BAHAGIAN PENYELIDIKAN & PEMBANGUNAN

CANSELORI

UNIVERSITI SAINS MALAYSIA



Laporan Akhir Projek Penyelidikan Jangka Pendek

1) Nama Penyelidik: **Prof. Madya Faridah Abdul Rashid**

Nama Penyelidik-Penyelidik Lain *(jika berkaitan)*:

**Prof. Wan Mohamad Wan Bebakar**

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

3) Tajuk Projek: **Characterization of Low Density Lipoprotein Subfraction Profile and Apo E Genotype Among Diabetic Patients**

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).


**Abstrak Bahasa Malaysia**

Diabetes mellitus ialah penyakit metabolik yang bercirikan hiperglisemia dan dislipidemia, yang memberi pesakit diabetes risiko tinggi untuk mendapat penyakit jantung. Adalah dipercayai bahawa LDL kolesterol (LDLC), trigliserida (TG) dan genotip apolipoprotein E (apo E) berkait rapat dalam menentukan risiko tinggi ini. Kajian ini bertujuan untuk mencirikan dislipidemia diabetes dengan penentuan saiz LDL dan genotip apo E. Seramai 30 subjek Normal dan 35 Diabetes mellitus jenis 2 dikaji. Analisis biokimia lipid ditentukan secara automatik menggunakan kit komersial. Saiz LDL ditentukan menerusi mikroskop elektron transmisi. LDL1 dan LDL2 adalah bersaiz besar manakala LDL3, LDL4, dan LDL5 adalah bersaiz kecil dan dianggap memudaratkan kesihatan. Genotip apo E pula ditentukan menerusi tindakbalas rantai polimerase dan polimorfisme kepanjangan fragmen restriksi.

**Penemuan**

- Pesakit diabetes mellitus jenis 2 yang berlebihan berat badan mempunyai lebih banyak LDL4 berbanding seseorang normal
- Kandungan LDL4 berkadar terus dengan aras TG plasma. Ini menunjukkan hipertrigliseridemia menyumbang kepada pembentukan LDL4 dalam kes diabetes yang dikaji
- Kandungan LDL1 berkadar songsang dengan LDL3. Ini menunjukkan LDL1 bertukar menjadi LDL lebih kecil, pertukaran ini berterusan dan mengakibatkan banyak LDL3 terbentuk
- Genotip ε4/2 bercirikan LDLC *paling rendah* dan HDLC *paling tinggi*. Genotip ini adalah baik bagi pesakit diabetes yang berlebihan berat badan kerana mempunyai risiko *paling rendah* untuk mendapat penyakit jantung iskemia
- Genotip ε4/3 bercirikan TG *paling tinggi*, LDLC *paling tinggi*, TC *paling tinggi*, dan HDLC *paling rendah*. Genotip ini tidak baik bagi pesakit diabetes yang berlebihan berat badan kerana mempunyai risiko *paling tinggi* untuk mendapat penyakit jantung iskemia
- Genotip ε3/3 (normal) mempunyai risiko *perantara* untuk mendapat penyakit jantung iskemia

**Kesimpulan**

Saiz LDL dan genotip apo E perlu ditentukan untuk pesakit diabetes mellitus jenis 2 yang berlebihan berat badan kerana mereka berpotensi tinggi untuk mendapat penyakit jantung iskemia. Semasa mengurus perawatan pesakit berkenaan, kedua-dua LDLC dan TG perlu dipantau rapi sehingga 4 jam postprandial jika boleh.



**Abstrak Bahasa Inggeris**

Diabetes mellitus is a metabolic disorder characterised by chronic hyperglycaemia and dyslipidaemia, which places diabetics at increased risk of cardiovascular disease. We strongly believed that LDL cholesterol (LDLC), triglycerides (TG) and apolipoprotein E (apo E) genotype are closely related in determining this high risk. This study aims to study the effect of LDL subfraction and apo E genotype on diabetic dyslipidaemia. Normal (n=30) and diabetes mellitus type 2 (n=35) subjects who were not on any drug treatment were studied. Lipid biochemical analysis was performed by automated methods using commercial kits. LDL size was determined by transmission electron microscopy. LDL1 and LDL2 are large particles while LDL3, LDL4, and LDL5 are smaller particles and are considered detrimental to health. Apo E genotype was determined by polymerase chain reaction and restriction fragment length polymorphism.

**Findings**

- Overweight diabetes mellitus type 2 subjects have significantly more LDL4 compared to normal
- LDL4 content varies directly with plasma TG. This indicates that hypertriglyceridaemia contributes to the formation of LDL4 in the diabetics who were studied
- LDL1 content varies indirectly with LDL3. This indicates that LDL1 is converted to smaller LDL, this conversion continues and results in abundance of LDL3
- ε4/2 genotype is characterised by *lowest* LDLC and *highest* HDLC. This genotype is considered good for overweight diabetics as it confers the lowest risk for ischaemic heart disease
- ε4/3 genotype is characterised by *highest* TG, *highest* LDLC, *highest* TC, and *lowest* HDLC. This genotype is considerd not good for overweight diabetics as it confers the highest risk for ischaemic heart disease
- Normal ε3/3 genotype confers intermediate risk for ischaemic heart disease

**Conclusion**

LDL size and apo E genotip should be obtained for overweight diabetes mellitus type 2 patients because they are at increased risk for getting ischaemic heart disease. When managing the treatment of these these patients, both LDLC dan TG should be closely monitored up to 4 hours postprandially if possible.

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

| Bahasa Malaysia | Bahasa Inggeris |
|---|---|
| Diabetes mellitus jenis 2 | Diabetes mellitus type 2 |
| hiperglisemia | hyperglycaemia |
| dislipidemia | dyslipidaemia |
| saiz LDL | LDL size |
| genotip apo E | apo E genotype |
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5) *Output* Dan Faedah Projek

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

| Jenis | Tajuk | Pengarang | Tahun Terbitan | Tempat Diterbit/ Dibentangkan |
|---|---|---|---|---|
| Full paper; poster presentation LAMPIRAN 1 | Preliminary studies on apolipoprotein E genotypes and allele frequency among healthy subjects | Shahrul BSH, Faridah AR | 29-30 April 2000 | Simposium Sains Kesihatan Kebangsaan Ke-3, FSKB, UKM |
| Abstract; poster presentation LAMPIRAN 2 | Relationship between genotype and allele of apolipoprotein E with the lipid status among Malays | Shahrul BSH, Mohd Rafi, Wan MWB, Faridah AR | 18-21 May 2001 | 1st ASEAN Conference on Medical Sciences, Renaissance Kota Bharu Hotel. Book of Abstracts, Abstract No. P-22, page 69 |
| Abstract; oral presentation LAMPIRAN 3 | A study of postprandial patterns of lipaemia and glycaemia in patients with ischaemic heart disease and those with glucose intolerance | Faridah Abdul Rashid, Shahrul Bariyah Sahul Hamid, Mafauzy Mohamed, Wan Mohamad Wan Bebakar, Nazmi Mohd Noori, and Ruhani Halim | 30 June – 1 July 2001 | IRPA Top Down Research Workshop 8. Concorde Inn, KLIA 43900 Sepang, Selangor |
| Abstract; poster presentation LAMPIRAN 4 | Palm olein load causes alteration of lipid kinetics during postprandial state in a paired study group | Shahrul BSH, Wan MWB, Mafauzy M, and Faridah AR | 18-20 April 2002 | 1st PENSMA National Congress, Renaissance Melaka Hotel. *Submitted* |
| Abstract; to request for oral presentation LAMPIRAN 5 | Characterization of low density lipoprotein subfraction profile and apo E genotype among diabetic patients | Shahrul Bariyah Sahul Hamid, Faridah Abdul Rashid and Wan Mohamad Wan Bebakar | 17-18 May 2002 | 7th National Conference on Medical Sciences, Universiti Sains Malaysia Health Campus, Kubang Kerian, Kelantan. *To be submitted* |
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(b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten
(Jika ada dan jika perlu, sila gunakan kertas berasingan)

(c) Latihan Gunatenaga Manusia

*i) Pelajar Siswazah:* 4 orang pelajar di bawah penyeliaan:

- Shahrul Bariyah Sahul Hamid – PhD
- Mohd. Rafi Mustapha – MSc / Pegawai Sains
- Eid Mohammad s/o Akhtar Mohammad – MSc
- Julia Omar – MMed (posting Makmal Lipid)

*ii) Pelajar Prasiswazah:* 6 orang pelajar DTMP Tahun 3 yang menjalani projek penyelidikan akhir kursus yang diselia:

- Ang Wooi Lee
- Kong Siaw Huong
- Joel Jeebaseelan a/l William
- Marsitah Omar
- Yong Yau Lee
- Zamani Mohd. Zain

*iii) Lain-lain:* 2 orang teknologis makmal perubatan dari Jabatan yang turut bersama atas jemputan:

- Zakaria Abu Samah
- Lau Yoke chie

6. Peralatan Yang Telah Dibeli: *(Vot 35000 sahaja)*
*(For Asset Management System Tracking)*

| Jenis | Keterangan | Pembekal | Serial Number | Harga | Tarikh beli | PO/DO/Invoice |
|---|---|---|---|---|---|---|
| Peralatan elektroforesis<br><br>LAMPIRAN 6 | Dual cooled vertical electrophoresis system, Model SE 600 Cat No: 80-6171-96 | Amersham Pharmacia Biotech, Hoefer | 20058937 | RM3,600 | 2 Oct 2000 | PO: A18103<br>DO: 21887<br>Invoice: 17872 |
| Peralatan elektroforesis<br><br>LAMPIRAN 7 | Gradient maker, Model SG 50 Cat No: 80-6197-99 | Amersham Pharmacia Biotech, Hoefer | None | RM999 | 2 Oct 2000 | PO: A18103<br>DO: 21887<br>Invoice: 17872 |
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| | | | JUMLAH | RM4,599 | | |

UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

TANDATANGAN PENGERUSI
JAWATANKUASA PENYELIDIKAN
PUSAT PENYELIDIKAN

Pascasidang, Simposium Sains Kesihatan Kebangsaan Ke-3, FSKB, UKM, 29-30 April 2000
(Full paper in proceedings page 360; poster presentation)

# KAJIAN AWAL MENGENAI GENOTIP APOLIPOPROTEIN E DAN FREKUENSI ALEL DI KALANGAN SUBJEK SIHAT

## PRELIMINARY STUDIES ON APOLIPOPROTEIN E GENOTYPES AND ALLELE FREQUENCY AMONG HEALTHY SUBJECTS

**Shahrul BSH, Faridah AR**
Department of Chemical Pathology, School of Medical Sciences,
Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan

ABSTRAK/ABSTRACT

Kajian ini menentukan genotip apolipoprotein E dan frekuensi allel dalam kalangan individu sihat. Penentuan genotip apo E dianalisa dengan kaedah polimorfisma rangkaian fragmen terpilih (RFLP). Kolesterol dan trigliserida ditentukan dengan kaedah berautomasi: Kolesterol lipoprotein berketumpatan rendah (LDL) diperoleh dari pengiraan Friedewald manakala kolesterol lipoprotein berketumpatan amat rendah (VLDL) ditentukan dengan membahagikan kepekatan trigliserida dengan pemalar 2.2. Kolesterol lipoprotein berketumpatan tinggi (HDL) dotentukan dengan kaedah pemendakan asid fosfotungstik dan ion Mg. Frekuensi alel epsilon 3 didapati lebih tinggi di kalangan wanita berbanding dengan lelaki sebanyak 5.2% manakala terdapat peningkatan sebanyak 8.3% dan 66.7% pada subjek lelaki dengan alel epsilon 4 serta 2. Kepekatan trigliserida dalam pembawa E3/3 adalah 1.79 mmol/L manakala dalam E4/2, E2/2, E3/2 dan E4/3 adalah lebih tinggi iaitu sebanyak 60.3%, 53.6%, 47.5% dan 10.6% dalam turutan. HDL adalah hampir sama bagi setiap genotip dan kepekatan LDL didapati meningkat sebanyak 46.2% pada subjek E2/2 dan sebaliknya pada subjek E4/2 sebanyak 13.8%. Kepekatan VLDL didapati lebih tinggi pada subjek E4/2, E2/2, E3/2 sebanyak 67.9%, 60.3% dan 53.8% dalam turutannya. Genotip E4/2 dicirikan oleh kepekatan trigliserida dan VLDL yang tinggi. Ciri ini juga diperhati pada subjek dengan E2/2 yang mempunyai kandungan kolesterol LDL yang tinggi berbanding dengan genotip lain.

