Insulin resistance correlates with maculopathy and severity of retinopathy in young adults with Type 1 Diabetes Mellitus.

Running head: Insulin resistance and retinopathy in young adults with Type 1 diabetes

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Word count: 2661

Funding: This work was funded by a John Hunter Hospital Charitable Trust Grant and the Hunter New England Local Health District Clinical Research Fellowship (to CR).

Conflicts of interest: Nil

Author contribution: CR conceived and designed the study with SA and KW; and drafted the manuscript. AH and DV interpreted retinal pathology. MJ and JA performed data analysis with CR and assisted with interpretation. All authors critically reviewed the manuscript and have approved the final version.

Structured Abstract

Aims: To assess the relationship between insulin resistance (IR), retinopathy and maculopathy in young adults with Type 1 Diabetes Mellitus.

Methods: A cross-sectional study at a regional Australian tertiary hospital. Retinal pathology assessed by colour fundus photography was correlated with two surrogate measures of IR: estimated glucose disposal rate (eGDR) and Insulin Sensitivity Score (ISS), where lower scores reflect greater IR.

Results: 107 patients were recruited, with mean age 24.7 years, 53% male, and mean duration of disease 10.8 years. Mean eGDR (5.6 vs 8.0 p<0.001) and ISS (4.7 vs 7.9, p<0.001) scores were lower in subjects having at least moderate non-proliferative diabetic retinopathy (NPDR; relative to nil/mild-NPDR). Similarly, mean eGDR (4.2 vs 6.2, p=0.001) and ISS (3.8 vs 6.1, p=0.003) were lower in patients with maculopathy. Multivariate logistic regression modelling was used to control for confounding. For retinopathy severity, a unit increase in eGDR or ISS (representing lower IR) was associated with a 50% decrease in odds of moderate-NPDR or worse (eGDR OR 0.5, 95%CI 0.32-0.77, p=0.002; ISS OR 0.49, 95%CI 0.29-0.84, p=0.01). A unit increase in eGDR or ISS was associated with a 46-56% decrease in odds of maculopathy (eGDR OR 0.54, 95%CI 0.37-0.81, p=0.003; ISS OR 0.44, 95%CI 0.22-0.88, p=0.02).

Conclusions IR correlates with more severe retinopathy in young adults with Type 1 DM. This is the first description of a correlation between IR and maculopathy in Type 1 DM, warranting further evaluation. Prospective studies examining whether reducing IR can improve microvascular complications are required.

Keywords: Type 1 Diabetes Mellitus; Insulin Resistance; Diabetic Retinopathy; Diabetic Maculopathy.

1. Introduction

Type 1 diabetes mellitus (Type 1 DM) is an autoimmune disease manifesting as hyperglycaemia, due to immune-mediated destruction of insulin-producing pancreatic beta-cells. Increasingly, a subset of patients develop insulin resistance (IR), or 'double diabetes', combining the pathophysiology of insulin deficiency in Type 1 DM and IR, more commonly associated with Type 2 DM [1].

IR in Type 1 DM is common, affecting 26% of children and adolescents with Type 1 DM [2]. Further, obesity in adolescents and adults with Type 1 DM has rapidly increased. A longitudinal study of 589 adults with Type 1 DM in Pittsburg found the age-specific prevalence of overweight and obesity in 40-49 year olds was 25% in 1986-88, and 68.2% in 2004-07, with a 10-fold increase in the proportion of obese subjects [3]. Intensification of insulin therapy is known to correlate with weight gain and central adiposity [4], with participants in the intensive arm of the Diabetes Control and Complications Trial (DCCT) gaining, on average, 4.6kg more than patients who received conventional therapy over 5 years [5]. Societal trends towards sedentary behaviors and calorie-dense nutrition are paralleled in patients with Type 1 DM.

HOMA-IR, frequently used in assessment of IR [6], is not well validated for use in subjects treated with insulin, precluding its use in patients with Type 1 DM [7]. The gold standard for assessing IR in Type 1 DM is rate of glucose disposal at euglycaemic-hyperinsulinaemic clamp. Clamp studies in young adults with Type 1 DM have associated IR with increased carotid media intimal thickness [8], and with dyslipidaemia across multiple age-groups [9]. However due to resource utilization and patient tolerability, clamp studies are impractical for large population studies. Regression modelling based on clamp data performed by two groups has independently derived similar formulae for estimating glucose disposal in Type 1 DM: the estimated glucose disposal rate (eGDR) [10], and the Insulin Sensitivity Score (ISS) [11].

