ORIGINAL ARTICLE

Retinal multispectral imaging of 'sub-clinical' capillary micro-aneurysms in non-diabetics correlates with insulin resistance

Kerry M. Gelb¹, Stuart P. Richer², Cheryl N. Zimmer^{3*}, Jerome Sherman⁴, Jeffrey M. Gold⁵

ABSTRACT

Insulin resistance (IR), short of diabetes mellitus, negatively impacts retinal vessel health. The purpose of this study was to evaluate the correlation between the number of sub-clinical retinal micro-aneurysms (MA#) identifiable by highly sensitive 580 nm multi-spectral retinal imaging (MSI 580 nm) and serological and calculated IR measures. Thirty (n=19 M; n=11 F) non-diabetic optometrists (n=54 eyes), 53.5 ± 7.6 years of age, were imaged at a professional conference using multispectral imaging (MSI) of the retina (RHA™, Annidis Corporation, Ottawa, Canada). A six parameter blood panel requisition: fasting glucose (FBS), 2 hr glucose (GTT) tolerance, HbA1c, fasting insulin, 2 hr insulin and 25 OH vitamin D liver reserve status were provided to each participant. MSI retinal images were reviewed and the MA# in the central 30 degrees were counted. The calculated clinical parameters used to diagnose IR were most highly correlated with retinal MA#, specifically insulin sensitivity. Subclinical MA#, less visible to non-spectral cameras but observed with multispectral imaging, correlate with insulin, pancreatic function and calculated measures of IR, more closely than FBS and vitamin D status. Future diabetes intervention research should focus upon MSI MA# and IR as actionable pre-diabetes and pre-retinopathy risk factors.

Keywords: *Insulin resistance, retinopathy, retinal micro-aneurysms, retinal imaging.*

Introduction

Insulin resistance (IR) is the inability of insulin to exert its metabolic functions on cells. It is considered the seminal initiating pathology of type 2 diabetes mellitus (DM) and metabolic syndrome.¹ In fact, IR underlies most modern chronic diseases including cardiovascular disease and cancer.² The earliest clinical IR serum markers are elevated fasting insulin and 2 hour insulin, following intake of a bolus of sugar. An even more accurate predictor for pre-DM is the homeostatic model assessment (HOMA) that accounts for fasting blood glucose (FBS) and fasting insulin.³ Yet, conventional medical treatment typically focuses on lowering blood sugar, and elevated insulin is considered irrelevant, even in patients with traditional risk factors of increased waist circumference, high BMI, family history, etc. Regrettably, the majority of practitioners wait for dysglycemia or its glycemic complications, ignoring the damage wrought by IR. (Figure 1.)

Hyperinsulinemia and IR directly and indirectly contribute to a vast array of metabolic diseases including most inflammatory conditions,

vascular diseases, gestational and type 2 DM, non-alcoholic fatty liver disease, obesity and certain cancers and dementias. Mechanistically, hyperinsulinemia effects the body by at least 6 processes including increased production of 1) growth factors i.e. IGF-1 / VEGF; 2) reactive oxygen species (ROS); 3) advanced glycation end products (AGEs); 4) triglycerides (TGs) /fatty acids; 5) plasminogen activator inhibitor-1 (PAL-1) i.e. fibrinolysis / thrombosis and 6) hormonal & cytokine mechanisms including hyper-leptinemia, adiponectin and estrogen. Some 60% of pancreatic beta cells are non-functional at diagnosis of type 2 DM⁴ where the mean latency for disease onset lags by 6 to 24 years depending on the study.^{4,5,6,7} As IR progresses, it is also aggravated by medications (i.e. corticosteroids, sulphonylureas, statins and certain anti-psychotics) that cause pancreatic beta cell attrition and/or promote hyperinsulinemia.^{8,9} This increases visceral fat deposition, thereby worsening IR.

Optometrists and ophthalmologists viewing the ocular fundus year over year are uniquely positioned to directly observe the systemic circulation, including capillary abnormalities reflecting observable

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²Eye Clinic, Captain James A Lovell FHCC, 3001 Green Bay Rd, North Chicago, IL, USA 60064. ³Annidis Corporation, 100 Maple Grove, Ottawa, ON, Canada K2V 1B8. ⁴SUNY College of Optometry 33 West 42nd Street New York, NY, USA 10036. ⁵Suburban Heart Group PA, 1000 Galloping Hill Rd Ste 107, Union, NJ, USA 07083. Copyright: © 2016 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License.

