Early life determinants of Beta-cell function in the sheep

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If we knew what it was we were doing, it would not be called research, would it?

Albert Einstein

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STATEMENT OF ORIGINALITY AND AUTHENTICITY

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signed,

Siti Aishah Sulaiman

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TABLE OF ABBREVIATIONS AND BIOCHEMICAL NAMES

ACTB	Beta cytoskeletal actin / β-actin
ADIPOQ	Adiponectin
ADP: ATP	Adenosine diphosphate: Adenosine triphosphate ratio
AGR	Absolute growth rate
ANOVA	Analysis of variance
arb. unit	Arbitrary unit
BMI	Body mass index
BUVL	Bilateral uterine artery ligation
CACNA1D	L-Type voltage-gated Ca^{2+} channel subunit
cDNA	Complementary DNA
CON	Control group
CpG island	Cytosine-Guanine base pairing rich regions
CRL	Crown-rump length
CSIRO	Commonwealth Scientific and Industrial Research Organisation
Ct	Cycle threshold
CUG	Catch-up growth
DAB	3,3'-Diaminobenzidine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
dNTP	Deoxyribonucleotide triphosphate
DPP-IV	Enzyme dipeptidyl peptidase IV
FGR	Fractional growth rate
FOXA2	Forkhead box protein A2

GCK	Glucokinase
GIR	Glucose infusion rate
GLP1	Glucagon-like-peptide 1
HAT	Histone acetyltranferase
HDAC	Histone deacetylase
HEC	Hyperinsulinaemic euglycaemic clamps
HMT	Histone methyltransferase
HOMA-IR	Homeostasis model, calculation of insulin sensitivity
IGF	Insulin-like growth factor
IGF1	Insulin-like growth factor 1
IGF1R	Type 1 insulin-like growth factor receptor
IGF2	Insulin-like growth factor 2
IGF2R	Type 2 insulin-like growth factor receptor
IMVS	Institute Medical and Veterinary Science, Adelaide, Australia
INS	Insulin
INSR	Insulin receptor
IUGR	Intrauterine growth restriction
IUGR+Ex-4	IUGR lambs treated with exendin-4 as neonates
IUGR+Veh	IUGR lambs treated with vehicle as neonates
IVGTT	Intravenous glucose tolerance test
KCNJ11	A subunit of ATP-sensitive K^+ channel
KRB/BSA	Krebs Ringer buffer supplemented with bovine serum albumin
LB-Broth	Luria-Bertani broth
miRNA/miR	Micro ribonucleic acid, microRNA
mRNA	Messenger ribonucleic acid

MTPN	Myotrophin
NADH	Reduced form of nicotinamide adenine dinucleotide
NSW	New South Wales
OCT	Optimum cutting temperature embedding substrate
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDX1	Pancreatic duodenal homeobox-1
PIK3CB	PI-3-kinase, catalytic subunit beta
PIK3R1	PI-3-kinase, regulatory subunit 1
PR	Placental restriction
QC	Quality control
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
RT PCR	Real Time PCR
SA	South Australia
s.c.	Subcutaneous
SGA	Small for gestational age
SLC2A2	Glucose transporter 2 / Glut2
SLC2A4	Glucose transporter 4 / Glut4
T2D	Type 2 Diabetes
TCA cycle	Tricarboxylic acid cycle,
USA	United States of America
V	Voltage
V_d	Volume density

PUBLISHED PEER REVIEWED JOURNAL ARTICLE AND CONFERENCE PRESENTATIONS ARISING FROM THIS THESIS

Neonatal exendin-4 reduces growth, fat deposition and glucose tolerance during treatment in the intrauterine growth-restricted lamb

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Neonatal exendin-4 intervention treatment normalises islet insulin secretion and expression of its molecular determinants in the intrauterine growth restricted lambs

Siti A Sulaiman, Kathryn L Gatford, Miles J De Blasio, Saidatul N Mohammad, Julie A Owens.

August 2011: *Faculty of Health Sciences 2011 Postgraduate Research Conference*, Adelaide, Australia. (Poster Presentation by Siti A Sulaiman)

September 2011: *ESA/APEG Annual Scientific Meeting*, Perth, Australia. (Oral presentation by Siti A Sulaiman)

November 2011: *7th Asia Pacific Congress in Maternal Fetal Medicine*, Kuala Lumpur, Malaysia. (Poster presentation by Siti A Sulaiman)

Neonatal exendin-4 treatment in the twin IUGR lamb normalises *in vitro* islet insulin secretion and expression of its molecular determinants

Siti A Sulaiman, Kathryn L Gatford, Miles J De Blasio, Saidatul N Mohammad, Julie A Owens.

