenesis marker, neurogenin-3, has also been reported in pancreas of pregnant rodents. Interestingly, recent studies have suggested that the apparent loss of β -cells occurring during development of diabetes may be due to de-differentiation rather than cell death, suggesting contributions from mechanisms going beyond neogenesis and replication. In summary, gestational diabetes (GDM) is associated with lack of appropriate adaptation of the β -cells that may be due to a reduced pre-pregnancy β -cell mass, lack of stimulating hormones and growth factors or appearance of β cytotoxic metabolites or factors. This chapter reviews the existing knowledge of multiple factors and put forward new mechanisms of pregnancy induced β -cell mass expansion, which are not yet completely understood.

Keywords: Diabetes, Growth hormone, Insulin, Pancreatic β -cells, Pregnancy, Prolactin.

INTRODUCTION

Pregnancy is a unique physiological condition associated with expansion of the β -cell mass in response to an increased insulin demand. As type 2 diabetes (T2D) is characterized by insulin insufficiency due to a lack of compensatory β -cell mass expansion, a better understanding of the mechanisms involved in β -cell adaptation during pregnancy, may help to elucidate the pathogenesis and lead to interventions in the treatment of T2D. Pregnancy is characterized by increased food intake, weight gain, changes in metabolism towards “facilitated anabolism” after food intake and “accelerated starvation” during fasting, in order to maintain optimal supply of nutrients to the fetus [1]. In normal pregnancy, these metabolic changes lead to an increase in the postprandial plasma glucose level which elicits an exaggerated insulin secretion in particular in late pregnancy. The physiological link between pregnancy and the mechanisms failing during development of T2D are further underscored by an increased risk of developing gestational diabetes (GDM). Interestingly, certain genetic forms of diabetes are often diagnosed during pregnancy [2]. As will be described in this chapter the adaptation of the β -cells to

pregnancy is a complex process triggered by a series of known, and yet unknown, systemic stimuli. β -cell adaptation is accomplished by increased β -cell function such as enhanced glucose stimulated insulin secretion (GSIS), as well as, by the formation of new β -cells most likely by a combination of replication and neogenesis. The changes in β -cell mass during normal and diabetic conditions are illustrated in Fig. (1) [3].

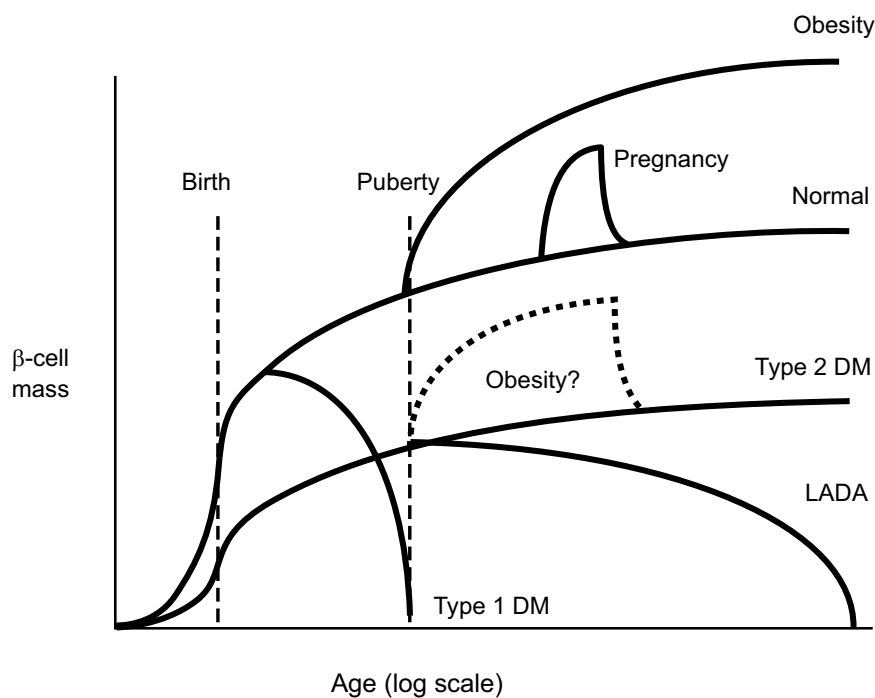


Fig. (1). Schematic illustration of changes in β -cell mass in response to increased insulin demand *i.e.* perinatal period, intrauterine growth retardation, obesity, pregnancy and type 1 diabetes (DM), type 2 diabetes (DM) and Latent Autoimmune Diabetes in Adults (LADA or type 1½ diabetes) [3].

Morphological Changes

Changes in the morphology of the endocrine pancreas during pregnancy in both humans and animals were described already in the early 20th century [4]. In 1930, Akehi [5] found hypertrophy, proliferation and newly formed islets during pregnancy in rabbits, which was followed by rapid atrophy and reduction of β -cells after parturition. In the same year, Macleod [6] described a diabetic woman that had a marked reduction in her insulin requirement in the last trimester,

Section 3: Generation of β -Cells and Future Applications

β -Cells from Embryonic and Adult Stem Cells and Progenitors

Christine A. Beamish*

Department of Surgery, Islet Transplantation Laboratory, The Methodist Hospital Research Institute, Houston Texas, USA

Abstract: Diabetes is a chronic autoimmune disease, causing the destruction of the insulin-producing β -cells of the pancreatic islet and leading to glycemic dysregulation. Exogenous insulin administration and glucose testing moderately rectifies hyperglycemia, but does not provide adequate fine tuning necessary for complete prevention of hypoglycemia acutely, nor micro- and macro-vascular complications in the long-term. Islet transplants have shown great promise for this dynamic glucose regulation, but a shortage of cadaveric-sourced cells, and lifelong immune suppression requirements vastly restrict this technique from being widely available to patients with the disease. Therefore alternative sources of insulin-producing cells are needed. In this chapter, the role of stem cell biology in the current context of diabetes therapy is discussed, including an assessment of human embryonic and human induced pluripotent stem cells for the restoration of β -cell mass. Additionally, the existence of putative resident stem cells, and possible fluidity in lineage fate determination within endocrine pancreas-related cell types is examined.