Figure 1. Multiple small retinal MAs in a 51 year old female. Her serology parameters are as follows: HbA1c 5.7 (39), FBS 99, 25 OH Vitamin D 32, fasting insulin 6.9, 2 hour insulin 95.7 and 2 hour GTT 188. 1A. Retinal vessel MAs circled within the central 30 degrees on the MSI-580 image using an Early Treatment Diabetic Retinopathy study (ETDRS) 7 standard field overlay. 1B. Magnified view of MSI-580 showing multiple small retinal MAs. The larger MAs, marked with asterisks, were determined to be 15-20 μm in size based on a Gaussian peak point spread function. 1C. Corresponding ultra-widefield SLO image. Most MAs are not visible.

and recordable IR within the retina of the eye. In DM, IR manifests as both capillary basement membrane thickening and the formation of micro-aneurysms (MAs) - weakened balloon-like capillary vessel wall outpouchings.¹⁰ The Hoorn Study, showed retinopathy prevalence positively associated with markers of inflammation and endothelial dysfunction.¹¹ Retinal optical coherence tomography (OCT) angiography, illustrates the full presence of MAs, in patients with DM, appearing as dilated saccular or discontinuous capillaries.¹² Early signs of DM-related ocular damage were recently detected on a microscopic level using retinal adaptive optics instrument. MAs differed in degree of dilation and appearance in early to moderate non-proliferative diabetic retinopathy, where corkscrew-shaped retinal capillaries, capillary remodeling and other capillary abnormalities were observed in patients with very early IR.¹³

Small blood vessel outpouchings and capillary dilations have also been observed clinically with non-invasive 580 nm wavelength multi-spectral imaging (MSI) (RHA™, Annidis Corporation, Ottawa, Canada).¹⁴ (Figure 1B.) The purpose of this pilot study was to investigate and compare micro-aneurysm number (MA#) against FBS, calculated IR and 25 OH vitamin D status. Vitamin D deficiency has recently been identified as an associated risk factor in early development of DM 1, DM 2 as well as more advanced stages of diabetic retinopathy.¹⁵

Materials and Methods

Study Subjects

Thirty (30) optometrists attending a Florida professional conference over a period of two consecutive days in October of 2014, volunteered to

be imaged and submit to a blood draw with a subsequent blood draw 2 hours later. Each participant provided "Informed Consent" regarding the nature and possible consequences of the research, and signed a HIPAA privacy authorization form. A commercially available, FDA approved, multispectral ophthalmic imaging system (RHA™, Annidis Corporation, Ottawa, Canada) was used to capture the images, as previously described.¹⁶

Calculated measures of IR were also determined. Homeostasis Model Assessment (1985) (HOMA1) estimates steady state beta cell function (%B) and insulin sensitivity (%S) as a percentage of the normal reference population using FBS and insulin.²⁰ It is equivalent to $\text{fasting glucose (mg/dL)} \times \text{fasting insulin (uIU/mL)} / 405$. Homeostasis Model Assessment (1998) (HOMA2) accounts for variations in hepatic and peripheral glucose resistance, increases in the insulin secretion curve for plasma glucose concentrations above 10 mmol/L (180 mg/dL) and the contribution of circulating proinsulin.^{21,22} Insulin resistance is the reciprocal of %S, equal to $100 / \%S$. The Quantitative Insulin Sensitivity Check Index (QUICKI) was also calculated.

Grading and Counting of MAs

The retinal MAs found on the MSI-580 nm images of each eye of the participants were analyzed in two ways. A masked observer (CZ) who was familiar with the RHA™, but had no background knowledge of any of the participants or the results of their blood work, graded the MAs as shown below. The MAs were distinguished from noise by their contrast, size and proximity to blood vessels:

Grade 0 - No obvious capillary dilations in either eye.

Grade 1 - Dilations in one or both eyes, but not wide spread; includes one profound area of dilations or multiple small areas.

