UNIVERSITI SAINS MALAYSIA

Laporan Akhir
Projek Penyelidikan USM Jangka Pendek
(No Akaun: 304/PPSP/6131320)

The insulin sensitivity of non-obese Malay subjects and the relationship between hyperlipidemia with insulin sensitivity

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Univesriti Sains Malaysia

Melalui
Timbalan Dekan Penyelidikan
Pusat Pengajian Sains Perubatan
Kampus Kesihatan
Universiti Sains Malaysia

Daripada
Dr. Mohd Hashim Mohd Hassan
Jabatan Perubatan Masyarakat

Tarikh 20 September 2005

Tuan/Puan

Laporan Akhir projek Penyelidikan USM Jangka Pendek
(No Akaun: 304/PPSP/6131320)

Sukacitnya perkara diatas dirujuk.
Bersama-sama ini disertakan laporan akhir yang telah disi bersama-sama penyata perbelanjaan dan ringkasan lapuran kajian yang telah dibuat.

Sekian terima kasih

BERSAING DI PERINGKAT DUNIA: KOMITMEN KITA

Dr. Mohd Hashim Mohd Hassan

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BAHAGIAN PENYELIDIKAN & PEMBANGUNAN
CANSELORI
UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

1) Nama Penyelidik:: Dr. Mohd. Hashim Mohd. Hassan

Nama Penyelidik-Penyelidik Lain (Jika berkaitan) : Dr. Abu Kholdun Al-Mahmood

2) Pusat Pengajian/Pusat/Unit : Pusat Pengajian Sains Perubatan

3) Tajuk Projek : The insulin sensitivity of non-obese Malay subjects and the relationship between hyperlipidemia with insulin sensitivity

4) (a) Penemuan Projek/Abstrak
(Perlu disediakan makluman di antara 100 – 200 perkataan di dalam Bahasa Malaysia dan Bahasa Inggeris. Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).

: Seperti di lampiran/mnuscript

(b) Senaraikan Kata Kunci yang digunakan di dalam abstrak:

Bahasa Malaysia	Bahasa Inggeris
Sensiviti insulin	Insulin sensitivity, HOMA%S, HOMA%R, HOMA-IR,

5) Output Dan Faedah Projek:

(a) Penerbitan (termasuk laporan/kertas seminar)
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

A. List of presentations based on this study

1. Title: Insulin sensitivity status of non-obese normoglycemic Malay subjects: Relationship between insulin sensitivity and lipid status.

   Authors: A. Kholdun Al-Mahmood *et al.*

   Conference: 9th National Conference on Medical Scinces

   Venue: Health Campus, USM

   Date: 22-23 May, 2004

2. Title: Insulin resistance in non-obese nondiabetic population

   Authors: A. Kholdun Al-Mahmood *et al.*

   Conference: 1st Post Graduate Research Colloquium

   Venue: Health Campus, USM

   Date: 14th August, 2004

3. Title: Insulin sensitivity status of non-obese normoglycemic Malay subjects.

   Authors: A. Kholdun Al-Mahmood *et al.*

   Conference: 29th Annual Conference of the Malaysian Society for Biochemistry and Molecular Biology

   Venue: KualaLumpur

   Date: 28-29 September, 2004

4. Title: Insulin sensitivity status of non-obese normoglycemic Malay subjects.

   Authors: A. Kholdun Al-Mahmood *et al.*

   Conference: 10th National Conference on Medical Scinces

   Venue: Health Campus, USM

   Date: 22-23 May, 2005

B. Articles send for publication

1. Title: Insulin sensitivity of non-obese non-diabetic Malay subjects: relationship with lipid status.

   Authors: Dr. AK Al-Mahmood *et al*

   Article is accepted for publication in the International Medical Journal (Japan).

2. Title:Insulin sensitivity and secretory status of healthy Malaysian subjects: Benefits of keeping BMI within limits

   Authors: Dr. AK Al-Mahmood *et al*

   Article is in process of review in the Malaysian Journal Of Medical Sciences (MJMS).

C. Articles waiting to send for publication or in preparation.

1. Title: Isolated hypercholesterolemia: Its relationship with insulin sensitivity

   Authors: Dr. AK Al-Mahmood *et al*

2. Title: Isolated hypertriglyceridemia: An insulin resistant state with or without low HDL cholesterol

   Authors: Dr. AK Al-Mahmood *et al*

3. Title: Mixed hyperlipidemia in non-obese Malay subjects: Its relationship with insulin sensitivity

Authors: Dr. AK Al-Mahmood *et al*

(b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.
(Jika ada dan jika perlu, sila guna kertas berasingan): Tiada

(c) Latihan Gunatenaga Manusia

Pelajar Siswazah: Dr. Abu Kholdun Al-Mahmood

ii) Pelajar Prasiswazah:

iii) Lain-Lain :

6. Peralatan Yang Telah Dibeli: Tiada

UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

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T/TANGAN PENGERUSI
J/K PENYELIDIKAN
PUSAT PENGAJIAN

PROFESSOR ABDUL AZIZ BABA
Chairman of Research & Ethics Committee
School of Medical Sciences
Health Campus
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan

UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN WANG PENYELIDIKAN GERAN USM(304)
PENYATA PERBELANJAAN SEHINGGA 31 OGOS 2005

Jumlah Geran:	RM	19,970.00	Ketua Projek:	DR. MOHD HASHIM MOHD HASSAN
Peruntukan 2004 (Tahun 1)	RM	9,000.00	Tajuk Projek:	The Insulin Sensitivity Status of Non-Obese Hyperlipidemic Subjects and the Relationship Between Hyperlipidemia with Insulin Sensitivity
Peruntukan 2005 (Tahun 2)	RM	10,970.00		
Peruntukan 2006 (Tahun 3)	RM	0.00	Tempoh:	01 April 04- 31 Mac 06
			No.Akaun:	304/PPSP/6131320

Kwg	Akaun	PTJ	Projek	Donor	Peruntukan Projek	Perbelanjaan T'kumpul Hingga Tahun Lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
304	11000	PPSP	6131320		-	-	-	-	-	-	-
304	14000	PPSP	6131320		-	380.72	(380.72)	-	146.78	146.78	(527.50)
304	15000	PPSP	6131320		-	-	-	-	-	-	-
304	21000	PPSP	6131320		-	169.00	(169.00)	-	669.80	669.80	(838.80)
304	22000	PPSP	6131320		-	-	-	-	-	-	-
304	23000	PPSP	6131320		200.00	201.76	(1.76)	-	481.70	-	(483.46)
304	24000	PPSP	6131320		-	-	-	-	-	-	-
304	25000	PPSP	6131320		-	-	-	-	-	-	-
304	26000	PPSP	6131320		-	30.00	(30.00)	-	10.00	10.00	(40.00)
304	27000	PPSP	6131320		10,970.00	509.00	10,461.00	3,100.00	593.70	3,693.70	6,767.30
304	28000	PPSP	6131320		-	-	-	-	-	-	-
304	29000	PPSP	6131320		8,800.00	6,945.90	1,854.10	1,800.00	4,706.40	6,506.40	(4,652.30)
304	32000	PPSP	6131320		-	-	-	-	-	-	-
304	35000	PPSP	6131320		-	-	-	-	-	-	-
					19,970.00	8,236.38	11,733.62	4,900.00	6,608.38	11,026.68	225.24

The insulin sensitivity of non-obese Malay subjects and the relationship between hyperlipidemia with insulin sensitivity

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ABSTRACT

Introduction: Hyperlipidaemia and insulin resistance may have a relationship. Most of the previous studies looked at insulin resistance in hyperlipidaemic subjects who were also obese. So influence of obesity and hyperlipidaemia acted simultaneously in the genesis of insulin resistance.

Objective: To determine insulin sensitivity and secretory status of non-obese normoglycemic Malay subjects, and to study the relationship between hyperlipidemia and insulin sensitivity in such population.

Methodology: A cross sectional study on 246 non-obese (BMI<25kg/m^2, waist circumference male<102cm, female <88cm.) and non-diabetic subjects age between 30-60 years was carried out. Fasting plasma glucose, fasting insulin and lipid profile were done. Insulin sensitivity and secretory status was calculated using homeostasis model assessment (HOMA) software (HOMA%S, HOMA%B and HOMA-IR). The subjects were divided into two groups according to their lipid status (128 normolipidemic and 118 hyperlipidemic) and their insulin sensitivity was compared.

Results: The hyperlipidemic subjects showed substantially lower insulin sensitivity and higher insulin resistance in comparison to normolipidemic subjects. The mean of HOMA%S of hyperlipidemic and normolipidemic subjects were 80 and 155 ($p<.0001$) respectively. The mean of relative insulin resistance HOMA-IR was 2.66 in hyperlipidemic subjects which was 1.05 in normolipidemic subjects. Insulin secretory status (HOMA%B) of two group were 178 and 116 respectively.

Conclusion: Insulin sensitivity of otherwise healthy non-obese hyperlipidemic subjects is lower than normolipidemic subjects. The B cells of hyperlipidemic subjects have to work more to compensate lowered insulin sensitivity.

Key words: insulin sesnsitivity, HOMA%S, HOMA%B, HOMA-IR

INTRODUCTION

Insulin resistance is a common phenomenon and plays a central role in the pathogenesis and clinical course of several human diseases. It is also associated with various metabolic and physiological abnormalities including obesity, hypertension, hyperlipidaemia and glucose intolerance (Boden, 2001). Its relation with obesity is established and a wealth of epidemiological studies has been done in exploring and explaining this relationship (Lillioja and Bogardus, 1988; Ferrannini *et al*, 1997; Kahn, 2003). It is also associated with hyperlipidaemia (Defronzo and Ferrannini, 1991). Hyperlipidaemia and insulin sensitivity have an interaction, whether insulin resistance is secondary to hyperlipidaemia or hyperlipidaemia follows insulin resistance the matter is yet to be known (Reaven, 1988; Doherty *et al*, 1997).

