AASCIT Journal of Health
2015; 2(5): 50-59
Published online September 20, 2015 (http://www.aascit.org/journal/health)

Keywords
Insulin Resistance,
Pre-Diabetes,
Diabetes,
Oral Glucose Tolerance Test,
OGTT,
Glucose Metabolic Index,
GMI,
Insulin for Type 2 Diabetes

Received: August 29, 2015
Revised: September 6, 2015
Accepted: September 7, 2015

Novel Use of Oral Glucose Tolerance Test Data in Pre-Diabetes and Diabetes

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Citation
Felician Stancioiu, T. McKenzie, M. Rodriguez, S. Ahmed. Novel Use of Oral Glucose Tolerance Test Data in Pre-Diabetes and Diabetes. *AASCIT Journal of Health*. Vol. 2, No. 5, 2015, pp. 50-59.

Abstract
Diabetes in its various forms is a global health problem and contributes directly and indirectly to a significant portion of healthcare expenses. 90% or more of diabetes cases are of the insulin-resistance variety and there is no known cure for the well-established type 2 diabetes except for cases of spontaneous remission after bariatric surgery. Pre-diabetic states (impaired glucose tolerance, IGT and impaired fasting glucose, IFG) are generally not treated although a significant proportion (about 1/3) progress to T2DM and they are associated with increased cardiovascular morbidity. Pre-diabetes can be reverted to normal much easier than diabetes. Our study proposes a novel way of assessing the pre-diabetic and diabetic states via oral glucose tolerance test – OGTT – and a new metric calculated from its 3 data points: the glucose metabolic index or GMI. Inferences about insulin secretion and sensitivity can be made accurately using GMI, which can be a very useful tool in guiding therapy in both new and established patients with diabetes and offers more prompt and ample information than the measurement of glycated hemoglobin.

1. Introduction

There is increased awareness about the rising prevalence of diabetes both in the developing and the developed nations. An estimate dating back to the turn of the millennia [1] has calculated that 100 million people will be afflicted worldwide by 2010; however shocking this was at the time it now seems a conservative number given that current estimations hover around 400 million people. While the number of type 1 diabetes (insulin-dependent) patients has not grown as much, this rise is mainly fueled by the very rapid increase in the number of patients with insulin – resistant or Type 2 Diabetes (T2DM); another observation being that in The United States the prevalence of T2DM is significantly higher in minority populations [2, 3]

Even though the pathophysiology of diabetes is not completely elucidated [4-6], it was shown that in many cases – 60% - diabetes is preceded by altered states of glucose metabolism that can be detected with simple tests [7]. Detection of the pre-diabetic state, categorized as either impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) - followed by early intervention were shown to have a positive impact in reducing the incidence of NIDDM (8-10). While screening for diabetes in general population is not recommended [6, 7], development of screening tools for populations deemed at high risk to develop diabetes becomes imperative [8-10] and a variety of methods and indices have been tested with various degrees of success [11-14]. Many of those indices developed have proved to be population-specific [6] and most of those indices are based on measurements of either a single sample of venous blood obtained after fasting or by administering an oral glucose tolerance test - OGTT.

Our study proposes a new way of interpreting the data from the 75-g OGTT, as an

inexpensive and readily available test which can be used both for screening for pre-diabetes in populations with high prevalence of diabetes and limited access to specialized laboratory testing (i.e. insulin levels), as well as a guiding tool for T2DM treatment in
such populations.

Also during this study we performed laboratory tests to determine blood levels of some inflammatory molecules (interleukins 1-beta, -4, -6,) and those linked to oxidative stress (oxidized low density lipoproteins, triglycerides, asymmetric dimethylarginine).

2. Materials and Methods

Participants in this study were enrolled while they were visiting the Outpatient Department of a community healthcare center in Northeast Arizona. Criteria of inclusion were adults over 18, non-pregnant, not currently on any oral hypoglycemiants or exogenous insulin. Exclusion criteria were random glucose levels below 100 mg/dL, current treatment with steroids, thiazide diuretics, phenytoin, estrogens, barbiturates, and lithium. While waiting for their primary complaints to be heard and addressed by a primary healthcare provider form the clinic, they were told about the study, what it entails for participation and the benefits. Potential participants were screened for random blood glucose levels via finger prick and measured with Accucheck and those with results above 100 mg/dL were asked to participate in the study. After a short verbal presentation, written information was given about the study – including the number of the contact person representing The Institutional Review Board.

Those who agreed to participate were scheduled for an appointment – usually within a week- at the clinic for a 2 hour Oral Glucose Tolerance Test after overnight fasting.

On the morning of the first study visit we measured patients' weight, height and waist circumference and collected venous blood samples by antecubital venipuncture after making sure that the patient was fasting. If the blood glucose level was less than 140 mg/dL the patient was given a standardized drink from Fisher Scientific that contained 75 g of anhidrous glucose with artificial citrus flavor and color. Patient's height, weight and waist circumference were measured and before completing the next two venipunctures – at 60 minutes and 120 minutes – the patient was given for completion a simple questionnaire on her/his eating habits. We have used 6 questions, both open-ended and with pre-determined choices in order to cover as many aspects of the diet as possible; there was some overlap in some of the questions to try to minimize bias from various backgrounds. The answer to each question was scored with 0, 1 or 2 points according to the implied use of saturated fats: 0 – never/none/very infrequent/very little; 1 – once or twice weekly/some; 2 – frequent/a lot.