Keywords: β -cell, Diabetes, Pancreas, Plasticity, Progenitor cell, Regeneration, Stem cell.

INTRODUCTION

Type 1 diabetes mellitus is an autoimmune disease of the endocrine pancreas, involving an interplay of immune, genetic, and environmentally-mediated factors

* **Corresponding author Christine A. Beamish:** Department of Surgery, Islet Transplantation Laboratory, The Methodist Hospital Research Institute, Houston Texas, USA; Tel: 713-363-9193; Fax: 713-441-3240; E-mail: cbeamish@houstonmethodist.org

[1 - 3]. While the overarching goal of diabetes therapy would ostensibly restrict the autoimmune destruction of insulin-producing β -cells and retain existing β -cell mass in susceptible individuals, the primary impediment to this approach is that the disease only presents with overt symptoms of hyperglycemia after β -cell mass is lost below a clinical threshold ($\sim 10\%$ remaining), and long after disease initiation [4]. Furthermore, while research advances actively target a variety of parameters including auto-antibody presence, diabetes-correlative genetics such as the presence of the HLA alleles DR3-DQ2/DR4-DQ8 [3], and viral exposure [2], $<20\%$ of patients have known risk factors prior to diagnosis (such as first-degree relatives with diabetes) [5], thus limiting the ability to predict diabetes incidence in all but few patients [6, 7]. Therefore, resolving the immune destruction is only part of the solution, and β -cell replacement strategies are imperative.

Since its advent in 1989 by Lacy, Ricordi, Scharp and associates [8, 9], islet transplants have improved drastically as a treatment for specific T1D patients. Much of the improvement came in the year 2000 with the Edmonton Protocol pioneered by Shapiro and colleagues [10], allowing exogenous insulin independence effected by a higher islet mass from two donors, and the use of steroid-free immunosuppression *via* sirolimus, tacrolimus, and daclizumab, an IL2-receptor antagonist antibody [11]. Grafts now last up to 5 years [12, 13]. In addition to freedom from injections, benefits from islet transplantation include significant decreases in micro- and macro-vascular complication rates, decreases in severe hypoglycemic episodes, increased circulating C-peptide titers, and improvement in HbA_{1c} [13, 14]. Importantly, these benefits are noted even if the graft fails. The obstacle to this therapy being offered more widely is primarily due to lack of islets, as well as risks associated with life-long immunosuppression for allogeneic grafts [13]. This shortage of islets is multi-factorial: many available pancreata do not meet minimum criteria for transplant, including donor age and metabolic profile; islets may be damaged irreparably during isolation; and tragically, the majority of islets die in the first days after transplant [15], primarily resulting from an instant blood mediated immune reaction (IBMIR) [16]. Some research has focused on mitigating the factors responsible for this process, such as modulating platelet-monocyte interactions [17] or by blocking complement activation [18]. The remaining islet death results from the loss of blood supply,

causing hypoxia, ischemia-reperfusion injury [19], and amyloid deposition as time progresses. Islet vascularization is a critical determinant of cell survival in the long-term [20]. Cost of the procedure is a final regional consideration, resulting from islet transplants being designated as “experimental therapy” in the United States, and hence only (and rarely) covered by private insurance or research funding, although this is not the case in other countries, such as Canada [21].

Alternate sources of insulin-producing β -cells would alleviate the need for cadaveric-sourced human tissue with their associated sequelae, and significantly alter morbidity and mortality outcomes in the future. The potential role of stem cell biology will be examined to this end, including assessments of both exogenous and endogenous stem and progenitor cell populations and their applications to human medicine.

GENERATION OF β -CELLS FROM STEM CELL SOURCE

The *de novo* generation of insulin-producing β -cells has long been a goal for diabetes therapy. Given the expansive data generated from lineage tracing experiments, it is now possible to ascertain the sequence of steps necessary in the developmental biology of the β -cell, from primitive gut endoderm through to functional, glucose-responsive, β -cell [22]. The generation of β -cells from stem cell source has generally co-opted these developmental procedures.

A stem cell may be defined by two functional properties, namely an unlimited ability for self-renewal, and the capacity to generate multiple cell types (*e.g.* multi-potentiality). This may be, but is not limited to, a response to injury or other stimulus. A progenitor cell, alternatively, demonstrates some restriction in self-renewal capability and which is usually uni-potent [23, 24]. The distinction between these two cell states is often fluid and vaguely indeterminate in the literature, but which may yield important and definitive outcomes for medicine. Similarly, subdivisions in definition exist for the somatic, or tissue-specific, stem cells *vs.* embryonic stem cells, namely that the latter demonstrates the capacity to generate progeny across all primary germ layers. However, precedents exist to suggest that this is not always rigidly obeyed, as has been demonstrated by hematopoietic and neural stem cells forming tissues from germ layers other than

Induction of β -Cell Regeneration by Human Postnatal Stem Cells

Tyler T. Cooper, Ruth M. Elgamal and David A. Hess*

Molecular Medicine Research Group, Krembil Centre for Stem Cell Biology, Robarts Research Institute; Department of Physiology & Pharmacology, Schulich School of Medicine & Dentistry, Western University, London, Ontario, Canada

Abstract: The International Diabetes Federation estimates 382 million people are currently living with diabetes mellitus worldwide; and with increasing rates of obesity in an aging population this number is predicted to increase to 592 million by 2035. The inability to ameliorate the causes of diabetes has motivated researchers to develop novel approaches aimed at providing curative therapies to replace current symptomatic management using exogenous insulin. Accordingly, postnatal or adult stem cell transplantation has recently emerged as a promising therapeutic strategy following reports detailing the stimulation of islet regeneration in preclinical and early clinical studies. Postnatal bone marrow (BM) and umbilical cord blood (UCB) sources contain progenitor cells of hematopoietic, endothelial, and mesenchymal lineages; and each have demonstrated islet regenerative functions in animal models of diabetes. In the context of this chapter, we summarize accumulating evidence from preclinical and clinical studies describing transplantation of these specific postnatal lineages to stimulate the regeneration of endogenous insulin secreting β -cells, and how these stem cells may be used to provide paracrine support alongside the transplantation of allogeneic islets.

