Review

Pancreatic islets and their roles in metabolic programming

Luiz Felipe Barella M.Sc.*, Júlio Cezar de Oliveira Ph.D., Paulo Cezar de Freitas Mathias Ph.D.

Laboratory of Secretion Cell Biology, Department of Cell Biology and Genetics, State University of Maringá, Maringá, PR, Brazil

A B S T R A C T

Experimental and epidemiologic data have confirmed that undernutrition or overnutrition during critical periods of life can result in metabolic dysfunction, leading to the development of obesity, hypertension, and type 2 diabetes, later in life. These studies have contributed to the concept of the developmental origins of health and disease (DOHaD), which involves metabolic programming patterns. Beyond the earlier phases of development, puberty can be an additional period of plasticity, during which any insult can lead to changes in metabolism. Impaired brain development, associated with imbalanced autonomous nervous system activity due to metabolic programming, is pivotal to the creation of pathophysiology. Excess glucocorticoid exposure, due to hypothalamic–pituitary–adrenal axis deregulation, is also involved in malprogramming in early life. Additionally, the pancreatic islets appear to play a decisive role in the setup and maintenance of these metabolic dysfunctions as key targets of metabolic programming, and epigenetic mechanisms may underlie these changes. Moreover, studies have indicated the possibility that deprogramming renders the islets able to recover their functioning after malprogramming. In this review, we discuss the key roles of the pancreatic islets as targets of malprogramming; however, we also discuss their roles as important targets for the treatment and prevention of metabolic diseases.

© 2014 Elsevier Inc. All rights reserved.

Introduction

Undernutrition early in life is one of the most important risk factors for the physiological and structural alterations that commonly appear in adulthood. Associated with nutritional conditions early in life, health disturbances in adulthood have become a burden on the governments of several developing countries around the world [1]. As demonstrated by both experimental [2,3] and epidemiologic data [4–6], metabolic diseases have become a worldwide problem, and the metabolic dysfunctions that are related to prenatal and postnatal nutritional insults are one of the triggers of the development of obesity, hypertension, and type 2 diabetes, among other disorders.

In agreement with several studies, the thrifty phenotype hypothesis has been offered, which postulates [7] that low birth weight is one of the major factors in the development of metabolic syndrome later in life, as well as in the developmental origins of health and disease (DOHaD), a matter that has been extensively discussed by many researchers at a variety of scientific institutions around the world. As part of defining the concept of DOHaD, DOHaD is expressed in adulthood if the body underwent some insult (e.g., malnourishment) during critical windows for body development, such as the perinatal phase. However, malnourishment’s effects on metabolism will be observed in adulthood if the diet improves or even if overnutrition occurs. It has been shown that a low-calorie diet increases lifespan and provides greater resistance in aged rats to the development of dysfunction of the pancreatic β cells, which establish a defense against the development of type 2 diabetes in these animals [8]. However, this effect in β cells is dependent on chronic food ingestion under caloric restrictions, begun immediately after adolescence. Considering the overall data, the concept of DOHaD can be defined as malprogramming of the metabolism that induces diseases when children become adults.

Concerning perinatal malprogramming, the meaning of the DOHaD concept today includes a focus of discussion not only on nutritional insult but also on the insults caused by several drugs, such as nicotine, which is one of the key triggers that leads to...
metabolic dysfunctions in adulthood [9]. Among other malfunctions, these insults include impaired brain development/function associated with imbalanced autonomous nervous system activity due to metabolic imprinting, which is pivotal to the genesis of critical pathophysiologies.

The long-term effects of malnutrition have been extensively studied in experimental protein–calorie-restricted animal models [3,10–12]. Despite central nervous system (CNS) malformation [13,14] due to this system's outstanding developmental sensitivity phase, the CNS is not the only affected system. Several other organs and tissues, such as hypothalamus nuclei, the adrenal medulla, the liver, and the endocrine pancreas in particular [12,15–17], are also functionally impaired by nutritional restriction during the early sensitive phases of development, with serious implications for body weight, energy regulation, and glucose homeostasis.

The functional role of the pancreatic islets has been well demonstrated as one of the key targets of metabolic programming [15,18,19], which might predispose the endocrine pancreas to exhaustion, resulting in a diabetic condition. Both overnutrition and undernutrition have been linked to pancreatic β-cell dysfunction induced by epigenetic changes, which appears to induce the onset of type 2 diabetes [20,21]. Among other molecular alterations, changes in microRNA also have been implicated in influencing diabetes [22,23]. Within this context, we aimed to conduct a brief review of some important data concerning pancreatic islet function as one of the major targets in the development of metabolic syndrome, which is associated with nutritional injuries in the early developmental phases of life.