Most of the previous studies on insulin sensitivity were performed on hyperlipidaemic subjects who were also obese. So influence of obesity and hyperlipidaemia acted simultaneously in the genesis of insulin resistance.

Importance of the present study:

It seems important to note that even modest change in lipid status may influence insulin sensitivity very much. Studies indicate that the metabolic syndrome is becoming a rapidly rising non-communicable disease around the world. Especially its prevalence rising with improvement of economic and social status of peoples of the developing countries as well as in developed countries. Study done by Mafauzy *et al* in 1999 shows that 57% of normal Malay subjects are hypercholesterloemic. Studies also show high prevalence of other features of metabolic syndrome in Malaysia, e.g. obesity (43-52% are either overweight or obese), hypertension (10-37%), hyperlipidemia (63-76%) (Mustaffa, 2004).

So far the metabolic syndrome as a whole is being discussed much. But it is still unsettled whether this syndrome represents a fortuitous cluster of metabolic features or whether insulin resistance itself is the common etiological factor behind all features of the syndrome (Sum *et al*, 1992). It is also unclear whether insulin resistance is present in each feature of the disease. If it is established by research we can come to conclusion that hyperinsulinemia or insulin resistance is the aetiological link between other features of metabolic syndrome. So we plan to isolate a group of people who are otherwise normal (i.e. free from all features of MS) but having only hyperlipidemia and then study their insulin sensitivity in order to find out the linkage between lipid abnormalities and insulin sensitivity.

METHODOLOGY

Study Design

This cross-sectional study was conducted from mid September 2003 to March 2005 which involved both outdoor and on-campus laboratory-based activities. Research volunteers were recruited from schools and public offices in Kota Bharu, the capital city of the state of Kelantan in northeastern peninsular West Malaysia. We circulated an open notice to all staffs at each location to invite them in our initial screening program.

Selection Criteria

Inclusion criteria were: 1) age between 30 to 60 years, 2) non-obese with BMI less than 25kg/m^2 and waist circumference in males less than 102cm and less than 88cm in females (NCEP ATPIII, 2001), 3) non-diabetic and non-hypertensive, 4) without family history of type 2 diabetes, and 5) non-smoker. Subjects suffering from chronic illnesses, ketosis, chronic liver and renal diseases, and pregnant women were excluded from the study. Subjects taking anti-hypertensive drugs, steroids or hormonal products were also excluded (Boden, 2001).

Ethical Clearance

The study was approved by the Research and Ethics Committee, School of Medical Sciences, Universiti Sains Malaysia (USM). Written informed consent was taken from every participant of the study. The study method adhered to the existing Malaysian guidelines for International Committee on Harmonization of Good Clinical Practice (ICH-GCP) Guidelines (GCP, 1999). All essential source documents required in this study were handled according to Malaysian GCP Guidelines (1999).

Recruitment of Subjects

We screened the subjects according to the selection criteria, anthropometric measurements (height, weight, waist circumference, BMI) and clinical history. Those who met the selection criteria were invited to come to the Department of Chemical Pathology in USM after overnight fasting (10-12 h) for oral glucose tolerance test (OGTT), liver function test (LFT), renal function test (RFT) followed by lipid levels and insulin sensitivity test in two separate visits.

Anthropometry and Blood Pressure

Body weight (in kilogram) was measured in patients wearing light clothing. Height in centimeter (cm) was measured using Standard ZT-120®, (Healthometer Inc., USA) with bare foot. Body mass index (BMI in kg/m^2) of the subjects was calculated as weight in kilogram divided by height in square meter. Waist circumference (in cm) was taken at the level of umbilicus (Meigs *et al*, 2003). Hip circumference was measured at the maximal extension of the buttocks (Ford *et al*, 2003). Waist-to-hip ratio (WHR) was calculated as the ratio of waist circumference to hip circumference.

Pulse and blood pressure of every subject were measured by the same physician. At least two readings of blood pressure were taken at 5 minutes interval on the right hand using a mercury sphygmomanometer (Baumanometer®, W.A. Buam Co, Inc., New York, USA) in the sitting position and the mean value was noted. A person was identified as hypertensive if he either had a systolic blood pressure at or above 140 mmHg (≥140 mmHg) and/or diastolic blood pressure at or above 90 mmHg (≥90 mmHg) (MOH,CPG, 2002).

Phlebotomy and Biochemical Tests

Blood specimens for LFT, RFT and lipids were collected in 5ml Vacutainer® tubes with SST® Gel and clot activator, that for insulin in 5ml plain Vacutainer® tubes, and for glucose in 2ml fluoride oxalate tubes (NAF OXALATE 2®). OGTT was performed using 75gm of anhydrous glucose made up to 250ml of solution with plain water. Diabetes and

Impaired Glucose Tolerance (IGT) were defined according to the criteria set by the WHO Expert Committee (WHO, 2003). Plasma glucose and lipid levels were performed on the same day of collection. Serum for insulin was frozen immediately at -80°C and was assayed within three months of specimen collection.

