The novel adiponectin-resistin (AR) and insulin resistance (IR_{AR}) indexes

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Serum hypoadiponectinemia and hyperrestinemia independently links insulin resistance to type 2 diabetes (T2DM) and metabolic syndrome (MS). Thus, the aim of this study was propose a novel adiponectin-resistin (AR) index by unifying the effect of adiponectin and resistin. Then, a novel insulin resistance (IR_{AR}) index was proposed by taking into account the AR index. Serum adiponectin and resistin levels as well as other insulin resistance, T2DM and MS risk factors were tested. Experimental results showed the AR index was more stronger correlated with insulin resistance risk factors and had stronger association (df=5; F=51.154; P<0.001) with T2DM and MS susceptibility rather than the serum adiponectin (df=5; F=15.680; P<0.001) and resistin (df=5; F=40.648; P<0.001) levels alone. Therefore, the AR index looks very strongly links insulin resistance to T2DM and MS. Meanwhile, the IR_{AR} index (df=5; F=78.396; P<0.001) is a potent useful index of insulin sensitivity in subjects with T2DM and MS.

The metabolic syndrome (MS) drives the twin global epidemics of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD)[1,2,3]. T2DM itself is accompanied by increased risk for CVD which is conferred by the concomitant risk factors of the MS[4,5]. Serum hypoadiponectinemia and hyperrestinemia independently are associated with insulin resistance in several prospective epidemiological studies across a variety of population groups[6,7,8,9]. Since, insulin resistance is a prerequisite root factor for

developing T2DM[10]. It is also the most unifying parameter to characterize the pathophysiology of the MS[1]. Thus, the investigation of the interaction effect of adiponectin and resistin in aetiological linking insulin resistance with T2DM and MS is looking forward. In addition, the modification of the existing insulin resistance indexes by taking into account the interaction effect of adiponectin and resistin merit a potential investigation. It hopefully to identify those with undiagnosed T2DM and MS due to insulin resistance in order to provide early treatment and prevent or delay the onset of long-term complications such as cardiovascular risk.

Adiponectin (also known as Acrp30, AdipoQ, GBP28, and ApM1) is a polypeptide hormone with molecular weight 30kDa (244 amino acids) which modulates a number of metabolic processes, including regulates energy homeostasis as well as glucose and lipid metabolism[11,12]. It is also a potent insulin sensitizer in muscle and liver[12]. It is exclusively secreted from adipose tissue into the bloodstream and is very abundant in plasma relative to many hormones which representing 0.01% of blood circulating proteins[13,14]. The hormone plays a principal role in the suppression of the metabolic derangements that may result in insulin resistance, type 2 diabetes, metabolic syndrome and cardiovascular diseases[7,10,11,12,13,15].

Resistin is also known as "*serine/cysteine-rich adipocyte-Specific Secretory Factor*" (ADSF and FIZZ3)[16]. It is a putative adipocyte-derived signalling polypeptide hormone with molecular weight 12.5kDa and the length is 108 amino acids in human[16]. It acts as a pathogenic factor contributing to insulin resistance by antagonizes insulin action, thereby increasing gluconeogenesis and impaired hepatic glucose uptake[17,18,19]. In contrast with adiponectin, resistin has low circulating levels[16]. However, the blood

circulating levels of resistin had been shown up-regulated in subjects with insulin resistance, type 2 diabetes, metabolic syndrome and cardiovascular diseases[8,9,20,21,22,23].

The novel adiponectin-resistin (AR) index. Experimental results showed fasting serum adiponectin (A_0) and resistin (R_0) levels were strongly correlated with insulin resistance indexes and risk factors (Tables 1-3). Besides, serum hypoadiponectinemia and hyperresistinemia were significantly associated with T2DM and MS susceptibility (Fig. 1-2). Therefore,

$$\alpha = \frac{1}{A_0} \tag{1}$$

$$\beta = R_0 \tag{2}$$

Since, fasting serum adiponectin (A_0) and resistin (R_0) levels were significantly negative correlated. Thus, (1) and (2) values are unifying by a multiplication as following

$$\gamma = \alpha\beta = \frac{1}{A_0} \times R_0 = \frac{R_0}{A_0} \tag{3}$$

Then (3) is logarithmically transformed for normalization,

$$\delta = log_{10}(\gamma) = \log_{10}\left(\frac{R_0}{A_0}\right) = \log_{10}(R_0) - \log_{10}(A_0) \tag{4}$$

Lastly, a numerical constant 1 added to the (4) to get a positive integer of the AR index

$$\text{AR Index} = 1 + \delta = 1 + \log_{10}(R_0) - \log_{10}(A_0) \tag{5}$$

Note: R_0 = fasting serum resistin levels in ng/mL;

A_0 = fasting serum adiponectin levels in μg/mL.

The novel adiponectin-resistin (AR) index showed stronger association (df=5; F=51.154; P<0.001) with type 2 diabetes (T2DM) and metabolic syndrome (MS) susceptibility rather than the serum adiponectin (df=5; F=15.680; P<0.001) and resistin (df=5; F=40.648; P<0.001) levels alone (Fig. 1-3). The value of the AR index was the lowest for the control subjects followed by the subjects with MS and T2DM. However, the value of the AR index reached the peak when T2DM concurrently with MS. In addition, the AR index showed stronger correlation with insulin resistance (IR) indexes and risk factors particularly the serum insulin, plasma glucose and whole blood HbA1C levels rather than the serum adiponectin and resistin levels alone (Tables 1-3). This indicated that the AR index very strongly links insulin resistance to T2DM and MS rather than the serum adiponectin and resistin levels alone.

The most plausible explanation for the interaction effect of adiponectin and resistin in linking insulin resistance with type 2 diabetes and metabolic syndrome is the overall multimeric assembly of the resistin is similar to that of adiponectin[24]. The comparable domain architecture of these two adipocyte-specific hormones, despite diametrically opposed physiological effects, suggest a common regulatory mechanisms for the glucose and lipid homeostasis as well as insulin signalling pathway[24].

