

# Insulin resistance detection using spectral analysis of arterial plethysmography versus Hyperinsulinemic Euglycemic Clamp

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## Abstract

**Background:** *Insulin resistance is a condition in which the body produces insulin but does not use it properly and usually this condition has no symptoms. Moreover, insulin resistance is a strong risk factor of impaired glucose tolerance (IGT), type 2 diabetes (T2DM) and cardiovascular diseases. Insulin resistance carried a greater risk for developing cardiovascular disease than smoking or age or total / HDLcholesterol ratio. Recent clinical trials have reported a reduction in the incidence of T2DM with lifestyle intervention, surgery and pharmacotherapy in subjects with IGT. Thus, early detection of insulin resistance could be useful to reduce the pandemic diabetes diseases and its complications such as retinopathy, nephropathy, hypertension and cardiovascular diseases.*

### **Material and method:**

*Thirty patients (23 women) in general good health of mean age 32 (range 22-55) years and BMI of 27.3 (range 19-49) Kg.m<sup>2</sup>, who were candidates for insulin resistance test were included in the study, and underwent hyperinsulinemic euglycemic clamp (HE clamp) test and examination with the ES Complex system. The ES Complex system is using a combination of technologies, but in this study with focus on signal processing analysis of the oximeter data in spectral analysis. We investigated the cross-sectional association between insulin resistance (M value, assessed using (HE clamp) and the spectral analysis of the total records of the photoelectrical plethysmograph (PTG).*

*Statistical analysis was performed to correlate M value and PTG Total Power (PTG TP) using Brand Altman Plot. Receiver-operating characteristic curves were also constructed to determine the specificity and sensitivity of PTG TP, Body Mass Index (BMI) and blood glucose in detecting M value < 4.5.*

### **Results:**

*The Spearman's coefficient of rank correlation ( $\rho$ ) was -0.624 (P. 0.001). PTG TP had a sensitivity of 90 % and specificity of 90% (cutoff # 370ms<sup>2</sup>) Area under the Roc curve (AUC) =0.95 to detect M value < 4.5 (P.0.0001). BMI had a sensitivity of 80 % and specificity of 60% (cutoff # 28.8 Kg.m<sup>2</sup>) AUC=0.752 to detect M value < 4.5 (P.0.01). Blood glucose had a sensitivity of 60 % and specificity of 95% (cutoff # 89.4) AUC=0.810 to detect M value < 4.5 (P.0.001).*

**Conclusion:** *PTG TP parameter has the best AUC (0.95) comparing with the other existing available tests to detect the M value < 4.5 of the HE clamp.*

*Therefore, PTG TP provided by the ES Complex system represents a novel parameter of screening and follow ups for insulin resistance on large scale population. This parameter is independent factor of risk for T2DM and cardiovascular diseases. Such a tool, which is easy to use, non-invasive, and cost-effective, would be of great benefit for the control of pandemic diabetes diseases and its complications. A new study is underway to confirm the results with 100 patients.*

## INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) has increased in recent decades to epidemic proportions. About 150 million individuals worldwide had T2DM in 2000, and this number is expected to increase to ~300 million by the year 2025<sup>1</sup>. Because of the chronic course of T2DM and the significant morbidity and mortality associated with the vascular complications of the disease, T2DM has become, not only a serious public health threat, but also a heavy economic burden on the health care system<sup>2</sup>. The total annual cost of diabetes care in the United States was estimated to be \$175 billion in the year 2007, and this number is expected to increase further with the increasing incidence of the disease<sup>2</sup>.

The association of obesity with T2DM has been recognized for decades, and the major basis for this link is the ability of obesity to engender insulin resistance. Insulin resistance is a fundamental aspect of the etiology of T2DM and is also linked to a wide array of other pathophysiologic sequelae including hypertension, hyperlipidemia, atherosclerosis (i.e., the metabolic syndrome, or syndrome X), and polycystic ovarian disease<sup>3,4</sup>.

Insulin resistance carried a greater risk for developing cardiovascular disease than smoking or age or total / HDLcholesterol ratio<sup>5,6,7,8</sup>.

There are also grounds for considering the related possibility that insulin resistance and hyperinsulinemia, in addition to being caused by obesity, can contribute to the development of obesity<sup>9</sup>.