Keywords: Allogeneic transplantation, Autologous transplantation, β -cells, Bone marrow, Diabetes mellitus, Endothelial progenitor cells, Hematopoietic progenitor cells, Hypoxia, Insulin, Islet angiogenesis, Islet neogenesis,

* **Corresponding author David A. Hess:** Molecular Medicine Research Group, Krembil Centre for Stem Cell Biology, Robarts Research Institute, Department of Physiology & Pharmacology, Western University, 1151 Richmond St, London, Ontario, Canada, N6A 5B7; Tel/Fax: 519.931.5777x.24118; E-mail: dhess@robarts.ca

Islets of langerhans, Islet regeneration, Multipotent stromal cells, Pancreas, Paracrine signals, Progenitor cells, Stem cells, Transplantation, Umbilical cord blood.

INTRODUCTION

Diabetes mellitus is generally characterized by the body's inability to maintain controlled glycemic levels due to pancreatic β -cell death or dysfunction [1]. Type 1 diabetes (T1D), also referred to as "juvenile onset diabetes" or "insulin-dependent diabetes", arises as a result of the autoimmune destruction of insulin-secreting β -cells in the islets of Langerhans. T1D is mediated by an inflammatory infiltrate composed of $CD4^+$ and $CD8^+$ T-lymphocytes, B-lymphocytes, macrophages, and NK-cells [2, 3]. β -cell-specific autoimmune depletion leaves T1D patients with an inadequate supply of endogenous insulin, requiring exogenous insulin to control hyperglycemia. In contrast, T2D, often referred to as "late-onset diabetes" or "non-insulin-dependent diabetes", occurs as a result of insulin insensitivity or resistance in peripheral tissues (skeletal muscle, liver, and adipose), and is associated with a combination of risk factors including obesity, sedentary lifestyle, environmental stimuli, and/or genetics [4]. In the early stages of T2D, prolonged hyperglycemia stimulates β -cells to over secrete insulin and results in β -cell exhaustion and apoptosis [5], culminating in reduced β -cell mass as T2D develops [6, 7]. Regardless of these pathological differences, T1D and T2D, ultimately result in the loss of β -cell mass over time; leading to inadequate insulin secretion within islets and the requirement for exogenous insulin therapy. Due to the insulin deficiency created by diabetes, restoration of physiological insulin secretion is essential to treating both T1D and T2D and its subsequent complications.

Dealing with the Complications and Consequences of Diabetes Mellitus: An Emerging Global Crisis?

In 2013, the International Diabetes Federation (IDF) estimated 382 million people are currently living with diabetes mellitus worldwide; and this number is predicted to increase to 592 million by 2035 [4, 8]. Specifically, >90% of diagnosed individuals have T2D, while <10% have T1D. Due to increasing rates of obesity and aging population demographics worldwide, diabetes mellitus has

reached pandemic proportions. Since the discovery of insulin by Banting and Best in the early 1920s [9], and despite the recent development of improved insulin administration (automated insulin pumps) [10], both T1D and T2D patients ultimately develop serious comorbidities and secondary complications associated with inadequate control of glycemia. The most severe complications include increased rates of cardiovascular diseases (heart attack, coronary and peripheral artery disease, stroke), vision loss/blindness, kidney failure, nerve damage, problems with pregnancy, and depression. According to the Public Health Agency of Canada, individuals with diabetes are 3 times more likely to develop cardiovascular disease, 12 times more likely to be hospitalized with end-stage renal failure, and 20 times more likely to require non-traumatic lower limb amputation [11]. Although it is difficult to assess the economic burden of diabetes worldwide, the American Diabetes Association estimated that the total cost of diabetes in 2012 exceeded \$245 billion in the USA (\$176 billion in direct medical costs and \$69 billion in reduced productivity) [12]. These staggering numbers have prompted researchers to aggressively investigate improved and potentially curative therapies to combat both T1D and T2D.

The Advent of Cellular Therapies for Diabetes: The Edmonton Protocol

Recently, cell-based therapies have emerged as a frontrunner to provide curative therapies for diabetes using islet replacement to restore functional β -cell mass [13]. In 2000, the pioneering efforts of the Edmonton protocol provided ‘proof-of-concept’ that portal vein transplantation of cadaveric human islets, combined with modern immunosuppressive therapy, could lead to insulin independence in patients with severe T1D [13, 14]. Although results at 1 year were initially promising with 7 of 7 patients achieving insulin-independence after the transplantation of islets from at least 2 donor pancreata, islet rejection and continued autoimmune assault resulted in the return to insulin therapy in the majority of patients [13]. In addition, islet survival and function was compromised by isolation procedures and engraftment in the hepatic site. Widespread application of this approach remains limited due to an extreme shortage of donor pancreas tissue available for transplantation. Finally, due to the requirement for life-long immunosuppression and increased risk of infections, islet transplantation is only indicated for brittle T1D patients [15].