Serum hypoadiponectinemia and hyperresistinemia probably coupling to mediate the dephosphorylation and deactivation of adenosine 5'-monophosphate-activated protein kinase (AMPK) both in muscle and liver with up-regulated gene expression of the gluconeogenic enzymes[11,25,26]. This resulting impaired in muscle free fatty acids oxidation and hepatic glucose uptake, dephosphorylation of coenzyme A carboxylase (ACC), increased of gluconeogenesis and glycogenolysis in the liver which eventually lead to free fatty acids and glucose levels *in vivo* inclined[7,11,24].

Furthermore, the transcription of peroxisome proliferator-activated receptor α (PPARα) might further down regulated by coupling the serum hypoadiponectinemia and hyperresistinemia[27,28]. This lead to decrease the levels of molecules involved in free

fatty acid transport and energy dissipation which eventually decreased the free fatty acids oxidation[11,27,28]. Up-regulated of resistin followed by down-regulated of PPARγ had been showed to inhibit adipocyte differentiation which lead to the reduction of the serum adiponectin levels dramatically[29]. Therefore, the interaction effect of adiponectin and resistin showed greater impact in the regulation of glucose and lipid homoeostasis rather than adiponectin and resistin alone. A simple schematic of the possible unifying mechanism of adiponectin and resistin in linking insulin resistance with type 2 diabetes and metabolic syndrome was proposed as shown in the Supplementary Figure S1. However, the complete mechanisms behind the interaction effect of the adiponectin and resistin on modulating steps in the regulation of energy, glucose and lipid metabolisms merit further investigation.

The novel insulin resistance (IR_{AR}) index. Experimental results showed the QUICKI index had the most stongest correlation with the adiponectin-resistin (AR) index among the existing insulin resistance indexes (Table 3). Therefore, the QUICKI index was chosen for a novel insulin resistance index formulation by taking into account the AR index.

$$\text{QUICKI Index} = \frac{1}{\log_{10}(I_0) + \log_{10}(G_0)} \quad (6)$$

$$\text{AR Index} = 1 + \log_{10}(R_0) - \log_{10}(A_0) \quad (5)$$

Since, the values of QUICKI and AR indexes were significantly negative correlated (Table 3). Therefore, (5) and (6) are unifying by a multiplicative inverse as following

$$IR_{AR}\ Index = \frac{\text{AR Index}}{\text{QUICKI Index}} = \frac{1}{\text{QUICKI Index}} \times \text{AR Index}$$

$$= [\log_{10}(I_0) + \log_{10}(G_0)] \times [1 + \log_{10}(R_0) - \log_{10}(A_0)]$$

$$= \log_{10}(I_0 G_0)\left(1 + \log_{10}\left(\frac{R_0}{A_0}\right)\right) \quad (7)$$

Lastly, (7) is simplify to become a finalized IR_{AR} index as following

$$IR_{AR}\ Index = \log_{10}(I_0 G_0) + \log_{10}(I_0 G_0)\log_{10}\left(\frac{R_0}{A_0}\right) \quad (8)$$

Note: I_0 = fasting serum insulin levels in μU/mL;

G_0 = fasting serum glucose levels in mg/dL;

R_0 = fasting serum resistin levels in ng/mL;

A_0 = fasting serum adiponectin levels in μg/mL.

The novel IR_{AR} index showed higher sensitivity of the insulin resistance (IR) assessment in subjects with type 2 diabetes (T2DM) and metabolic syndrome (MS) compared to the other existing IR indexes (Table 4). Besides, the subjects with T2DM and MS showed higher values of the IR_{AR} index compared to the control subjects (Fig. 4). This indicated that the insulin resistance is predisposing to T2DM and MS susceptibility. In addition, the severity of the insulin resistance using the IR_{AR} index reached the peak when T2DM concurrently with MS (Fig. 4). Therefore, this indicated that the insulin resistance is play a principle role in predisposing T2DM to MS which trigger the developing of the diabetic complications[4,5].

These might be explained by the interaction of adiponectin and resistin on modulating steps in the insulin-signalling pathway and inducing insulin resistance[6,9,11,12,22]. Serum hypoadiponectinemia and hyperrestinemia antagonizes insulin signalling via inhibition of the insulin-induced phosphorylation of Akt independent of AMPK pathway, thereby increasing gluconeogenesis and hepatic glucose output[9,11,18,26,30]. The recent study showed it occurred by stimulated expression of glucose-6-phosphate (G6Pase), phosphoenolphyruvate carboxykinase (PEPCK), and suppressor of cytokine signalling 3 (SOC-3), repressed the expressions of insulin receptor substrate 2 (IRS-2) and glucose transporter 2 (GLUT2)[9,31]. Therefore, the coupling of serum hypoadiponectinemia and hyperresistinemia showed stronger induction of insulin resistance rather than serum hypoadiponectinemia and hyperresistinemia alone.

However, these study just a preliminary evaluation for the sensitivity and specificity of the novel insulin resistance (IR_{AR}) index. For the validation purposes, the euglycemic hyperinsulinemic clamp technique should be used instead as a gold standard index of insulin resistance to evaluate the sensitivity and specificity of the IR_{AR} index in the assessment of insulin resistance. Therefore, the potential of the novel IR_{AR} index as

an alternatif useful index for insulin resistance assessment in general population merit further investigation.

As a conclusion, the novel adiponectin-resistin (AR) index showed stronger association in linking insulin resistance with type 2 diabetes (T2DM) and metabolic syndrome (MS) rather than the serum adiponectin and resistin levels alone. Meanwhile, the novel insulin resistance (IR_{AR}) index is a potent useful index of insulin sensitivity in subjects with T2DM and MS.