IR, as measured by eGDR, has been associated with development of microvascular and macrovascular disease in the Pittsburg EDC Study [12-14], microvascular disease in a reanalysis of

DCCT data [15], and with microvascular disease in cross sectional studies [16-18]. IR, measured by ISS is correlated with risk factors for cardiovascular disease [19].

However, few studies have evaluated the relationship specifically between IR and retinal complications in Type 1 DM [20, 21]. Retinopathy (DR) is an important cause of morbidity in Type 1 DM, and strongly correlates with glycaemic control [5]. Further, the relationship between IR and diabetic maculopathy (DMc), referring to retinopathy affecting the macula, has not been examined in Type 1 DM. DMc may occur at any stage of DR and is the leading cause of visual loss in patients with diabetes [22].

This study aimed to explore the relationship between validated surrogate measures of IR (eGDR and ISS) and the presence of DR and DMc in a cohort of adolescents and young adults with Type 1 DM.

2. Subjects

Patients attending a regional tertiary hospital young adult outpatients clinic for routine care of Type 1 DM were recruited. The clinic provides multidisciplinary care for a mixed urban and rural catchment of predominantly Caucasian ethnicity. The Human Research Ethics Committee approved a waiver of consent for retrospective review of an historical cohort. All prospectively enrolled participants provided written informed consent. From August 2015 – March 2016, clinic attendees were invited to participate in a prospective cross-sectional study. Additionally, a medical records database was searched to identify all clinic attendees in the 2014 calendar year aged 18-30 years. The clinical records gave us baseline population characteristics, which were compared to the participants in the cross-sectional study to identify potential selection bias.

3. Materials and Methods

Consenting participants had demographic and disease specific information recorded by a study nurse. Waist circumference was measured according to WHO criteria as a measurement taken parallel to the floor at the midpoint between the top of the iliac crest and lower margin of the last

palpable rib in the mid-axillary line [23]. Colour fundus (retinal) photography was chosen as the outcome measure for microvascular disease as a simple, reproducible and objective measure of early stage retinal disease. Clinic-based non-dilated fundus photographs were obtained by a trained retinal photographer and qualified orthoptist using a Topcon retinal camera *(TRC NW8; Topcon Corporation, Tokyo, Japan*). Two single field 45° images centred on the macula and optic disc were taken in each eye, with additional images taken in the presence of significant pathology.

Retinal photographs were graded by two independent and experienced ophthalmology fellows who were blinded to patient characteristics. The eye with the worst grade of disease determined the score. Discordant grading was adjudicated by consensus. Images were classified in two ways: (i) degree of DR and (ii) presence or absence of DMc. The level of DR was graded as no DR; mild, moderate or severe non-proliferative DR (NPDR); and proliferative DR, based on the International Classification of DR (ICDR) Disease Severity Scale [24] (Supplementary Table 1). Mild or minimal DR was defined as microaneurysms only; moderate DR as microaneurysms with additional signs of background retinopathy (i.e. intraretinal haemorrhages or exudates) but to a lesser extent than severe DR; severe DR as any intraretinal vascular abnormalities (IRMA), venous beading and/or extensive intraretinal haemorrhages assessed as more than 20 in each quadrant in the absence of proliferative DR; and proliferative DR as any evidence of new vessel growth.

DMc was graded as absent or present based on the presence of hard exudates within one disc diameter (1500 microns) of the macula in images with DR, a surrogate measure that has been validated in several studies [25].