Thus, the detection of insulin resistance could be useful to identify the health factor risks.

The diagnosis of insulin resistance requires performing of the gold standard euglycemic hyperinsulinemic clamp (HE Clamp) which is costly, time consuming and inconvenient in routine clinical setting<sup>10</sup>.

Therefore, many tools based on algorithms of the laboratory tests results have been developed and compared to the HE clamp. The best algorithm using Receiver-operating characteristic curves (ROC) was Homeostasis model assessment –insulin resistance (HOMA-IR) with Area under the ROC curve (AUC) at 0.946<sup>11</sup>.

Study had been published using ES Complex system (LD Technology-Florida -USA) to detect the insulin resistance versus HOMA-IR. ES Complex-Insulin resistance (ESC-IR) algorithm was correlated with HOMA-IR ( $r=0.84$ )<sup>12</sup>.

In this study, we use ES Complex system to compare a new parameter calculated with the Fast Fourier Transforms of the oximeter wave form (plethysmograph) to detect insulin resistance versus HE Clamp.

### **Materials:**

The ES Complex system is using a combination of oximeter, Bioimpedance device and a blood pressure device.

The oximeter placed on the right index finger displays in real time the photoelectrical plethysmography which represents the arterial blood volume changes during the cardiac cycle. Signal processing analysis of the waveform allows determining the heart rate, the heart rate variability analysis and stiffness or Aging Index which is inversely proportional to the arterial compliance.

Bioimpedance device measures within 4 electrodes the electrical resistance of the body and uses common algorithm of peer reviews for estimating the Fat mass.

The spectral analysis using the Fast Fourier Transforms (FFT) of the first derivative of total records of the plethysmograph provides 3 frequencies High, low and very low frequencies, the sum of the 3 frequencies is the total Power of the spectral analysis. We named this parameter Plethysmograph Total Power (PTG TP).

### **SPECIFIC AIMS**

The specific aim of this study is to examine the ability of PTG TP to identify subjects with insulin resistance comparing to the M value of the HE clamp.

### **RESEARCH PLAN**

#### **Subjects:**

Thirty patients (23 women) of mean age 32 (range 22-55) years, of mean of BMI of 27.3 (range 19-49) Kg.m<sup>2</sup>, who were candidates for insulin resistance testing were included in the study, and underwent HE clamp tests and examination with the ES Complex system. Subjects must be in good general health as determined by physical exam, medical history, blood pressure measurement and blood glucose assessment.

Table 1 describes the subject characteristics.

The study was approved by the regional ethics committee, and was conducted according to the ethical principles of the Declaration of Helsinki. All of the subjects provided written informed consent, and confidentiality was maintained for all subjects.

**Inclusion criteria:**

1. Age > 18 years.
2. Ability to provide written informed consent.
3. To be in general good health

**Exclusion criteria**

1. Taking drugs known to affect glucose homeostasis.
2. Major organ disease involving the heart, lung, kidney or the nervous system.

Also, patients were excluded if they had any contraindication to use of the ES Complex system. Use of the ES complex system is contraindicated (1) wore an automatic external defibrillator device; (2) had erratic, accelerated, or mechanically-controlled irregular heart rhythms; (3) had arterial fibrillation/flutter; (4) had atrioventricular block; ((5) had any implanted electronic device.

**Table 1. Subject characteristics**

<b>General Demographic Table</b>	<b>Means</b>
Men/Women	23/7
Age (years) range	32 (range 22-55)
BMI (Kgm <sup>2</sup> ) range	27.3 (range 19-49)
Systolic pressure (mmHg)	109.5 (range 90-140)
Diastolic pressure (mmHg)	70.7 (range 58-95)
Blood glucose	86.3 (range 78-99.6)

**Experimental Design**

All patients received the following tests:

- 1) Screening Visit which includes medical history, physical exam, blood pressure and blood glucose assessment. An ES Complex measurement also was performed at this visit.
- 2) Every subject received HE clamp test.