After blood collection in the respective Vacutainer tubes – EDTA or no additive – the specimens were further prepared by centrifugation within 15 minutes of collection and the respective plasma or serum was frozen and kept in storage at -30 F until shipment to the laboratories for testing; shipment was done same-day or overnight on dry ice. Using a 7 digit-and-letter code identified the specimens and the correspondence table was kept confidential.

Also, each patient was provided with a 14 day supply of dietary supplements in the following daily dosages:

1. Essential amino acids (4 capsules/day of with 1 g of protein/capsule from soy and whey hydrolysate manufactured by Optimum Nutrition)
2. Polyunsaturated fatty acids softgels; 1 g total – SuperOmega Complex – by Spring Valley: Eicosapentanoic Acid 180 mg; Docosahexanoic Acid 120 mg; Gamma-Linolenic Acid 11 mg; Linoleic Acid 176 mg, Oleic Acid 43 mg, Alpha-Linolenic Acid 75 mg;
3. Antioxidant Formula (Vitamin A 10,000 IU, Vitamin C 250 mg, Vitamin E 200 IU, Zinc 7.5 mg, Selenium 15 mcg, Copper 1 mg, Manganese 1.5 mg) 1 softgel – Spring Valley
4. S- adenosylmethionine; 200 mg x 2 tablets – Spring Valley and Nature Made
5. L-Arginine, 500 mg, 1 capsule/day – Nature Made
6. L- Glutamine, 500 mg – Rexall, 1 tablet
7. Ester- C, 500 mg, 2 tablets (1000 mg) – Spring Valley.

2 pill organizers were given to each patient with the supplements mentioned above; for most patients those organizers were filled in their presence from the supplement containers and a brief explanation about their role was given and questions were answered. Emphasis was placed on not eating saturated fats for the 2 weeks between the clinic visits; to help enforce the use of unsaturated fats for cooking, each patient was also given 1 container with 2 Quarts of Olive Oil.

The second study visit for each patient was scheduled at 14 days from the first visit, and the patient was again instructed to come at the clinic after fasting overnight for the 2 hr OGTT. We followed the same protocol as in the initial visit; in addition a second and final questionnaire was given to each patient containing complaints on adverse reactions and the timing of their menstrual cycle in female participants.

Blood glucose levels were measured in the facility laboratory; the equipment used was tested for accuracy according to CLIA standards. Insulin levels were measured from plasma samples that were kept frozen at –30 degrees and tested at ARUP Laboratories in Salt Lake City, Utah.

We have tested for IL-1β, IL-4, IL-6 and Il-1A in the Research Department of TriCore Laboratories in Albuquerque, NM. Testing was performed with the respective EIA kits from Cayman Chemical (Ann Arbor, MI) and read at 405 nm with the Meridian Premier plate reader.

For oxLDL testing we have used first-generation EIA kits from Mercodia (Research Triangle Park, North Carolina) and most of the testing was performed at TriCore Labs Research Department. Additionally 30 specimens were tested at IBT Reference Labs using the same type of Mercodia kits; 5 of these specimens were tested in both labs and yielded similar

values; in the analysis we have used the average of those results.

Additionally we have sent some specimens to be tested for Homocysteine, asymmetric dimethylarginine (ADMA) which were done on frozen plasma and a Metabolic Syndrome Panel (insulin, glucose, HDL, triglycerides, eicosapentanoic acid, arachidonic acid) performed from serum that was stored frozen and shipped overnight on dry ice to Metametrix Laboratories in Norcross, Georgia.

The weight of each patient was recorded at the beginning of each of the 2 visits separated by two weeks. Waist circumference was measured while patient was seated on a chair or exam table with feet grounded and breathing normally; we recorded the biggest abdominal circumference in a horizontal plane in inches.

For each patient we calculated a Weight Distribution Index (WDI) by dividing the weight of the patient (lbs) by the waist circumference (in inches). Patients with more abdominal fat will have a lower WDI than patients with less abdominal bulk. We also calculated patients' Body Mass Index (BMI) as well as another index that includes patient's height which was calculated by multiplying WDI with patients' height measured in centimeters – we called this Constitutional Index – CI (cm*lbs/in). We surmise that both WDI and CI, because they include waist circumference in their calculation, are better representing central obesity and its risks than the widely used BMI.

Based on the results of the OGTT testing, we have grouped the patients in 5 categories:

1. *Normal* (N) – normal blood glucose levels and insulin levels on OGTT;
2. *Insulin Resistant* (IR) - normal glucose levels and elevated insulin levels;
3. *Insulin Resistant Type 2* (IR2) with normal glucose levels, elevated insulin levels and inverted shape of the glycemic curve: H2>H1;
4. *Impaired Glucose Tolerance* (IGT): those with blood glucose > 140 mg/dL at 2 hours and elevated insulin levels; and
5. *Diabetes* (D); patients with glucose levels >200mg/dL on two distinct measurements (at both H1 and H2 or in both testing days).

Based on the glucose values from the 2 hr OGTT we have calculated a glucose metabolic index - GMI – which is defined as the area under the curve plotted with the 3 glucose values from the OGTT: fasting (F), 1 hour (H1) and 2 hours (H2); as shown in Figure 1.