This study ascertained apolipoprotein (apo) E genotypes and allele frequency among healthy subjects. The apo E genotype was analyzed by restriction fragment length polymorphism (RFLP). Cholesterol and triglycerides were measured by automated method. Low density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula and very low density lipoprotein (VLDL) cholesterol determined by dividing triglycerides by a constant of 2.2. High density lipoprotein (HDL) cholesterol was determined by the phosphotungstic acid and Mg ion sedimentation method. The allele frequency of epsilon 3 was found to be higher in females compared to males by 5.2% whereas there was an increase of 8.3% and 66.7% in the percentage of epsilon 4 and epsilon 2 among males. Triglycerides concentration in E3/3 carriers was 1.79 mmol/L while in E4/2, E2/2, E3/2 and E4/3, the concentrations were higher by 60.3%, 53.6%, 47.5% and 10.6% respectively, HDL cholesterol concentration was found to be almost similar for each of the genotypes and LDL cholesterol concentration was raised by 46.2% in the E2/2 carrier and reduced in the E4/2 carrier by 13.8%. VLDL cholesterol concentration was found to be higher in the E4/2, E2/2, E3/2 by 67.9%, 60.3% and 53.8% respectively. The E4/2 genotype carriers were characterized by the higher triglyceride and VLDL cholesterol concentration. This was also seen in the E2/2 carrier whose LDL cholesterol was seen to be higher compared to the other genotypes.

Correspondence:
Shahrul Bariyah Sahul Hamid
sbariyah@yahoo.com

*Original* Research Publication & Presentation 2001
1st ASEAN Conference on Medical Sciences, 18-21 May 2001, Book of Abstracts, Abstract No. P-22, page 69

Research Publication 2001
The Malaysian Journal of Medical Sciences July 2001; 8(2): 91-92

# RELATIONSHIP BETWEEN GENOTYPE AND ALLELE OF APOLIPOPROTEIN E WITH THE LIPID STATUS AMONG MALAYS

Shahrul BSH, Mohd Rafi, Wan MWB[a], Faridah AR
Department of Chemical Pathology, School of Medical Sciences, University Sains Malaysia, Kelantan, Malaysia
[a]Department of Medicine, School of Medical Sciences, University Sains Malaysia, Kelantan, Malaysia

Objective: This study ascertained apolipoprotein (apo) E genotypes and allele frequency among Malays and its influences towards the lipoprotein classes.

It is the major protein involved in catabolism of triglyceride rich lipoproteins (VLDL and remnants). Apo E has three common alleles which are ε2, ε3, and ε4 which code for three major apoE isoforms and 6 genotypes.

A number of 189 volunteers aged 20 to 65 years where recruited in the study. Blood was collected in tubes containing EDTA and the leucocytes from the buffy coat layer were used to extract DNA. Restriction fragment length polymorfism (RFLP) method was used to identify the apoE genotype. Lipid profile was determined using automated method to measure total cholesterol, triglyceride, and high density lipoprotein (HDL-C). The low density lipoprotein (LDL-C) level was calculated using the Friedewald formula.

The E3/3 genotype had the highest frequency among the the 6 genotypes. Female subjects had higher frequency for the ε4/2 genotype and lower ε4/4 frequency value. Male subjects had higher ε4/4 frequency. Epsilon 3 and epsilon 2 alleles frequency was high among females whereas the male subjects had high epsilon 4 allele frequency. Generally individuals with the ε4/2 genotype had higher total cholesterol and those with the ε2/2 had higher triglyceride concentration. In males the ε4/3 genotype group had higher triglyceride concentration while among the females the ε2/2 genotype individuals had the highest triglyceride concentration. The ε4/4 genotype caused the raised cholesterol content among females.

Our data suggest that male subjects have high epsilon 2 allele frequency which is associated with the high triglyceride. Females with epsilon 2 homozygous genotype had higher triglyceride concentration while the ε4/4 genotype had increased cholesterol level.

Correspondence:
Shahrul Bariyah Sahul Hamid
sbariyah@yahoo.com

IRPA Top Down Research Workshop 8 "Promotion of Healthy Lifestyles in Malaysia", Concorde Inn, KLIA 43900 Sepang, Selangor, 30 June – 1 July 2001. Project 7, oral presentation on 30 June 2001.

# A STUDY OF POSTPRANDIAL PATTERNS OF LIPAEMIA AND GLYCAEMIA IN PATIENTS WITH ISCHAEMIC HEART DISEASE AND THOSE WITH GLUCOSE INTOLERANCE

Faridah Abdul Rashid[1], Shahrul Bariyah Sahul Hamid[1], Mafauzy Mohamed[2*], Wan Mohamed Wan Bebakar[2*], Nazmi Mohd Noori[2*], and Ruhani Halim[3]

[1]Department of Chemical Pathology, [2]Department of Medicine, [*]Clinical Trials Unit, School of Medical Sciences, and [3]Nutrition and Dietetics Unit, Hospital Universiti Sains Malaysia, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

Project 7 as entitled above, is a 3-year project from March 1999 to March 2002. This project has 3 objectives, one for each year or stage. *Stage 1* is to identify a Malaysian standard meal for postprandial lipid profile quantification. *Stage 2* was completed and reported last year. *Stage 2* is to determine the pattern and prevalence of postprandial lipaemia and glycaemia in different subgroups of patients in response to standard fat load. I will report on the progress of stage 2 today. *Stage 3* is to determine the effects of 3 and 6 month of dietary intervention on postprandial lipaemia. *Stage 3* will be completed next year.

In *Stage 1*, 3 types of test meals in the form of milkshake A, B, and C, were made according to formula. The 3 test meals each contain the same amount of milk powder (50 g), glucose (57 g), one medium egg (50 g) and either low (22 g) oil A, medium (42 g) oil in B, and high (62 g) oil in C. All ingredients were blended in water to a final volume between 200 to 250 ml. Then, 10 normal subjects with no history of medical illness were fasted overnight for 12 hours and given either test meal A, B, or C on separate occasions. Blood was collected using a butterfly needle at 6 time-points: 0, 2, 4, 6, 8, and 9h, and analysed for lipids (TC, TG, HDLC, LDLC, VLDLC) and glucose. The results of the study in stage 1 show that in the 10 normal subjects studied, test meal B gave the worst postprandial triglycerides response. Test meal B was therefore selected for further study in *Stage 2*.

In *Stage 2*, 64 male and female subjects aged between 18-75 years were recruited. Subjects fasted overnight for 12h and were given only test meal B. Blood was similarly collected for automated biochemical analysis of lipids and glucose. Based on the biochemical results obtained, subjects were categorised into 3 groups: Normal Control, Hyperlipidaemia, and Impaired Glucose Tolerance (IGT). A minimum of 30 subjects is needed in each category. The biochemical results for serum lipids and plasma glucose were plotted.

The serum lipid results for Normal Control are as follows: TC <5.2 mmol/L, TG <2.25 mmol/L with maximum at 4h, HDLC >0.9 mmol/L, LDLC <3.4 mmol/L, and VLDLC <1.0 mmol/L with maximum at 4h (0.5-2.5 mmol/L). The serum lipid results for Hyperlipidaemia are as follows: TC >5.2 mmol/L, TG >2.25 mmol/L with maximum between 4-6h, HDLC <0.9 mmol/L, LDLC >3.4 mmol/L, and VLDLC >1.0 mmol/L. The serum lipid results for IGT are as follows: TC 4-6 mmol/L, TG maximum at 6h, HDLC 0.5-1.2 mmol/L, LDLC 2.8-4.1 mmol/L, and VLDLC 0.5-1.2 mmol/L with maximum at 6h (0.6-3.1 mmol/L).

Fasting glucose is <7.0 mmol/L in Normal Control, and 4-7 mmol/L in IGT. In all 3 groups studied, Normal Control, Hyperlipidaemia, and IGT, two peak glucose values are observed, with the first peak at 2h (2hPG) and a second peak at 6h (6hPG). The 2hPG is <7.8 mmol/L in Normal Control and Hyperlipidaemia. In IGT, the 2hPG is 7.8-11.1 mmol/L, and the 6hPG is 4.5-7.5 mmol/L.

In addition, postprandial lipoprotein particles are also studied using transmission electron microscopy (TEM). The isolated LDL particles were stained negatively with phosphotungstic acid at pH 5.04. The lipoprotein particle size can be determined more rapidly using a particle size analyser. The 2-year funding and current account of Project 7 was presented (A/C 305/PPSP/6140020 balance at time of reporting = RM348,742.70).

Some of the problems faced included difficulty recruiting the IGT and IHD subjects from USM alone. Two subjects were recruited per week, and a total of 8 subjects per month. Samples collected so far in this study are 30 Normal Control, 28 Hyperlipidaemia and 6 IGT. No IHD was detected. There is still a need for 2 Hyperlipidaemia, 24 IGT and 30 IHD. Another problem faced was the long 9h collection time which exceeded office hours. Subjects spent the entire time in the Clinical Trials Unit. Students participated when they were free. However, working subjects preferred to go back to work in between blood sampling. Considerable time was wasted walking back and forth between the Lipid Lab in Chemical Pathology and the Clinical Trials Unit. Distance also affected the daily routines of subjects who participated.

1st PENSMA National Congress, Renaissance Melaka Hotel, 18-20 April 2002

**Title** **Palm olein load causes alteration of lipid kinetics during postprandial state in a paired study group**

**Authors** Shahrul B.S.H., Wan M.W.B.*, Mafauzy M.*, and Faridah A.R.

**Institution** Department of Chemical Pathology, *Department of Medicine, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia

**Introduction** Palm olein is the major edible oil in Malaysia. Based on this basis this study on fat metabolism was conducted to clarify the optimum fat load in maintaining chylomicron and VLDL remnant clearance. Individuals are frequently in the postprandial state throughout a day and the differences in fat load along with postprandial lipaemia could predispose individuals to an increase in atherosclerosis development.

**Method** Ten volunteers were given 3 test meals at separate times. Test meals contained fat, carbohydrate and protein sources but differed in the amount of palm olein. The amount of palm olein was increased from 22 g in test meal A to 42 g in test meal B and subsequently to 62 g in test meal C. Blood was drawn pretest and followed by 2 hour, 4 hour, 6 hour, 8 hour, and finally 9 hour, into plain tubes. Triglyceride (GPO-PAP) and total cholesterol (CHOD-PAP) were measured using automated enzymatic colorimetric method.

**Results** The kinetics of triglyceride and total cholesterol differed when the amount of palm olein was increased. The significance of differences between the 3 test meals was tested using repeated measure multiple linear regression and results obtained indicate no significant diffence between the test meals. However when the differences within the test meals during the 8 hour sampling time was tested using Kruskal Wallis it was noted that only test meal A ($p=0.035$) showed significant differences in the changes of triglycerides contents during postprandial state. There was no significant change in cholesterol content between and within the group.

**Discussion** Palm olein contains saturated fatty acid palmitic acid (39.8%), monounsaturated fatty acid oleic acid (42.5%), and polyunsaturated fatty acid linoleic acid (11.2%). As the amount of fatty acids was increased the concentration of triglycerides also increased whereas the was a decline in the clearance rate of triglycerides and chylomicron remnants by remnant receptors in liver. Longer retention time of triglyceride-rich remnants could lead to an increase in the LDL cholesterol concentration which is correlated with the development of atherosclerosis.

**Conclusion** Palm olein does not cause a significant change in cholesterol content but the amount of fat load (palm olein) consumed have to be reduced to less than 60 % calories (26.4% calories) to enable normal metabolism of dietary fat.

Correspondence:
Shahrul Bariyah Sahul Hamid
sbariyah@yahoo.com

7th National Conference on Medical Sciences, 17-18 May 2002, Universiti Sains Malaysia Health Campus, Kubang Kerian, Kelantan, Malaysia.