SUBJECT INDEX

A

Acetate 115, 116
 Acetylation 68, 69
 Acetylcholine 95, 96, 99, 110, 116
 Acinar cells 5
 Activity, methyltransferase 30
 Adipocytes 201, 202
 Adiponectin 127
 Aging 20, 24, 25, 29, 33, 58, 70, 71, 73
 cellular 58, 70, 71, 73
 effects of 20, 24, 25, 29, 33
 ALDHhi cells 197, 198, 199, 203
 fresh UCB 199
 human 198
 isolated 199
 iv-injected 198
 ALDHhi UCB cells 199
 Allogeneic HSCT 200, 201
 Allogeneic islets 190, 208
 mismatched 208
 Allogeneic transplantation 190, 207
 Alterations in islets mass 87
 Amino acids 7, 72, 107, 109
 Anorexigenic neuropeptides 97, 98
 Anti-diabetogenic effects 128
 Architecture 46, 84
 altered islet 84
 human islet 46
 Artificial rearing technique 86, 87
 ATP, cytosolic 91, 92
 Autocrine signaling 112, 113, 115
 Autoimmune diabetes 48, 148
 adult-onset 48
 Autologous BM cells 199
 Autologous transplantation 190

B

B-cell ablation 25, 28, 197
 B-cell apoptosis 21, 22
 glucose-induced 21
 B-cell death, pancreatic 191
 B-cell dedifferentiation 176, 179

B-cell destruction, autoimmune 207, 208
 B-cell development 3, 7, 8, 9, 10, 11, 13, 69
 B-cell differentiation 8, 9, 10
 B-cell Function 11, 21, 49, 174, 204
 abnormal 11
 declining 21
 increased 21, 204
 reduced 49
 retained 174
 B-cell growth 20, 24, 26, 28, 29, 58
 adaptive 24, 26
 B-cell injury 209, 210
 B-cell/islet density 44
 B-cell loss 48
 B-cell lymphoma 144
 B-cell mass 7, 21, 30, 33, 42, 48, 60, 64, 70, 72,
 169, 179, 191, 198, 207
 increased 21, 30, 33
 increased pancreatic 64
 loss of 179, 191
 pancreatic 21, 42
 recovery of 60, 198
 reduced 7, 21, 48, 70, 191
 reductions in 70, 72
 restoration of 169, 207
 B-cell mass quantification 42, 45
 B cell neogenesis 85, 108, 126, 143
 B-cell overexpression 29, 30
 B-cell pathophysiology 42
 B-cell phenotypes 69, 72, 178
 B-cell plasticity 64, 67
 B-cell progenitor cells 61
 B-cell proliferation 22, 23, 24, 25, 26, 27, 28,
 29, 30, 31, 32, 64, 195, 199, 212
 adaptive 23, 25, 28
 age-restricted 29
 basal 26
 compensatory 32
 decreased 28, 29, 30, 31
 glucose-induced 27, 28, 29
 human 22, 26
 increased 26
 increasing 25, 32
 induced 27, 28, 29
 inhibited 64

- limit 27
 - measured 22
 - reducing 27
 - residual 212
 - stimulate 23, 195
 - stimulated 25, 26, 28, 199
 - suggested increased 24
 - B-cell proliferation rates 22
 - B-cell regeneration 24, 25, 208, 212, 213
 - endogenous 208, 212, 213
 - models of 24, 25
 - B-cells 3, 6, 9, 10, 11, 12, 13, 14, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 42, 44, 45, 46, 47, 48, 49, 51, 58, 60, 61, 62, 63, 64, 66, 67, 69, 73, 107, 112, 113, 114, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 190, 191, 193, 194, 198, 200, 202, 204, 206, 207, 211, 213
 - adult 177
 - aged 25, 26, 28
 - cell-derived 3
 - clonal 113
 - derived 173
 - differentiated 6
 - dysfunctional 42, 49, 51
 - endogenous 32, 200, 211
 - endogenous insulin secreting 190
 - freeing 33
 - functional 13, 69, 173, 178
 - generating 13
 - glucose-responsive 194
 - hypoxic 211
 - insulin-producing 48, 169, 170, 171
 - insulin-secreting 191
 - neogenic 179
 - novel 114
 - old 20
 - positive replicating 23
 - proliferating insulin+ 193
 - rare 8
 - residual 48, 193
 - responsive 33, 172
 - rodent 113
 - zero replicating 23
 - B-cells development 9
 - B-cells function, altered 58
 - B-cells/islets 42, 47, 193
 - exogenous 193
 - B-cell survival 28, 64, 71
 - B-cell transplantation 210, 211
 - B-cell turnover 22, 23, 26, 28, 47
 - Betatrophin 26, 27, 123, 144
 - B-like cells, insulin-producing 202
 - Blood glucose levels 107, 108, 139
 - BM cells 194
 - BM-derived hematopoietic stem and progenitor cells 195
 - BM-derived MSC 205, 208
 - BM-derived stem and progenitor cells 194
 - Bone marrow stem cells initiate islet regeneration 194
- ## C
- Ca²⁺, extracellular 94
 - Carbohydrate-derived calories 84, 86, 87, 92, 100
 - Cell adaptation 124, 125, 133, 136, 139, 151
 - Cell apoptosis 70, 117
 - Cell-based therapies 192, 194, 204
 - Cell cycle 20, 32
 - Cell cycle genes 29, 30, 67
 - Cell cycle inhibitors 20, 27, 30, 32, 33, 144
 - Cell cycle progression 20, 29, 32
 - Cell death 22, 109, 124, 136, 146, 149
 - Cell effect 109, 110
 - Cell fate 149, 175, 179
 - Cell function 84, 100, 107, 110, 115, 116, 117, 123, 125, 134, 148, 149
 - Cell growth 68, 90, 91, 123, 133, 145, 148
 - Cell mass expansion 108, 123, 124, 126, 141, 144, 146, 147
 - Cell proliferation 10, 21, 32, 63, 64, 85, 90, 91, 107, 108, 109, 110, 114, 117, 123, 130, 131, 133, 134, 137, 138, 139, 140, 142, 143, 144, 145, 147, 150, 198
 - augmenting 107, 109, 110, 117, 198
 - Cell proliferation and Vascularization 198
 - Cell regeneration 129, 147, 148
 - Cell replication 88, 116, 124, 139, 140, 141, 142, 144, 145
 - Cells 12, 13, 28, 61, 66, 71, 143, 147, 169, 172, 173, 175, 177, 178, 179, 193, 194, 196, 199, 201, 209
 - allogeneic 199