Selection of Groups for Comparing Insulin Sensitivity

Based on the results of lipid profile of the subjects, they were divided into two groups, hyperlipidemic and normolipidemic groups, based on the criteria of NCEP ATP III (2001). A person was defined as hyperlipidemic if his blood cholesterol level was ≥5.18mmol/L and/or his triglyceride (TG) level was ≥1.71mmol/L.
The insulin sensitivity of both groups was compared based on HOMA parameters.

Laboratory Analyses

Laboratory analyses were performed in the Department of Chemical Pathology Laboratory and Department of Medicine Endocrine Laboratory. Both laboratories are ISO-9001 certified.
Plasma glucose was estimated by the glucose oxidase (GOD-PAD) method using commercial kits from ROCHE®, Switzerland and Roche Cobas Integra 400® automated chemistry analyzer (Switzerland), with an inter-assay coefficient of variation (CV) of 2.45%.
Serum total cholesterol (interassay CV 1.27%), HDL cholesterol (HDL-C) (interassay CV 1.26%) and triglyceride (interassay CV 1.38%) were measured by automated fully enzymatic colorimetric method using commercial kits from ROCHE® and using Roche Cobas Integra 400® automated chemistry analyzer. The LDL cholesterol (LDL-C) level in serum was calculated by using the Friedewald formula (Friedwald, 1972). Very low density lipoprotein (VLDL) cholesterol was calculated by dividing TG level by 2.2.
Serum insulin was measured by chemiluminescence method using commercial reagent IMMULITE (Diagnostics Products Corporation EURO/DPC, United Kingdom) using IMMULITE® analyzer (interassay CV 1.39%).

Homeostasis Model Assessment (HOMA)

Homeostasis model assessment (HOMA) software was used to calculate insulin sensitivity (HOMA%S) and insulin secretory capacity (HOMA%B) of the subjects. Fasting insulin levels (in pmol/L) and fasting plasma glucose(FPG) (in mmol/L) were keyed into the computer using the HOMA software to calculate HOMA%S and HOMA%B (Matthews *et al*, 1985; Hermans *et al*, 1999).
HOMA for insulin resistance (HOMA-IR) was also calculated manually using the following formula in order to compare the results of this study with those of previous studies.

HOMA–IR = (fasting insulin in μIU/ml X fasting glucose)/22.5

Statistical Analyses

Statistical analysis were done using statistical package for social sciences (SPSS) for Windows version 11.0 (SPSS Inc, 2000).
Subjects demographic, anthropometric and biochemical (lipid, FPG, insulin sensitivity) baseline descriptive statistics were presented as percentage, mean (sd) and median (iqr)

whenever appropriate.Independant t test or MannWhitney test was used for comparing numerical data. Correlation between insulin sensitivity and different lipid types were expressed as Pearsons/Spearman coefficient according to data distribution. The relationship between lipid status and insulin sensitivity was determined with ANCOVA to adjust the possible effect of age, sex, BMI and waist circumference.

RESULTS

Characteristics of study subjects

From a total of 890 people of different school and public office of KotaBharu a total of 561 subjects participated (Participation rate 63%) in the random screening program according to the selection criteria of this study. Of this, 246 person fulfilled the selection criteria; 128 (52.03%) were normolipidemic and 118 (47.97%) were hyperlipidemic. Mean BMI, waist and hip circumference and WHR of the hyperlipidemic group was significantly higher than the normolipidemic group (Table 1).

Lipid levels of two different groups

TG, LDL-C, VLDL and total cholesterol of the hyperlipidemic group were significantly higher than in the normolipidemic group. However, the HDL cholesterol of the female hyperlipidemics was not significantly lower than that of the normolipidemic subjects (Tables 2 and 3).

Fasting glycemia and insulin sensitivity status

The median insulin sensitivity of normolipidemic male Malay subjects aged between 30-60 years expressed as HOMA%S was 141.70% compared to 68.30% ($p<0.001$) in hyperlipidemic subjects. HOMA%S for female normolipidemic and hyperlipidemic subjects was 151.30% and 70.10% respectively ($p<0.001$).

The median insulin resistance expressed by HOMA-IR was 1.05 for male non-obese, normolipidemic Malay subjects. For hyperlipidemic subjects, this value was as high as 2.17 ($p<0.001$). For female normolipidemic and hyperlipidemic subjects these two value were 0.95 and 2.21, respectively ($p<0.001$).

Fasting insulin level was significantly higher in the hyperlipidemic male and female subjects compared to normolipidemics (Tables 4 and 5).

Correlation between insulin sensitivity and lipid status

Statistical correlation tests were performed between insulin sensitivity with TG, HDL-C, LDL-C, VLDL and total cholesterol. Insulin sensitivity (HOMA%S) showed negative correlation with total cholesterol ($r= -0.533$, $p<0.001$) and TG ($r= -0.313$, $p<0.001$), LDL cholesterol ($r= -0.407$, $p<0.001$) and VLDL cholesterol ($r= -0.311$, $p<0.001$), positive correlation with HDL cholesterol ($r=0.260$, $p<0.001$) (Table 6).