Methods Summary

The subjects classify into three main groups included controls, type 2 diabetes mellitus (T2DM) and metabolic syndrome (MS). T2DM and MS subjects defined according to World Health Organization (WHO) 1999 diagnostic criteria and International Diabetes Federation (IDF) 2005 diagnostic criteria respectively. Serum adiponectin and resistin levels were determined using the ELISA assays. The other insulin resistance (IR) risk factors such as the serum fasting insulin, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, plasma glucose and whole blood HbA1C levels were tested. The clinical parameters included the blood pressure, body mass index (BMI), waist, and waist-to-hip ratio (WHR) also were measured and calculated. The indirect method of insulin resistance assessment performed by using the HOMA, QUICKI, Bennett, McAuley (1) and McAuley (2) indexes. Then, followed by the formulation of a novel adiponectin-resistin (AR) index by unifying the serum adiponectin and resistin levels. A novel insulin resistance (IR_{AR}) index was proposed by integrate the AR index into a chosen existing insulin resistance index for the modification. Lastly, statistical analysis were performed to evaluate the novel AR and IR_{AR} indexes in linking insulin resistance with T2DM and MS respectively.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Table 1 The correlation of the single insulin resistance risk factors.

IR Risk Factors	Adiponectin [J] (μg/mL) (n = 809) P_a (r_a) P_b (r_b)	Resistin [J] (ng/mL) (n = 809) P_a (r_a) P_b (r_b)	Adiponectin [J] + Resistin [J] (n = 809) P_c (r_c) P_d (r_d)	AR Index [J] (n = 809) P_a (r_a) P_b (r_b)
BMI (kg/m^2) [J]	0.000*** (-0.160) [P]	0.000*** (+0.123) [P]	0.000*** (0.192)	0.000*** (+0.185) [P]
	0.000*** (-0.146)	0.000*** (+0.129)	0.000*** (0.195)	0.000*** (+0.184)
Waist (cm) [J]	0.000*** (-0.190) [P]	0.000*** (+0.175) [P]	0.000*** (0.246)	0.000*** (+0.245) [P]
	0.000*** (-0.186)	0.000*** (+0.177)	0.000*** (0.256)	0.000*** (+0.245)
WHR [B]	0.000*** (-0.210) [S]	0.000*** (+0.212) [S]	0.000*** (0.278)	0.000*** (+0.270) [S]
	0.000*** (-0.235)	0.000*** (+0.187)	0.000*** (0.300)	0.000*** (+0.278)
Systolic BP (mmHg) [B]	0.414 (-0.029) [S]	0.134 (+0.053) [S]	0.142 (0.069)	0.562 (+0.020) [S]
	0.629 (-0.017)	0.104 (+0.057)	0.060 (0.060)	0.398 (+0.030)
Diastolic BP (mmHg) [B]	0.204 (-0.045) [S]	0.397 (+0.030) [S]	0.743 (0.027)	0.054 (+0.068) [S]
	0.356 (-0.033)	0.580 (+0.019)	0.310 (0.038)	0.373 (+0.031)
Total cholesterol (mmol/L) [J]	0.032* (+0.076) [P]	0.124 (-0.054) [P]	0.041* (0.089)	0.013* (-0.087) [P]
	0.004** (+0.101)	0.163 (-0.049)	0.000*** (0.113)	0.005** (-0.099)
HDL cholesterol (mmol/L) [J]	0.000*** (+0.362) [P]	0.006** (-0.097) [P]	0.000*** (0.366)	0.000*** (-0.276) [P]
	0.000*** (+0.352)	0.004** (-0.102)	0.000*** (0.367)	0.000*** (-0.274)
LDL cholesterol (mmol/L) [J]	0.112 (+0.056) [P]	0.260 (-0.040) [P]	0.177 (0.065)	0.093 (-0.059) [P]
	0.033* (+0.075)	0.316 (-0.035)	0.008** (0.083)	0.054 (-0.068)
Triglyceride (mmol/L) [B]	0.000*** (-0.221) [P]	0.459 (+0.026) [P]	0.000*** (0.221)	0.000*** (+0.138) [P]
	0.000*** (-0.205)	0.362 (+0.032)	0.000*** (0.209)	0.000*** (+0.133)
Glucose (mmol/L) [B]	0.000*** (-0.173) [S]	0.000*** (+0.307) [S]	0.000*** (0.302)	0.000*** (+0.328) [S]
	0.000*** (-0.143)	0.000*** (+0.280)	0.000*** (0.316)	0.000*** (+0.302)
HbA1C (%) [B]	0.000*** (-0.226) [S]	0.000*** (+0.328) [S]	0.000*** (0.359)	0.000*** (+0.372) [S]
	0.000*** (-0.211)	0.000*** (+0.311)	0.000*** (0.376)	0.000** (+0.359)
Insulin (μU/mL) [B]	0.000*** (-0.237) [S]	0.000*** (+0.160) [S]	0.000*** (0.256)	0.000*** (+0.239) [S]
	0.000*** (-0.223)	0.000*** (+0.131)	0.000*** (0.259)	0.000*** (+0.222)
Adiponectin (μg/mL) [J]	NA	0.003** (-0.105) [P]	NA	NA
	NA	0.002** (-0.110)	NA	NA
Resistin (ng/mL) [J]	0.003** (-0.105) [P]	NA	NA	NA
	0.002** (-0.110)	NA	NA	NA