Grade 2 - Obvious wide spread dilations in both eyes; large areas containing dilations and/or multiple moderate dilations.

Grade 3 - Multiple, obvious widespread dilations in both eyes; too many to count or circle

The MAs were also reviewed by a second masked observer (KG) using the Early Treatment Diabetic Retinopathy Study (ETDRS) 7 standard field overlay, to count the number of small blood vessel outpouchings seen in the central 30 degrees. (Figure 1A) The observer was familiar with MSI, but had no background knowledge of any of the participants or the results of their blood work. The MAs were distinguished from noise by their contrast, size and proximity to blood vessels.

Statistical analysis

Correlation coefficient analysis (Microsoft Excel) was used to determine the relationship between grade, MA# and the blood test results. A p value less than 0.05 was considered statistically significant, based on regression analysis. The correlation between the grade and MA# was 0.40 with a p value of 0.003. The minimum MA# found in this study was 24 and the maximum was 112 within the central 30 degrees area. To test for repeatability, two other masked observers counted a sampling of four images. The counting system was considered repeatable with a correlation of 0.69 with a standard deviation of ± 18 MAs.

Results

The mean age ($\pm$ SD) was 53.5 ± 7.6 years with the majority of participating optometrists ($n=19$) of male gender. Four participants were hypertensive, three hypercholesteraemic and one was under treatment for both conditions. Participants were mildly overweight with a mean BMI of 26.0 ± 4.4 kg/m².¹⁷ Thirty six (36) subjects completed the study, both imaging and blood work within the allotted 3-month post imaging timeframe. Three optometrists were excluded due to previously diagnosed and treated type 2 DM.

Two more participants were excluded due to the discovery of untreated DM, based upon serum positive blood sugar results. The remaining eye care professionals were relatively healthy by conventional measures. Six images, including the right and left eyes of one participant were- excluded due to poor image

quality as a result of cataracts, keratoconus or small pupil size. This resulted in the final grading of 54 eyes from 30 subjects completing both imaging and blood work within the allotted 3-month post imaging timeframe.

Table 1. BMI and Laboratory Blood Tests

BMI and Laboratory Blood Tests	Mean (SD)	Normal ¹⁸
Body Mass Index (BMI)	26.0 (4.5) n=16	18.5-24.9
HbA1c (%; mmol/mol)	5.61 (0.3), 38 (3.3) n=30	4.8-5.6
Fasting blood glucose (mg/dL)	93.0 (9.2) n=30	65-99
2 hour glucose tolerance test (mg/dL)	100.0 (37.1) n=27	65-139
Fasting insulin (uIU/mL)	8.2 (4.5) n=30	2.6-24.9
2 hour insulin (uIU/mL)	67.7 (49.2) n=20	0.0-145.4
25 hydroxy vitamin D (ng/mL)	40.7 (21.7) n=29	30.0-100.0

Table 2 tabulates the linear correlation coefficients and statistical significance for the blood tests and computed values relative to MA#. Interestingly, our data failed to correlate with the traditional physical exam clinical measures of diabetes risk: BMI and HbA1c. Additionally, both FBS and Vitamin D status were each weakly correlated with MA# ($r=0.32$; $p=0.02$), and less correlated than measures of IR.

Table 2. Measured and Calculated Indicators of Insulin Resistance

Indicators of Insulin Resistance (n=30)	Correlation Coefficient (r)	Statistical Significance (p<0.05)
Insulin Sensitivity (%S)	- 0.51	0.00008
QUICK-I	- 0.47	0.0004
HOMA1	0.40	0.002
HOMA2	0.41	0.002
Insulin Resistance (100 / %S)	0.41	0.002
Fasting Insulin	0.40	0.003
FBS	0.32	0.02
25 OH vitamin D	- 0.32	0.02
Beta cell function (%B)	0.32	0.02
2 hr Insulin	0.29	0.08 (not significant)
2 hr GTT	-0.12	0.39 (not significant)
HbA1c	-0.03	0.84 (not significant)

Insulin sensitivity (%S) as a percentage of the normal reference population, a derived value, had the highest correlation coefficient of -0.51 ($p < 0.0001$) relative to MA#. Figure 2. shows %S plotted relative to the MA#. IR is the reciprocal of insulin sensitivity ($100 / \%S$) where normal sensitivity is considered 100%.¹⁹ IR showed a strong positive correlation with MA# of +0.41 ($p = 0.002$). Note that sensitivity and resistance are the inverse of one another, hence the negative correlation coefficient for %S.