Association of lipid status with insulin sensitivity

There were significant difference in insulin sensitivity, relative insulin resistance and insulin secretory capacity between two groups based on lipid status ($p<0.001$) (Table 7). It was also seen that age, sex, BMI and waist circumference was not significant predictor of insulin sensitivity status among non-obese subjects.

DISCUSSION

We found that the insulin sensitivity of hyperlipidemic Malay subjects was significantly lower and their relative insulin resistance was significantly higher than normolipidemic (adjusting for age, sex, BMI and waist circumference).

Previous studies on insulin sensitivity involved peoples of all BMI range, so the insulin sensitivity or resistance we got from those studies not always mention the insulin sensitivity of a healthy population (Tai *et al*, 2000; Tan *et al*, 1999; Haffner *et al*, 1997). In studies involving Malay partcipants in Singapore (Tai *et al*, 2000; Tan *et al*, 1999), the mean HOMA-IR was 1.48 for male (n = 254) and 1.63 for female (n = 254) participants, obese people was also included in the study. The median HOMA-IR of Bruneck study (Bonora *et al*, 1988) people was 2.51 (n=888, including diabetic and obese people too). In another study (Haffner *et al*, 1997) involving Mexican-Americans and Non-Hispanic whites mean HOMA-IR of Mexican-Americans were3.83 and non-Hispanic whites were 2.56 (population included diabetic, IGT and obese people also). In comparison to these studies our study represents non-obese, normoglycemic Malay subjects and adjusted for the possible confounders of age, sex, BMI, waist circumference. So the insulin sensitivity of normolipidemic population of this study represents the insulin sensitivity of normal Malay population and it's different than Malays of Singapore, probably difference between lifestyle may responsible behind this difference.

We found that lipid status is associated with insulin sensitivity in non-obese subjects other than age, sex, BMI and waist circumference. Previous studies relating BMI with insulin sensitivity have simply used BMI as a measure of relative body size or obesity without considering that people with similar BMI may have widely varying distribution of their adipose tissue (Yoshitomi *et al*, 2005). Though there were many studies showing higher insulin resistance among male but when it was adjusted for other confounders than it showed no influence of sex, Ferrannini *et al* and Barbieri *et al* also reported of founding no difference in insulin sensitivity and resistance between male and female. Ferrannini *et al* also conclude that in healthy Europeans, age per se is not a significant cause of insulin resistance.

The insulin secretory capacity as expressed by HOMA%-B of hyperlipidemic subjects was higher than the normolipidemic subjects. It indicates that they face a higher challenge to meet the lowered insulin sensitivity so they need to secret more insulin to compensate lowered insulin sensitivity.

Our observation was that even moderate change in lipid status in non-obese subjects influence insulin sensitivity and secretion. These findings have clinical importance as these clinical conditions in non-obese subjects are often ignored and they remain untreated.

CONCLUSION

Insulin sensitivity of otherwise healthy non-obese hyperlipidemic subjects is lower, and their insulin secretion is more to compensate for the lowered insulin sensitivity, in comparison with normolipidemic subjects. Insulin sensitivity decreases with increasing TG, LDL-C and total cholesterol. It has positive correlation with HDL-C level. Even modest dyslipidemia in non-obese people should be managed with priority for the prevention of metabolic syndrome in future.

Table 1. Demographic and anthropometric characteristics of 246 Malay study subjects

Demographic variable		Normolipidemic (n = 128)	Hyperlipidemic (n = 118)	p[a]
Mean age (yrs)				
	Male	39.43 (6.42)	43.03 (7.79)	0.014
	Female	37.75(6.36)	41.65 (7.12)	0.001
Sex				
	Male	44 (43.14%)	58 (56.86%)	
	Female	84 (58.33%)	60 (41.67)	
BMI*				
	Male	22.41 (2.16)	23.77 (2.12)	<0.001[b]
	Female	21.83 (2.50)	23.91 (2.14)	<0.001[b]
Waist				
	Male	81.28 (7.34)	89.74 (7.30)	<0.001
	Female	72.34 (8.17)	78.40 (7.85)	<0.001
Hip				
	Male	94.73 (5.45)	100 (6.03)	<0.001
	Female	94.04 (8.40)	97.95 (7.81)	<0.001
WHR				
	Male	0.85 (0.05)	0.89 (0.04)	<0.001
	Female	0.77 (0.06)	0.80 (0.05)	<0.001

[a]Independent Student's t test, *Median (iqr), [b]Mann-Whitney test

Table 2. Lipid profile of the different groups of Malay males

Parameter	**Normolipidemic** n=44	**Hyperlipidemic** n=58	**p-value**[a]
Cholesterol*	4.89 (0.35)	6.52 (0.96)	<0.001[b]
Tg	1.10 (0.33)	2.40 (1.74)	<0.001
HDL-C	1.40 (0.25)	1.31 (0.43)	0.014
LDL-C	2.98 (0.38)	4.18 (1.01)	<0.001
VLDL	0.50 (0.15)	1.09 (0.79)	<0.001