Early nutritional insults malprogram brain development: the intrauterine and suckling phases as very sensitive windows for metabolic programming

Multidisciplinary studies, focusing on the influences of perinatal and postnatal protein restriction on the development of the CNS, have been performed by Morgane's group for four decades [13,14,24]. Within this paradigm, these researchers hoped to establish a parallel between the many typical chronic human diseases correlated with protein restriction early in development and disturbances in brain connections. In humans, the development of CNS connections occurs primarily during the embryonic phase. During this phase, the ontogenesis, growth, proliferation, migration, and differentiation of neurons and glial cells in many brain areas occur more prominently, with the peak occurring around the time of birth. In contrast, this process occurs in rodents primarily from the last third of gestation through approximately the first 2 wk of suckling [25–27].

Physiological control of metabolism depends on CNS modulation. Impairment by calorie/protein diet restriction, both in human and animal models, has been linked to several chronic diseases later in life [14,25,26,28]. A link was recently reviewed between the CNS and pancreatic islet function with regard to undernutrition early in life [29]. Similarly, we have shown that the vagus nerve (the direct CNS connection to the pancreas) is less active in adult rats from dams fed a low-protein diet during lactation. These rats displayed glucose intolerance and weak insulin secretion, as well as a reduced cholinergic response in their pancreatic islets [18,30].

The early phases of brain development have been implicated as the more prominent developmental stages in which environmental influences render the brain highly sensitive to metabolic programming. However, as previously reported [31,32], it should be noted that puberty and the beginning of adulthood (unpublished data) also may constitute stages in which nutritional insults induce long-lasting metabolic dysfunction.

Early nutritional insults malprogram pancreatic islet function

Glucose homeostasis has long been considered essentially controlled by the ability of pancreatic β cells to secrete insulin and by the effects of insulin on peripheral tissues. Our understanding of the appropriate function of the pancreatic islets has increased regarding some types of metabolic pathophysiology later in life related to pancreatic β-cell malfunction early in development. Endocrine dysfunction of the pancreatic islets has been demonstrated using multiple experimental models of metabolic programming [10,12,19,33], making caloric-protein undernutrition early in life a well-established model for studying these changes. Glucose uptake, insulin secretion, insulin sensitivity, and increased glucocorticosteroid levels are among the most common metabolic parameters disturbed by caloric/protein restriction during either pregnancy or breastfeeding [15,18,19,34]. As a consequence, this restriction might exhaust the pancreatic β cells and result in subsequent susceptibility to diabetic conditions.

In rodents, it has been extensively reported that the first steps in the biological development of the endocrine pancreas are observed as soon as the beginning of the second week of the embryonic stage, which is approximately 9.5 embryonic d in mice and 11 embryonic d in rats, equivalent to 25 to 26 gestational d in humans [35]. Under the influence of a large number of transcriptional and growth factors, multipotent endodermal progenitor cells are generated in one of several different endocrine cell lineages [36]. In a not-yet mature stage of the endocrine pancreas, rodents present few cells that produce glucagon, which appears to induce the development of insulin-coproducing cells; however, these cells do not appear to be responsible for the formation of mature glucagon-and insulin-secreting cells. Subsequently, at approximately 13 to 14 embryonic d, the insulin-producing cells are expressed in large numbers, resulting in endocrine cell subtype differentiation. Finally, from the unification of multiple undefined endocrine cells, the pancreatic islets are formed at approximately 18 to 19 embryonic d [37,38]. In rodents, the ability of the pancreatic β cells to secrete insulin begins during the last third of pregnancy, whereas in humans, it occurs during the first trimester [39,40]. However, the final characteristics of the endocrine cells in the pancreatic islets are not completely established until the end of the gestational phase, and the maturation process continues even after birth, through 2 wk postnatally [37], making these stages of life a target of nutritional and/or hormonal insults. Such insults induce later functional disturbances in rodents.