To estimate IR, two independently derived models of glucose disposal were used. Estimated glucose disposal rate (eGDR, $mg.kg^{-1}.min^{-1}$), was calculated using the formula validated by Williams in an adult, multi-ethnic population of patients with Type 1 DM [10, 18]: eGDR = 21.158 + (-0.09*WC) + (-3.407*HTN) + (-0.551*HbA1c), where WC is waist circumference measured in centimeters; HTN is hypertensive status, defined as systolic blood pressure >140mmHg or diastolic blood pressure

>90mmHg or treatment with antihypertensive pharmacotherapy; and HbA1c is percent glycosylation of haemoglobin (to convert % to mmol/mol, multiply by 10.93 and subtract 23.5). Insulin Sensitivity Score (ISS) was calculated using Dabela's equation, validated in an adolescent, majority non-Hispanic white population of Type 1 DM [2, 11]: ISS = exp[4.64725 – (0.02032 *WC) – (0.09779*HbA1c) – (0.20815*TG), where TG is triglycerides measured in mmol/L (to convert to mg/dL, divide by 88.57396); other parameters are as for eGDR. Lower scores for both parameters reflect increased IR.

3.1 Statistical Analysis

To establish that the prospective cohort was not substantively different from the broader group of clinic patients, we compared demographic and clinical characteristics to the historical cohort.

For the outcomes of interest, namely presence of retinopathy ('nil'/'present'), severity of retinopathy ('nil or mild'/'moderate or worse') and diabetic maculopathy ('nil'/'present'), we provide group level means for eGDR, ISS and other biomarkers of retinal pathology. We compared group means with Student's t tests and a Bonferroni correction for multiple comparisons.

We examined the relationship between eGDR and ISS using a generalized linear model (Gaussian family, log link).

To explore the association between DR, DMc, and biomarkers of retinal pathology we fitted separate multivariate logistic regression models for eGDR and ISS with additional covariates for duration of diabetes, age, smoking and gender. Analyses were based on complete cases.

We assessed the discriminative value of eGDR and ISS for DR and DMc against duration of disease, HbA1c, BMI and hypertension with receiver operating characteristic (ROC) curves. The analyses were performed using Stata (version 14.1, Statacorp, Texas USA).

4. Results

From August 2015 to March 2016, 157 clinic attendees were prospectively enrolled in the cross-sectional study (58% of total clinic attendees over that period), of whom 107 completed the study visit. Demographic characteristics for the prospective cohort and historical retrospective cohort are presented in Table 1. We found no significant differences (at the 0.05 level) between the 107 participants used in this study and the historical cohort of 163 clinic attendees in 2014 (duplicate participants were excluded) with respect to important potential confounders of gender (p=0.87), weight (p=0.48), BMI (p=0.36), HbA1c (p=0.07) and duration of disease (p=0.37). Differences were observed in age, blood pressure and triglycerides. While we could compute eGDR for all 107 participants, we were able to compute ISS for only 97 participants due to missing data.

Table 2 show the means and standard deviations of established biomarkers by group membership for presence of DR (nil/present), severity of DR (nil or mild/moderate or worse) and DMc (nil/present). Significant differences (at the family-wise 0.05 level = 0.05/18) in the eGDR means were found for the severity of DR (8.0 vs 5.6, p<0.001) and DMc (7.9 vs 4.7, p<0.001) comparisons. We found analogous differences in the ISS means for the severity of DR (6.2 vs 4.2, p=0.001) and DMc (6.1 vs 3.8, p=0.003) comparisons. Differences were also found in the means for HbA1c (8.1% (65mmol/mol) vs 9.2% (77mmol/mol), p=0.003) and duration of disease (15.8 vs 9.9 years, p=0.002) for severity of DR and waist circumference (109.9cm vs 91.9cm, p=0.001) and duration of disease (17.4 vs 10.2 years, p=0.005) for DMc comparisons.

Fig. 1 shows the relationship between eGDR and ISS based on the participants in this study. While eGDR and ISS are, by definition, closely related through a log transform, a generalized linear model (Gaussian family, log link) regressing ISS on eGDR showed a strong association between the two measures. The exponentiated parameter estimate for eGDR was 1.197 (p<0.001) suggesting that a one unit increase in eGDR was associated with a 1.2 unit increase in ISS (95%CI 1.17-1.22). Adjusting the model for hypertension status was supported by a likelihood ratio test ($p < 0.001$) and changed

the estimate for eGDR such that a unit increase in eGDR was associated with a 1.25 unit increase in ISS (95%CI 1.23-1.27), holding hypertension status constant.