### **HE Clamp procedure**

The HE clamp technique according to De Fronzo<sup>10</sup> was used, with a slight modification to suppress hepatic glucose production, for estimation of in vivo sensitivity to insulin. Insulin (Actrapid Human; Novo, Copenhagen, Denmark) was infused in a primary dose for the first 10 min and then as a continuous infusion (56 mU /min per body surface area in meters squared), during which De Fronzo used 40 mU/min per body surface area in meters squared, for 2 h to maintain steady-state hyperinsulinemia. The target plasma glucose level was 5.1 mmol/l and maintained by measuring plasma glucose every 5 min. The glucose infusion rate during the last hour was used as a measure of insulin sensitivity. The total M value derived from the glucose clamp was normalized to body area surface. An M value < 4.5 mg/kg · min per μU/ml is considered as a diagnosis of Insulin resistance<sup>10</sup>.

### **DATA ANALYSIS:**

The primary objective of the study is to examine the ability of PTG TP to detect insulin resistance (M value < 4.5). Statistical analysis was performed to correlate M value and PTG Total Power (PTG TP) using Brand Altman Plot. Receiver-operating characteristic curves were also constructed to determine the specificity and sensitivity of PTG TP, body Mass Index (BMI) and blood glucose in detecting M value < 4.5.

### **Adverse Events**

No adverse events are reported with the use of the device and during the H.E clamp tests.

### **Sample Size Calculation and Statistical Analysis**

Based upon the preliminary studies results to have 90% power to detect a significant difference between Insulin resistance subjects with the above mean and standard deviation at alpha =0.05, 30 subjects had been included. The sample size ( $n \geq 30$  and  $\alpha = 0.05$  %) was determined using MedCalc software (<http://www.medcalc.org/publications/journals.php>).

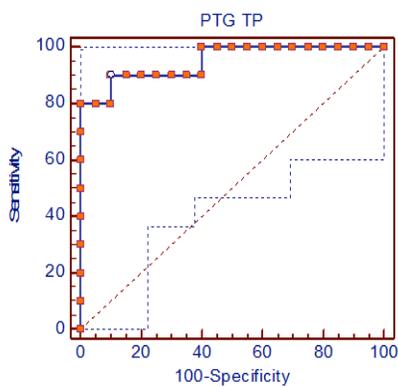
## Results

MedCalc software was used to perform the statistics.

PTG TP had a sensitivity of 90 % and specificity of 90% (cutoff # 370ms2) to detect M value < 4.5 (P.0.0001). Figure 1

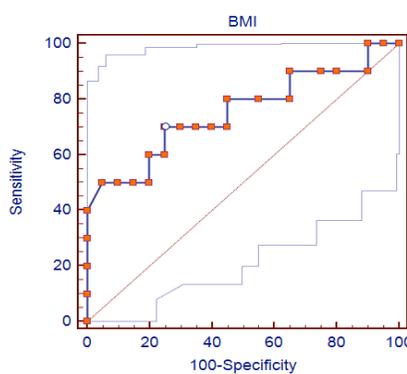
BMI had a sensitivity of 80 % and specificity of 60% (cutoff # 28.8) to detect M value < 4.5 (P.0.01). Figure 2

Blood glucose had a sensitivity of 60 % and specificity of 95% (cutoff # 89.4) to detect M value < 4.5 (P.0.001). Figure 3



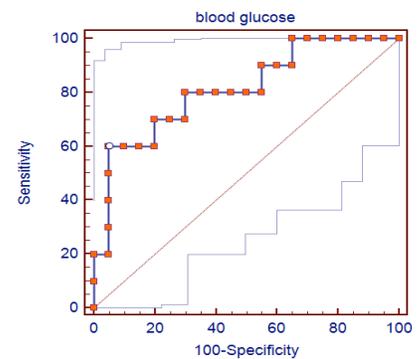
Area under the ROC curve (AUC)	
Area under the ROC curve (AUC)	0.950
Standard Error <sup>a</sup>	0.0426
95% Confidence interval <sup>b</sup>	0.803 to 0.996
z statistic	10.555
Significance level P (Area=0.5)	<0.0001

<sup>a</sup> Hanley & McNeil, 1982  
<sup>b</sup> Binomial exact



Area under the ROC curve (AUC)	
Area under the ROC curve (AUC)	0.752
Standard Error <sup>a</sup>	0.105
95% Confidence interval <sup>b</sup>	0.562 to 0.891
z statistic	2.409
Significance level P (Area=0.5)	0.0160