February 2011: *Fetal Neonatal Workshop of Australia and New Zealand*, Hobart, Australia. (Oral presentation by Kathryn L Gatford)

Neonatal Exendin-4 Treatment Increases β-cell Mass and Alters Islet Gene Expression in the IUGR Lamb

SA Sulaiman, KL Gatford, SN Mohammad, ML Harland, MJ De Blasio, RA Simmons, JS Robinson, JA Owens

September 2010: *ESA/SRB Scientific Meeting*, Sydney, Australia (Oral presentation by SA Sulaiman)

Neonatal exendin-4 treatment increases insulin secretion, beta-cell mass and decreases fat deposition in the IUGR lamb

Siti A Sulaiman, Kathryn L Gatford, Saidatul N Mohammad, M Lyn Harland, Miles J De Blasio, Rebecca A Simmons, Julie A Owens

June 2010: *ASMR SA Scientific Meeting*, Adelaide, Australia. (Poster presentation by Siti A Sulaiman)

Intervention strategies against programming of diabetes following IUGR

KL Gatford, SN Mohammad, SA Sulaiman, ML Harland, MJ De Blasio, AL Fowden, JS Robinson, JA Owens

2010: *PSANZ Scientific Meeting*, Wellington, New Zealand. (Oral presentation by KL Gatford)

Effects of exendin-4 on the growth-restricted twin lamb

KL Gatford, SA Sulaiman, MJ De Blasio, TA How, ML Harland, SN Mohammad, JA Owens

2009: *PSANZ Scientific Meeting*, Darwin, Australia. (Oral presentation by KL Gatford)

ABSTRACT

Low birth weight or intrauterine growth restriction (IUGR) consistently predict increased risk of Type 2 diabetes (T2D) through impairment of glucose tolerance, insulin resistance and inadequate insulin secretion in humans (1, 2), as well as in many experimental studies in other species (3, 4). IUGR due to insufficient supply of fetal nutrients, decreased oxygen supply and elevated exposure to stress hormones are thought to 'program' the impairment of β-cell mass, function and plasticity which then contributes to development of diabetes later in life, as observed in humans (5-7) and animals (4, 8). Interestingly, administration of the glucagon-like-peptide 1 (GLP1) analogue exendin-4 to neonatal IUGR rats normalised subsequent β-cell mass and insulin secretion and prevented later development of T2D (9), thus providing a possible intervention strategy to prevent T2D following IUGR. However, there are differences in the timing of pancreatic and β-cell development between species and therefore in the developmental stages during exposure to IUGR and neonatal interventions. In humans and sheep, most pancreatic and β-cell development occurs before birth (10-17). In contrast, rodents undergo later development of β-cells than sheep or humans, with the majority of pancreatic remodelling occurred at postnatal ages (18-20). It is therefore necessary to test the efficacy of neonatal exendin-4 treatment in animal models such as sheep that share similar profile of pancreatic development and growth with humans (9, 21). Therefore, this thesis will address the effects of IUGR on β-cell mass and function, expression of their molecular determinants, as well as epigenetic modifications, and the possible involvement of altered circulating adiponectin abundance and expression in adipose tissue in the young lamb from birth to 16 d of age. The efficacy of neonatal exendin-4 treatment as a postnatal intervention to prevent these adverse effects of IUGR on metabolic outcomes will also be assessed.

Here, natural twin pregnancies were used as a model of IUGR in progeny and unrestricted singleton lambs as the controls. In each twin set, sibling twin lambs with high and low birth weights were alternately allocated to either vehicle or exendin-4 treatment. Effects of IUGR due to twinning and of neonatal exendin-4 treatment of the twin lambs on neonatal growth, pancreatic β-cell *in vivo* and *in vitro* insulin secretory function, β-cell mass and islet expression of key regulatory genes including microRNAs, epigenetic regulators, and adiponectin, and on adiponectin abundance were analysed.