For descriptive purposes, the sign of correlation coefficient (+/-) are presented using untransformed variables.
For hypothesis testing purposes, the values of correlation coefficient (r) is presented using transformed variables.
Skewed variables or outliers dealing by using Johnson transformation (J) [1] or Box-Cox Power Transformation (B) [1].
Proceed with non-parametric test for skewed variables and/or if the outliers keep emerging after the data transformation.
r_a = Pearson Product Moment Correlation Coefficient (P) [2] / Spearman's Rho Rank Correlation Coefficient (S) [2]
r_b = Partial Correlation Coefficient using 10,000 Stratified Bootstrap Samples with Bias Corrected and Accelerated 95% CI [2]
(multiple testing bias corrections with controlling for covariate ages and stratified ethnics, T2DM and MS status)
r_c = Multiple Correlation Coefficient [3]
r_d = Partial Redundancy Correlation Coefficient with permutation 10,000X [4]
(controlling for covariate ages, ethnics, T2DM and MS)
[1] Minitab 15 Program [2] PASW Statistics 18 [3] statistiXL 1.8 Program [4] XLSTAT Program
Significant levels: P*<0.05, P**<0.01, P***<0.001
Note: IR = insulin resistance; BMI = body mass index; WHR = waist-to-hip ratio; BP = blood pressure; NA = not applicable

Table 2 The correlation of the multiple insulin resistance risk factors.

IR Risk Factors	Adiponectin [J] (μg/mL) (n=809) P_a (r_a)	Resistin [J] (ng/mL) (n=809) P_a (r_a)	Adiponectin [J] + Resistin [J] (n=809) P_b (r_b)	AR Index [J] (n=809) P_a (r_a)
2 Variables Combination :				
BMI [J] + HDL [J]	0.000*** (0.374)	0.000*** (0.145)	0.000*** (0.384)	0.000*** (0.309)
BMI [J] + TG [B]	0.000*** (0.253)	0.002** (0.123)	0.000*** (0.260)	0.000*** (0.214)
BMI [J] + Insulin [B]	0.000*** (0.242)	0.000*** (0.147)	0.000*** (0.270)	0.000*** (0.248)
Waist [J] + HDL [J]	0.000*** (0.381)	0.000*** (0.186)	0.000*** (0.396)	0.000*** (0.337)
Waist [J] + TG [B]	0.000*** (0.271)	0.000*** (0.175)	0.000*** (0.290)	0.000*** (0.265)
Waist [J] + Insulin [B]	0.000*** (0.249)	0.000*** (0.181)	0.000*** (0.288)	0.000*** (0.275)
SBP [B] + DBP [B]	0.720 (0.029)	0.021* (0.098)	0.065 (0.103)	0.216 (0.062)
TC [J] + LDL [J]	0.072 (0.081)	0.254 (0.058)	0.121 (0.095)	0.020* (0.098)
HDL [J] + LDL [J]	0.000*** (0.362)	0.015* (0.102)	0.000*** (0.367)	0.000*** (0.278)
HDL [J] + TG [B]	0.000*** (0.373)	0.020* (0.098)	0.000*** (0.377)	0.000*** (0.279)
HDL [J] + Insulin [B]	0.000*** (0.385)	0.000*** (0.141)	0.000*** (0.395)	0.000*** (0.316)
TG [B] + Insulin [B]	0.000*** (0.288)	0.002** (0.126)	0.000*** (0.298)	0.000*** (0.244)
Glucose [B] + HbA1C [B]	0.000*** (0.221)	0.000*** (0.315)	0.000*** (0.364)	0.000*** (0.365)
Glucose [B] + Insulin [B]	0.000*** (0.265)	0.000*** (0.288)	0.000*** (0.357)	0.000*** (0.358)
HbA1C [B] + Insulin [B]	0.000*** (0.295)	0.000*** (0.313)	0.000*** (0.397)	0.000*** (0.395)
3 Variables Combination:				
BMI [J] + HDL [J] + TG [B]	0.000*** (0.383)	0.001** (0.147)	0.000*** (0.390)	0.000*** (0.309)
BMI [J] + HDL [J] + Insulin [B]	0.000*** (0.387)	0.000*** (0.158)	0.000*** (0.399)	0.000*** (0.326)
BMI [J] + TG [B] + Insulin [B]	0.000*** (0.292)	0.001** (0.147)	0.000*** (0.305)	0.000*** (0.259)
Waist [J] + HDL [J] + TG [B]	0.000*** (0.389)	0.000*** (0.188)	0.000*** (0.401)	0.000*** (0.338)
Waist [J] + HDL [J] + Insulin [B]	0.000*** (0.390)	0.000*** (0.188)	0.000*** (0.406)	0.000*** (0.345)
Waist [J] + TG [B] + Insulin [B]	0.000*** (0.298)	0.000*** (0.181)	0.000*** (0.317)	0.000*** (0.285)
HDL [J] + LDL[J] + TG [B]	0.000*** (0.374)	0.036* (0.103)	0.000*** (0.378)	0.000*** (0.281)
HDL[J] + LDL [J] + Insulin [B]	0.000*** (0.385)	0.001** (0.143)	0.000*** (0.395)	0.000*** (0.317)
HDL [J] + TG [B] + Insulin [B]	0.000*** (0.391)	0.001** (0.143)	0.000*** (0.400)	0.000*** (0.316)
Glucose [B] + HbA1C [B] + Insulin [B]	0.000*** (0.295)	0.000*** (0.322)	0.000*** (0.400)	0.000*** (0.400)
4 Variables Combination:				
BMI [J] + HDL[J] + TG [B] + Insulin [B]	0.000*** (0.393)	0.000*** (0.161)	0.000*** (0.403)	0.000*** (0.326)
Waist [J] + HDL[J] + TG [B] + Insulin [B]	0.000*** (0.396)	0.000*** (0.191)	0.000*** (0.409)	0.000*** (0.345)
HDL[J] + LDL[J] + TG [B] + Insulin [B]	0.000*** (0.392)	0.002** (0.145)	0.000*** (0.400)	0.000*** (0.317)

Skewed variables or outliers dealing by using Johnson transformation (J) [1] or Box-Cox Power Transformation (B) [1].
Factor Analysis with Principle Component Extraction Method [3] used to identify factors combination for multiple correlations.
r_a = Multiple Correlation Coefficient [2] r_b = Canonical Correlation Coefficient [2]
[1] Minitab 15 Program [2] statistiXL 1.8 Program [3] XLSTAT Program
Significant levels : P*<0.05, P**<0.01, P***<0.001
Note: IR = insulin resistance; BMI = body mass index; WHR = waist-to-hip ratio; BP = blood pressure; NA = not applicable

Table 3 The correlation of the insulin resistance indexes.