# Characterization of Low Density Lipoprotein Subfraction Profile and Apo E Genotype Among Diabetic Patients

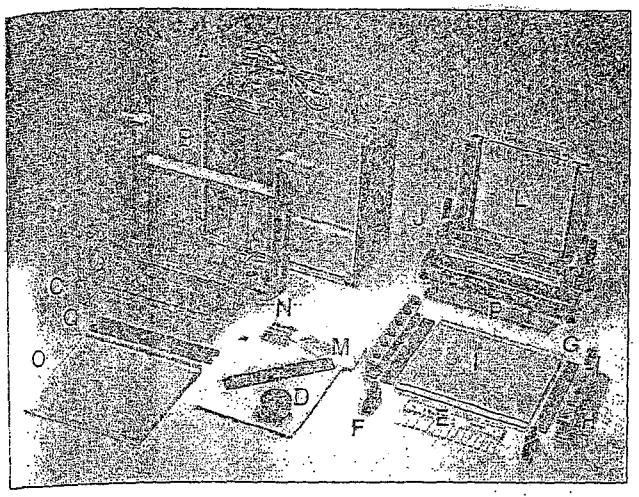
**Shahrul Bariyah Sahul Hamid, Faridah Abdul Rashid and Wan Mohamad Wan Bebakar***
Dept. of Chemical Pathology and *Medicine, School of Medical Sciences
Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

The present study aimed to examine the association between diabetes mellitus type 2 with low density lipoprotein particle size distribution and the influence of apolipoprotein E genotype in altering lipid profile. A total of 35 subjects (19 males, 16 females, mean age 50±11 years, mean BMI 27±4 kg/m$^2$) with diabetes mellitus type 2 who were overweight and without any drug treatment were enrolled in this study. Results obtained were compared with that of 30 normal control subjects (14 males, 16 females, mean age 27±5 years, mean BMI 23±3 kg/m$^2$). Plain blood samples were taken after an overnight fast of 10-12 hours. Serum biochemical analysis was done using automated enzymatic methods (Hitachi 912) for the determination of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and glucose concentration. HDL cholesterol was performed after chemical precipitation. LDL cholesterol was calculated if triglycerides was less than 4.5 mmol/L. Otherwise, direct LDL cholesterol estimation was done. LDL subfraction area under curve percentage (% AUC) was determined by using non-denaturing 2-16 % polyacrylamide gel electrophoresis. APOE gene analysis was by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The lipid profil test results showed that male diabetics had higher triglycerides and LDL cholesterol whereas female diabetics had higher total cholesterol, triglycerides, HDL cholesterol, VLDL cholesterol and glucose. There was a positive and significant correlation between triglycerides and AUC of LDL 4. Triglycerides also correlated positively and significantly with glucose. This indicates that hypertriglyceridaemia could possibly lead to the formation of small LDL particles. The possibility of step-wise conversion of bigger LDL into smaller LDL was studied by looking into the correlation between the LDL subfractions. The AUC of LDL 1 correlated negatively with the AUC of LDL 3. Diabetics generally were found to have higher AUC values for the smaller LDL particles which comprise of LDL 3, LDL 4, and LDL 5. The study on APOE gene showed that ε3 and ε4 diabetics had elevated total cholesterol and glucose. Diabetics with the ε2 allele did not have any significant difference with the ε3 and ε4 subjects when the triglycerides concentration was compared with that of the 3 allele carrier. Allele frequency obtained for diabetics was ε2 (0.143), ε3 (0.714) and ε4 (0.143). The frequency distribution obtained was similar to the findings from the study on diabetic mellitus type 2 subjects by Boemi *et. al* (1995). Frequency comparison with the normal control showed that the ε2 allele frequency was higher in diabetics. Overweight ε4/3 diabetics portrayed the worst atherogenic profile with the highest triglycerides, LDL cholesterol and total cholesterol, and the lowest HDL cholesterol. Insulin resistance is associated with increased non-esterified fatty acid flux to the liver, increased hepatic output of large VLDL which is not suppressed postprandially, hyperlipidaemia and increased cholesterol ester transfer protein activity. All these factors could act in concert and possibly give rise to the formation of small LDL particles observed in this study. The mechanism responsible for the relationship of hyperglycaemia or hyperinsulinaemia with elevated triglycerides and elevated LDL cholesterol is due to reduced enzymatic activity of lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) involved in lipid metabolism. Differences in plasma insulin level or insulin action may affect the activity of both these enzymes. The presence of glycosylated LDL and small LDL increases the contribution of LDL to total cholesterol estimation. This is explained by the reduced binding ability of both these abnormal LDL particles to LDL receptors compared to normal LDL. We therefore conclude that a decrease in LDL size with high propensity for small LDL 4 therefore confers additional atherosclerotic risk to overweight individuals with diabetes mellitus type 2, especially those with ε4/3 genotype.

**Reference:** Boemi, M., Sirolla, C., Amadio, L., Fumelli, P., Pametta, D., and James, R. W.(1995). Apolipoprotein E polymorphism as a Risk Factor for Vascular Disease in Diabetic Patients. *Diabetes Care*. 18(4): 504-508

Correspondence:
Shahrul Bariyah Sahul Hamid sbariyah@yahoo.com

# Hoefer SE 600 Components and Accessories




SE 600 Standard Vertical Unit Components

A. Lid assembly, 80-6176-52
B. Lower buffer chamber, 80-6182-98
C. Upper buffer chamber, 80-6176-33
D. Gel seal, 80-6421-43
E. Comb, various
F. Cams, 80-6174-24
G. Spacer, various
H. Clamp 16 cm, 80-6173-29
I. Glass plate 18 × 16 cm, 80-6178-99
J. Casting stand, 80-6175-00
K. Heat exchanger, 80-6183-74
L. Level, 80-6194-19
M. Wonder Wedge separator, 80-6127-88
N. Spacer-Mate, 80-6181-65
O. Buffer dam, 80-6175-19
P. Laminated rubber gasket, 80-6174-62
Q. Slotted rubber gasket, 80-6174-43

ORDERING INFORMATION

| Product | Quantity | Code Number |
|---|---|---|
| **SE 600 and SE 660 Replacement Parts and Accessories** | | |
| Wonder Wedge plate separation tool | 1 | 80-6127-88 |
| Slotted silicone rubber gaskets for upper buffer chamber | 2 | 80-6174-43 |
| Laminated silicone rubber gaskets for casting stand | 2 | 80-6174-62 |
| Buffer dam | 1 | 80-6175-19 |
| Upper buffer chamber for SE 600, SE 660 | 1 | 80-6176-33 |
| Lid with power cables for SE 600, SE 660 | 1 | 80-6176-52 |
| High-voltage safety lead set | 1 | 80-6177-09 |
| Lower buffer chamber for SE 600 | 1 | 80-6182-98 |
| Heat exchanger for SE 600 and SE 660 | 1 | 80-6183-74 |
| Lower buffer chamber for SE 660 | 1 | 80-6191-15 |
| Gel Seal | 1 | 80-6421-43 |
| Level | 1 | 80-6194-19 |
| **Clamps and Cams** | | |
| Clamp assemblies, 16 cm | 2 | 80-6173-29 |
| Clamp assemblies, 8 cm | 2 | 80-6187-35 |
| Clamp and cam kit, four 16 cm clamps and 8 black cams | 1 | 80-6173-67 |
| Cams, white, for old-style clamps without cam holes | 4 | 80-6174-05 |
| Cams, black, for new-style clamps with cam holes | 4 | 80-6174-24 |

| Technical Bulletins | Code Number |
|---|---|
| #116 – Preparative Electrophoresis in the SE 600 Vertical Slab Unit: A Simple Modification | 80-6009-89 |
| #117 – Vertical Agarose Gel Electrophoresis of Plasmid DNA | 80-6010-08 |

# Hoefer SE 600 / SE 400 Combs and Spacers

- *All combs listed can be used in all SE 600 and SE 400 units.*
- Preparative combs with single or dual reference lanes.
- Spacers (16 cm) and most SE 600 accessories can be used with SE 400.
- Spacers (24 cm) and most SE 660 accessories can be used with SE 410.

ORDERING INFORMATION

| Product | Quantity | Code Number |
|---|---|---|
| **Comb Accessory** | | |
| Adjustable comb back (Convert any 25 mm deep comb to 10 or 15 mm depth.) | 1 | 80-6163-22 |

| Length (cm) | Thickness (mm) | Width (cm) | Quantity | Code Number |
|---|---|---|---|---|
| **Spacers for Vertical Gel Units (set of 2)** | | | | |
| 8 | 1.00 | 1 | 2 | 80-6443-09 |
| 8 | 1.50 | 1 | 2 | 80-6443-28 |
| 8 | 0.75 | 2 | 2 | 80-6187-73 |
| 8 | 1.00 | 2 | 2 | 80-6187-92 |
| 8 | 1.50 | 2 | 2 | 80-6188-11 |
| 8 | 1.50 | 2 | 2 | 80-6180-51 |
| 16 | 1.00 | 2 | 2 | 80-6180-70 |
| 16 | 1.50 | 2 | 2 | 80-6180-89 |
| 24 | 0.75 | 2 | 2 | 80-6190-58 |
| 24 | 1.00 | 2 | 2 | 80-6190-77 |
| 24 | 1.50 | 2 | 2 | 80-6190-96 |
| 16 | 1.00 | 1 | 2 | 80-6179-94 |
| 16 | 1.50 | 1 | 2 | 80-6180-13 |

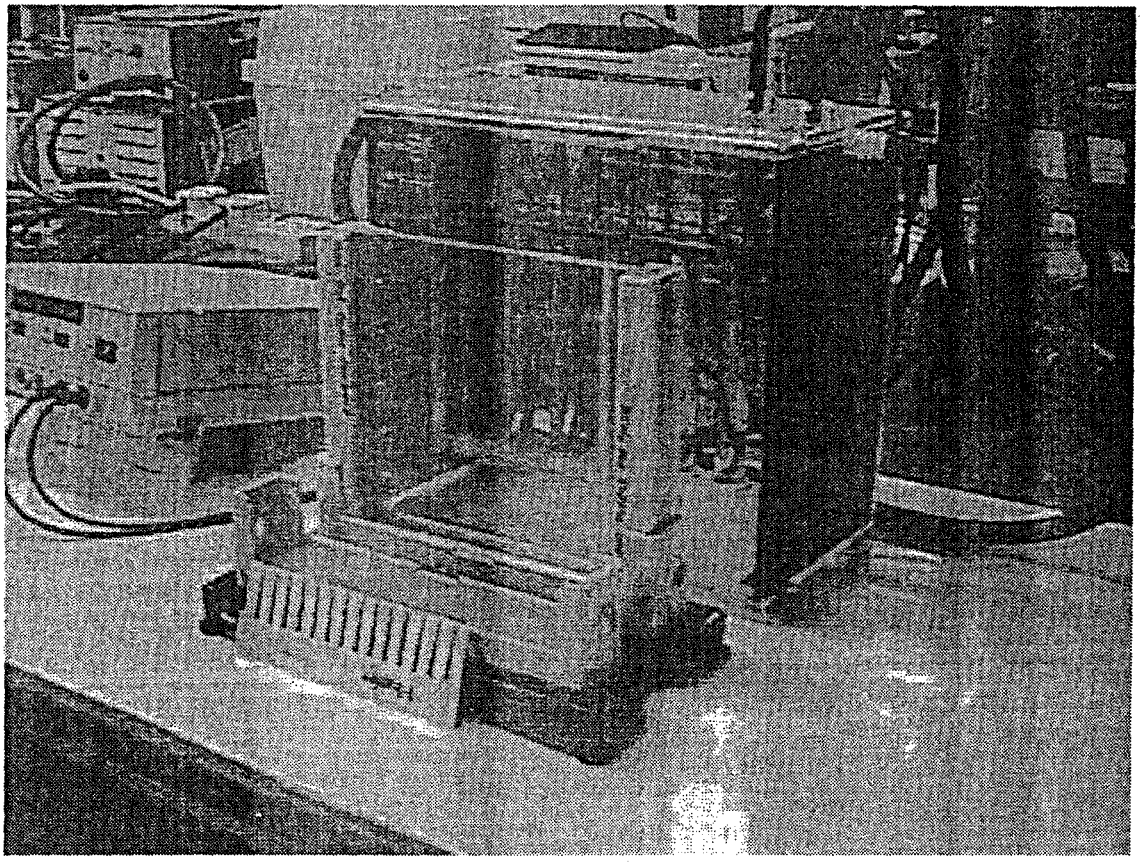
ORDERING INFORMATION

| No. Wells Prep/rel | Thickness (mm) | Width (mm) | Quantity | Code Number |
|---|---|---|---|---|
| **Preparative Combs for SE 400 and SE 600 Series** | | | | |
| 1/1* | 0.75 | 121/6 | 1 | 80-6164-17 |
| 1/1* | 1.00 | 121/6 | 1 | 80-6164-36 |
| 1/1* | 1.50 | 121/6 | 1 | 80-6164-55 |
| 1/2* | 0.75 | 113/6 | 1 | 80-6163-41 |
| 1/2* | 1.00 | 113/6 | 1 | 80-6163-60 |
| 1/2* | 1.50 | 113/6 | 1 | 80-6163-79 |
| **Teflon Combs for SE 400 and SE 600 Series** | | | | |
| 10 | 0.75 | 8.3 | 1 | 80-6159-99 |
| 10 | 1.00 | 8.3 | 1 | 80-6160-18 |
| 10 | 1.50 | 8.3 | 1 | 80-6160-37 |
| 12 | 0.75 | 7.6 | 1 | 80-6160-75 |
| 12 | 1.50 | 7.6 | 1 | 80-6160-94 |
| 15 | 0.75 | 5.7 | 1 | 80-6161-13 |
| 15 | 1.00 | 5.7 | 1 | 80-6161-32 |
| 15 | 1.50 | 5.7 | 1 | 80-6161-51 |
| 20 | 0.75 | 4.1 | 1 | 80-6161-70 |
| 20 | 1.00 | 4.1 | 1 | 80-6161-89 |
| 20 | 1.50 | 4.1 | 1 | 80-6162-08 |
| 28** | 0.75 | 2.7 | 1 | 80-6162-27 |
| 28** | 1.00 | 2.7 | 1 | 80-6162-46 |
| 28** | 1.50 | 2.7 | 1 | 80-6162-65 |