Table 3 provides the odds-ratios, 95% confidence intervals and p-values from multivariate logistic regression models examining the association between the retinopathy and maculopathy outcomes and the eGDR and ISS biomarkers adjusted for potential confounders of duration of disease, age, gender and smoking status. For retinopathy severity, a unit increase in eGDR (representing lower IR) was associated with a 50% decrease in the odds of moderate NPDR or worse (OR 0.5, 95%CI 0.32-0.77, p=0.002). Similarly a unit increase in ISS was associated with a 51% decrease in the odds of moderate NPDR or worse (OR 0.49, 95%CI 0.29-0.84, p=0.01). For the maculopathy outcome, a unit increase in eGDR was associated with a 46% decrease in the odds of moderate NPDR or worse (OR 0.54, 95%CI 0.37-0.81, p=0.003). Similarly a unit increase in ISS was associated with a 56% decrease in the odds of moderate NPDR or worse (OR 0.44, 95% CI 0.22-0.88, p=0.02). These results were consistent with the comparison of means in Table 2.

The Area Under the ROC curve (AUROC) for eGDR (0.81, 95% CI 0.70-0.92) and ISS (0.78, 95% CI 0.66–0.91) were not significantly different (at the 0.05 level) when compared to the AUROC for duration of disease (0.78, 0.64 – 0.88, p=0.64). IR also was similar in discrimination of DMc (AUROC 0.84 (0.68 – 1) and 0.83 (0.70-0.95) for eGDR and ISS respectively, compared to 0.80 (0.64 – 0.95) for duration of disease (p = 0.45) (Table 4, Fig. 2).

5. Discussion

This study identifies a strong association between severity of DR and IR, based on two previously established surrogate markers of IR in a cohort of young adults with Type 1 DM, controlled for potential confounders. This finding is in agreement with other studies, although most studies have defined retinopathy presence/absence as a categorical variable [15-18]. While this study confirmed

this trend (borderline significant at the 0.05 level), we noted a stronger relationship between severity of DR and IR in adolescents and young adults, confirming findings from two cross-sectional studies of older adults (mean age 45-46 years) in Romania [20, 21]. The reproducibility of these findings across ages and ethnicities supports the generalizability of this association.

A new finding from our study is the association between DMc and IR in Type 1 DM. The presence of DMc was highly associated with IR using both the eGDR and ISS scores, with a 1 unit decrease in IR reducing the odds of maculopathy by 45% and 57% respectively (Table 3). Macular edema in diabetes has a complex pathophysiology, with disruption of the blood-retinal barrier resulting in oxidative stress, inflammation and vascular dysfunction as the final common pathway [26]. Hypertension and hyperlipidaemia, key features of metabolic syndrome, represent established risk factors for DMc [22], and it is possible that IR contributes to the underlying pathophysiology of this condition. IR has been associated with maculopathy in Type 2 DM [27], and DMc may be more common in Type 2 DM than Type 1 DM [28], supporting a possible causal link between IR and the development of DMc. To our knowledge, this is the first study demonstrating an association between IR and DMc in Type 1 DM.

This study highlights that IR was comparable, and perhaps slightly better, in its discriminative ability (measured by AUROC) for DR and DMc as traditional biomarkers such as duration of diabetes and HbA1c (Fig. 2). This has several implications. First, it suggests a potential role for eGDR or ISS as a biomarker to identify patients appropriate for more intensive macular screening by an ophthalmologist, which may allow for earlier detection and appropriate treatment to prevent visual loss. Second, the possibility of minimising progression of DR and DMc by reducing IR must be considered, and follow up studies addressing this issue prospectively through weight loss or exercise strategies are required.

One strength of this study is the use of two independently derived algorithms for IR. Not only did the study demonstrate consistency of association between DR, DMc and IR using both algorithms,

but it was also able to show a high degree of correlation between both measures (Fig. 1). Both models were created as a 'best fit' for glucose disposal measured under hyperinsulinaemic-euglycaemic clamp, and were not developed as predictive scores for complications [10, 11]. Hence, the observation in this study that both ISS and eGDR are highly correlated with each other is an important confirmation that these scores, which were developed and validated in different populations of Type 1 DM and incorporate different variables, may represent the same underlying biological variable. One previous study has examined this relationship in older adults with Type 1 DM in Brazil; the group did not stratify for hypertension and found a lower degree of correlation between the two scores [29].