- cadaveric-sourced 169
 - circulating 209
 - differentiated 12
 - duct 147
 - exocrine 178
 - glucose-unresponsive 179
 - hES 172, 193
 - human 194
 - insulin+ 61, 173
 - insulin-expressing 175, 177, 178
 - mononuclear 71, 196
 - mTOR-positive 66
 - multipotent 178
 - multipotent-stromal 201
 - nonendocrine 179
 - producing 13, 172
 - proliferating 28, 143
 - regenerative 193
 - Cell types, pancreatic 175
 - Cellular composition 46, 49
 - Cellular therapies 193, 200, 201, 202, 205, 210, 211
 - Cellular therapies for diabetes 192
 - Chondrocytes 201, 202
 - Chorionic gonadotropin (CG) 126
 - Chromatin remodeling 30, 31
 - Complications and consequences of diabetes mellitus 191
 - Consequences of diabetes mellitus 191
 - Cord blood, umbilical 190, 191
 - Curative therapies 190, 192, 193, 206, 213
 - Cyclin D2 27, 28, 136, 137
 - Cyclophilin 123, 146
- D**
- Days post-transplantation 198, 203
 - Demethylase 31
 - De novo* β -cells 174
 - Developmental genes 13, 14
 - Development of techniques to study islet function 129
 - Dexamethasone 136, 137
 - Diabetes 58, 85, 128, 129, 138, 191, 193
 - aggravated 138
 - established 193
 - insulin-dependent 191
 - insulino-dependent 11
 - juvenile onset 191
 - late-onset 191
 - maternal 58, 85
 - non-insulin-dependent 191
 - permanent 128, 129
 - Diabetes and islet transplantation 11
 - Diabetes care 18
 - Diabetes incidence 170
 - Diabetes mellitus, gestational 108
 - Diabetes progression 179
 - Diabetes therapies 20, 21, 22, 23, 33, 169, 170, 171, 174, 201
 - developing 22
 - human 23
 - Diabetic-induced nephropathy 205
 - Diabetic mothers 62, 71
 - Diabetic nephropathy 203
 - Diabetic pancreas 193
 - Diabetic patients, diagnosed 207
 - Diabetic symptoms 136, 201
 - severe 136
 - Diabetic therapies 207
 - Diabetic woman 125
 - Diabetogenic effects 128, 137
 - reported 128
 - Diacylglycerol 95, 96
 - Differentiation, controls β -cells 13
 - Diseases, cardiovascular 58, 59, 192
 - Distribution, β -cell/islet 42
 - DNA contents 138
 - DNA methylation 68
 - DNA synthesis 130, 138, 139, 148
 - Donor cells 194, 197, 200
 - transferred 200
 - transplanted eGFP+ 194
 - Donor islets 24, 210, 211
 - human 24
 - Donor pancreata, human 173
 - Drugs, antidiabetic 11
 - Ductal epithelium cells, positive 88
 - Ducts, pancreatic 43, 46, 61, 143
- E**
- Early on-set hyperinsulinemia in HC pups 91
 - EGFR signalling 139, 140

- Embryogenesis, pancreatic 68
Embryonic pancreases 5, 8, 10
Endocrine cells 46, 111, 114, 143, 172, 173, 178, 210
 functional 178
 pancreatic 143
 single 46
Endocrine hormones, pancreatic 14
Endocrine pancreas, developing 59
Endocrine pancreas maturation 172
Endocrine pancreas morphology 60
Endothelial cells 196, 202, 209, 211
 functional 209
Endothelial colony forming cells (ECFC) 209
Endothelial progenitor cells (EPC) 61, 190, 194, 195, 208, 209, 210, 211, 212
Endothelium, fenestrated islet 60
Environment, gestational 58, 59, 70
Epigenetic changes 32, 33, 58, 68, 70
Epigenetic marks 30, 31, 68, 69, 70
Epigenetic modifications 67, 69, 73
Epigenetics 20, 59
Epithelial cells 89, 203
Estrogens, role of 138
Euglycemia 107, 108, 211
Exocrine pancreas 69, 143
Exogenous insulin administration and glucose 169
Expansion of β -cell mass 21, 108, 174, 204
- F**
- Fatty acids 109, 110, 142
Females, age-matched non-pregnant 23
Fetal pancreases 9, 69
Fetal rodent islets 92
Fluid, amniotic 147, 148
Fluorescence activated cell sorting (FACS) 197
Foregut endoderm 4, 68, 69, 209
Foreign body responses (FBR) 173
Formation, islet capillary 195
Functional islet repair 194
Functional loss of β -cell mass 48
- G**
- Gene expression 3, 6, 20, 30, 67, 88, 90, 98, 123
Genetics, diabetes-correlative 170
Genetics of pancreas embryogenesis 13
Gene transcription 30, 31, 33, 89, 90
 preproinsulin 89, 90
Gestational diabetes 24, 59, 123, 124, 148, 149
 developing 24, 124
Gestational diabetes mellitus (GDM) 108, 124, 149, 150
GH-induced diabetes 128
GH-induced diabetic cats 128
GH-induced diabetic dogs 128
GHR and PRLR mRNA in islets 131
Glucocorticoid-regulated kinase 64, 65
Glucocorticoids 58, 123, 126, 134, 137
Glucokinase 28, 90, 93, 95, 123, 130, 137, 142
Glucose-dependent insulinotropic polypeptide 109
Glucose homeostasis 108, 128, 133
Glucose insulin secretion 93
Glucose intolerance 7, 129, 134, 141, 143
Glucose metabolism 68, 70, 90, 95, 108, 115, 142
Glucose-stimulated insulin secretion (GSIS) 46, 69, 84, 91, 92, 93, 96, 98, 109, 110, 113, 115, 116, 117, 125, 130, 133, 138, 139, 142, 146, 147
Glucose tolerance 21, 58, 64, 72, 112, 115, 133, 144, 198
 impaired 58, 112
 improved 64, 115, 198
Glucose transporter 70, 89, 95
Glycolysis 27, 28, 93
G protein-coupled receptor (GPCRs) 109, 110, 111, 113, 115, 116
Gross phenotypes 99
Growth factors 5, 6, 13, 26, 27, 28, 29, 63, 64, 113, 123, 124, 130, 141, 172, 173, 175, 197, 204, 209
 keratinocyte 13, 172, 173
Growth hormone 123, 124, 126, 127
 placental 123, 126, 127
Growth hormone (GH) 123, 124, 126, 127, 128, 130, 133, 134, 136
GSIS promote 110
GSIS stimulate 109, 110