IR Indexes	Adiponectin [(J)] (n = 809) P_a (r_a) P_b (r_b)	Resistin [(J)] (n = 809) P_a (r_a) P_b (r_b)	Adiponectin [(J)] + Resistin [(J)] (n = 809) P_c (r_c) P_d (r_d)	AR Index [(J)] (n = 809) P_a (r_a) P_b (r_b)
HOMA Index [(B)]	0.000*** (-0.258) [P] 0.000*** (-0.243)	0.000*** (+0.224) [P] 0.000*** (+0.232)	0.000*** (0.325) 0.000*** (0.340)	0.000*** (+0.319) [P] 0.000*** (+0.313) [P]
QUICKI Index [(J)]	0.000*** (+0.290) [P] 0.000*** (+0.275)	0.000*** (-0.247) [P] 0.000*** (-0.241)	0.000*** (0.389) 0.000*** (0.364)	0.000*** (-0.364) [P] 0.000*** (-0.358)
Bennett Index [(J)]	0.000*** (+0.276) [P] 0.000*** (+0.263)	0.000*** (-0.221) [P] 0.000*** (-0.229)	0.000*** (0.336) 0.000*** (0.351)	0.000*** (-0.330) [P] 0.000*** (-0.329)
McAuley (1) Index [(J)]	0.000*** (+0.286) [P] 0.000*** (+0.271)	0.002** (-0.109) [P] 0.001** (-0.117)	0.000*** (0.297) 0.000*** (0.298)	0.000*** (-0.242) [P] 0.000*** (-0.235)
McAuley (2) Index [(J)]	0.000*** (+0.288) [P] 0.000*** (+0.272)	0.001** (-0.118) [P] 0.000*** (-0.126)	0.000*** (0.301) 0.000*** (0.303)	0.000*** (-0.250) [P] 0.000*** (-0.244)

For descriptive purposes, the sign of correlation coefficient (+/-) are presented using untransformed variables.
For hypothesis testing purposes, the values of correlation coefficient (r) is presented using transformed variables.
Skewed variables or outliers dealing by using Johnson transformation (J) [1] or Box-Cox Power Transformation (B) [1].
r_a = Pearson Product Moment Correlation Coefficient (P) / Spearman's Rho Rank Correlation Coefficient (S) [2]
r_b = Partial Correlation Coefficient using 10,000 Stratified Bootstrap Samples with Bias Corrected and Accelerated 95% CI [2]
(multiple testing bias corrections with controlling for covariate ages and stratified ethnics, T2DM and MS status)
r_c = Multiple Correlation Coefficient [3]
r_d = Partial Redundancy Correlation Coefficient with permutation 10,000X [4]
(controlling for covariate ages, ethnics, T2DM and MS)
[1] Minitab 15 Program [2] PASW Statistics 18 [3] statistiXL 1.8 Program [4] XLSTAT Program
Note: IR = insulin resistance; HOMA = Homeostasis Model Assessments index;
QUICKI = Quantitative Insulin Sensitivity Check index Significant levels: P*<0.05, P**<0.01, P***<0.001

Table 4 The sensitivity and specificity of the insulin resistance indexes.

IR Indexes	T2DM Sensitivity (%) 95% CI (%)			MS Sensitivity (%) 95% CI (%)		
	Without MS (208 controls) (171 cases) (n = 379)	With MS (208 controls) (256 cases) (n = 464)	Total (208 controls) (427 cases) (n = 635)	Without T2DM (208 controls) (174 cases) (n = 382)	With T2DM (208 controls) (256 cases) (n = 464)	Total (208 controls) (430 cases) (n = 638)
HOMA Index (IR ≥ 4.520)	36.30 (29.40, 43.70)	65.60 (59.60, 71.20)	53.90 (49.10, 58.50)	34.50 (27.80, 41.80)	65.60 (59.60, 71.20)	53.00 (48.30, 57.70)
QUICKI Index (IR ≤ 0.307)	36.30 (29.40, 43.70)	65.60 (59.60, 71.20)	53.90 (49.10, 58.50)	34.50 (27.80, 41.80)	65.60 (59.60, 71.20)	53.00 (48.30, 57.70)
Bennett Index (IR ≤ 0.202)	40.40 (33.30, 47.80)	71.90 (66.10, 77.00)	59.30 (54.50, 63.80)	33.90 (27.30, 41.20)	71.90 (66.10, 77.00)	56.50 (51.80, 61.10)
McAuley (1) Index (IR ≤ 5.360)	14.60 (10.10, 20.80)	53.10 (47.00, 59.10)	37.70 (33.20, 42.40)	54.00 (46.60, 61.30)	53.10 (47.00, 59.10)	53.50 (48.80, 58.10)
McAuley (2) Index (IR ≤ 5.660)	12.30 (8.10, 18.10)	57.40 (51.30, 63.30)	39.30 (34.80, 44.10)	57.50 (50.00, 64.60)	57.40 (51.30, 63.30)	57.40 (52.70, 62.00)
IR_{AR} Index (IR ≥ 4.683)	47.40 (40.00, 54.80)	73.80 (68.10, 78.80)	63.20 (58.60, 67.70)	42.50 (35.40, 50.00)	73.80 (68.10, 78.80)	60.70 (56.00, 65.20)