During the embryonic stage of pancreatic organogenesis and cell development, several transcription factors are indispensable to appropriate pancreatic cell formation, maturation, and future function. As extensively reviewed [41–44], the expression of transcription factors belonging to the families of paired box (Pax) 4 and 6, homeodomain (Nkx) 6.1 and 2.2, forkhead box (Fox) O1 and A2, neurogenin (Ngn) 3 and pancreatic and duodenal homeobox-1 (Pdx-1), among several others, produces crucial markers in pancreatic β-cell budding and differentiation and in pancreas development, as well as in the maintenance of pancreatic endocrine cell function throughout life.
The effects of nutrients, hormones, and growth factors on the transcriptional regulators involved in pancreatic cell function are commonly impaired by maternal metabolic insults during the phase in which the pancreas, particularly the β cells, are developing [15]. Intrauterine growth retardation (IUGR) has been linked to the development of type 2 diabetes mellitus in rat offspring when they become adults, suggesting that among other alterations in the transcription factors that promote pancreatic β-cell development and function, it is primarily associated with lower expression of the Pdx-1 gene [45]. Similar data were found in the islets of rat offspring from dams that underwent protein restriction during pregnancy and lactation [19]. Similarly, the mRNA expression levels of Pdx-1, Nkx6.1, and Pax-6, and other important transcriptional factors involved in this process, were decreased in rats from dams that underwent nicotine exposure during pregnancy. Interestingly, in adulthood, these rat offspring, in addition to being obese, glucose intolerant, and insulin resistant, displayed lower numbers and sizes of pancreatic islets, with less ability to secrete insulin [46].

Beyond other reported stressor insults that disturb the intrauterine and/or suckling phase environment, high levels of glucocorticoids, either due to exogenous administration of dexamethasone, a synthetic analog of glucocorticoids, or functional changes through prenatal calorie restriction [34, 47, 48], have been identified among the main causes of the growing pandemic of metabolic syndrome. Indeed, glucocorticoid excess in later gestation appears to play a crucial role in insulin biosynthesis and secretion, and in β-cell mass impairment in rat offspring [49–51]. As previously reported, excessive levels of glucocorticoids and their effects have been associated with disrupted expression of transcription factors, such as increases in the expression of the mitogen-inducible gene (Mig6) in the islets of rodents and humans [52], and a decrease in Ngn3, which is involved in β-cell proliferation [50]. Regarding the glucocorticoids’ action, the FoxO1 gene and proteins that are crucial to the processes of Pdx-1 activation of the endocrine pancreas and β-cell proliferation, maintenance, and function throughout development were found to be increased in rat islets treated with dexamethasone [53]. Although the implications of the environmental and/or genetic factors involved in the effects of stressful insults early in life on the mechanisms associated with gene transcription and their actions on β-cell proliferation and function have been described, these factors are not yet completely understood. Glucocorticoid action on transcriptional factors might be one important trigger in endocrine pancreas malprogramming early in life and its associated insulin secretion and action disturbances. Nevertheless, further investigations are necessary to improve our knowledge on this topic.

Epigenetic roles in pancreatic β-cell malprogramming

A growing body of evidence has emerged that has confirmed the role of epigenetic factors in healthy development. In humans, the children of mothers who were pregnant during the Dutch famine of 1944 (occurred in the Netherlands at the end of World War II) displayed less DNA methylation of the Igf2 gene, a key factor in human growth and development, in blood cells in adulthood [54]. Epigenetic modifications also were found in a rodent model of IUGR, in which low levels of Pdx-1 were expressed in the islets of the offspring in adulthood due to complete silencing of this transcription factor. This silencing resulted from a self-propagating epigenetic cycle, in which a repressor complex named mSin3A/HDAC, which catalyzes histone deacetylation, is first recruited to the Pdx-1 promoter, then histone tails are exposed to deacetylation, and extensive DNA methylation leads to locking in a transcriptionally silent state of Pdx-1. This repressed expression of Pdx-1 is linked to the development of type 2 diabetes in adulthood [45]. Similarly, dietary management schemes, such as low-protein or food-restriction diets, which are used to induce IUGR in rats, can alter the epigenetic markers that regulate gene expression through DNA methylation and/or histone modifications; such changes have been reported in a rat model of uteroplacental insufficiency, which showed decreased Pdx-1 mRNA expression associated with the development of type 2 diabetes mellitus in adulthood [55]. This Pdx-1 mRNA decrease was explained by progressive epigenetic silencing of the Pdx-1 gene locus, secondary to proximal promoter methylation [55]. Pancreatic β-cell development and maintenance of function are pivotally dependent on the control of these factors [56], which make these observations importantly suggestive of the malprogramming of pancreatic β cells early in development and the possibility of inducing metabolic disease onset in adulthood (Fig. 1).