H

HC islet hyperinsulinemia 91
 HC pups, hypothalamus of 97
 HC rats, weaning of 84, 97
 Hematopoiesis 196, 200
 Hematopoietic cells 209
 Hematopoietic progenitor cells (HPC) 190, 194, 196, 197, 209, 212
 Hematopoietic stem cells 9
 Hematopoietic stem cell transplantation (HSCT) 200, 212
 Hepatocyte growth factor (HGF) 60, 123, 140, 175
 Heterogeneity, inter-individual 42, 49, 51
 HGF receptor expression in murine mammary cells 140
 HIF1-alpha 9
 High ALDH-Activity 197, 199
 High ALDH-activity promote regenerating islet cell proliferation 198
 High fat diet (HFD) 24, 25, 29, 31, 32, 63, 136, 204
 HLA-matched islet 201
 HOMA- β -cell function 62
 Hormonal changes 127
 Hormonal Changes During Pregnancy 126
 Hormones 126, 128, 129, 132, 134, 137, 142, 172, 173
 glucocorticoid 137
 lactogenic 132, 134, 142
 pancreatic 126
 pituitary 128, 129
 thyroid 172, 173
 HPAP+ Ins- cells 177
 HPAP+Ins- cells 177, 178
 HPAP+ insulin- cells 177
 HPC for islet regeneration 196
 HSCT for diabetes therapy 201
 Human BM 196, 197
 Human endocrine pancreas 193
 Human fetal pancreas cells 175
 Human intranslet 211
 Human leukocyte antigen (HLA) 48
 Hydrocortisone 137

Hyperglycemia 11, 25, 28, 59, 62, 63, 64, 69, 108, 112, 128, 137, 149, 170, 172, 198, 201, 203
 maternal 62, 63
 Hyperinsulinemia 21, 84, 85, 87, 90, 92, 97, 98, 129
 development of 84, 87
 programming of 92, 97
 Hyperphagia 84, 97, 98
 Hypothalamus 59, 84, 85, 86, 89, 92, 96, 98, 99
 Hypoxia 8, 9, 10, 171, 190, 210, 213

I

IGF-II immunostaining in islets cells 88
 Immunohistochemical localization of mTOR in islets 66
 INS-1 cells 136
 protected 136
 Instant blood mediated immune reaction (IBMIR) 170
 Insulin 4, 12, 13, 23, 27, 28, 49, 58, 61, 62, 65, 70, 91, 92, 93, 94, 98, 99, 107, 108, 109, 111, 112, 113, 124, 128, 130, 133, 138, 144, 150, 175, 177, 178, 190, 191, 192, 200
 exogenous 113, 190, 191
 obtained glucose-responsive mono-hormonal 13
 secret 93
 synthesize 42, 49
 Insulin biosynthesis 69, 73, 89, 130
 Insulin deficiency 42, 191
 Insulin demand, increased 23, 108, 123, 124, 125
 Insulin exocytosis 92, 93
 Insulin expression 175, 194, 198
 Insulin injections 11, 12
 Insulin levels 99, 150
 Insulin-positive cells, single 203
 Insulin receptors 27, 28, 112
 Insulin receptor substrate (IRS) 28, 65
 Insulin release 62, 73, 94, 130, 131
 demonstrated glucose-sensitive 178
 glucose-stimulated 60, 64, 70, 72
 reduced glucose-stimulated 58

- Insulin resistance 21, 46, 63, 71, 123, 127, 128, 129, 140, 149
increased 24, 49
peripheral 148, 150
- Insulin secreting cells 3
- Insulin secretion
deficient 108
enhanced 97, 116
glucose-sensitive 62
glucose-stimulated 46, 84, 91, 92, 146
impaired 129, 133
impaired glucose-stimulated 69
inhibiting glucose-stimulated 113
physiological 191
stimulated 11, 96, 112, 117, 123, 125
- Insulin secretory response by isolated islets 94
- Insulin sensitivity 70, 71, 108
increased 150, 204
- Insulin signaling pathway 28, 91
- Insulin therapy 21, 192
- International Diabetes Federation (IDF) 191
- Intra-islet cell proliferation 22
- Intra-islet-derived factors 107
- Intra-islet environment 112
- Intra-islet signaling pathways 117
- Islet adaptation 133
- Islet allografts 208, 210
co-transplanted 196
- Islet allograft survival 210
- Islet Amyloid Polypeptide (IAPP) 110, 113, 114
- Islet angiogenesis 190
- Islet Architecture and Cellular Composition 46
- Islet cell differentiation downstream, direct 14
- Islet cell lysates 66
- Islet cell neogenesis 87
- Islet cells 132
dissociated 130
human 131
- Islet cell type 117
- Islet cell types 111
- Islet content 67
- Islet density 91
- Islet derived factors, identified 110
- Islet derived precursors 194
- Islet endothelium HGF expression 140
- Islet environment 114
- Islet expression 145, 146
increased 140
stimulated 29
- Islet formation 195, 203, 212
- Islet function 70, 194, 197
improving 194
pancreatic 70
- Islet graft rejection 208
- Islet hormone genes 149
- Islet hyperplasia 142
- Islet hypertrophy 63
- Islet injury 210
- Islet mass 44, 46, 51, 88, 170
higher 170
- Islet microenvironment 107, 111
- Islet microvascular density 60
- Islet morphometry 63
- Islet neogenesis 61, 190, 194
compromised compensatory 61
initiating 194
- Islet ontogeny 87, 88
modified pancreatic 88
- Islet outward 112
- Islet perfusion 129
- Islet recovery 194, 204
- Islet regeneration 67, 190, 191, 193, 194, 195, 196, 198, 199, 203, 206, 210
cell-induced 198
cell-stimulated 194
endogenous 193, 194, 203
suggesting 193
- Islet regeneration occur 193
- Islet-regenerative functions 195
- Islet regenerative functions by expanded HPC subsets 199
- Islet regenerative mechanisms 205, 206
- Islet-regenerative subsets 213
- Islet rejection 192
- Islet replacement 192
- Islets 12, 24, 25, 26, 29, 46, 48, 63, 88, 97, 125, 126, 144, 147, 190, 194, 199, 200, 203, 204, 205, 206, 208, 209, 210, 211
adult human 26, 29
compromising 199
cultured STZ-treated 204
demonstrated 190
developing 203
diabetic 210
endogenous 204, 205, 209