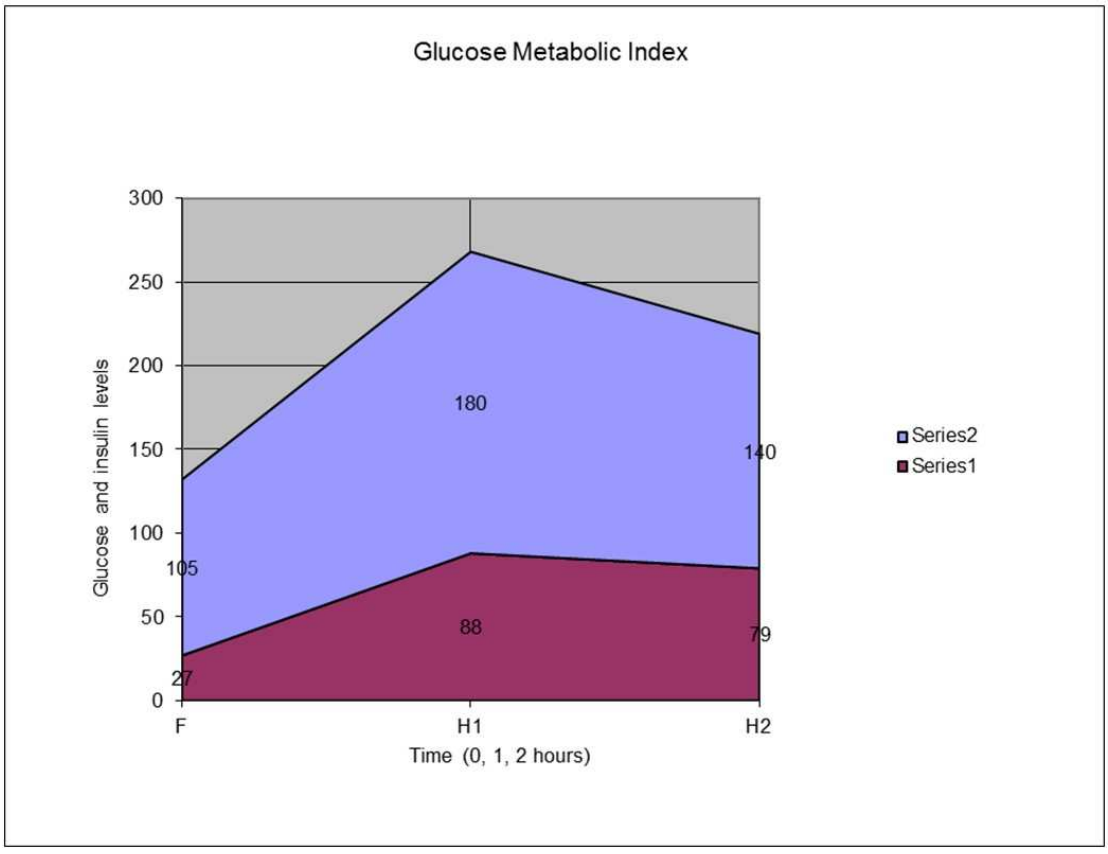

Figure 1. *Glucose Metabolic Index (GMI) and Insulin Metabolic Index (IMI).*

$$GMI = F + 2 \times H1 + H2$$

The data obtained from testing was then tabulated and analyzed with Microsoft Excel and NCSS. The study was approved by the corresponding Institutional Review Board and each patient has signed an approved Informed Consent Form after agreeing to participate in the study.

3. Results

155 patients were screened for random blood glucose levels; 56 met the enrolment criteria and agreed to participate in the study; of these 45 completed the first OGTT testing. 38 of these patients were given another OGTT 2 weeks after the initial OGTT. Finally, 4 patients were tested for the 3rd time after 2 more weeks (total of 4 weeks).

Regarding the initial screening, of 19 patients who had random blood glucose values in excess of 100 mg/dL, upon the OGTT testing, 10 had Diabetes or IGT, 5 had Insulin Resistance defined as below and 4 had normal test results.

All patients with Impaired Fasting Glucose (F>105 mg/dL) also met the criteria for Diabetes, IGT or IR and were not considered as a separate group in our analysis. Finally, even though there were 2 patients showing insufficient pancreatic insulin secretion (by the low levels of insulin compared to their glucose levels), we could not group them together due to different etiologies: one patient with pancreatitis secondary to ethanol abuse had IGT (blood glucose 191 mg/dL at H2) which was normalized after taking the supplements (final H2 = 133 mg/dL), while the other patient had overt diabetes with low insulin values and the OGTT values did not change with taking of the supplements.

The demographic and anthropometric data is summarized in Tables 1-4.

Table 1. *Demographics, Groups and GMI values on initial visit.*

Group Categories	N	IR	IR 2	IGT	D	Total n (%)
Females	6	11	2	6	4	29 (74.4)
Males	3	3	0	2	2	10 (25.6)
Native American	7	14	2	8	6	37 (94.9)
Caucasian	2	0	0	0	0	2 (5.1)
GMI* (mg/dL)	379 +/- 47	491 +/- 42.6	450 +/- 19.4	592.1 +/- 46.9	837.7 +/- 117.4	

* GMI – Glucose Metabolic Index (mg/dL)