<sup>a</sup> Hanley & McNeil, 1982  
<sup>b</sup> Binomial exact



Area under the ROC curve (AUC)	
Area under the ROC curve (AUC)	0.810
Standard Error <sup>a</sup>	0.0857
95% Confidence interval <sup>b</sup>	0.626 to 0.929
z statistic	3.615
Significance level P (Area=0.5)	0.0003

<sup>a</sup> Hanley & McNeil, 1982  
<sup>b</sup> Binomial exact

Figure 1 .ROC and AUC PTG TP

Figure 2 .ROC and AUC BMI

Figure 3 .ROC and AUC blood Glucose

Brand Altman Plot for PTG TP and M value is Figure 4

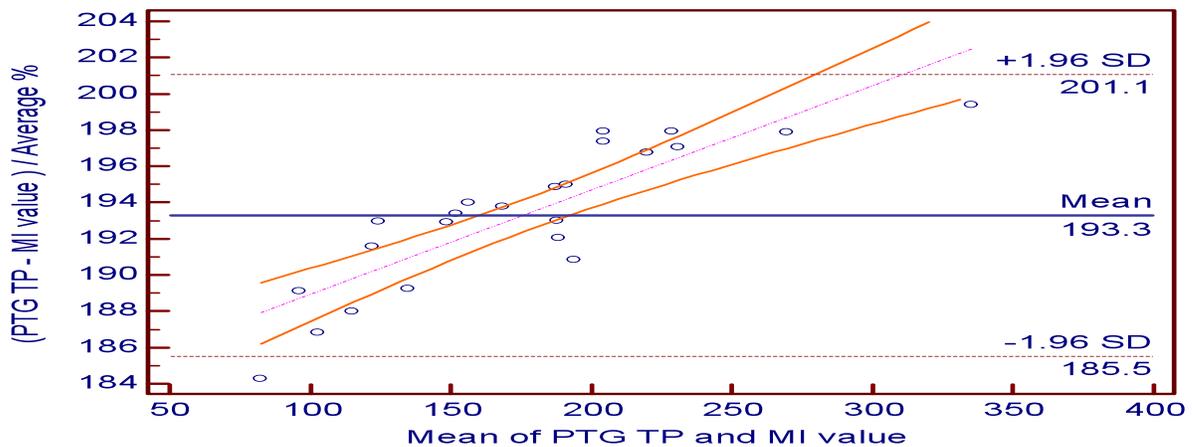


Figure 4. Brand Altman Plot for PTG TP and M value

Table 2. Summary table of results.

Items	Positive group	Negative group	P value M/I < 4.5
M value (range)	2.84 (1.04-4.4)	6.15 (4.7-8.9)	Diagnostic
Age in years(range)	33.8 (25-55)	31(21-40)	NS
PTG TP in ms <sup>2</sup> (range)	450.7(344-669)	291.8 (158-379)	0.0001
Blood Glucose in mg/dl (range)	87.01(78.2-99.6)	85.9 (78-94.6)	0.001
BMI in Kg.m <sup>2</sup> (range)	30.9 (23.6-49.2)	25.4(19.3-30.5)	0.01
Systolic Pressure in mmHg (range)	118.2 (96-140)	105.2 (90-128)	0.27
Diastolic Pressure in mmHg (range)	75.3(60-95)	68.5(58-84)	0.19

### Discussion

How PTG TP is related to the insulin resistance? The explanation could be that PTG TP is calculated from the change of arterial blood volume during the cardiac cycle.

These changes were investigated and are related to the arterial stiffness<sup>13</sup>.

There are changes in responsiveness in small to medium sized artery compliance in patients with early insulin resistance syndrome. There is increased responsiveness to angiotensin II (AT2) mediated by both AT1 and AT2 receptors producing decreased and increased compliance respectively. There is also increased basal nitric oxide activity producing an increase in compliance when measured as the reduction in compliance to nitric oxide synthase inhibition.<sup>14</sup>

American Diabetes Association, the European Association for the Study of Diabetes and the International Diabetes Federation recommended that T2DM screening include Fasting blood glucose (FBG) test and Oral glucose tolerance (OGTT) test<sup>15</sup>.

However, UK Prospective Diabetes Study suggests that loss of beta cell function can start at least 10 years before diagnosis of diabetes, and that mean beta cell function may already be less than 50% at diagnosis<sup>16</sup>.