Another mechanism that might explain reduced β-cell formation is related to early life differences in exposure to glucocorticoids. Blondeau et al. (2001) showed that the glucocorticoid receptor is critical for developing pancreatic architecture and survival, including β-cell expansion (i.e., a negative correlation has been observed between glucocorticoids, fetal weight and insulin content in rat fetuses with normal nutrition); moreover, fetal β-cell mass has been shown to increase after maternal adrenalectomy or inhibition of steroid production [57]. The glucocorticoid influence might act by modification of the expression of some transcription factors, primarily binding of the Pdx-1 promoter, resulting in the suppression of fetal β-cell differentiation [58]. Given this link, Pdx-1 is a key target candidate for epigenetic modifications. Along the same line of the stress axis, IUGR offspring present different levels of T1β-HSD type 1 and 2 activity, and these enzymes are responsible for converting inactive glucocorticoids into corticosterone and vice versa, respectively [34, 59]. Studies have shown evidence of epigenetic modulation of the T1β-HSD2 gene in aldosterone target tissues, leading to gene repression [60, 61]. Although there are no studies on β cells, epigenetic modulation of the T1β-HSD2 gene could play an important role in pancreatic islet function.

Hepatocyte nuclear factor 4-α (Hnf4α) is implicated in β-cell differentiation and glucose homeostasis. To date, it is known that both nutrition and aging induce changes in promoter-enhancer interactions (i.e., Hnf4α is epigenetically regulated in islets). Exposure of dams to a low-protein diet leads to epigenetic silencing in offspring at the enhancer region, resulting in a permanent reduction in Hnf4α expression, whereas aging induces progressive epigenetic silencing of the entire Hnf4α locus in islets. This silencing is even more pronounced in rats exposed to poor maternal diets [62].

Recently, one study has shown that epigenetic changes in the offspring’s pancreatic islets can also be induced by high-fat paternal nutrition, programming β-cell dysfunction in rat female offspring. The father’s chronic high-fat diet consumption led his female offspring to have impaired insulin secretion and glucose tolerance, along with altered islet expression of many genes involved in glucose homeostasis, but with no changes in the adipose tissue accumulation. This study also indicates that these changes can be explained, at least in part, by modification of the offspring epigenome, specifically the gene Il13ra2, which is responsible for part of some key metabolic networks [63]. Regardless of how, whether maternal or paternal, epigenetic changes may be transmitted to offspring. Beyond transcription
factors directly involved in gene activation or repression due to metabolic malprogramming, several types of microRNA have been modiﬁed in response to nutritional deprivation [64]. This finding could suggest one intrinsic association between most epigenetic changes induced by the effects of early metabolic disturbances. MicroRNAs have also been strongly implicated in influencing the type 2 diabetes output by silencing different types of genes involved in β-cell development and function [22].

Notably, early environmental influences also appear to induce morphologic, rather than epigenetic, development, resulting in permanent changes in organ structure and adult metabolism (i.e., not all developmental plasticity can be explained by epigenetics) [65]. Nevertheless, epigenetic mechanisms might underlie several examples of metabolic imprinting, as shown in this review.

Puberty as a window for metabolic malprogramming

Similar to during gestation and lactation [18,66,67], any insult that occurs during adolescence, when new neuronal connections are being made in the brain, can induce changes in metabolism [68,69]. Adolescence is critical for the final maturation of most neuroendocrine circuits, including those regulating energy expenditure [70]. We support the hypothesis that rats are also very sensitive to nutritional insults during the peripubertal period.

Recently, our group undertook studies that supported this hypothesis. Rats fed a high-fat diet during the peripubertal period exhibited more drastic effects, such as increased adipose tissue accretion, severe glucose intolerance, and diminished insulin sensitivity compared with rats fed a high-fat diet only during adulthood [32]. When rats are fed a low-protein diet during adolescence, dysfunction in the β cells related to changes in the activity of the muscarinic receptors is observed in addition to imbalanced metabolism homeostasis with concomitant high vagal nerve activity [31].