Table 2. *Additional Demographic data (initial visit).*

	Females (n = 29)	Males (n=10)
Age (y), mean +/- SD, (CI)*	41.1 +/- 13.2 (36.3 - 45.9)	44.1 +/- 17.1, (33.5 –54.7)
Height (cm), mean +/- SD, (CI)	161.3 +/- 6.1 (158.6 – 164)	175.2 +/- 6.59 (171.2-179.2)
Weight (lbs), mean +/- SD, (CI)	180 +/- 33.4 (167.6 – 192.4)	199.3 +/- 53.03 (166.4 – 232.2)
Waist (in), mean +/- SD, (CI)	43.4 +/- 5.7 (41.2 – 45.5)	44 +/- 9.49 (38.1 – 49.9)
BMI, mean +/- SD, (CI)	31.4 +/- 5.1 (29.6 – 33.2)	29.49 +/- 7.51 (24.8-34.1)
WDI mean +/- SD, (CI)	4.1 +/- 0.43 (3.73 – 4.29)	4.50 +/- 0.39 (4.3 – 4.7)
CI mean +/- SD, (CI)	670.9 +/- 90.1 (638.5 – 703.3)	789.8 +/- 90.1 (734 - 845.7)
GMI mean +/- SD, (CI) (mg/dL)	560.5 +/- 169.3 (509 – 611.9)	536.5 +/- 160.3(441.3 – 634.7)

F = total females (n=29); M = total males (n=10); (CI) – 95% Confidence Interval
BMI – Body Mass Index in kg/m^2; WDI (2) – Weight Distribution Index; lb/in
CI – Constitutional Index, cm*lb/in

Table 3a. *Initial and final patient visit results.*

Variable, units	Initial	Final	p value*
Weight, mean, (SD), lbs	184.97 (39.49), 12.4	184.05 (39.29) 12.4	0.0028
Waist, mean, (SD), (in)	43.5 (6.75), 2.13	43.53 (6.91), 2.19	0.42
BMI, mean, (SD), (kg/m2)	30.9 (5.74), 1.80	30.69 (5.71), 1.81	0.003
WDI, mean, (SD), lb/in	4.23 (0.44), 0.13	4.21 (0.42), 0.13	0.132
CI, mean, (SD), cm*lb/in	701.40 (103.30), 32.42	698.24 (100.86), 32.06	0.129
GMI, mean, (SD), (mg/dL)	554.69 (164.35), 51.47	520.2 (150.3), 53.68	P < 0.001

* p values of paired T test between Initial and Final results in same patients

Table 3b. *Distribution of weight modification of patients.*

Δ Lbs	+4	+3	+2	+1	0	-1	-2	-3	-4	-5	-6	N/R	Total
No of patients	1	1	1	5	9	5	5	2	2	2	3	3	39
Δ GMI	+33	-72	+14	+3	-27	-3	-21	-10	+4	-8	-31		

N/R – not recorded; Δ GMI delta – change in GMI between initial and final visit (mg/dL);
Δ Lbs – change in patients' weight between initial and final visit (lbs)

BMI was not statistically different among the 5 groups; however we have found such a difference when using constitutional indices that consider the distribution of fat: WDI and CI. There were significant differences for the WDI and CI between N vs. IR2 (p = 0.009 and 0.008 respectively), N vs. IGT (p=0.022 and 0.017 respectively) however there was no statistically significant difference between the N vs D (p=0.092 and 0.062 respectively) nor N vs IR groups (p=0.126 and 0.137 respectively). The only other statistically significant differences between the groups in study involving

constitutional indices was found in height: N vs IR2: p = 0.002; N vs. IGT: p = 0.009 and N vs D: p = 0.018, as well as in weight: IR vs. IR2: p = 0.044 for initial visit and IR vs. IGT: p = 0.006 for the final visit. However, because there were 3 men in the N group compared to 2 in the IR group, 1 in the IGT and D groups and none in the IR2 groups we cannot conclude that constitutional measures play a significant role in the observed differences. Not only the height of men was higher on average and thus introduced its own bias, but also gender differences in themselves are significant in diabetes prevalence [3].

There was a significant difference in the correlation of GMI and constitutional indexes between women within reproductive cycle (n=8) and women without (n=21). It is difficult to quantify this effect because the former tended to be also were significantly older (mean age 50.8 vs. 37.4; p = 0.02), however the combined effect of age and diminished estrogen (tended to correlate in a opposite way when compared with both women with reproductive cycle and men.

The total score on the questionnaire (Table 4, first variable) was significantly higher in the D (diabetes) group, p = 0.029, as well as in the IGT group, p = 0.021 – when compared to the N (normal) group, indicating that the diet of people with diabetes and IGT contained a significantly higher amount of saturated and or trans-fats when compared to people who have normal glucose metabolism.

Comparing the total scores of the IR and IR2 groups with that of D group was not statistically significant (p = 0.065 and p = 0.056 respectively), however the difference was sizeable; it is possible that the sample size may have reduced the statistical significance of the difference.

It is worth observing (Table 3a and 3b) that using this 2-week of diet modifications and dietary supplements regimen without modifying physical activity, there was significant weight loss overall as well a significant improvement in glucose metabolism.

By grouping the patients in the 5 categories mentioned above (N, IR2, IR, IGT and D) as a function of their glucose and insulin levels, we have obtained averages (Table 4) that are statistically different from one another (p < 0.01).

We have categorized patients that have a flat or inverted shape for their glycemic curve (the H2 value is similar or bigger than H1) in a separate subgroup of the IR group (IR2) for cross-sectional comparison. The rise in glucose values at 2 hours compared to the 1 hr value may be due to delayed absorption of glucose from the gut or delayed insulin secretion or hepatic gluconeogenesis due to increased cytokine release or saturated/trans fats, which can be pursued in a separate study.