The linear homeostasis model assessment for insulin resistance (HOMA1), a mathematically derived

model for IR, had a statistically significant correlation with MA# ($r = + 0.40$, $p = 0.002$). HOMA2 is determined to be more accurate than HOMA1 because it accounts for hepatic and peripheral glucose resistance, for plasma glucose concentrations above 180 mg/dL and the contribution of circulating proinsulin.^{20,21,22} The correlation between HOMA2 and MA# was also statistically significant ($r = +0.41$, $p = 0.002$). The QUICKI score, a variation on HOMA1 utilizing the logarithm and reciprocal of glucose and insulin had an inverse correlation coefficient of -0.47 ($p < 0.001$).²³

Figure 2. Insulin sensitivity as a percentage of the normal reference population (%S) vs. Micro-aneurysm number (MA#)*. Insulin sensitivity (%S) had the highest correlation coefficient of -0.51 ($p = 0.00008$) relative to the micro-aneurysm grading results for the group. *Considered to be repeatable with a correlation of 0.69 ± 18 MAs.

Discussion and Conclusions

Tirsi et al. found elevated insulin and IR, short of DM, negatively impacted retinal vessel health.²⁴ In this pilot study, MAs highlighted with non-invasive MSI-580 imaging, failed to correlate with fasting glucose or BMI but did correlate with IR. This is also congruent with the transitory nature of fasting glucose and the emerging importance of percentage body fat and adiposity as compared to simple BMI. Retinal microvascular abnormalities are associated with inflammation and oxidative stress, resulting in endothelial dysfunction.²⁵ MAs are not generally found in normal healthy eyes.²⁶ The presence of MAs in

participants with IR could result from other multiple factors including undiagnosed hypertension, anemia, atherosclerosis, carotid occlusive disease or obstructive sleep apnea.^{27,28} It has recently been speculated that endothelial insulin receptors are located in the small vessels of biopsied human atherosclerotic plaques. Such vessels are susceptible to angiogenesis.²⁹

Larger MAs, marked with asterisks in Figure 1b. were determined to be 15-20 μm in size based on a Gaussian peak point-spread function. MAs typically range in size from 12-100 μm in diameter and those less than 30 μm are not usually visible clinically.³⁰ Previous in vivo MA counting studies have shown an

association between counts and progression towards advanced diabetes.³¹ Histologically, MAs in DM occur within the inner retinal capillary plexus with counts varying from 0 to 26 per 0.41 mm².²⁶ It is possible that the graders in this pilot study were aggressive and considered the occasional artifact as an MA, as no participants scored zero. An alternative explanation is the older age of the subjects, coupled with the recent finding that 50% of the American population is either pre-diabetic or diabetic, resulted in at least a minimal number of MAs in everyone in this cohort.³²

A follow up study that investigates MAs by size and the presence of sharp margins rather than number is required to confirm whether all of the suspected MAs are definitively MAs. It's anticipated that MSI-580 images from a younger, healthier population should reveal significantly fewer, if any MAs. Additionally, an image comparative study of MSI-580 to fundus photography and OCT angiography may help further establish and illustrate the clinical utility of MSI-580 images with respect to early resolution of MAs.

Based upon the relationship found in this pilot study between IR and MA#, MSI 580 nm retinal imaging has the potential to identify subclinical retinal vasculopathy associated with pre-diabetes. This would mitigate the time, expenditure and this would mitigate the time, expenditure and multi-hour serum insulin testing and multi-hour insulin testing needed to diagnose IR, because ostensibly, only selectively suspect patients would be further evaluated. This study is also important because few people with IR are actually aware that they suffer from this pre-diabetic condition. Basic preventative lifestyle changes such as avoidance of simple carbohydrates and intermittent fasting have been shown to prevent or postpone DM and associated life-threatening disease processes.³³

The MSI-580 is capable of providing clear static retinal vascular detail by showing oxygenated hemoglobin in the retinal vasculature and hence compromised retinal tissue health in this sample population.¹⁶ Our data suggests a positive association between insulin sensitivity and MA#. Multi-spectral retinal imaging of MAs is thus a potential non-invasive, inexpensive, periodically accessible, and instantaneous indicator of the pre-diabetic state of IR, for which no current technology has been widely adopted.