Our study has some limitations. First, macular assessment was limited non-stereoscopic fundus photography without optical coherence tomography. As such, it is possible the number of patients with true DMc were underestimated. Reduced visual acuity is associated with DMc, and is a useful adjunct to retinal examination to help prioritise ophthalmic referral [30], although was not assessed in this study. Second, some enrolled participants did not complete the study visit and were not included in analysis, potentially introducing bias. However, analysis of completers and non-completers showed no significant difference in baseline characteristics between these groups. Finally, given the cross-sectional nature of our design, our study is not able to infer a causal link between IR and development of retinal pathology, or to show that reducing IR can modify the progression of DR and DMc over time.

7. Conclusions

This study suggests for the first time that IR may be associated with maculopathy in Type 1 DM, and extends evidence for a correlation between IR and DR into young adults. The relationship between IR and maculopathy must be confirmed in larger prospective studies. Further studies are needed to determine if reducing IR can impact complications.

Acknowledgements: The authors would like to thank Mr Jeff Stormer (Research Nurse); Mr Chris Gialouris (Orthoptist); Dr Kiran Manku (Ophthalmologist and Medical Retinal Subspecialist) and the staff of the John Hunter Hospital Young Persons Clinic for invaluable assistance with this study.

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Table 1: Baseline characteristics of entire clinic cohort (historical) and prospectively recruited sample. P values represent the probability of a true difference in the measured parameter between the two populations.

Parameter	Historical Cohort (2014)	Prospective Cohort (2015/2016)	p
n	163	107	
Age (mean, SD)	23.5 (3.61)	24.7 (6.0)	0.04
Male:Female Ratio	0.53:0.47	0.54:0.46	0.87
Years since Diagnosis (mean, SD)	10.10 (5.9)	10.8 (6.8)	0.37
Subcutaneous insulin pump users (n, %)	38 (0.23)	33 (0.31)	0.14
Weight, kg (mean, SD)	77.3 (17.3)	78.9 (20.0)	0.48
BMI, kg/m^2 (mean, SD)	26.2 (5.04)	26.8 (5.5)	0.36
Current tobacco use (n, %)	16 (0.10)	11 (0.10)	0.95
HbA1c, %; mmol/mol (mean, SD)	8.6 (2.0); 70 (21)	8.2 (1.4); 66 (15)	0.07
Systolic BP, mmHg (mean, SD)	118 (14)	126 (15)	<0.01
Diastolic BP, mmHg (mean, SD)	73 (11)	79 (9)	<0.01
Cholesterol, mmol/L (mean, SD)	4.9 (1.1)	4.7 (1.1)	0.15
Triglycerides, mmol/L (mean, SD)	1.4 (1.0)	1.13 (0.70)	0.01
Albuminuria (n, %)	17 / 128 (0.13)	13/96 (0.14)	0.82
Retinopathy (n, %)			
Nil	97 (0.60)	65 (0.61)	0.87
NPDR, mild	30 (0.18)	16 (0.15)	
NPDR, moderate	20 (0.12)	9 (0.08)	
NPDR, severe	3 (0.02)	2 (0.02)	
Proliferative DR	2 (0.01)	4 (0.04)	
Missing data	11 (0.07)	11 (0.1)	
Other retinal pathology (n, %)			
Diabetic maculopathy	9/152 (0.06)	8/96 (0.08)	0.54
Prior Photocoagulation	1/152 (0.01)	3/96 (0.03)	0.24

NPDR: Non-proliferative diabetic retinopathy. DR: Diabetic Retinopathy

Table 2: Group means and comparison of means for established biomarkers for retinopathy and maculopathy. Lower scores of eGDR and ISS represent increased insulin resistance.