[a]Independent Student's t test, *Median (iqr), [b]Mann-Whitney test, (values are in mmol/L)

Table 3. Lipid profile of the different groups of Malay females

Parameter	Normolipidemic n=84	Hyperlipidemic n=60	p-value[a]
Cholesterol*	5.08 (0.52)	6.30 (1.26)	<0.001[b]
Tg	0.85 (0.33)	1.59 (0.92)	<0.001
HDL-C	1.73 (0.45)	1.62 (0.37)	0.157
LDL-C	2.81 (0.58)	3.96 (0.81)	<0.001
VLDL	0.39 (0.15)	0.72 (0.42)	<0.001

[a]Independent Student's t test, *Median (iqr), [b]Mann-Whitney test, (values are in mmol/L)

Table 4. Fasting glycemia and insulin sensitivity status of Malay males

Parameter	Normolipidemic n=44 Mean (SD)	Hyperlipidemic n=58 Mean (SD)	p-value[a]
FPG (mmol/L)	4.35 (0.87)	4.50 (0.83)	0.05
Insulin (pmol/L)	33.60 (12.90)*	72.60 (65.40)*	<0.001[b]
HOMA%S	141.70 (59.87)*	68.30 (69.20)*	<0.001[b]
HOMA%B	97.60 (61.52)*	166.70 (123.50)*	<0.001[b]
HOMA-IR	1.05 (0.54)*	2.17(2.24)*1	<0.001[b]

[a]Independent Student's t test, *Median (iqr), [b]Mann-Whitney test

Table 5. Fasting glycemia and insulin sensitivity status of Malay females

Parameter	Normolipidemic n=84 Mean (SD)	Hyperlipidemic n=60 Mean (SD)	p-value[a]
FPG (mmol/L)	4.09 (0.56)	4.45 (0.66)	<0.001
Insulin (pmol/L)	32.40 (19.50)*	68.10 (43.05)*	<0.001[b]
HOMA%S	151.30 (80.40)*	70.10 (36.45)*	<0.001[b]
HOMA%B	106.55 (51.52)*	151.60 (89.72)*	<0.001[b]
HOMA-IR	0.95 (0.52)*	2.21(1.89)*	<0.001[b]

[a]Independent Student's t test, *Median (iqr), [b]Mann-Whitney test

Table 6. Correlation between insulin sensitivity index (HOMA%S) with lipids in 246 non-obese, normoglycemic Malay subjects.

Parameter	n	r	p-value
Total cholesterol	246	-0.533*	<0.001
LDL-C[a]	239	-0.407	<0.001
HDL-C	246	0.26	<0.001
VLDL	246	-0.311	<0.001
Tg	246	-0.313	<0.001

*Spearman's correlation coefficient, [a]LDL-C of 7 subjects was not calculated using Friedwald formula because TG was higher than 4.5 mmol/L.

REFERENCES

Barbieri M, Rizzo MR, Manzella D, Paolisso G. Age related insulin resistance: is it an obligatory finding? The lesson from healthy centenarians. *Diabetes Metab Res Rev.* 17 (2001): 19-26.

Boden G (2001). Pathogenesis of type 2 diabetes. *Endocrinology and Metabolism Clinics of North America.* **30**(4), 801-13.

Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Targher G *et al.* Prevalence of insulin resistance in metabolic disorders: The Bruneck study. *Diabetes.* 47 (1988):1643-49.

Defronzo RA, Ferrannini E (1991). Insulin resistance a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia and atherosclerotic cardiovascular disease. *Diabetes Care.* **14**, 173-94.

Doherty RO, Stein D, Foley J (1997). Insulin resistance. *Diabetologia.* **40**, B10-B15
Ferannini E, Natali A, Bell P, Cavallo-perin P, Lalic N, Mingrone G (1997). Insulin resistance and hypersecretion in obesity. *J Clin Invest.* **100**, 1166-1173.

Ferrannini E, Vichi S, Beck-Nielsen H, Laakso M, Paolisso G and Smith U. Insulin action and age. *Diabetes.* 45 (1996), 947-953.

Ford ES, Giles WH. A comparison of the prevalence of the metabolic syndrome using two proposed definitions. *Diab Care.* 26 (3) (2003):575-81.

Friedwald WT. Estimation of serum low density lipoprotein. *Clin Chem.*18 (1972):499.
Kahn SE (2003). The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia.* **46**, 3-19.

Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio heart study. *Diabetes Care* 20 (7) (1997):1087-1092

Hermans MP, Levy JC, Morris RJ & Turner RC. Comparison of insulin sensitivity tests across a range of glucose tolerance from normal to diabetes. *Diabetologia.* 42 (1999):678-687.