Data are expressed as values (95% Confident Interval) in % using XLSTAT Program.
For the purposes of the sensitivity comparison, the specificity for all insulin resistance (IR) indexes was maintained at 90%.
Test data values threshold (X ≤ IR or IR ≥ X) indicated the positive event of insulin resistance (IR) assessment in subjects.
Note: T2DM = type 2 diabetes; MS = metabolic syndrome; IR = insulin resistance

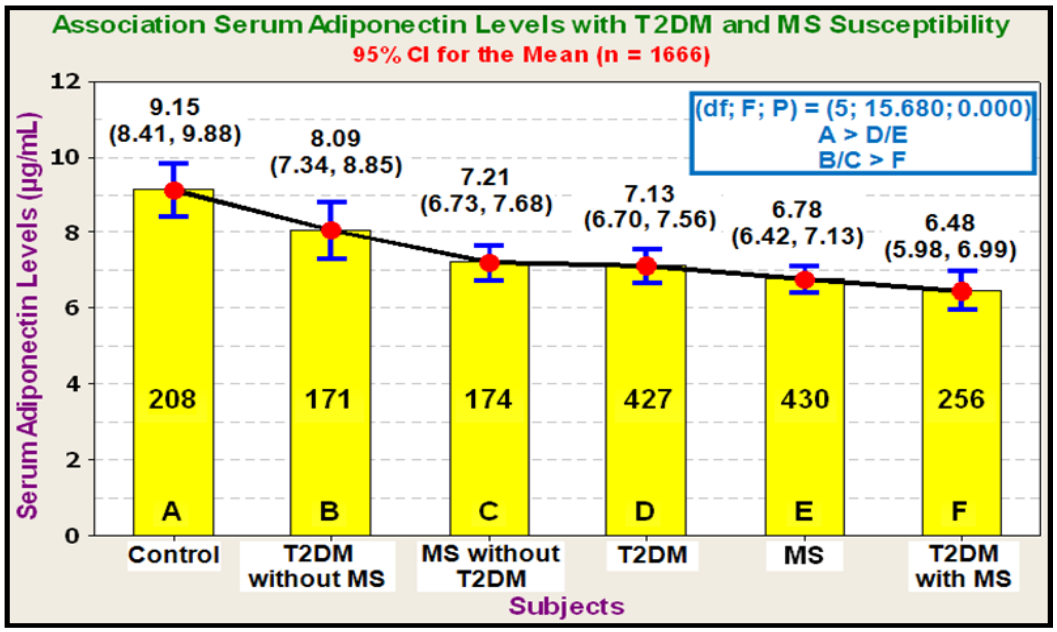

Figure 1 | **The association of the serum adiponectin levels with T2DM and MS susceptibility.** For descriptive purposes, the mean values with 95% CI are presented using untransformed and unadjusted variables. For hypothesis testing purposes, the variable was transformed using Box-Cox Power Transformation [1]. ANCOVA Test [2] in General Linear Model was used with adjustment the covariate effect of ages and ethnics. Pairwise Comparisons using 1,000 Stratified Bootstrap Samples with Bias Corrected and Accelerated (BCa) 95% CI [2] for multiple testing bias corrections with adjusted covariate effect of ages and stratified ethnic's status. The location of statistically significant differences are showed in the legend of the Figure 1 as A > D/E and B/C > F. Two-tailed p-value was used for the tests. [1] Minitab 15 Program [2] PASW Statistics 18 Program Note: T2DM = type 2 diabetes; MS = metabolic syndrome.

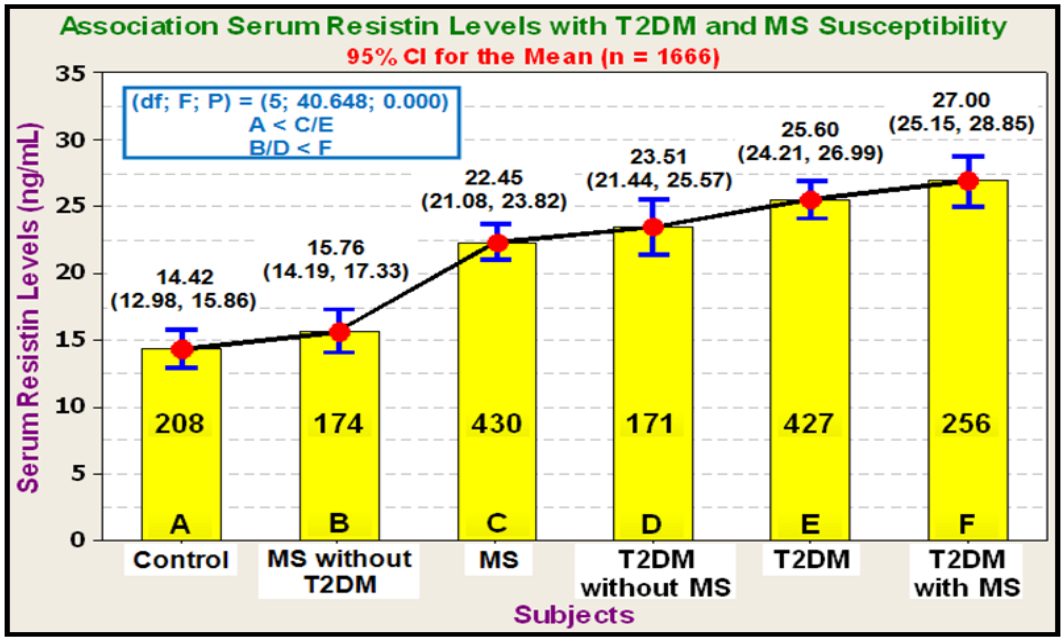

Figure 2 | The association of the serum resistin levels with T2DM and MS susceptibility. For descriptive purposes, the mean values with 95% CI are presented using untransformed and unadjusted variables. For hypothesis testing purposes, the variable was transformed using Johnson Transformation [1]. ANCOVA Test [2] in General Linear Model was used with adjustment the covariate effect of ages and ethnics. Pairwise Comparisons using 1,000 Stratified Bootstrap Samples with Bias Corrected and Accelerated (BCa) 95% CI [2] for multiple testing bias corrections with adjusted covariate effect of ages and stratified ethnic's status. The location of statistically significant differences are showed in the legend of the Figure 2 as A< C/E and B/D < F. Two-tailed p-value was used for the tests. [1] Minitab 15 Program [2] PASW Statistics 18 Program Note: T2DM = type 2 diabetes; MS = metabolic syndrome.