* *These combs are 25 mm deep, adjustable to 10 or 15 mm.*
** *Comb depth 15 mm; all others 25 mm.*

| Jenis | Keterangan | Pembekal | Serial Number | Harga | Tarikh beli | PO/DO/Invoice |
|---|---|---|---|---|---|---|
| Peralatan elektroforesis<br><br>LAMPIRAN 6 | Dual cooled vertical electrophoresis system, Model SE 600 Cat No: 80-6171-96 | Amersham Pharmacia Biotech, Hoefer | 20058937 | RM3,600 | 2 Oct 2000 | PO: A18103<br>DO: 21887<br>Invoice: 17872 |
| **Lokasi terkini peralatan** | | | Makmal Lipid, Jabatan Patologi Kimia<br>**Tarikh:** 7 April 2002 | | | |
| **Staf bertanggungjawab ke atas perlatan berkenaan** | | | Shahrul Bariyah Sahul Hamid (calon PhD)<br>sbariyah@yahoo.com | | | |

Gambar perlatan yang dibeli:

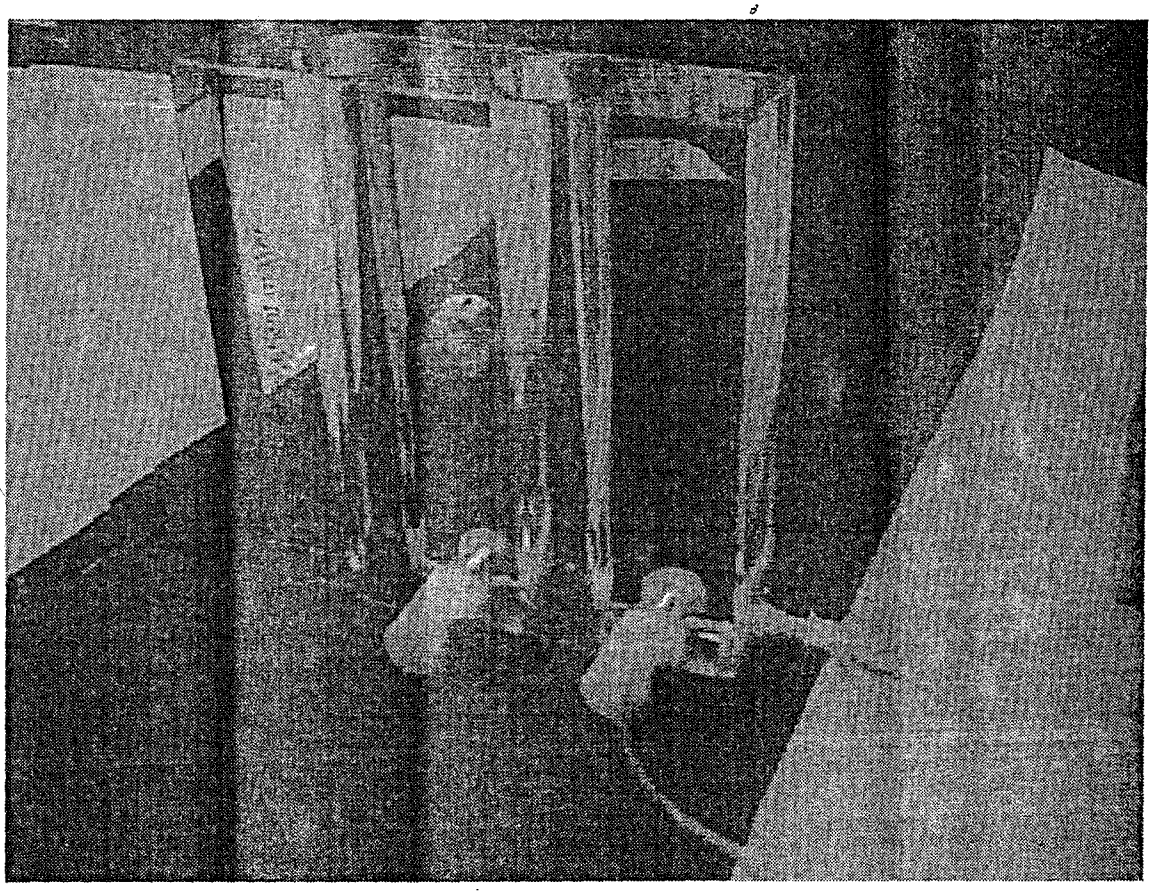


Special note:

1. This system is complete for 18x16 cm slab gels 2000
2. Please refer to Amersham Pharmacia Biotech~~Hoefer~~ catalogue for details
3. Does not include power supply unit
4. Does not include Julabo circulator and hoses

| Jenis | Keterangan | Pembekal | Serial Number | Harga | Tarikh beli | PO/DO/Invoice |
|---|---|---|---|---|---|---|
| Peralatan elektroforesis<br><br>LAMPIRAN 7 | Gradient maker, Model SG 50<br>Cat No: 80-6197-99 | Amersham Pharmacia Biotech, Hoefer | None | RM999 | 2 Oct 2000 | PO: A18103<br>DO: 21887<br>Invoice: 17872 |
| **Lokasi terkini peralatan** | | | Makmal Lipid, Jabatan Patologi Kimia<br>**Tarikh:** 7 April 2002 | | | |
| **Staf bertanggungjawab ke atas perlatan berkenaan** | | | Shahrul Bariyah Sahul Hamid (calon PhD)<br>sbariyah@yahoo.com | | | |

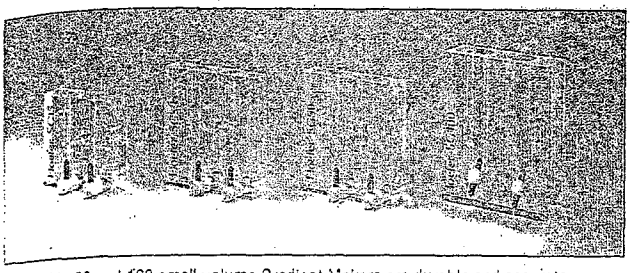
Gambar peralatan yang dibeli:



Special note:

1. Includes/does not include magnetic stir bar
2. Includes/does not include silicon tubing
3. Does not include mounting clamp for mounting gradient maker

# Hoefer SG Gradient Makers



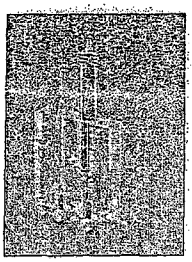
SG 15, 30, 50 and 100 small volume Gradient Makers are durable and accurate.

- *Form gradients for acrylamide gel electrophoresis; liquid chromatography elution; and sucrose, caesium, and preparative zonal centrifugation.*
- Generate concave or convex exponential gradients with accessory plungers (50 and 100 ml models).
- Choose from five sizes between 15 ml and 500 ml total volume.
- Ring stand support rod included with the SG 15, SG 30, SG 50 and SG 100.

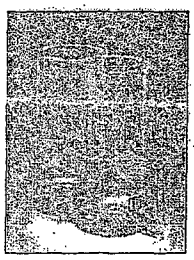
For gradients up to 2.2 litres, refer to DALT Gradient Maker, see page 361.

ORDERING INFORMATION

| Product | Quantity | Code Number |
|---|---|---|
| SG 15 Gradient maker, 15 ml total volume | 1 | 80-6197-61 |
| SG 30 Gradient maker, 30 ml total volume | 1 | 80-6197-80 |
| SG 50 Gradient maker, 50 ml total volume | 1 | 80-6197-99 |
| SG 100 Gradient maker, 100 ml total volume | 1 | 80-6196-09 |
| SG 500 Gradient maker, 500 ml total volume | 1 | 80-6198-18 |
| Accessories and Replacement Parts | | |
| Plunger for exponential gradient, SG 50 | 1 | 80-6198-75 |
| Plunger for exponential gradient, SG 100 | 1 | 80-6197-23 |
| White 3 mm outlet fitting for SG 15, SG 30, SG 50 and SG 100 | 1 | 80-6196-85 |
| Valve, salt gradient push-pull, for SG 500 | 1 | 80-6198-56 |
| Outlet fitting, red, 4 mm, for SG 500 | 4 | 80-6226-30 |



SG 50 Gradient Maker with plunger.

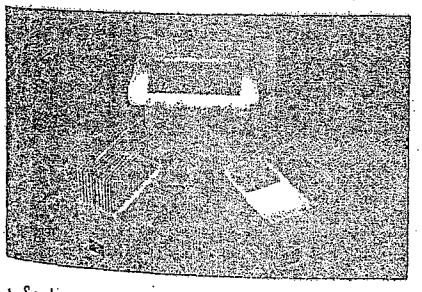


SG 500 Large Volume Gradient Maker.

Separation Guide – Gradient Volumes for Gel Formats

| Approximate Gradient Volumes | | | | | |
|---|---|---|---|---|---|
| Electrophoresis Unit | No. of Gels | Hoefer Gel Caster | Gel Thickness 0.75 mm | 1.0 mm | 1.5 mm |
| SE 600, SE 400 | 10 | Multiple Gel Caster, 16 cm, 80-6182-79 | 22 ml | 29 ml | 44 ml |
| SE 600, SE 400 | | Gel Caster Kit, 16 cm, 80-6131-34 | 63 ml | 85 ml | 127 ml |
| SE 250 | 10 | Mighty Small II Multiple Gel Caster, 8 cm, 80-6142-51 | 55 ml | 73 ml | 110 ml |
| SE 250 | | Mighty Small II Gel Caster, 10 × 8 cm, 80-6151-06 | 22 ml | 29 ml | 44 ml |
| SE 260 | | Mighty Small II Gel Caster, 10 × 10 cm, 80-6145-12 | 28 ml | 37 ml | 55 ml |

# Hoefer SE 100 Plate Washer and Storage Unit



SE 100 Plate Washer and Storage Unit simplifies glass plate handling.

- *Soak, rinse and store multiple gel plates with convenient rack and covered tank.*
- Protects gel plates from chipping and cracking during handling and storage.
- Adjusts for plates from 8 × 10 cm to 18 × 16 cm.
- Holds ten 10-18 cm plates or twenty 8 cm plates.

SE 100 Plate Washer is an ideal accessory for all Hoefer vertical units.

ORDERING INFORMATION

| Product | Quantity | Code Number |
|---|---|---|
| SE 160 Plate Washer and Storage Unit<br>Includes: 2 moulded plate holders with handles, 2 long plate adaptors, 2 dust covers and 1 polypropylene washing tank with lid. | 1 | 80-6116-29 |
| Long plate adaptor for 10 cm and 12 cm long plates | 2 | 80-6116-48 |
| Moulded plate holder with handle and dust cover | 1 | 80-6116-67 |
| Moulded plate holders with handles and dust covers | 4 | 80-6116-86 |
| Polypropylene washing tank with lid | 1 | 80-6117-05 |
| Dust cover for plate holder | 1 | 80-6117-24 |

# Manual DNA Sequencing Equipment

See main product entries, Chapter 7.

Senarai Penerbitan Geran Penyelidikan USM Jangka Pendek "**Characterisation of LDL subfraction profile and apolipoprotein E genotype among diabetic patients**" A/C No: 304/PPSP/6131113 Tempoh penyelidikan: 01.06.2000 – 31.05.2001; disambung hingga 30.11.2001; Jumlah geran: RM19,575

1. Tesis PhD:

    - Nama pelajar: Cik Shahrul Bariyah bt. Sahul Hamid
    - Tajuk tesis PhD:
        i. BM: **Pembangunan Kaedah Genotip Apolipoprotein E, Profil Pecahan Lipoprotein Densiti Rendah** dan Kajian Lipemia Pascaprandial
        ii. BI: **Development of Apolipoprotein E Genotyping, Low Density Lipoprotein Subfraction Profiling** and Study of Postprandial Lipaemia
    - Tesis telah dihantar ke IPS, USM
    - Tarikh hantar tesis: Mei 2002

2. Kajian awal mengenai genotip apolipoprotein E dan frekuensi alel di kalangan subjek sihat. Shahrul BSH, Faridah AR. Pascasidang: Simposium Sains Kesihatan Kebangsaan Ke-3 FSKB, UKM, 29-30 April 2000, Kuala Lumpur. Ms 360

3. Relationship between genotype and allele of apolipoprotein E with the lipid status among Malays. Shahrul BSH, Mohd Rafi, Wan MWB, Faridah AR.