Hosker JP, Rudenski AS, Burnett MA, Matthews DR & Turner RC. Similar reduction of first and second phase B-cell response at three different glucose levels in type II diabetes and the effect of gliclizide therapy. *Metabolism.* 38 (1989), 767-772.

Lillioja S & Bogardus C (1988). Obesity and insulin resistance: lessons learned from the Pima Indians. *Diabetes and metabolism review.* **4**, 517-540

Mafauzy M, Mokhtar N, Wan Mohamad WB, Musalmah M (1999). Diabetes Mellitus and associated cardiovascular risk factors in north-east Malaysia. *Asia Pac J Public Health.* **11** (1): 16-19.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC. Homeostasis model assessment: insulin resistance and Beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 28 (1985):412-419.

Meigs JB, Wilson PW, Nathan DM, D'Agostino RBSr, Williams K, Haffner SM. Prevalence and characteristics of the metabolic syndrome in the San Antonio Heart and Framingham offspring studies. *Diabetes.* 52 (8) (2003): 2160-67.

Ministry of Health Malaysia. Malaysian Guidelines for Good Clinical Practice(1999).

Ministry of Health Malaysia.Clinical Practice Guideline on Management of Hypertension (2002).

Mustapha BE (2004). Diabetes epidemiology in Malaysia. *Med J Malaysia* **59** (3): 295-6.

National Cholesterol Education Program (NCEP). Expert Panel on Detection, Evaluation and treatment of High Blood Cholesterol in Adults (Adult treatment panel III): Executive summary of the third report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation and treatment of High Blood Cholesterol in Adults (Adult treatment panel III). *Circulation.* 106 (2002).

Reaven GM (1988). Role of insulin resistance in human disease: Banting lecture 1988. *Diabetes.* **37**,1595-1607.

Rudenski AS, Matthews DR, Levy JC & Turner RC. Understanding "insulin resistance" both glucose resistance and insulin resistance are required to model human diabetes. *Metabolism.* 40 (1991), 908-917.

SPSS Inc.. Statistical Package for Social Sciences software for Windows, relaese 11.1 Chicago: SPSS Inc (2000).

Sum CF, Wang KW, Tan CE, Fok Kc, Chew LS and Tan YT (1992). Hyperinsulinemia in non-obese subjects with hypertriglyceridemia: a preliminary report. *Ann Acad Med Singapore.* **21** (1) 10-13.

Tai ES, Lim SC, Chew SK, Tan BY and Tan CE. Homeostasis model assessment in a population with mixed ethnicity: the 1992 Singapore National Health Survey. *Diabetes Res Clin Pract.* 49 (2000):159-68.

Tan CE, Emmanuel SC, Tan BY and Jacob E. Prevalence of diabetes and ethnic differences in cardiovascular risk factors. The 1992 Singapore National Health Survey. *Diabetes Care* 22 (2) (1999):241-47

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus Report of the expert committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*. 20 (7) (2002):1183-1197.

Yoshitomi Y, Ishii T, Kaneki M, Tsujibayashi T, Sukarai S, Nagakura C, Miyauchi A. Realationship between insulin resistance and effect of Atrovastatin in non-diabetic subjects. *J Atheroscler Thromb*. 12 (1) (2005): 9-13

Title: Insulin sensitivity of non-obese non-diabetic Malay subjects: relationship with lipid status.

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Abstract

The relationship between insulin sensitivity and hyperlipidemia in normal bodyweight normoglycemic subjects is not well-studied. We were interested to study the insulin sensitivity of non-obese normoglycemic Malay subjects free from other confounders that may influence insulin sensitivity (i.e. obesity, glucose intolerance and hypertension) and to find its relation with lipid status. A cross-sectional study was performed on 246 non-obese and non-diabetic subjects aged between 30-60 years. Fasting plasma glucose, fasting insulin and lipid profile were determined. Insulin sensitivity and secretory status were calculated using the homeostasis model assessment (HOMA) software (HOMA%S, HOMA%B and HOMA-IR). The subjects were divided into normolipidemic and hyperlipidemic groups based on their lipid status and their insulin sensitivity was compared. The insulin sensitivity (HOMA%S) of hyperlipidemic subjects was 80.72%, HOMA-IR was 2.66 and HOMA%B was 178.51%, these values were significantly different in normolipidemic subjects, their HOMA%S was 155.17%, HOMA-IR was 1.05 and HOMA%B was 116.65% ($p<0.001$)(values adjusted for age, sex, BMI and waist circumference). Our observation was that the insulin sensitivity of otherwise healthy non-obese hyperlipidemic subjects was lower in comparison with normolipidemic subjects. We conclude that dyslipidemia in non-obese people should be managed with priority for the prevention of metabolic syndrome in future.