Parameter	eGDR	p	ISS	p	BMI kg/m2	p	HbA1c %	p	Waist circ. (cm)	p	Duration (years)	p
Presence of Retinopathy		0.07		0.09		0.2		0.03		0.41		0.0001
Nil	7.9 (2.2)		6.2 (2.1)		26.6 (5.2)		8.0 (1.4)		92.5 (15.4)		9.0 (7.0)	
Present	7.0 (2.5)		5.4 (2.2)		28.3 (6.4)		8.7 (1.3)		95.3 (15.9)		14.7 (4.9)	
Severity of Retinopathy		0.0002		0.001		0.21		0.003		0.07		0.002
Nil or Mild NPDR	8.0 (2.2)		6.2 (2.1)		26.7 (5.2)		8.1 (1.4)		92.0 (15.0)		9.9 (6.9)	
Moderate NPDR or worse	5.6 (2.3)		4.2 (1.5)		29.4 (7.6)		9.2 (1.1)		100.9 (16.7)		15.8 (4.6)	
Diabetic Maculopathy		0.0001		0.003		0.11		0.22		0.001		0.005
Nil	7.9 (2.2)		6.1 (2.1)		26.6 (5.1)		8.2 (1.4)		91.9 (6.2)		10.2 (6.7)	
Present	4.7 (2.5)		3.8 (1.3)		32.5 (9.0)		8.9 (0.9)		109.9 (1.5)		17.4 (5.2)	

Data is mean (SD). NPDR: Non-proliferative diabetic retinopathy. PDR: Proliferative diabetic retinopathy. eGDR (estimated glucose disposal rate; mg/kg/min). ISS: Insulin sensitivity score. DM: Diabetes Mellitus. Duration: duration of diabetes. To convert HbA1c to mmol/mol multiply by 10.93 and subtract 23.5.

Table 3: Crude and adjusted odds ratio estimates from logistic regression models examining the association between retinal pathology and insulin sensitivity. Higher values of eGDR and ISS reflect increased insulin sensitivity

Outcome	Model	Odds Ratio and 95% CI		p-value
		Crude	Adjusted	Adjusted OR)
Any Retinopathy	eGDR	0.84 (0.71-1.02)	0.80 (0.64-1.0)	0.05
	ISS	0.83 (0.66-1.03)	0.84 (0.64-1.10)	0.2
Moderate NPDR or worse	eGDR	0.67 (0.54-0.85)	0.50 (0.32-0.77)	0.002
	ISS	0.54 (0.37-0.81)	0.49 (0.29-0.84)	0.01
Diabetic Maculopathy	eGDR	0.61 (0.45 – 0.82)	0.54 (0.37 - 0.81)	0.003
	ISS	0.46 (0.26 – 0.81)	0.44 (0.22 - 0.88)	0.02

Crude model: IR parameter (eGDR or ISS) only.
Adjusted model accounts for effects of age, gender, duration of disease and smoking status.

Table 4: AUROC for diagnostic performance of biomarkers for discrimination of retinal pathology.

	Any Retinopathy		Moderate NPDR or worse		Diabetic Maculopathy	
Biomarker	AUROC	95% CI	AUROC	95% CI	AUROC	95% CI
eGDR	0.65	0.52 - 0.77	0.81	0.70 - 0.92	0.84	0.68 - 1.0
ISS	0.62	0.49 - 0.75	0.78	0.66 - 0.91	0.83	0.70 - 0.95
Duration of disease (years)	0.75	0.64 - 0.85	0.78	0.64 - 0.88	0.8	0.64 - 0.95
HbA1c	0.68	0.57 - 0.63	0.74	0.61 - 0.86	0.67	0.50 - 0.83
Waist circumference	0.58	0.45 - 0.71	0.7	0.56 - 0.83	0.79	0.61 - 0.96
BMI	0.58	0.44 - 0.71	0.6	0.41 - 0.78	0.68	0.41 - 0.95

Figure Captions:

Figure 1: Relationship between eGDR and ISS, stratified by the presence (triangles) or absence (circles) of diagnosed hypertension (HTN). Lines show predicted values from generalized linear model.

Figure 2: ROC Curves for discriminative value of eGDR and ISS (Panel A) and conventional risk factors (Panel B) in prediction of Diabetic Maculopathy. Legend shows AUROC with 95% CI.