We are proposing to use GMI as a useful estimate in evaluating the overall metabolism of glucose in individuals whose pancreatic function is uncompromised (i.e. in the absence of pancreatitis or impaired insulin secretion seen in the later stages of NIDDM). Based on the results of this study, we obtained the following categories of impairment of glucose metabolism when correlated with the GMI values:

1. GMI < 430 mg/dL – normal; normal glucose and normal insulin levels
2. 430 < GMI < 540 mg/dL – Insulin Resistance (FI > 27 and/or H1I > 88 and/or H2I>79)
3. 540 < GMI < 650 mg/dL – Impaired Glucose Tolerance: abnormal glucose, high insulin
4. 650 < GMI < 900 mg/dL - NIDDM by ADA criteria (non-insulin dependent)
5. 900 < GMI - IDDM by ADA criteria (insulin-dependent)

Table 4. *Constitutional variables among the groups.*

Patient ID	N	IR	D	IGT	IR2
Total Score	6.67 +/- 1.41	6.77 +/- 1.991	9 +/- 2	8.5 +/- 1.215	6.75 +/- 1.708
Age	40 +/- 9.7	41.33 +/- 16.43	45 +/- 8.97	42.5 +/- 16.96	48 +/- 12.68
Height	170 +/- 8.62	166.4 +/- 6.123	162 +/- 5.07	158.2 +/- 7.477	159 +/- 2.63
Initial Weight	183 +/- 35.8	182.6 +/- 32.96	180 +/- 35	167.2 +/- 18.67	154 +/- 12.02
Initial Waist Circumference	40 +/- 6.03	42.39 +/- 5.231	42.5 +/- 5.29	41.92 +/- 3.639	40 +/- 1.414
BMI	28.0 +/- 5.92	29.94 +/- 5.047	31.2 +/- 6.09	30.32 +/- 2.182	27.4 +/- 2.39
WDI	4.57 +/- 0.46	4.288 /- 0.408	4.21 +/- 0.37	3.997 +/- 0.404	3.83 +/- 0.165
CI	781 +/- 117	715.5 +/- 87.92	682 +/- 73.6	633.8 +/- 86.52	612 +/- 23.6
Final Weight	189 +/- 32.9	187.4 +/- 21.13	178 +/- 34	153.3 +/- 16.7	160 +/- 20.37
Final Waist Circumference	41.2 +/- 6.93	44.1 +/- 3.753	42.9 +/- 5.66	40.5 +/- 4.813	40.5 +/- 4.655
BMI	28.9 +/- 5.59	31.87 +/- 2.848	30.9 +/- 5.77	29.74 +/- 4.788	28.8 +/- 3.251
WDI	4.58 +/- 0.29	4.23 +/- 0.349	4.14 +/- 0.36	3.794 +/- 0.298	3.94 +/- 0.149
CI	791 +/- 70.9	693.2 +/- 77.26	670 +/- 68.8	580.3 +/- 64.6	465 +/- 311.4
Initial GMI	371 +/- 37.1	511.9 +/- 42.32	879 +/- 132	646 +/- 77.8	461 +/- 24.04
IFi	8.4 +/- 3.58	12.56 +/- 4.72	24.2 +/- 8.9	21.5 +/- 12.52	12 +/- 4.243
Final GMI	389 +/- 49.2	479.6 +/- 13.24	799 +/- 129	596.3 +/- 59.88	440 +/- 6.11
IFf	9.57 +/- 2.88	12.75 +/- 15.21	21.8 +/- 7.12	19.67 +/- 9.345	15.3 +/- 11.06

WDI – weight distribution index; CI – constitutional index; GMI – glucose metabolic index; IF – level of fasting insulin (initial is IFi, final is IFf); BMI – Body Mass Index; Total Score – sum of points on the diet questionnaire

We have categorized patients that have a flat or inverted shape for their glycemic curve (the H2 value is similar or bigger than H1) in a separate subgroup of the IR group (IR2) for cross-sectional comparison. The rise in glucose values at 2 hours compared to the 1 hr value may be due to delayed absorption of glucose from the gut or delayed insulin secretion or hepatic gluconeogenesis due to increased cytokine release or saturated/trans fats, which can be pursued in a separate study.

Using the T test for differences among groups, we have

obtained p values of less than 0.05 for comparison of initial and final values of weight, BMI, GMI, fasting glucose levels and at 1 hr and 2 hrs, but not for waist circumference, constitutional indexes or insulin levels: Table 4. Also from this table it is also interesting to observe that glucose metabolism (fasting glucose, glucose levels at 1 and 2 hours, GMI) was improved while insulin levels were decreased, which clearly shows that insulin resistance was improved with the dietary supplements and vegetal fat.

Table 5. *Results for Blood Glucose and Insulin Levels during OGTT testing.*

	Initial	Final	T test p value
F, mean +/-SD, mg/dL	98.26 +/- 13.7	96.4 +/- 11.3	0.02
H1, mean +/-SD, mg/dL	162 +/- 52.4	158.7 +/- 53.6	0.02
H2, mean +/-SD, mg/dL	132.2 +/- 60.5	126 +/- 55.2	0.02
FI, mean +/-SD, mg/dL	18.5 +/- 11.5	17.2 +/- 10.2	0.46
H1I, mean +/-SD, microU/mL	156.9 +/- 156.4	132 +/- 119	0.26
H2I, mean +/-SD, microU/mL	105.3 +/- 139.8	85.9 +/- 73	0.06
GMI, mean (SD) mg/dL	554.7 +/- 164.3	536.52 +/- 160.32	0.0003

F = fasting glucose level; H1, H2 = blood glucose levels during OGTT at 1 hour and 2 hours; FI = fasting insulin level; H1I, H2I = insulin levels at 1 hour and 2 hours; GMI = glucose metabolic index

In our supplement formula the most serious side effect encountered with longer term (4 weeks) administration of this formula in patients who were simultaneously on other medication was hypokalemia. We speculate this is due to increased K-Na diuresis in the distal tube of the nephron and that it may be addressed by individual adjustment of supplements (lowering the amount of arginine or of all components) or administering over-the counter magnesium oxide and/or potassium if necessary. On the same account, administering arginine to a patient with concomitant diabetes and hypertension may be an efficient intervention that may reduce the need of administering different types of drugs simultaneously.