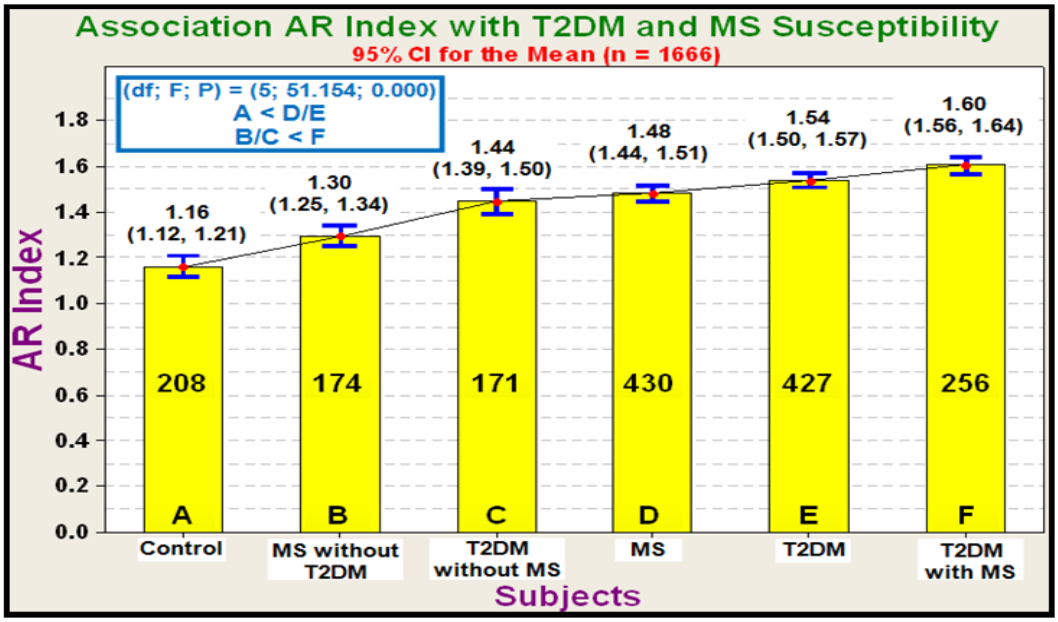

Figure 3 | The association of the adiponectin-resistin (AR) index with T2DM and MS susceptibility. For descriptive purposes, the mean values with 95% CI are presented using unadjusted variables. No transformation of the variables had been done since variable is normally distributed, no outlier detected and homogeneity of variances for each group of subject. ANCOVA Test [2] in General Linear Model was used with adjustment the covariate effect of ages and ethnics. Pairwise Comparisons using 1,000 Stratified Bootstrap Samples with Bias Corrected and Accelerated (BCa) 95% CI [2] for multiple testing bias corrections with adjusted covariate effect of ages and stratified ethnic's status. The location of statistically significant differences are showed in the legend of the Figure 3 as A< D/E and B/C < F. Two-tailed p-value was used for the tests. [1] Minitab 15 Program [2] PASW Statistics 18 Program Note: T2DM = type 2 diabetes; MS = metabolic syndrome.