Primary periodic eye exams are the ideal venue for screening microvascular health, as the retinal vessels are a surrogate reflection of the systemic circulation. Some 120 million persons visit an optometrist each year compared to 45 million people who have a systemic health 'primary care' evaluation.³⁴

Presbyopic adults with pre-diabetes, needing reading eyeglasses are certainly an important public health sub-population of typical patients who present in their 40's and 50's. With early detection being the key to prevention, the discovery of MAs using MSI-580 imaging holds substantial clinical promise in preventing damage to the miniscule capillaries within other vital end organs. Future studies will validate the sensitivity and specificity of this rapid noninvasive test against traditional time consuming and expensive IR determinations.

Micro-aneurysms, easily documented with non-invasive 580 nm wavelength multi-spectral retinal imaging correlate strongly with %S, a measurement of insulin sensitivity and the inverse of IR. Multispectral digital retinal imaging of MAs during periodic routine eye examinations, is a valuable asset to the health care system for evaluating IR, early DM and preventing associated systemic disease complications. Broad adoption of MSI 580 nm retinal imaging, combined with rapid objective computational image processing could lead to prevention, earlier treatment, lessened diabetic morbidity and premature mortality from accelerated cardiovascular disease and glycemic associated epithelial cancers.

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K.G. gathered and analyzed the study data as well as researched information to contribute to the discussion. K.G. is the guarantor. S.R. researched information to contribute to the discussion and authored/edited the manuscript. C.Z. designed the study, analyzed the data and authored/edited the manuscript. J.S. analyzed the study data. J.G. contributed to the discussion.

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Conflict of interest

Cheryl Zimmer is an employee of Annidis Corporation (Ottawa, Canada). The remaining authors have no conflicts of interest to disclose.

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ORIGINAL ARTICLE

Retinal multispectral imaging of 'sub-clinical' capillary micro-aneurysms in non-diabetics correlates with insulin resistance

Kerry M. Gelb¹, Stuart P. Richer², Cheryl N. Zimmer^{3*}, Jerome Sherman⁴, Jeffrey M. Gold⁵

Appendix 1

Using Dr. Joseph Kraft's threshold of insulin resistance based on a change from fasting to 2 hour insulin of greater than 40 uIU/mL, microaneurysm grading correlates moderately strongly to the blood test results and computed values for the subjects of this study. (35,36) Note that the results include only those study participants that completed a 2 hour insulin test as shown in Table A1. When plotted, Figure A1, three distinct groups emerge. Normals have an average grade of 0.92 out of 3. Those people with insulin resistance have an average grade of 1.47 and have an average grade of 2.25.

Table A1. Correlation of Blood Tests and Computed Values Relative to Grade

Laboratory Blood Test and Computed Values	Correlation Coefficient (p value) p value < 0.05 was considered statistically significant n=24 participants
Insulin sensitivity (%S)	-0.629 (0.001)
Insulin resistance (100 / %S)	0.623 (0.001)
HOMA2	0.622 (0.001)
Fasting insulin	0.618 (0.001)
HOMA1	0.613 (0.001)
2 hour insulin	0.583 (0.003)

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²Eye Clinic, Captain James A Lovell FHCC, 3001 Green Bay Rd, North Chicago, IL, USA 60064. ³Annidis Corporation, 100 Maple Grove, Ottawa, ON, Canada K2V 1B8. ⁴SUNY College of Optometry 33 West 42nd Street New York, NY, USA 10036. ⁵Suburban Heart Group PA, 1000 Galloping Hill Rd Ste 107, Union, NJ, USA 07083. Copyright: © 2016 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License.

Figure A1. Insulin Over Time Based on the Kraft Threshold for IR of > 40uIU/mL

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