The results of the cytokine and oxLDL testing are summarized in Tables 6-8, along with ADMA, fasting insulin, glucose, HDL, Triglycerides, EPA, Arachidonic Acid, AA:EPA ratio and GMI.

Table 6. *Test Results for oxLDL and Metabolic Syndrome Panel.*

	Group N, mean +/- SD (n)	Group IR, mean +/- SD (n)	Group IR 2, mean +/- SD (n)	Group IGT, mean +/- SD (n)	Group D, mean +/- SD (n)	T test, lowest p value
Ox LDL, mU/mL	56.35 +/- 15.13 (11)	54.97 +/- 12.54 (12)	50.34 +/- 17.4 (4)	50.6 +/- 6.32 (7)	48.87 +/- 16.07 (8)	N vs D p = 0.160
ADMA nmol/L	201.3 +/-38.71 (8)	231.1 +/-62.14 (9)	206.7 +/-11.55 (3)	236.11 +/-37.82 (5)	226.7 +/-33.5 (6)	N vs IGT p = 0.073
Homocysteine nmol/L	N/R	5.4 +/- 1.1 (5)	N/R	5.8 +/- 1.9 (4)	7.2 +/- 1.5 (7)	D vs. IR p = 0.027
HDL mg/dL	48.2 +/- 4.76 (5)	46.8 +/- 8.23 (5)	35.7 +/- 17 (3)	49.5 +/- 10.2 (4)	N/R	IGT vs. IR2 p = 0.014
Triglycerides mg/dL	84.4 +/- 22.8 (5)	199 +/- 84.1 (5)	85 +/- 26.6 (3)	159 +/- 57.59 (4)	N/R	N vs. IR/IGT p = 0.018/0.037
GMI mg/dL	379 +/- 47.4 (8)	491.1 +/- 42.6 (14)	450 +/- 19.4 (4)	592.1 +/- 46.9 (9)	837.7 +/- 117.4 (11)	IR vs. N/IR2 p = 0.002/0.009
Fasting Insulin µIU/mL	8.84 +/- 1.95 (7)	19.2 +/- 8.06 (10)	14.1 +/- 3.52 (4)	16.3 +/- 8.6 (8)	25.6 +/- 11.1 (11)	N vs. IGT p = 0.023 D vs. IGT = 0.031

N/R – not recorded; HDL – high density lipoprotein; GMI – glucose metabolic index; ADMA – asymmetric dimethylarginine, oxLDL – oxidized Low Density Lipoprotein

Table 7. *Test Results for Cytokines.*

Cytokine (pg/mL)	Group N (n = 7)	Group IR (n =10)	Group IR 2 (n = 5)	Group IGT (n = 4)	Group D (n = 13)	T test lowest p value
IL-1 Beta, mean (SD)	3.22 (2.02)	4.3 (2.16)	2.67 (0.81)	2.61 (1.04)	2.54 (0.56)	D vs. IR; p = 0.015 IR2 vs. IR; p = 0.027 IGT vs. IR; p = 0.036
IL-4, mean (SD)	10.16 (5.10)	11.9 (5.92)	34.1 (30.4)	7.83 (2.30)	12.68 (8.63)	D vs. IGT; p = 0.040 IR vs. IGT; p = 0.044
IL-6, mean (SD)	4.9 (2.14)	5.47 (1.72)	7.09 (2.86)	4.34 (2.14)	5.71 (3.52)	N vs. IR2; p = 0.047
*IL-1 Alpha, mean (SD)	6.17 (3.10) [*2]	11.40 (1.57) [*2]			5.86 (1.36) [*4]	D vs. IR; p = 0.029

* Interleukin 1 alpha was tested on a smaller number of patients; number of patients tested in each group is given in square brackets

For cytokines there were statistically significant differences between some of the groups, however there was not an identifiable pattern (in the manner N<IR<IGT<D or inversely), probable because the IGT group was smaller in number. In triglycerides we have seen a statistically significant difference between the N vs IR and N vs, IGT groups, which is in

concordance with the literature. When compared to the initial visit testing, oxLDL values were higher on average - Table 3a however their absolute values were not significantly different (p=0.08). Similarly, when we considered only those patients who had improved their GMI after the 2 weeks (n=16), their average oxLDL were lower after the intervention, but not significantly different (p=0.08). It is possible that after adjusting for age we will see a statistically significant difference, which will be in concordance with results obtained by Kopprasch et al [36-39].