    - 1st ASEAN Conference on Medical Sciences, 18-21 May 2001, Book of Abstracts, Abstract No. P-22, page 69
    - The Malaysian Journal of Medical Sciences, July 2001; 8(2): 91-92

4. Characterisation of low density lipoprotein subfractio profile and apo E genotype among diabetic patients. Shahrul Bariyah Sahul Hamid, Faridah Abdul Rashid and Wan Mohamad Wan Bebakar. 7th National Conference on Medical Sciences, 17-18 May 2002, USM. Abstract Code O-62, page 99

Maklumat disediakan oleh:
PROF. MADYA FARIDAH ABDUL RASHID
28 November 2002

Nama pelajar PhD:
Cik Shahrul Bariyah bt. Sahul Hamid

Tesis PhD dalam Bahasa Malaysia:
**Pembangunan Kaedah Genotip Apolipoprotein E, Profil Pecahan Lipoprotein Densiti Rendah** dan Kajian Lipemia Pascaprandial

Tesis PhD dalam Bahasa Inggeris:
**Development of Apolipoprotein E Genotyping, Low Density Lipoprotein Subfraction Profiling** and Study of Postprandial Lipaemia

Submit tesis PhD kepada:
USM

Tarikh submit tesis PhD:
Mei 2002

Maklumat geran yang digunakan untuk penyelidikan PhD:

| Name | Position | Title of Research Project | Grant | Start | End | Amount (RM) |
|---|---|---|---|---|---|---|
| Assoc. Prof. Faridah Abdul Rashid | PI | **Characterisation of LDL subfraction profile and apolipoprotein E genotype among diabetic patients**<br><br>A/C No: 304/PPSP/6131113 | USM Short Term | 01.06.00 | 31.05.01; extended to 30.11.01 | 19,575 |
| Assoc. Prof. Faridah Abdul Rashid | CI | Promotion of Healthy Lifestyles in Malaysia:<br>I. A study of postprandial patterns of lipaemia and glycaemia in normal controls and patients with IGT and IHD<br>II. Diabetes Control and Dyslipidaemia<br>A/C No: 304/PPSP/6140020 | IRPA RM7 | 1999 | 09.02 | 1999:215,950<br>2000: 141,700<br>2001: 119,890<br>2002: 283,549<br>(PI=Prof. Mafauzy) |

PI=Principal Investigator; CI=Co-Investigator

# PEMBANGUNAN KAEDAH GENOTIP APOLIPOPROTEIN E, PROFIL PECAHAN LIPOPROTEIN DENSITI RENDAH DAN KAJIAN LIPEMIA PASCAPRANDIAL

SHAHRUL BARIYAH SAHUL HAMID

UNIVERSITI SAINS MALAYSIA

MEI 2002

## ABSTRAK

### PEMBANGUNAN KAEDAH GENOTIP APOLIPOPROTEIN E, PROFIL PECAHAN LIPOPROTEIN DENSITI RENDAH DAN KAJIAN LIPEMIA PASCAPRANDIAL

Matlamat kajian ini adalah untuk menentukan peranan polimorfisme apolipoprotein E terhadap profil lipid yang merangkumi perubahan lipidemia dan glisemia semasa pascaprandial. Genotip ditentukan melalui kaedah polimorfisme panjang fragmen restriksi (RFLP) teroptimum menggunakan DNA dari leukosit yang diekstrak melalui kaedah penggaraman keluar. Frekuensi genotip yang diperoleh adalah sama dengan populasi kawasan Asia yang normal ($\chi^2 < 11.07$). Tahap kolesterol jumlah, LDL kolesterol, dan trigliserida adalah tinggi dalam kalangan pembawa ε3 dan ε4 manakala HDL kolesterol adalah tinggi hanya dalam kalangan subjek genotip ε3/2. Apabila perbandingan dilakukan dengan frekuensi populasi keseluruhan, didapati bahawa frekuensi alel ε2 (0.05) dan ε3 (0.83) lebih tinggi dalam kalangan subjek perempuan manakala ε4 (0.15) pula adalah lebih tinggi dalam kalangan subjek lelaki. Pemencilan partikel lipoprotein berdensiti rendah dilakukan dengan kaedah pemendakan yang ringkas dan cepat menggunakan penimbal natrium sitrat berheparin yang mempunyai pH 5.16. Seterusnya, LDL dicirikan dengan cara subpecahan menggunakan elektroforesis gel poliakrilamida cerun tanpa penyahaslian selepas proses pengoptimuman. Pewarna lipid (Oil Red O dan Sudan Black B) dan protein (Coomassie Brilliant Blue R-250) telah digunakan dalam kajian ini. Pempiawaian gel dilakukan dengan menggunakan piawai yang diketahui diameter yang terdiri dari butiran lateks polisterin, albumin tulen, HDL, globulin, dan serum tanpa lipoprotein (LPDS). Peratus keluasan kawasan bawah graf (AUC) subpecahan LDL untuk setiap puncak ditentukan dengan menggunakan perisian ImageMaster (TotalLab versi 1.0).

Kajian ke atas subjek pesakit diabetes mellitus jenis 2 menunjukkan bahawa pembawa ε3 mempunyai tahap LDL kolesterol yang lebih tinggi berbanding dengan kumpulan kawalan normal ($p=0.003$). Hiperglisemia juga didapati mempengaruh mekanisme penyingkiran remnan dalam kalangan pembawa alel ε3 ($p<0.001$) dan ε4 ($p<0.001$). Korelasi positif antara trigliserida dengan glukosa ($p<0.001$) menunjukkan bahawa individu yang mempunyai kawalan glisemia yang rendah berkecenderungan untuk mempunyai partikel LDL kecil. Ini kerana peratus AUC subpecahan LDL 4 adalah berkorelasi secara hampir signifikan dengan trigliserida ($p=0.05$). Pemberian sumber lemak sebanyak 58.1 % iaitu lebih tinggi dari kapasiti penyingkiran lemak normal (47.7 %) yang didapati semasa kajian awal dipilih sebagai minuman piawai dalam kajian pascaprandial. Terdapat perbezaan yang ketara dalam metabolisme LDL kolesterol dan trigliserida dalam tempoh 9 jam di antara subjek kawalan, hiperlipidemia, dan tolerans glukosa tidak terkawal (IGT). Perbandingan selanjutnya terhadap kepekatan kedua-dua parameter ini di antara masa permulaan dengan masa penyampelan menunjukkan terdapat perbezaan signifikan selepas 4 jam pascaprandial. Perbandingan taburan alel APOE di antara 3 kumpulan menunjukkan bahawa terdapat perbezaan yang ketara di antara subjek dengan alel ε2 ($\chi^2 < 7.815$) dan ε4 ($\chi^2 < 7.815$).

## ABSTRACT

## DEVELOPMENT OF APOLIPOPROTEIN E GENOTYPING, LOW DENSITY LIPOPROTEIN SUBFRACTION PROFILING AND STUDY OF POSTPRANDIAL LIPAEMIA

The aim of the present study was to determine the role of apolipoprotein E polymorphism in lipid profile including lipidaemic and glycaemic changes in the postprandial state. Genotyping was done by optimized restriction fragment length polymorphism (RFLP) method using DNA from leucocytes extracted by salting out method. The frequency of genotypes obtained are the same as for the normal Asian population ($\chi^2$<11.07). The total cholesterol, LDL cholesterol, and triglyceride level was high among the ε3 and ε4 carriers whereas HDL cholesterol was high only in the ε3/2 genotype subjects. The ε2 (0.05) and ε3 (0.83) allele frequency among the female subjects were higher whereas the ε4 (0.15) allele was higher in the male subjects when comparison was done with the overall population frequency. Low density lipoprotein (LDL) particles were isolated by a simple and rapid precipition method using sodium citrate heparinol buffer, pH 5.16. LDL was further characterized by subfractionating it using 2-16 % non denaturing gradient polyacrylamide gel electrophoresis after optimization. Lipid (Oil Red O and Sudan Black B) and protein (Coommassie Brilliant Blue R-250) stains were used in this study. Gel was calibrated using standards of known diameter which comprised of latex polystyrene beads, purified albumin, HDL, globulin, and lipoprotein deficient serum (LPDS). The LDL subfraction area under curve percentage (AUC) for each peak was determined using the ImageMaster (TotalLab version 1.0) software. Study on the diabetes mellitus type 2 subjects showed the ε3 carriers had higher LDL cholesterol level compared to the normal control group (p=0.003). Hyperglycaemia is seen to affect the remnant removal mechanism also

among the ε3 ($p<0.001$) and ε4 ($p<0.001$) allele carriers. Positive correlation between triglyceride and glucose ($p<0.001$) indicates that individuals with poor glycaemic control have the tendency to have small LDL particles. This is due to the marginally significant correlation of LDL 4 AUC with triglyceride ($p=0.05$). A total fat load of 58.1 % which is higher than the normal fat removal capacity (47.7%) seen in the pilot study was chosen to be the standard milkshake in the postprandial study. There was significant differences in LDL cholesterol and triglyceride metabolism within the 9$^{th}$ hour period between the control, hyperlipidaemic, and impaired glucose tolerance subjects. Further comparison of changes between baseline and sampling hour shows that both the parameters were significant ($p<0.001$) between the groups at 4 hour postprandial. Comparison of each APOE allele distribution between the 3 groups showed that there was a significant difference among the ε2 ($\chi^2 > 7.815$) and ε4 ($\chi^2 > 7.815$) allele subjects.

# Kajian Awal Genotip Apo E and Frekuensi Alel Di Kalangan Subjek Normal

## *Preliminary Studies on Apolipoprotein E Genotypes and Allele Frequency Among Healthy Subjects*

Shahrul BSH ,Faridah AR

Department of Chemical Pathology
School of Medical Sciences
Universiti Sains Malaysia
16150 Kubang Kerian, Malaysia

**Abstrak**

Apo E dikaitkan dengan penyakit jantung (Kardia *et al.*, 1999 ). Tiga alel iaitu epsilon 2, epsilon 3 dan epsilon 4 menentukan 6 genotip apo E. Penentuan genotip dilakukan dengan kaedah polimorfisma rangkaian fragmen terpilih (RFLP). Kolesterol dan trigliserida ditentukan dengan kaedah berautomasi. Kolesterol lipoprotein berketumpatan rendah (LDL) diperolehi dari pengiraan Friedewald manakala kolesterol lipoprotein berketumpatan amat rendah (VLDL) ditentukan dengan membahagikan kepekatan trigliserida dengan pemalar 2.2. Kolesterol lipoprotein berketumpatan tinggi (HDL) ditentukan dengan kaedah pemendakan asid fosfotungstik dan ion Mg. Frekuensi alel epsilon 3 didapati lebih tinggi di kalangan wanita berbanding dengan lelaki sebanyak 5.2 % manakala terdapat peningkatan sebanyak 8.3 % dan 66.7 % pada subjek lelaki dengan alel epsilon 4 serta 2. Kepekatan trigliserida dalam pembawa E 3/3 adalah 1.79 mmol/L manakala dalam E 4/2, E 2/2, E 3/2 dan E 4/3 adalah lebih tinggi iaitu sebanyak 60.3 %, 53.6 %, 47.5 % and 10.6 % dalam turutan. Kepekatan lipoprotein berketumpatan tinggi (HDL) adalah hampir sama bagi setiap genotip dan kepekatan LDL didapati meningkat sebanyak 46.2 % pada subjek E 2/2 dan sebaliknya pada subjek E 4/2 sebanyak 13.8 %. Kepekatan VLDL didapati lebih tinggi pada subjek E 4/2, E 2/2, E 3/2 sebanyak 67.9 %, 60.3 % and 53.8 % dalam turutannya. Genotip E 4/2 dicirikan oleh kepekatan trigliserida dan VLDL yang tinggi. Ciri ini juga diperhati pada subjek dengan E 2/2 yang mempunyai kandungan kolesterol LDL yang tinggi berbanding dengan genotip lain.