IUGR due to twinning reduced size at birth and increased neonatal growth, without altering insulin sensitivity, *in vivo* insulin action, β-cell mass or islet mRNA expression of β-cell mass molecular determinants when compared to CON lambs. However, *in vitro* glucose-stimulated insulin secretion was increased in the IUGR twin lamb relative to controls (+420%, P = 0.081), consistent with up-regulation of islet mRNA expression of *GCK* in this group (+80%, P = 0.017), thus suggesting up-regulated β-cell function at this age. Interestingly, IUGR twin lambs also had increased islet mRNA expression of *DNMT3B* relative to CON lambs (+96%, P = 0.027), which is responsible for *de novo* DNA methylation (22, 23). Islet mRNA expression of *GCK* was positively correlated with that of *DNMT3B* in the IUGR twin group, suggesting that altered islet *GCK* mRNA expression and β-cell function after IUGR may occur in part via epigenetic changes that may persist throughout life. In conjunction with enhanced β-cell function, up-regulation of adiponectin mRNA expression in omental fat (+72%, P = 0.008) and increased circulating adiponectin levels (P = 0.012) were also observed in the IUGR twin lamb group. Omental adiponectin mRNA expression and circulating adiponectin correlated positively with insulin secretion and β-cell mass

in combined control and IUGR twin lamb groups, suggesting that this adipokine may play a role in regulating neonatal insulin secretion.

Daily exendin-4 treatment of IUGR twin lambs during neonatal life prevented accelerated neonatal growth or catch up growth (CUG) and fat accumulation (-57%, $P < 0.001$), and normalised *in vitro* insulin secretion and *GCK* and *DNMT3B* mRNA expression in their islets, relative to vehicle-treated IUGR twins. This may retain adaptive capacity of β-cell function for later life. Glucose tolerance of twin IUGR lambs was impaired during exendin-4 treatment (+156%, $P = 0.003$) reflecting decreased insulin sensitivity (-46%, $P = 0.002$) in this group, despite having normal *in vivo* insulin secretion. This may be due to central actions of exendin-4 to inhibit food intake and insulin sensitivity (24-26). β-cell mass in IUGR twin lambs treated with exendin-4 tended to be higher than in their IUGR counterparts (+28%, $P = 0.083$), and consistent with this, islet mRNA expression of *IGF1* and *IGF2R* was increased in this group (+62%, $P = 0.058$ and +63%, $P = 0.005$ respectively) when compared to controls. Moreover, in the IUGR+Ex-4 lambs, islet mRNA expression of *PDX1* correlated positively with that of *IGF1R*, while *IGF1* mRNA expression correlated positively with β-cell volume density, which may suggest hyperplastic effects of the *IGF* axis on β-cell mass during exendin-4 treatment. Despite the profound reduction in visceral fat mass induced by neonatal exendin-4 treatment, circulating adiponectin concentrations were not reduced in exendin-4-treated lambs, possibly due to up-regulation of adiponectin expression in subcutaneous fat in these animals (+91%, $P = 0.007$). Nevertheless, the reduction in fat accumulation and normalised *in vitro* β-cell action of IUGR lambs during neonatal exendin-4 treatment suggest that neonatal exendin-4 might have beneficial effects on insulin-regulated glucose homeostasis in later life. These outcomes also demonstrate the biological activity of exendin-4 for the

first time in the sheep, at least in the context of individuals who had undergone growth-restriction before birth.

In conclusion, IUGR due to twinning induced CUG, early life up-regulation of *in vitro* β-cell insulin secretion and islet expression of its determinant, *GCK*, but did not alter *in vivo* insulin action, glucose tolerance or β-cell mass in young lambs at 16 d of age. These metabolic and molecular changes may be partly mediated by increases in circulating adiponectin and its expression in omental fat, as part of an adipose tissue response during neonatal fat deposition. Consistent with our hypothesis, neonatal exendin-4 treatment prevented this IUGR-induced CUG and decreased visceral fat deposition, increased 2^{nd} phase insulin secretion *in vivo*, normalised *in vitro* insulin secretion and islet expression of its determinant, *GCK*, at the end of treatment in the IUGR twin lambs. Although exendin-4 treatment only tended to increase β-cell mass in young IUGR lamb, the up-regulation of islet expression of β-cell mass determinants after 16 days of exendin-4 treatment may suggest beneficial effects of exendin-4 to subsequently expand β-cell mass. This may protect the exendin-4-treated IUGR individual from a need to increase β-cell function, and preserve the capacity of β-cells for later plasticity of insulin secretion in response to the development of insulin resistance with ageing. Hence, a long term investigation is required to address how these changes following IUGR and neonatal exendin-4 treatment at 16 d of age will affect β-cell function and mass and insulin action and their regulation in the IUGR sheep to adulthood.