Table 8. *Comparison between initial vs final test values in same patient.*

	Initial Visit	Follow-up	T test, p value
`GMI, mean (SD) mg/dL	554.7 +/- 164.3	536.52 +/- 160.32	0.0003
^oxLDL, mean (SD) mU/mL	55.27 +/- 15.31	50.03 +/- 14.61	0.087
IL-1Beta, mean (SD) pg/mL	3.01 +/- 1.39	3.18 +/- 1.63	0.18
IL-4, mean (SD) pg/mL	16.05 +/- 10.53	14.90 +/- 10.65	0.316
IL-6, mean (SD) pg/mL	5.80 +/- 1.59	7.04 +/- 3.46	0.078
*ADMA, mean (SD), nmol/L	222.2 +/- 45.1	205 +/- 28.7	0.09

` GMI – Glucose Metabolic Index; ^ oxLDL – oxidized Low Density Lipoprotein;
* ADMA – asymmetric dimethyl arginine

4. Discussion

The importance of detecting IFG and IGT was underscored by the repeated findings of increased mortality rate of patients with IGT as well as the rate of cardiovascular morbidity [6, 15, 16]. Having a good screening procedure to detect such asymptomatic conditions in general population is thus a very important undertaking.

There are multiple methods used to estimate insulin resistance, insulin secretion and sensitivity, starting with the minimal model proposed by Bergman [17] and further developed as mathematical models and indices [18-20] or improved experimental protocols that are closer to real life or offer more simplicity [21-24]. However, the use of insulin-based indices (insulin secretion index, insulin disposition index, insulin sensitivity index) has limited practical value for use in asymptomatic, healthy people as well as in differentiating outside of the laboratory the various types of impairments of glucose metabolism [25-29].

This is another strong argument in employing OGTT in high-risk populations as a screening procedure as well as in helping to guide the treatment of T2DM with the use of GMI.

The objections raised towards a more widespread use of OGTT are inconvenience (time and multiple sampling required); higher cost when compared to a single fasting plasma glucose sampling; and questions about test retest reproducibility of the 2 hour value (based on which the diagnosis of IGT or diabetes can be made).

The first two objections (inconvenience and cost) can be addressed by using the finger prick OGTT performed with a handheld glucometer as a screening procedure, which has the added advantage that it can be done in any ambulatory setting (public spaces such as schools, pharmacies, meeting halls, etc.) so that the availability of this screening procedure is greatly improved when compared to the usual procedure of having apparently healthy people come to a clinic or hospital or laboratory for testing. The accuracy of those portable glucometers nowadays is very high, and their results are as good as a laboratory chemistry analyzer.

The last objection may be overcome by using the newly proposed index (the glucose metabolic index or GMI) which uses 3 data points as opposed to a single one which we think that significantly improves the reproducibility of data obtained by performing an OGTT. Additionally GMI gives more information more promptly than glycated hemoglobin in assessing the efficacy of the treatment and the need for adding insulin to the patients' regimens.

From a clinical practice standpoint, looking beyond the interplay between the sensitivity of beta-cells to glucose and the insulin resistance displayed by hepatic and peripheral tissues we propose the use of GMI as a overall estimate of individual glucose metabolism.

The most acute intervention based on this index can be employed when a patient who is currently on oral hypoglycemiants does not show an improvement in either glycated hemoglobin (A1c) or random blood glucose values after 3 or more months of treatment, which may be due to either low compliance or inefficient intervention. Without performing tests which measure dynamic insulin levels and based solely on the GMI values, the practitioner can add insulin to the regimen (if GMI is more than 900) or enforce compliance measures (when GMI is less than 900) with the help of the nutritionist and diabetes clinic personnel.

Another scenario in which GMI show its usefulness is represented by the newly diagnosed patient with diabetes. Even though the majority of new T2DM cases are properly treated and improve with oral medication, if the diagnosis was made relatively late during the progression of the disease (more than 5 years of insulin resistance status), the function of the pancreatic beta cell in some of those patients may be severely compromised; such a patient will benefit only marginally, if any from oral hypoglycemiants. The side effects and failed intervention may reduce the compliance of the patient; also precious months and in some cases years may be passing before this patient can properly control glucose levels with insulin. A OGTT which shows a GMI of over 900 may help put this new patient faster on needed insulin.

Conversely, adding insulin in a patient who already has high levels of endogenous insulin (in the early phase of NIDDM) but is rather non-compliant (not taking oral medication properly) due to side effects or other factors may not be helpful, especially as a long-term treatment strategy.

For performing an OGTT in a patient already diagnosed with diabetes and who has a fasting glucose over 140 mg/dL, a short trial (2 weeks) of oral hypoglycemiant - or in our case dietary supplements – can be administered in order to get the fasting glucose levels below 140 mg/dL, when administering glucose is acceptable. We encountered this situation once, when a patient had fasting glucose of 152 mg/dL, postponed the OGTT and after 2 weeks of taking supplements the testing could be completed given the new fasting glucose of 126 mg/dL.

Perhaps the most important use of the finger prick OGTT and GMI is for early detection of the status of impaired metabolism of glucose. By normalizing blood glucose values on OGTT in patients with IGT it is possible that we will preclude progression to diabetes in the estimated 20-30 % of such patients that are estimated to progress to diabetes. The cost of such intervention (approx $25/month/patient using dietary supplements) is much less than that of treating diabetes and less than that of metformin ($50/ month), which requires prescription (adding to the cost of medication) probably has more side effects than dietary supplements and does not address important factors shown to induce insulin resistance – fatty acid imbalance and oxidative stress - but is rather acting by mitigating their effects.