**Abstract**

Apo E is correlated with heart disease (Kardia *et al.*, 1999). Three alleles, epsilon 2, epsilon 3 and epsilon 4, determine 6 apo E genotypes. The apo E genotype was analyzed by restriction fragment length polymorphism (RFLP). Cholesterol and triglyceride were measured by automated method. Low density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula and very low density lipoprotein (VLDL) cholesterol determined by dividing triglyceride by a constant of 2.2. High density lipoprotein (HDL) cholesterol was quantitated by first precipatating using phosphotungstic acid and Mg ions. The allelic frequency of epsilon 3 was found to be higher in females compared to males by 5.2 % whereas the there was an increase of 8.3 % and 66.7 % in the percentage of epsilon 4 and epsilon 2 among the males. Triglyceride concentration in E 3/3 carriers was 1.79 mmol/L while in E 4/2, E 2/2, E 3/2 and E 4/3 the concentrations were higher by 60.3 %,

53.6 %, 47.5 % and 10.6 % respectively. High density lipoprotein (HDL) cholesterol concentration was found to be almost similar for each of the genotypes and LDL cholesterol concentration was raised by 46.2 % in the E 2/2 carrier and reduced in the E 4/2 carrier by 13.8 %. VLDL cholesterol concentration was found to be higher in the E 4/2, E 2/2, E 3/2 by 67.9 %, 60.3 % and 53.8 % respectively. The E 4/2 genotype carriers are characterized by the higher triglyceride and VLDL cholesterol concentration. This was also seen in the E 2/2 carrier whose LDL cholesterol was seen to be higher compared to the other genotypes.

## Introduction

Apo E is one of the several protein components that occur normally on VLDL, chylomicrons and certains classes of high density lipoprotein (HDL). Apo E mediates the rapid and efficient clearance of chylomicron remnants from the circulation via the liver. Apo E was discovered to be a major ligand for the LDL receptor and is responsible for mediating the cellular uptake of chylomicron remnants and VLDL remnants. A certain portion of these hepatic very low density lipoprotein (VLDL) remnants (~50 percent) is also removed from circulation via the liver before reaching the final stage in the cascade for the formation of low density lipoprotein (LDL). Hepatic uptake of VLDL remnants including intermediate denstity lipoprotein (IDL) is mediated by apo E. This resulting in progressively smaller and more cholesterol-rich lipoprotein.

## Materials and Method

Healthy volunteers 54 women and 16 men 20 to 65 years from Kelantan were recruited in the study. Blood was collected after an overnight fast of 10 to 12 hours. Blood samples were drawn in tubes containing sodium EDTA, 1mg/mL, centrifuged at $4^0$C and plasma was separated. DNA was extracted from the buffy coat layer. The apo E genotype was analyzed by restriction fragment length polymorphism (RFLP). Polyacrylamide gel electrophoresis was used to separate the restriction fragments, followed by ethidium bromide staining and visualized on an ultraviolet transilluminator. Lipid profile was determined from plasma obtained. Cholesterol and triglyceride were measured by automated method using colorimetri enzymatic tests CHOD-PAP Boehringer Mannheim and GPO-PAP Boehringer Mannheim respectively. LDL cholesterol was calculated by the Friedewald formula and VLDL cholesterol was determined by dividing triglyceride by a constant of 2.2.

## Results

In our sample of, we observed 61.1 % of the women and 62.5 % of the men are of the E 3/3 genotype. Combined (male and female) the distribution of apo E genotype was 61.4 % were E 3/3 carriers, 21.4 % (E 4/3), 11.4 % (E3/2), 4.3 % (E 4/2), 1.4 % (E 2/2) and none were E 4/4. The epsilon 3 allele frequency was found to be the highest which accounted for 78 % compared to 13 % for the epsilon 4 allele and 9 % for the epsilon 2 allele. Comparison between the male and female group showed that in the males almost 63 % of them had the normal E 3/3 genotype followed by 25 % having E 4/3 and 12.5 % having E 3/2. Data obtained from the female subjects were almost similar to the males, where 61.1

% of them were E 3/3, 20.4 % E4/3, 11.1 % E 3/2 and 5.5 % E 4/2. The allelic frequency of epsilon 3 was found to be higher in females compared to males by 5.2 % whereas there was an increase of 8.3 % and 66.7 % in the percentage of epsilon 4 and epsilon 2 among the males. Plasma lipid analysis for the combined group showed the cholesterol concentration among the normal E 3/3 genotype was 5.77 mmol/L and the concentration was raised by 4.8 % in the E 3/2 carriers. Triglyceride concentration was found to be 1.79 mmol/L in E 3/3 carriers while in, E 4/2, E 2/2, E 3/2 and E 4/3 the concentrations were higher by 60.3 %, 53.6 %, 47.5 % and 10.6 %, respectively. HDL cholesterol concentration was found to be almost the similar for each of the genotypes, which was about 1.3 mmol/L. The LDL cholesterol concentration for the E 3/3 genotype was found to be 3.55 mmol/L and raised by 46.2 % in the E 2/2 carrier (1 person) and reduced in the E 4/2 carrier by 13.8 %. Finally the VLDL cholesterol was found to be higher in the E 4/2, E 2/2, E 3/2 by 67.9 %, 60.3 % and 53.8 % respectively.

**Discussion**

The results indicate that most of the normal subjects were epsilon 3 allele carriers. A similar allele frequency was found in the population study done by Boemi et al., 1995. In our study the frequency of the E 3/3 genotype was the highest followed by E 4/3, E 3/2, E 4/2 and E 4/4 among the Kelantanese population. Compared to the study by Boemi et al., the difference seen in the Kelantanese population was in the male subjects carrying E 4/3 and E 3/2 genotype, where the frequencies were higher by 38.9 % and 20 % respectively. The epsilon 3 allele frequency was similar (78 %) but the epsilon 4 frequency was only 0.5 % lower (13%). The frequency of subjects with epsilon 2 was higher among Kelantanese polulation by 26.7 %. The E 4/2 genotype carriers were characterized by the higher triglyceride and VLDL cholesterol concentration. This was also seen in the E 2/2 carrier whose LDL cholesterol was seen to be higher compared to the other genotypes. The epsilon 2 and 4 were associated with some degree of impaired clearance of triglyceride-rich chylomicrons and VLDL. Further research will have to be done to demonstrate that the abnormal apo E genotypes influence the development of coronary artery disease among diabetic patients.

**References**

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Kardia S.L.R., Haviland M.B., Ferrell R.E. and Sing C.F. (1999). The relationship Between Risk Factor Levels and Presence of Coronary Artery Calcification is Dependent on Apolipoprotein E Genotype. *Arteriosclerosis, Thrombosis and Vascular Biology*. 19(2): 427-435

## Abstract

Apo E gene is correlated with heart disease (Kardia et al., 1999). Three alleles, epsilon 2, epsilon 3 and epsilon 4, determine 6 apo E genotypes. The apo E genotype was analyzed by restriction fragment length polymorphism (RFLP). Cholesterol, triglyceride and HDL cholesterol were measured by automated method. Low density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula and very low density lipoprotein (VLDL) cholesterol determined by dividing triglyceride by a constant of 2.2. The allelic frequency of epsilon 3 was found to be higher in females compared to males by 5.2 % whereas the there was an increase of 8.3 % and 66.7 % in the percentage of epsilon 4 and epsilon 2 among the males. Triglyceride concentration in E 3/3 carriers was 1.79 mmol/L while in E 4/2, E 2/2, E 3/2 and E 4/3 the concentrations were higher by 60.3 %, 53.6 %, 47.5 % and 10.6 % respectively. High density lipoprotein (HDL) cholesterol concentration was found to be almost similar for each of the genotypes and LDL cholesterol concentration was raised by 46.2 % in the E 2/2 carrier and reduced in the E 4/2 carrier by 13.8 %. VLDL cholesterol concentration was found to be higher in the E 4/2, E 2/2, E 3/2 by 67.9 %, 60.3 % and 53.8 % respectively. The E 4/2 genotype carriers are characterized by the higher triglyceride and VLDL cholesterol concentration. This was also seen in the E 2/2 carrier whose LDL cholesterol was seen to be higher compared to the other genotypes.

Apo E is one of the several protein components that occur normally on VLDL, chylomicrons and certains classes of high density lipoproteins (HDL).

Apo E was discovered to be a major ligand for the LDL receptor and is responsible for mediating the cellular uptake of chylomicron remnants and VLDL remnants.

Apolipoprotein E polymorphism is one of the common genetic factor for interindividual differences in lipid and lipoprotein levels.

Three alleles, epsilon 2, epsilon 3 and epsilon 4, determine 6 apo E genotypes. The sequence of apo E 2, E 3 and E 4 are identical except residue 112 and residue 158.

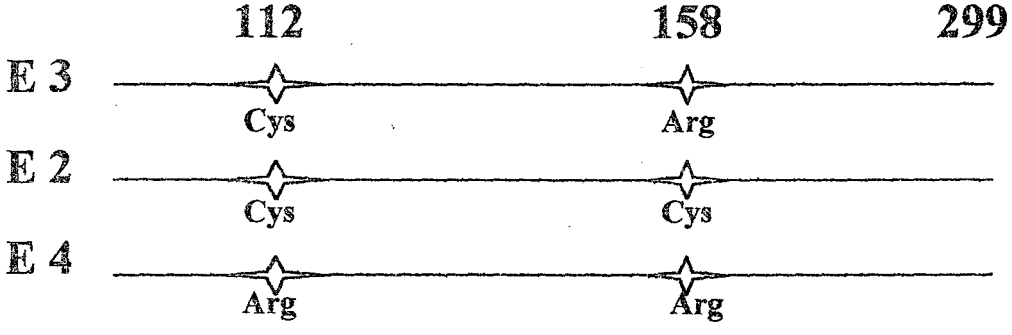


**FIGURE 1 : Apo E isoforms**

## Materials and Method

Healthy volunteers, 54 women and 16 men aged 20 to 65 years from Kelantan were recruited in the study. Blood was collected after an overnight fast of 10 to 12 hours.

↓

Blood samples were drawn in tubes containing sodium EDTA ( 1mg/mL ), centrifuged at $4^0$C and plasma was separated.

↓

DNA was extracted from the buffy coat layer. The apo E genotype was analyzed by restriction fragment length polymorphism (RFLP).

↓

Lipid profile was determined from plasma obtained. Cholesterol and triglyceride were measured by automated methods using colorimetric enzymatic tests, CHOD-PAP Boehringer Mannheim and GPO-PAP Boehringer Mannheim respectively. LDL cholesterol was calculated by the Friedewald formula and VLDL cholesterol was determined by dividing triglyceride by a constant of 2.2.