formed 125
large 46, 48, 63, 88, 126
larger 46
larger human 46
long-lasting 199
measurable 203
mRNA level in 97, 144, 147
murine 206
non-human 12
obtained human 12
porcine 12
possessed 194
recipient 194
regenerated 200
transplanted 25, 208, 210, 211
transplanted human 24
Islets cells 88
Islet serotonin production 145
 arrested 145
Islets expansion 126
Islets experience 210
Islets inpercent 88
Islet sizes ranging 46
Islets mass 87
Islet structure 100
 pancreatic 84
Islet survival 192
Islet topology 111, 117
 human 117
Islet transplantation 190, 210, 211
 allogeneic 190, 211
 human 210
Islet transplantation models 209
Islet transplants 169, 170
Islet vascularization 7, 29, 63, 171
Islet vasculature 27, 29, 72, 112
Islet volume, total 126
Isolated human islets 180
Isolated islets 21, 24, 61, 62, 67, 91, 94, 96, 99,
 138, 139, 148

K

Keratinocyte growth factor (KGF) 13, 172,
 173, 175
Kinases, serum and glucocorticoid-regulated
 64, 65

L

Leukemia inhibitory factor (LIF) 175
Lineage-restricted CD34⁻ cells 196
Loss of β -cell mass in diabetes 47
Lower blood glucose and insulin levels 150

M

Malprogramming 84
Maturity onset diabetes of the young (MODY)
 13
Measured intra-islet proliferation 22
Membrane depolarization 27, 28
Mesenchymal cells 3, 5
 pancreatic 3
Mesenchymal stem cells (MSC) 194, 195, 201,
 202, 203, 204, 205, 207, 208, 212
Metabolic changes 123, 124
Metabolic coupling 111
Metabolic programming 85, 86
Metabolites 92
Methylation 68, 69
Microvasculature 29, 60, 61
 supportive islet 29
Mobilized-peripheral blood (MPB) 197
MRNA levels 89, 96, 97, 144, 147
MSC 203, 204, 206, 207, 208
 immunomodulatory properties of 207, 208
 transplanted 203, 204, 206
MSC and regenerating β -cells 204
MSC for diabetic-induced nephropathy 205
MTOR, activation of 64, 65
Multipotent/mesenchymal stromal cells 195
Multipotent mesenchymal stromal cells 201
Multipotent stromal cells 191
Murine hematopoietic cells 194
Murine mammary cells 140

N

N-acetyl cystein (NAC) 10
Neogenesis 21, 23, 88, 108, 124, 125, 126, 133,
 139, 143, 150
Neonatal diabetes 14
 severe form 14
Nerve growth factor (NGF) 123, 140

- Nervous system, sympathetic 96
Neural lineage cells 178
Nicotonic acid adenine dinucleotide phosphate (NAADP) 115
Nutrient-mediated insulin secretion 91, 96
Nutritional supplementation 72, 73
- O**
- Obesity, adult-onset 84
Offspring of diabetic mothers 62, 71
Organogenesis, influence pancreas 3
Oxidative stress 9, 71, 72, 73
Oxygen, partial pressure of 3, 8
- P**
- Pancreas embryogenesis 13
Pancreatectomy, partial 24, 25, 26, 29, 128, 149, 179, 203
Pancreatic agenesis 4
Pancreatic β -cells 23, 30, 58, 59, 63, 72, 194, 211
Pancreatic buds, dorsal 6, 45, 209
Pancreatic cancer 45, 146
Pancreatic cells 6, 175, 213
 human embryonic 6
Pancreatic content of insulin 92
Pancreatic epithelial precursor cells 5
Pancreatic insulin-producing β -cells 20
Pancreatic islet adenocarcinomas 141
Pancreatic islets 12, 44, 62, 71, 108, 123, 169, 200, 210
 isolated 123
Pancreatic organogenesis 89, 90
Paracrine 107, 109, 110
Parturition 60, 63, 70, 125, 127
Pdx-1 gene expression 85
PDX1-positive cells 6
Peptides, glucose-dependent insulinotropic 27
Peripheral blood, human 209
Permit endogenous islet regeneration 200
Physiology, β -cell 51
Pituitary adenylate cyclase activating polypeptide (PACAP) 110
Pituitary extracts 128
Placental lactogens (PL) 123, 126, 127, 130, 133, 134, 136, 139, 148
Plastic adherent cells 201
Platelet derived growth factor (PDGF) 26, 27
Polycomb-repressive complexes (PRC) 31
Polypeptide, pancreatic 42, 179
Postnatal stem cells 193, 211, 212
Post-natal stem cells stimulate 212, 213
Postweaning period, immediate 97
Precursor cells 4, 6, 209
 endothelial 209
 epithelial 6
Pregnancy 124, 127, 137, 138, 142
 late 124, 127, 137, 138, 142
 normal 124
Pregnancy estrogens 127
Pregnancy hormones 148, 149
Pregnancy-induced changes 129
Pregnancy serum 148
Preproinsulin genes 90, 97
Primary graft function, optimal 12
Primitive gut tube (PGT) 173
PRL, pituitary 133
PRL treatment 129, 130, 148
Progenitor cells 7, 9, 61, 143, 150, 169, 171, 173, 174, 177, 178, 179, 190, 191, 194, 195, 197, 199, 208
 early pancreatic 173, 174
 endothelial 61, 190, 194, 195, 208
 human 7
 human hematopoietic 194, 197
 long-lived 197
 pancreatic 173
 pro-regenerative 199
 putative 143, 150
 resident 179
 transplanting 195
Progesterone 123, 126, 127, 137, 138, 139
Prolactin 124, 127, 129, 143, 145, 146
Prolactin receptor 144, 146
Proliferation, islet-cell 198
Proliferative effects 144
Proliferative program 198, 199
Prolyl hydroxylases (PHDs) 9
Promote Islet Regeneration 206
Putative islet neogenic mechanism 203