None of the therapies used in the UK Prospective Diabetes Study (sulfonylureas, metformin, and insulin) were able to prevent or delay the progressive deterioration of beta cell function and complications. On average, beta cell function declines by 1% per year with normal aging, compared with 4% per year in diabetes<sup>17</sup>.

Thus, T2DM screening could not use only the conventional diagnostic (FBG and OGTT) and it would include the screening of insulin resistance using the gold standard HE clamp.

However, the gold standard is costly, time consuming and impossible in routine clinical setting and in large scale screening.

Several tests are offered to detect insulin resistance using blood laboratory tests and algorithms. The study of Lindsey George et al.<sup>11</sup> Compared the different available tests versus euglycemic hyperinsulinemic shows the area under the ROC curve (AUC) for each index ranged from 0.888–0.946.

The ratio of glucose AUC to insulin AUC (GlucAUC/InsAUC) was the least useful discriminator of insulin resistance (AUC=0.888), whereas the AUC was higher for all of the other surrogates measures, indicating high sensitivity and specificity for detection of M value < 4.5 as follow:

- ✓ Quantitative insulin sensitivity check index (QUICKI) AUC=0.938
- ✓ Fasting glucose to insulin ratio (GF/IF) AUC = 0.942
- whole-body insulin sensitivity index (WBISI) AUC=.0.945
- ✓ Insulin ratio (1/IF) AUC= 0.946
- ✓ Homeostasis model assessment-Insulin resistance (HOMA-IR) AUC= 0.946

Therefore, with AUC =0.95, PTG TP has the best sensitivity and specificity for detection of M value < 4.5. Recent clinical trials have reported a reduction in the incidence of T2DM with lifestyle intervention, surgery and pharmacotherapy in subjects with IGT<sup>18-23</sup>.

As regards to the above facts, with the best AUC (0.95) comparing with the other existing available tests to detect the M value < 4.5 of the HE clamp PTG TP Parameter measured from non-invasive, fast and effective cost, will be useful in insulin resistance early detection in large scale population and could be an effective strategy to restrain the epidemic increase in the disease prevalence and reduce the economic burden it poses on the health care system.

The new ADA and ESDA guidelines<sup>24, 25</sup> show the complexity of diabetes treatment and to prevent the diabetes complications.

Long-term glycemic control is one of the most important treatment goals for type T2DM, and the authors of the current position statement provide a broad overview of research into different levels of glycemic control among patients with type 2 diabetes. Tighter glycemic control has been most associated with a lower risk for microvascular complications, such as retinopathy and nephropathy. However, very tight glycemic control appears to be less beneficial regarding the risk for cardiovascular disease, with some research suggesting that tight control actually increases the risk for cardiovascular mortality<sup>25</sup>.

Therefore A1C goal may vary depending on age and various other factors and the new recommended level was increased at 7%<sup>1</sup> and the algorithm treatments<sup>1</sup> based only on 1Ac are controversy<sup>24,25</sup>.

In addition to the A1C test, periodically are necessary to check cholesterol levels, thyroid function, liver function and kidney function. Regular eye and foot exams also are important<sup>1</sup>. As regards to the study results, PTG TP parameter could be also add to the above exams EASD president Andrew J.M. Boulton also commended the new guidelines for their patient-specific approach<sup>25</sup>.

Dr. Boulton said in a news release. "Diabetes is a condition which affects people in a multitude of ways: the new guidelines take a more holistic approach, focusing on treating the patient as an individual and understanding that treatments need to be 'made to measure,' an approach that will likely improve not only patient care, but also quality of life."

**Conclusion:**

PTG TP parameter is independent factor of risk for T2DM and cardiovascular diseases  
PTG TP parameter has the best AUC (0.95) comparing with the other existing available tests to detect the M value < 4.5 of the HE clamp.

Therefore, PTG TP provided by the ES Complex system represents a novel screening parameter and follow ups for insulin resistance on a large scale. Such a tool, which is easy to use, non-invasive, and cost-effective, would be of great benefit for the control of pandemic diabetes diseases and its complications.

A new study is underway to confirm the results with 100 patients.

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**Conflict of Interest:** This study was not sponsored. The authors have no conflicts of interest to declare.

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