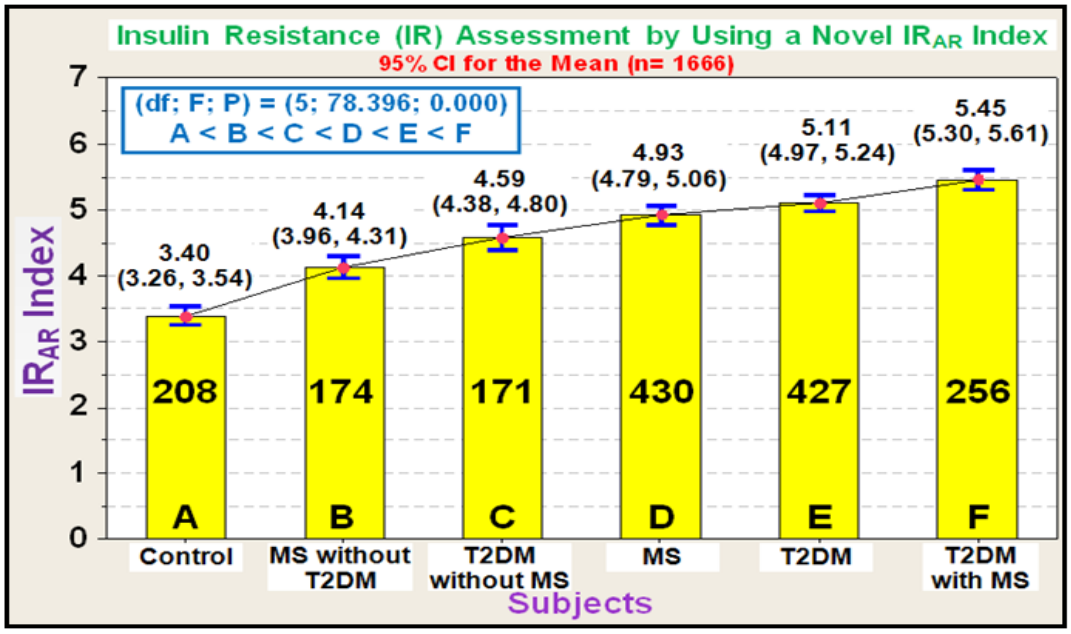

Figure 4 | The assessment of insulin resistance in subjects with T2DM and MS by using a novel IR_{AR} index. For descriptive purposes, the mean values with 95% CI are presented using unadjusted variables. No transformation of the variables had been done since variable is normally distributed, no outlier detected and homogeneity of variances for each group of subject. ANCOVA Test [2] in General Linear Model was used with adjustment the covariate effect of ages and ethnics. Pairwise Comparisons using 1,000 Stratified Bootstrap Samples with Bias Corrected and Accelerated (BCa) 95% CI [2] for multiple testing bias corrections with adjusted covariate effect of ages and stratified ethnic's status. The location of statistically significant differences are showed in the legend of the Figure 4 as A< B < C < D < E < F. Two-tailed p-value was used for the tests. [1] Minitab 15 Program [2] PASW Statistics 18 Program Note: IR = insulin resistance; T2DM = type 2 diabetes; MS = metabolic syndrome.

Methods

Subjects. All subjects were native to Malaysia. The ages for all subjects were restricted to 40-70 years old and males in gender for the homogeneity of the subjects purposes. The subjects comprised three primary ethnic groups of Malaysian subjects which were Malay, Chinese and Indian. All the subjects were from the University Malaya Medical Centre (UMMC). The study design used was randomized case-control study. Ethical clearance (612.17) to undertake this study was obtained from the UMMC Ethics Committee and informed consent was obtained from each subject. Each subject received a detailed questionnaire about the personal and family disease history followed by the demographic data measurement.

The subjects classify into three main groups which were controls, type 2 diabetes mellitus (T2DM) and metabolic syndrome (MS). T2DM and MS subjects defined according to World Health Organization (WHO) 1999 diagnostic criteria[32] and International Diabetes Federation (IDF) 2005 diagnostic criteria[33] respectively. T2DM subjects further divided into subgroups without MS and with MS. Meanwhile, MS subjects further divided into subgroups without T2DM and with T2DM. The criteria for each group of subjects defined with clearly in the Supplementary Methods. The minimum samples size for each group of subjects calculated by using the PS Power and Sample Size Calculation Version 3.0.12 Program[6]. The justification for the samples size calculation explained with detail in the Supplementary Methods.

Serum adiponectin and resistin levels measurement. Serum adiponectin levels were determined using the AssayMax Human Adiponectin ELISA Kit (AssayPro, USA) while serum resistin levels were detected using the AssayMax Human Resistin Elisa Kit (AssayPro, USA). For the adiponectin assayed, the intra-assay and inter-assay coefficients of variation were 4.1% and 7.2% respectively with a sensitivity of 0.5ng/ml.

Meanwhile, the intra-assay and inter-assay coefficients of variation were 4.0% and 7.2% respectively with a sensitivity of <100pg/mL for the resistin assayed.

Other insulin resistance risk factors measurement. The other biochemical parameters including serum fasting insulin, total cholesterol, LDL cholesterol[34], HDL cholesterol, triglycerides, plasma glucose and whole blood HbA1C levels were tested. The clinical parameters including the blood pressure, body mass index (BMI), waist, and waist-to-hip ratio (WHR) also were measured and calculated. The procedure of the measurements for these biochemical and clinical parameters are shown detailed in the Supplementary Methods.

Insulin resistance assessment. The indirect methods of the insulin sensitivity evaluation included the Homeostasis Model Assessments index (HOMA)[35], Quantitative Insulin Sensitivity Check index (QUICKI)[35], Bennett index[36], McAuley (1) index[35] and McAuley (2)[36] index were used to assess the insulin resistance for each subjects. The mathematical equations for each insulin resistance indexes with its corresponding components' units are shown in the Supplementary Table S1.

The formulation and evaluation of the novel AR and IR_{AR} indexes. The experimental results used to derive a novel adiponectin-resistin index (AR) by unifying the effect of serum adiponectin and resistin levels. Then, a novel insulin resistance (IR_{AR}) index was proposed by integrate the AR index into a chosen existing insulin resistance index for the modification. The sensitivity and specificity of the novel IR_{AR} indexes as compared to the other existing insulin resistance indexes were evaluated by XLSTAT program (Addinsoft, USA). Lastly, statistical analysis were performed to evaluate the novel AR and IR_{AR} indexes in linking insulin resistance with type 2 diabetes and metabolic syndrome respectively.

Statistical analysis. Classical statistical analysis included the normality test (Anderson-Darling test followed by P-P and Q-Q plots), equality of variance test (Barlett's test or Levene's test), outliers' detection (Box plot) and data transformation were performed using the Minitab 15 Program (Minitab Inc, USA). The best fitted transformation of variables perfomed with Johnson transformation or Box-Cox transformation. Meanwhile, the resampling statistical analysis included the analysis of covariance (ANCOVA) and Partial Correlation test with bootstrapping method using Bias Corrected and Accelerated (BCa) 95% CI were performed using the PASW Statistics 18 Program (SPSS Inc, USA). The statistiXL 1.8 Program (Addinsoft, USA) was used for the parametric Pearson Product Moment Correlation test, nonparametric Spearman's Rho Rank Correlation test, Multiple Correlation test and Factor Analysis. Meanwhile, the XLSTAT Program (Addinsoft, USA) was used for the Canonical Correlation and Partial Redundancy Correlation tests. Data are expressed as mean (95% confident interval) for parametric test and median (95% confident interval) for nonparametric test. All p-values were two-tailed, and p-values below 0.05 were considered statistically significant. The details of statistical analysis used with its justification are shown in Supplementary Notes.

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33. The IDF consensus worldwide definition of the metabolic syndrome. 1-23 (International Diabetes Federation (IDF), Belgium, 2006).
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