Key words:

Insulin sensitivity, insulin secretory status, HOMA%S, HOMA%B, HOMA-IR

INTRODUCTION

Insulin sensitivity is the sensitivity of the tissues (especially hepatic and skeletal) to the actions of insulin. It is influenced by age, body mass index (BMI), abdominal obesity, level of physical activity and different metabolic and endocrine factors [1]. Originally it was discussed in connection with the pathogenesis of type 2 diabetes but in his Banting lecture [2], Reaven connected it with many metabolic disorders such as glucose intolerance, dyslipidemia, hyperuricemia and hypertension. Further research established this concept with more evidence [1-4]. Its relation with obesity is established and a wealth of epidemiological studies has been done in exploring this relationship [1,5-7]. It is also associated with hyperlipidemia [4]. Hyperlipidemia and insulin resistance have a complex interaction. Whether insulin resistance is secondary to hyperlipidemia or hyperlipidemia follows insulin resistance the matter has yet to be resolved [2, 8]. Most of the previous studies on insulin sensitivity were performed on hyperlipidemic subjects who were also obese [9] as a result both effect of obesity and hyperlipidemia acted simultaneously in genesis of insulin resistance. In an attempt to study the relationship between insulin sensitivity and lipid status, we were interested to isolate a group of subjects who were free from possible factors (e.g., obesity, glucose intolerance, hypertension) which influenced insulin sensitivity and to compare their insulin sensitivity according to their lipid status.

METHODOLOGY

(a) Study Design

This cross-sectional study was conducted from mid September 2003 to March 2005 which involved both outdoor and on-campus laboratory-based activities. Research volunteers were recruited from schools and public offices in Kota Bharu, the capital city of the state of Kelantan in northeastern peninsular West Malaysia. We circulated an open notice to all staffs at each location to invite them in our initial screening program.

(b) Selection Criteria

Inclusion criteria were: 1) age between 30 to 60 years, 2) non-obese with BMI less than 25kg/m^2 and waist circumference in males less than 102cm and less than 88cm in females [10], 3) non-diabetic and non-hypertensive, 4) without family history of type 2 diabetes, and 5) non-smoker. Subjects suffering from chronic illnesses, ketosis, chronic liver and renal diseases, and pregnant women were excluded from the study. Subjects taking anti-hypertensive drugs, steroids or hormonal products were also excluded [1].

(c) Ethical Clearance

The study was approved by the Research and Ethics Committee, School of Medical Sciences, Universiti Sains Malaysia (USM). Written informed consent was taken from every participant of the study. The study method adhered to the existing

Malaysian guidelines for International Committee on Harmonization of Good Clinical Practice (ICH-GCP) Guidelines [11]. All essential source documents required in this study were handled according to Malaysian GCP Guidelines (1999).

(d) Recruitment of Subjects

We screened the subjects according to the selection criteria, anthropometric measurements (height, weight, waist circumference, BMI) and clinical history. Those who met the selection criteria were invited to come to the Department of Chemical Pathology in USM after overnight fasting (10-12 h) for oral glucose tolerance test (OGTT), liver function test (LFT), renal function test (RFT) followed by lipid levels and insulin sensitivity test in two separate visits.

(e) Anthropometry and Blood Pressure

Body weight (in kilogram) was measured in patients wearing light clothing. Height in centimeter (cm) was measured using Standard ZT-120®, (Healthometer Inc., USA) with bare foot. Body mass index (BMI in kg/m^2) of the subjects was calculated as weight in kilogram divided by height in square meter. Waist circumference (in cm) was taken at the level of umbilicus [12]. Hip circumference was measured at the maximal extension of the buttocks [13]. Waist-to-hip ratio (WHR) was calculated as the ratio of waist circumference to hip circumference.

Pulse and blood pressure of every subject were measured by the same physician. At least two readings of blood pressure were taken at 5 minutes interval on the right hand using a mercury sphygmomanometer (Baumanometer®, W.A. Buam Co, Inc.,

New York, USA) in the sitting position and the mean value was noted. A person was identified as hypertensive if he either had a systolic blood pressure at or above 140 mmHg (≥140 mmHg) and/or diastolic blood pressure at or above 90 mmHg (≥90 mmHg) [14].

(f) Phlebotomy and Biochemical Tests

Blood specimens for LFT, RFT and lipids were collected in 5ml Vacutainer® tubes with SST® Gel and clot activator, that for insulin in 5ml plain Vacutainer® tubes, and for glucose in 2ml fluoride oxalate tubes (NAF OXALATE 2®). OGTT was performed using 75gm of anhydrous glucose made up to 250ml of solution with plain water. Diabetes and Impaired Glucose Tolerance (IGT) were defined according to the criteria set by the WHO Expert Committee [15]. Plasma glucose and lipid levels were performed on the same day of collection. Serum for insulin was frozen immediately at -80°C and was assayed within three months of specimen collection.

(g) Selection of Groups for Comparing Insulin Sensitivity

Based on the results of lipid profile of the subjects, they were divided into two groups, hyperlipidemic and normolipidemic groups, based on the criteria of NCEP ATP III [10]. A person was defined as hyperlipidemic if his blood cholesterol level was ≥5.18mmol/L and/or his triglyceride (TG) level was ≥1.71mmol/L.

The insulin sensitivity of both groups was compared based on HOMA parameters.