## Results

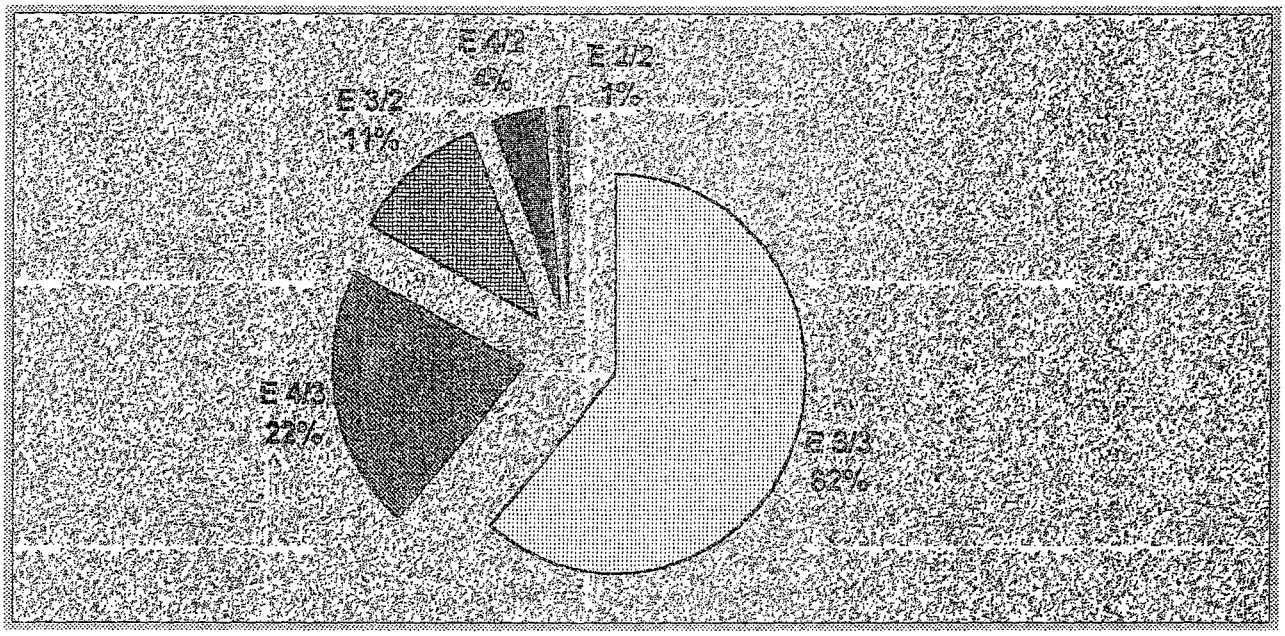


FIGURE 2: Percentage of normal individuals in each of the 5 phenotypes

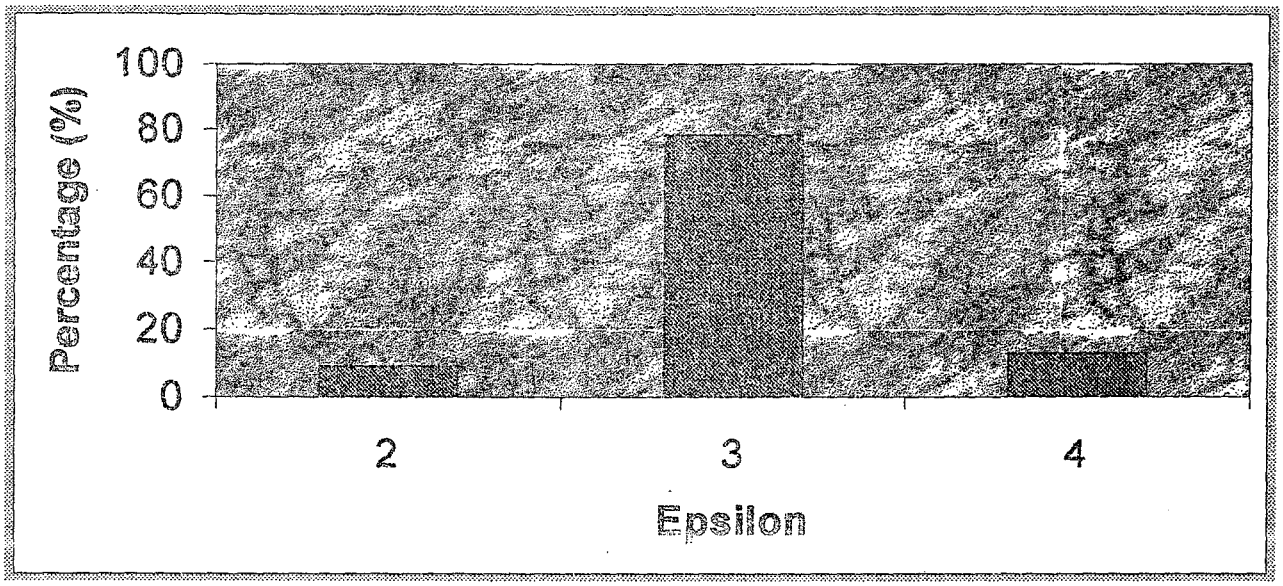


FIGURE 3: Percentages of each epsilons in normal individuals

TABLE 1: Percentage of phenotype in normal males and females

| Phenotype | Male (%) | Female (%) |
|---|---|---|
| E 3/3 | 63 | 61 |
| E 4/3 | 25 | 20 |
| E 3/2 | 12 | 11 |
| E 4/2 | - | 6 |
| E 2/2 | - | 2 |
| E 4/4 | - | - |

The allelic frequency of epsilon 3 was found to be higher in females compared to males by 5.2 % whereas there was an increase of 8.3 % and 66.7 % in the percentage of epsilon 4 and epsilon 2 among the males.

Plasma lipid analysis for the combined group showed the cholesterol concentration among the normal E 3/3 genotype was 5.77 mmol/L and the concentration was raised by 4.8 % in the E 3/2 carriers.

Triglyceride concentration was found to be 1.79 mmol/L in E 3/3 carriers while in, E 4/2, E 2/2, E 3/2 and E 4/3 the

concentrations were higher by 60.3 %, 53.6 %, 47.5 % and 10.6 %, respectively.

HDL cholesterol concentration was found to be almost the similar for each of the genotypes, which was about 1.3 mmol/L.

The LDL cholesterol concentration for the E 3/3 genotype was found to be 3.55 mmol/L and raised by 46.2 % in the E 2/2 carrier (1 person) and reduced in the E 4/2 carrier by 13.8 %.

Finally the VLDL cholesterol was found to be higher in the E 4/2, E 2/2, E 3/2 by 67.9 %, 60.3 % and 53.8 %, respectively.

## Discussion

The results indicate that most of the normal subjects were epsilon 3 allele carriers. A similar allele frequency was found in the population study done by Boemi et al., 1995. In our study the frequency of the E 3/3 genotype was the highest followed by E 4/3, E 3/2, E 4/2 and E 4/4 among the Kelantanese population. Compared to the study by Boemi et al., the difference seen in the Kelantanese population was in the male subjects carrying E 4/3 and E 3/2 genotype, where the frequencies were higher by 38.9 % and 20 %, respectively.

The frequency of subjects with epsilon 2 was higher among Kelantanese polulation by 26.7 %. The E 4/2 genotype carriers were characterized by the higher triglyceride and VLDL cholesterol concentration. This was also seen in the E 2/2 carrier whose LDL cholesterol was seen to be higher compared to the other genotypes.

The epsilon 2 and 4 were associated with some degree of impaired clearance of triglyceride-rich chylomicrons and VLDL. Further research will have to be done to demonstrate that the abnormal apo E genotypes influence the development of coronary artery disease among diabetic patients.

## References

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Pascasidang;
Simposium Sains Kesihatan Kebangsaan Ke-3,
FSKB, UKM, 29-30 April, 2000, Kuala Lumpur

# KAJIAN AWAL MENGENAI GENOTIP APOLIPOPROTEIN E AND FREKUENSI ALEL DI KALANGAN SUBJEK SIHAT

# PRELIMINARY STUDIES ON APOLIPOPROTEIN E GENOTYPES AND ALLELE FREQUENCY AMONG HEALTHY SUBJECTS

**Shahrul BSH, Faridah AR**
Department of Chemical Pathology, School of Medical Sciences.
Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan

## ABSTRAK/ABSTRACT

Kajian ini menentukan genotip apolipoprotein E dan frekuensi allel dalam kalangan individu sihat. Penentuan genotip apo E dianalisa dengan kaedah polimorfisma rangkaian fragmen terpilih (RFLP). Kolesterol trigliserida dan ditentukan dengan kaedah berautomasi. Kolesterol dan trigliserid diukur dengan kaedah berautomasi. Kolesterol lipoprotein berketumpatan rendah (LDL) diperolehi dari pengiraan Friedewald manakala kolesterol lipoprotein berketumpatan amat rendah (VLDL) ditentukan dengan membahagikan kepekatan trigliserid dengan pemalar 2.2. Kolesterol lipoprotein berketumpatan tinggi (HDL) ditentukan dengan kaedah pemendakan asid fosfotungstik dan ion Mg. Frekuensi alel epsilon 3 didapati lebih tinggi di kalangan wanita berbanding dengan lelaki sebanyak 5.2 % manakala terdapat peningkatan sebanyak 8.3 % dan 66.7 % pada subjek lelaki dengan alel epsilon 4 serta 2. Kepekatan trigliserid dalam pembawa E 3/3 adalah 1.79 mmol/L manakala dalam E 4/2, E 2/2, E 3/2 dan E 4/3 adalah lebih tinggi iaitu sebanyak 60.3 %, 53.6 %, 47.5 % dan 10.6 % dalam turutan. HDL adalah hampir sama bagi setiap genotip dan kepekatan LDL didapati meningkat sebanyak 46.2 % pada subjek E 2/2 dan sebaliknya pada subjek E 4/2 sebanyak 13.8 %. Kepekatan VLDL didapati lebih tinggi pada subjek E 4/2, E 2/2, E 3/2 sebanyak 67.9 %, 60.3 % dan 53.8 % dalam turutannya. Genotip E 4/2 dicirikan oleh kepekatan trigliserid dan VLDL yang tinggi. Ciri ini juga diperhati pada subjek dengan E 2/2 yang mempunyai kandungan kolesterol LDL yang tinggi berbanding dengan genotip lain.

This study ascertained apolipoprotein (apo) E genotypes and allele frequency among healthy subjects. The apo E genotype was analyzed by restriction fragment length polymorphism (RFLP). Cholesterol and triglyceride were measured by automated method. Low density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula and very low density lipoprotein (VLDL) cholesterol determined by dividing triglyceride by a constant of 2.2. High density lipoprotein (HDL) cholesterol was determined by the phosphotungstic acid and Mg ion sedimentation method. The allelic frequency of epsilon 3 was found to be higher in females compared to males by 5.2 % whereas the there was an increase of 8.3 % and 66.7 % in the percentage of epsilon 4 and epsilon 2 among the males. Triglyceride concentration in E 3/3 carriers was 1.79 mmol/L while in E 4/2, E 2/2, E 3/2 and E 4/3 the concentrations were higher by 60.3 %, 53.6 %, 47.5 % and 10.6 % respectively. HDL cholesterol concentration was found to be almost similar for each of the genotypes and LDL cholesterol concentration was raised by 46.2 % in the E 2/2 carrier and reduced in the E 4/2 carrier by 13.8 %. VLDL cholesterol concentration was found to be higher in the E 4/2, E 2/2, E 3/2 by 67.9 %, 60.3 % and 53.8 % respectively. The E 4/2 genotype carriers were characterized by the higher triglyceride and VLDL cholesterol concentration. This was also seen in the E 2/2 carrier whose LDL cholesterol was seen to be higher compared to the other genotypes.

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## RESULTS

In our sample of, we observed 61.1 % of the women and 62.5 % of the men are of the E 3/3 genotype. Combined (male and female) the distribution of apo E genotype was 61.4 % were E 3/3 carriers, 21.4 % (E 4/3), 11.4 % (E3/2), 4.3 % (E 4/2), 1.4 % (E 2/2) and none were E 4/4. The epsilon 3 allele frequency was found to be the highest which accounted for 78 % compared to 13 % for the epsilon 4 allele and 9 % for the epsilon 2 allele. Comparison between the male and female group showed that in the males almost 63 % of them had the normal E 3/3 genotype followed by 25 % having E 4/3 and 12.5 % having E 3/2. Data obtained from the female subjects were almost similar to the males, where 61.1 % of them were E 3/3, 20.4 % E4/3, 11.1 % E-3/2 and 5.5 % E 4/2. The allelic frequency of epsilon 3 was found to be higher in females compared to males by 5.2 % whereas there was an increase of 8.3 % and 66.7 % in the percentage of epsilon 4 and epsilon 2 among the males. Plasma lipid analysis for the combined group showed the cholesterol concentration among the normal E 3/3 genotype was 5.77 mmol/L and the concentration was raised by 4.8 % in the E 3/2 carriers. Triglyceride concentration was found to be 1.79 mmol/L in E 3/3 carriers while in, E 4/2, E 2/2, E 3/2 and E 4/3 the concentrations were higher by 60.3 %, 53.6 %, 47.5 % and 10.6 %, respectively. HDL cholesterol concentration was found to be almost the similar for each of the genotypes, which was about 1.3 mmol/L. The LDL cholesterol concentration for the E 3/3 genotype was found to be 3.55 mmol/L and raised by 46.2 % in the E 2/2 carrier (1 person) and reduced in the E 4/2 carrier by 13.8 %. Finally the VLDL cholesterol was found to be higher in the E 4/2, E 2/2, E 3/2 by 67.9 %, 60.3 % and 53.8 % respectively.

## DISCUSSION

The results indicate that most of the normal subjects were epsilon 3 allele carriers. A similar allele frequency was found in the population study done by Boemi et al., 1995. In our study the frequency of the E 3/3 genotype was the highest followed by E 4/3, E 3/2, E 4/2 and E 4/4 among the Kelantanese population. Compared to the study by Boemi et al., the difference seen in the Kelantanese population was in the male subjects carrying E 4/3 and E 3/2 genotype, where the frequencies were higher by 38.9 % and 20 % respectively. The epsilon 3 allele frequency was similar (78 %) but the epsilon 4 frequency was only 0.5 % lower (13%). The frequency of subjects with epsilon 2 was higher among Kelantanese polulation by 26.7 %. The E 4/2 genotype carriers were characterized by the higher triglyceride and VLDL cholesterol concentration. This was also seen in the E 2/2 carrier whose LDL cholesterol was seen to be higher compared to the other genotypes. The epsilon 2 and 4 were associated with some degree of impaired clearance of triglyceride-rich chylomicrons and VLDL. Further research will have to be done to demonstrate that the abnormal apo E genotypes influence the development of coronary artery disease among diabetic patients.