R

Receptor activity-modifying proteins (RAMP)
114

Receptor expression 26, 27

Receptors 59, 114, 140
calcitonin 114
lacking pancreatic HGF 140
tissue glucocorticoid 59

Reduction, progressive 69

Regenerating β -cells 204

Regenerating islets 196, 197, 198, 210, 213
surrounded 198

Regeneration 20, 25, 29, 32, 169, 174, 176, 177,
180, 190, 207, 209, 211
endogenous β -cell mass 209

Regenerative capacity 24, 25, 180, 194, 199,
203, 205

Regenerative functions 190, 199, 200

Regenerative niche for new islet formation 203

Regenerative therapies 20, 197, 198, 202

Regions, gene promoter 68

Regulation, epigenetic 20, 27, 30, 31

Resident stem/progenitor cells 177

Restriction, intrauterine growth 58, 71, 72

Retinoic acid (RA) 172, 173, 197, 199

Reverse diabetogenic effects 128

Reverse hyperglycemia 199, 200

Risk, postnatal diabetes 70

S

Short chain fatty acids (SCFAs) 115, 116

Signals, mitogenic 20, 26, 28, 32

Sirt1-mediated glucose stimulate insulin
release 21

Somatolactogenic hormones 123, 127, 129,
131, 132, 133, 135, 137, 150

Sources, stem cell 171, 173, 180

Stem cells 3, 12, 169, 171, 173, 194, 195, 197,
201, 211
embryonic 12, 171, 173
human induced pluripotent 169
induced pluripotent 3
marrow stromal 201
mesenchymal 194, 201
neural 171

pancreatic 178
post-natal 194, 195, 211
purify 197
putative resident 169

Stem cells biology 9

Stimulate islet regeneration 213

Stimulus, important physiological 91

Streptozotocin diabetes 139

Stress, physiological 71, 149

STZ-induced diabetic rats 208

Supplementation, leucine 7, 8

Support β -cell regeneration 206, 207

T

Tagged non- β -cells 179

T-cells 208

Telomere length 71

Temporal diabetes 128

Theophylline 138

Thymidine 22, 23

Tissues 46, 47, 61, 70, 107, 108, 109, 129, 191,
192
donor pancreas 192
endocrine 46, 47
non-pancreatic 61
pancreatic 129
peripheral 70, 107, 108, 109, 191

T-lymphocytes, regulatory 200, 201, 207, 212

Toxins, environmental 58, 70

Transcription factors 3, 4, 8, 32, 68, 69, 151

Transdifferentiation 176, 194

Trans-differentiation 175

Transplantation, direct intra-pancreatic 198

Transplantation of freshly-isolated BM
ALDHhi cells 198

Transverse mesocolon 43

Trefoil factors (TFFs) 147

Trimethylates 31

Trimethylation 30, 31

TrxG proteins 31, 33

Tryptophan 145

TSC2 gene in β -cells 64

Tuberous sclerosis 64, 65

Tumors, pancreatic 45

U

Ubiquitination, decreased H2A 30
UCB ALDHhi cells 199
UCB cells, total 199
UCB-derived ALDHhi hematopoietic cells 198
UCB-derived stem cells 208
Umbilical cord blood (UCB) 190, 191, 197

V

Vascular endothelial growth factor (VEGF) 9,
60, 204, 211
Vasoactive intestinal polypeptide (VIP) 109
Vessels, superior mesenteric 43
VGF-derived peptides 116, 117



David J. Hill

David Hill (D.Phil., FCAHS) is Scientific Director of the Lawson Health Research Institute and Integrated Vice President, Research London Health Sciences Centre and St. Joseph's Health Care London, Ontario, Canada. He holds the Lawson Professorship in Diabetes Research, and is a Professor in the Departments of Medicine, Physiology and Pharmacology, and Paediatrics at Western University, Canada. Educated at the Universities of Nottingham and Oxford, Professor Hill is a past Chair of the National Board of the Canadian Diabetes Association, and is presently a Board Member for Research Canada and of Health Care Canada. He is a recipient of the Canadian Diabetes Association Frederick G. Banting Award, the Medal of the Society for Endocrinology from the UK, and is a Fellow of the Canadian Academy of Health Sciences. Dr. Hill has published some 250 research papers, and has most recently focused on the targeted generation of new insulin producing β -cells in the pancreas, the fetal programming of type 2 diabetes, and strategies to prevent gestational diabetes and early-onset type 2 diabetes in obese youth.