Other authors also have hypothesized that the pubertal period is a critical window for the development of metabolic changes. A body of evidence has shown that stressors during puberty can alter the activity of the hypothalamus–pituitary–adrenal (HPA) axis, which is the major neuroendocrine axis that controls stress responses, with lasting effects later in life [71]. Some studies have indicated that increased dietary fat during the prepubertal and pubertal periods can affect behavioral responses following exposure to stressful conditions, as well as modify HPA axis function and alter the homeostatic responses of metabolic and neuroendocrine circuits, thus promoting persistent alterations in adulthood [72,73].

Pancreatic islets and deprogramming

Although the term malprogramming has been postulated, it is also possible to apply the term deprogramming: however, it is necessary to determine whether there is a possibility to intervene to attenuate the perinatal/pubertal metabolic programming that causes metabolic dysfunctions later in life. Some non-pharmacologic interventions, such as functional food, surgical maneuvers, and exercise training, among others, have been postulated as important tools to prevent metabolic diseases (Fig. 1).

Functional nutrition can be a powerful tool for attenuating the effects of metabolic programming due to malnutrition during gestation. Supplementation of diets with folic acid during pregnancy can prevent or attenuate IUGR-induced increases in reactive oxygen species and steroid hormone responses [74], and as a methyl donor, folic acid might act through an epigenetic mechanism [75]. However, these nutritional supplements should be used with care, because different stages of life can be affected
in different ways; folic acid supplementation during the pubertal period induces distinct changes in the phenotype and epigenotype of offspring from protein-restricted dams during pregnancy [76].

Concerning epigenetic studies, DNA methyltransferases (Dnmts) have a great affect on epigenetic regulation, and they are used with histone methylation and acetylation to regulate gene expression and to maintain genomic integrity and chromosome structure [77]. One study showed that Dnmts might influence the reprogramming of cells toward the fate of pancreatic β cells [77]. Another study suggested that α-cell to β-cell reprogramming could be achieved by histone methylation signature manipulation of pancreatic islets, resulting in colocalization of Pdx-1 in islets through the inhibition of histone methyltransferase; this epigenomic manipulation could be effective as a therapy for diabetes [78,79].

Our group has already shown that islets from adult rats fed low-protein diets during lactation undersecrete insulin; however, these same islets can reduce glycemia by the same magnitude as normal islets when they are grafted into hyperglycemic diabetic rats [80]. These results suggest that metabolic programming due to perinatal protein restriction provokes long-term changes in pancreatic β cells; however, it may be possible for these cells to recover their function (i.e., insulin secretion control is not permanent).

Physical activity is a well-studied factor in improving the metabolism of patients with metabolic syndrome; however, the mechanisms that underlie this effect are not yet completely understood. Exercise training may attenuate metabolic dysfunction through the epigenetic regulation of gene expression [81].

Moreover, when rat mothers are both fed a low-protein diet and trained during the perinatal period, the effects of the poor diet, such as altered glucose homeostasis and leptin concentrations in skeletal muscle, are attenuated in their offspring [82]. We have also shown that exercise training, when begun early in life, exerts potential benefits in monosodium l-glutamate-obese mice through the attenuation of metabolic dysfunction via the modulation of autocrine insulin action [83].

Conclusion

Pancreatic islets are among the preferential targets for mal-programming, and they collaborate significantly in the development of metabolic malfunctions. Altered mechanisms controlling insulin secretion can disrupt metabolic homeostasis, resulting in the development of conditions that lead to the genesis of metabolic diseases. Furthermore, pancreatic islets may constitute an important target for prevention and treatment. Additionally, unveiling and understanding the mechanisms underlying the programming and deprogramming of pancreatic islets might be crucial in reducing the worldwide epidemic of metabolic diseases.

Acknowledgments

This work was supported by the following Brazilian Federal Foundations: Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Support was also received from the Paraná Science Foundation (Fundação Araucária).

References

[17] Alltmann S, Murani E, Schwierin M, Metges CC, Wimmers K, Ponsuksili S. Physical activity is a well-studied factor in improving the metabolism of patients with metabolic syndrome; however, the mechanisms that underlie this effect are not yet completely understood. Exercise training may attenuate metabolic dysfunction through the epigenetic regulation of gene expression [81].

Moreover, when rat mothers are both fed a low-protein diet and trained during the perinatal period, the effects of the poor diet, such as altered glucose homeostasis and leptin concentrations in skeletal muscle, are attenuated in their offspring [82]. We have also shown that exercise training, when begun early in life, exerts potential benefits in monosodium l-glutamate-obese mice through the attenuation of metabolic dysfunction via the modulation of autocrine insulin action [83].