The use of dietary supplements to improve glucose metabolism in pre-diabetes and diabetes was successfully done previously with a formula containing lipoic acid, magnesium, aspirin, vitamins and minerals. However this does not address the role of fatty acids in pancreatic secretion and insulin resistance that we believe plays an important role in the pathogenesis of diabetes in some minorities. By removing saturated fats of animal origin and trans fats from diet or significantly reducing it, it is possible that the need for additional supplements will be precluded altogether. In making our formula we considered results from a previous study in this population [30] which showed that hypoalbuminemia (along with elevated globulins) was found in more than 50% of patients with GI problems, as well as anecdotal evidence suggesting malnutrition as a relatively common clinical encounter.

We were prompted to look for non-genetic factors involved in the diabetes endemic in this community based on multiple considerations. One observation is a historical reference from a 1939 study in which there was only 1 case of diabetes in a Native American population out of 6000 patients that were treated in that hospital [40]; in 2000 at the same hospital there are at least 500 patients with diabetes among any 6000 randomly chosen patients. Such hundreds-fold increase in the diabetes prevalence in 3 generations cannot be explained by any genetic transmission; at best an autosomal dominant transmission of diabetes genes – the one with the most aggressive phenotype expression – may have been followed by less than 50 fold increase in the disease prevalence in 3 generations. Another study [41] shows that in Pima Indians, changes in their diet and exercise patterns over time were accompanied by an increase in diabetes prevalence. Also, Pima Indians living in Mexico had lower obesity rates associated with increased physical labor and consumption of less animal fat compared to their counterparts living in Arizona.

Results on oxLDL show that the lowest values were obtained in the D group, although there were not significantly different from the other groups ($p > 0.1$). Even though this finding was surprising given the literature [36,42 - 44] which would lead to expect higher oxLDL values on average in the D group, the results may be due to the testing itself, which is very sensitive from a technical standpoint. First we need to mention that lipid oxidation was shown to continue in frozen samples that are kept at temperatures of less than –70 degrees (and our only choice was storage at –30 degrees). This may falsely elevate oxLDL values in samples that were stored longer, which may have been our case given that samples from the D group were collected 4-6 weeks later compared to the N group. This may have been compounded by the use of first-generation EIA kits, the fact that oxLDL testing was the only one performed in two completely different laboratories (IBT and TriCore) and the lack of experience with handling oxLDL testing, which is not a widespread procedure.

The role of estrogen in insulin resistance through its action on the inflammatory pathway is established [45] and may explain the observed differences in our study between women with and without current reproductive cycle.

Finally, there seems to be a strong interdependence between inflammatory factors, oxidative capacity and circulating lipids [46-51] both in their generation and also in the pathways of their action (through NFkappaB, IRS-1 and IRS2), and to have a complete picture of their action is probably better to study them at the same time rather than separately, which we have attempted with some degree of success in our pilot study.

One important consideration is that in our study we have used glucose values measured from whole venous blood; blood glucose levels determined by finger prick are about 15% more than those obtained from testing whole venous blood [4]. Furthermore, the use of capillary blood from finger prick in itself introduces further measuring bias because of the variations in the quality of the sample obtained. However, those considerations may not preclude using the finger prick OGTT as a screening test rather than a diagnosing tool in high-risk populations. Even though larger studies will be necessary for validation, the finger prick OGTT and the resulting GMI may be an adequate screening test for insulin resistance and pre-diabetes given its simplicity to perform, its low cost and minimally invasive characteristics. This testing offers the flexibility of being used outside of a dedicated healthcare facility, for example at Native American Chapter Houses, Health Fairs, Schools, Diabetes Clinics and in other settings and may be employed on a large scale as a prevention tool against progression to diabetes.

In a dedicated healthcare facility the use of OGTT from venous blood can give the clinician instant and important information about a patients' insulin resistance status as well as some estimate of insulin secretion, which will help in devising a more efficient intervention.

5. Conclusion

As much as T2DM is a metabolic disturbance which depends on the balance of multiple factors [4, 5, 35, 46-51], it is very unlikely that one can muster a "cure" in the form of an oral or injectable medication which can apply to all patients. At the same time we know that the progression of events leading to diabetes is not sudden but rather a continuum of various degrees of impairment in glucose metabolism [35]. With early detection of insulin resistance, IFG and IGT - and subsequent intervention we can normalize or at least significantly improve glucose metabolism and such we can essentially "cure" diabetes by preventing it [5].

Our study pleads for the detection and better treatment of the insulin resistant states with a relatively simple tool – GMI - and adds dietary supplements to the arsenal of interventions employed in fighting the diabetes endemic. Exercise [31, 32] and diet [33, 34] are perhaps the best interventions towards improving glucose metabolic status, however for the patients who are losing the battle on this front we are proposing the use of dietary supplements, which are shown to improve glucose metabolism by acting on multiple pathways (inflammation, oxidative stress, mitochondrial function, etc.).

This type of efforts is likely to improve both the healthcare status of a given population as well as reduce the healthcare costs associated with treating chronic diseases.

Acknowledgements

The study was financed by the Navajo Nation Health Department in Window Rock and Sage Memorial Hospital in Ganado, AZ.

The authors want to thank the following institutions and individuals who have kindly contributed to this study: Navajo Nation Human Research Review Board – Mrs. Beverly Becenti Pigman, Dr. Martia Glass – Navajo Area IHS, Dr. Greg Vannoy and Richard Buschor, PA, RN – Sage Memorial Hospital, Sage Memorial Hospital Laboratory staff, TriCore Laboratories of Albuquerque, NM.

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