## REFERENCES

1. Boemi M, Sirolla C, Amadio L, Fumelli P, Pametta D, James RW. 1995. Apolipoprotein E polymorphism as a Risk Factor for Vascular Disease in Diabetic Patients. Diabetes Care 18(4): 504-508.
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SECOND & FINAL ANNOUNCEMENT

# 3RD NATIONAL SYMPOSIUM ON HEALTH SCIENCES

# HEALTH SCIENCES FOR THE NEW MILLENNIUM

Organized by:

Faculty of Allied Health Sciences
Universiti Kebangsaan Malaysia

29 - 30 APRIL 2000

Venue:
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## INTRODUCTION
Presentation

The health sciences research activity has grown rapidly over the past few years in responds to its increasingly importance in dealing and promoting health standard in Malaysia. Following this, the faculty of Health Sciences, UKM is pleased to offer a platform for all local and international scientist and doctors to present and exchange ideas on the latest development of the current and challenging future health issues of the 21st century.

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The symposium will be held at the Ayer Keroh D' Village, Ayer Keroh, Melaka.

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# Instruction For Full Paper Submission for the 3rd National Symposium on Health Sciences

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2. Font: Times New Roman
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5. Total pages: not exceeding 6 sheets of A4 size paper, including table, illustration

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Faculty of Allied Health Sciences
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50578 Kuala Lumpur, Malaysia

*The text should be presented under the following sections:*

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@ Malay.
@ Each not exceeding 200 words.
@ Do not use abbreviation.

*The following section may be in Malay or English*

Introduction
Materials and Method
Results
Discussion
Acknowladgement
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1. Text: (Yew and Thomas 1999), (Aishah *et al.* 1997), (Mischa 1999a, 1999b)
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1. Kirnpal-Kaur BS, Megat RAzi, Yap KL. 1997. The Comperetive sensitivity of immune electron microscopy in the detection of adenovirus in feacal specimen. In: proc First ASEAN Microscopy Conference, p237-9. Electron Microscopy Society of Malaysia & Microscopy Society of Singapore. Senai, Johor.
2. Yap KL, Lam SK. 1994. Infectivity titration of the fast-replicating and cytophatic hepatitis A virus strain HM175A.2 by an in situ enzyme immunoassay. J. Virological Methods 15:119-23.
3. Yap KL, Aziz AH. 1985. Infective diarrhoea in Malaysian children. In: Infectious Diarrhoea in the Young (ed. Tzipori S) p 42-5. Amsterdam: Elsevier Science Publishers.

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Assoc. Prof. Dr. KL Yap
Jabatan Sains Bioperubatan
Fakulti Sains Kesihatan Bersekutu
Universiti Kebangsaan Malaysia
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*Original* Research Publication & Presentation 2001
1st ASEAN Conference on Medical Sciences, 18-21 May 2001, Book of Abstracts, Abstract No. P-22, page 69

Research Publication 2001
The Malaysian Journal of Medical Sciences July 2001; 8(2): 91-92

# RELATIONSHIP BETWEEN GENOTYPE AND ALLELE OF APOLIPOPROTEIN E WITH THE LIPID STATUS AMONG MALAYS

Shahrul BSH, Mohd Rafi, Wan MWB[a], Faridah AR
Department of Chemical Pathology, School of Medical Sciences, University Sains Malaysia, Kelantan, Malaysia
[a]Department of Medicine, School of Medical Sciences, University Sains Malaysia, Kelantan, Malaysia

Objective: This study ascertained apolipoprotein (apo) E genotypes and allele frequency among Malays and its influences towards the lipoprotein classes.

It is the major protein involved in catabolism of triglyceride rich lipoproteins (VLDL and remnants). Apo E has three common alleles which are ε2, ε3, and ε4 which code for three major apoE isoforms and 6 genotypes.

A number of 189 volunteers aged 20 to 65 years where recruited in the study. Blood was collected in tubes containing EDTA and the leucocytes from the buffy coat layer were used to extract DNA. Restriction fragment length polymorfism (RFLP) method was used to identify the apoE genotype. Lipid profile was determined using automated method to measure total cholesterol, triglyceride, and high density lipoprotein (HDL-C). The low density lipoprotein (LDL-C) level was calculated using the Friedewald formula.

The E3/3 genotype had the highest frequency among the the 6 genotypes. Female subjects had higher frequency for the ε4/2 genotype and lower ε4/4 frequency value. Male subjects had higher ε4/4 frequency. Epsilon 3 and epsilon 2 alleles frequency was high among females whereas the male subjects had high epsilon 4 allele frequency. Generally individuals with the ε4/2 genotype had higher total cholesterol and those with the ε2/2 had higher triglyceride concentration. In males the ε4/3 genotype group had higher triglyceride concentration while among the females the ε2/2 genotype individuals had the highest triglyceride concentration. The ε4/4 genotype caused the raised cholesterol content among females.

Our data suggest that male subjects have high epsilon 2 allele frequency which is associated with the high triglyceride. Females with epsilon 2 homozygous genotype had higher triglyceride concentration while the ε4/4 genotype had increased cholesterol level.

Correspondence:
Shahrul Bariyah Sahul Hamid
sbariyah@yahoo.com

factors involved in maintaining weight changes in urban areas including Kuala Lumpur and Kota Bharu, Kelantan. This paper reports on the preliminary findings of the prevalence and sociodemographic components of overweight among primary school children between 9-11 years in Kota Bharu, Kelantan.

This is a multicentre study involving two urban areas in Malaysia namely Kuala Lumpur and Kota Bharu. A total of 14 primary schools from two main zones in Kuala Lumpur and 21 primary schools from Kota Bharu were chosen randomly from the list of primary schools provided by the Ministry of Education. All children in year 3, 4 and 5 (age 9,10 and 11 years old) in these selected schools were recruited in the study. Their weight and height were recorded by trained research assistants using digital seca balance and microtoise to the nearest decimal reading. A set of screening questionnaires regarding sociodemographic, psychosocial, food intake habit and physical activity were administered. The data were collected between March to November 2000. For the purpose of this study, overweight problem includes children who are obese and at risk of obesity based on Body Mass Index (BMI) measurement (WHO,1995). Obesity is defined as those whose BMI is equal or more than 95th percentile BMI-for-age whereby at risk of overweight is defined based on BMI which is more and equal 85th percentile and less than 95 percentile BMI-for-age6. The data is analysed using the SPSS version 9.0. Chi square tests and Student's t-tests were employed and p value of less than 0.05 is considered significant.

A total of 5047 students in Kuala Lumpur and 7476 students in Kota Bharu between 9-11 years old were selected for the study. From the study, the results show that the overall prevalence of children having overweight problems in Kuala Lumpur is 912 (18.1%) of which 437 (8.7%) are obese and 475 (9.4%) are at risk of obesity. In Kota Bharu a total of 924 (12.4%) children have overweight problem whereby 435 (5.8%) are obese and 489 (6.5%) are at risk of obesity.

This study provides new information on the overall prevalence of obesity among primary school children 9 to 11 years old in Kota Bharu is 12.6% of which 13.5% are boys and 11.7% are girls. The increase in age is an important factor for an increase in prevalence in both sexes. Since obesity is a major risk factor for many important preventable diseases in later life, so proper early effective intervention program should be implemented. Intervention package will be prepared based on this study to address the appropriate problems.

*POSTER SESSION 1*

## NUTRITION KNOWLEDGE, ATTITUDE AND PRACTICES OF MOTHERS WITH CHILDREN 6 - 72 MONTHS OLD: A COMPARISON STUDY BETWEEN MALNOURISHED AND WELL-NOURISHED CHILDREN IN BALING DISTRICT, KEDAH

*Nurhayati Mohamad [1], Fatimah Arshad[1], Poh Bee Koon[1], Normah Hashim[2], Md. Idris Mohd Nor[3], Wan Roshani Wan Mohammed[1] Norlela Hussein4, Khor Geok Lin[2]*
*[1]Department of Nutrition and Dietetics, Faculty of Allied Healt h Sciences, University Kebangsaan Malaysia; [2]Department of Nutrition and Health Sciences, Faculty of Medicine and Health Sciences, University Putra Malaysia; [3]Department of Community Health, Faculty of Medicine, University Kebangsaan Malaysia; [4]Department of Family Health Development, Ministry of Health, Malaysia.*

A cross sectional study on knowledge, attitude and practice was carried out on mothers having children 06-72 months in Baling Kedah. The objective of this study was to determine the nutritional status of the children 6-72 months and to relate nutrition knowledge, attitude and practice of mothers towards their nutritional status.

A total of 94 mothers with children aged 6-72 months from 7 villages were taken as study subjects using universal sampling method. Only one child from each mother was randomly selected for the study. Anthropometric measurements (weight, height and mid-arm circumference) were taken. A sub sample of 60 children (30 malnourished and 30 well-nourished) was selected for a 24-hour dietary recall for three consecutive days. Socio-economic factors, nutrition knowledge, attitude and practices of mother were collected using a set of pre-tested and validated questionnaire. The data were analyzed using A Statistical Package for The Social Sciences (SPSS) Program, version 9.0. The Anhtro 1 program was used to analyze anthropometric data and the Nutrical Program version 1.02 was used to analyze 24-hour dietary recall.

The socio-demographic data showed that 41.5 % mothers attended primary school and 58.5 % went to secondary schools. About 67% of the mothers are housewives and 33% are employed. Based on the per-capita poverty line income of RM 100.00, it was found that 13.8% of the households earned less than RM 50.00 which can be considered as hard-core poor, while 41.5% were poor (monthly per-capita income between RM 50.00 and RM100.00). The results of anthropometric assessments showed that 31.9% of the children were underweight, 23.4% stunted and 6.4 % wasted when compared to the NCHS reference. The mean percent score of KAP achieved by all mothers is 77.91%, 73.82% and 61.11%, which is considered moderate nutrition knowledge, attitude and practices. The result of the dietary study showed that, protein intake of the children is (169.8% of RDA) 43.34 g, iron 10.67 mg (106.7% of RDA) and Vitamin A 482.27 ug (178.8% of RDA) over the Recommended Daily Allowances (RDA). However, the mean intake of calories 1275.65 kcal (81.38% of RDA), calcium 284.58 mg (63.19% of RDA) and vitamin C 19.18 mg (95.92% of RDA) were inadequate, that is below 100% RDA. There is no significant difference between the nutrition knowledge, attitude and practice, socio-demographic factor and nutrient intake of children of mothers with malnourished children versus mothers of well-nourished children using the Mann-Whitney U test, $c^2$ and T-test respectively. This could be due to the fact that the backgrounds of these mothers were homogenous.

In conclusion, inadequate nutrition knowledge, attitude and practice are important to ensure the welfare of children. A nutrition education intervention program was prepared to increase the knowledge and awareness of mothers on nutrient intake of their children with emphasis on family dynamic and social culture aspect.

*POSTER SESSION 1*

## RELATIONSHIP BETWEEN GENOTYPE AND ALLELE OF APOLIPOPROTEIN E WITH THE LIPID STATUS AMONG MALAYS

*Shahrul BSH, Mohd R, Wan MWB[a], Faridah AR*
*Department of Chemical Pathology, School of Medical Sciences, University Sains Malaysia, Kelantan, Malaysia*
*[a]Department of Medicine, School of Medical Sciences, University Sains Malaysia, Kelantan, Malaysia*

Objective: This study ascertained apolipoprotein (apo) E genotypes and allele frequency among Malays and its influences towards the the lipoprotein classes.

It is the major protein involved in catabolism of triglyceride-rich lipoproteins (VLDL and remnants). Apo E has three common alleles which are e 2, e 3, and e 4 which code for three major apo E isoforms and 6 genotypes.

A number of 189 volunteers aged 20 to 65 years where recruited in the study. Blood was collected in tubes containing EDTA and the leucocytes from the buffy coat layer were used to extract DNA. Restriction fragment length polymorfism (RFLP) method was used to identify the apo E genotypes. Lipid profile

was determined using automated method to measure total cholesterol, triglyceride, and high density lipoprotein (HDL-C). The low density lipoprotein (LDL-C) level was calculated using the Friedewald formula.

The E 3/3 genotype had the highest frequency among the 6 genotypes. Female subjects had higher frequency for the e 4/2 genotype and lower e 4/4 frequency value. Male subjects had higher e 4/4 frequency. Epsilon 3 and epsilon 2 alleles frequency was high among females whereas the male subjects had high epsilon 4 allele frequency. Generally inviduals with the e 4/2 genotype had higher total cholesterol and those with the e 2/2 had higher triglyceride concentration. In males the e 4/3 genotype group had higher triglyceride concentration while among the females the e 2/2 genotype individual had the highest triglyceride concentration. The e 4/4 genotype caused the raised cholesterol content among females.

Our data suggest that male subjects have higher epsilon 2 allele frequency which is associated with the high triglyceride. Females with epsilon 2 homozygous genotype had higher triglyceride concentration while the e 4/4 genotype had increased cholesterol level.

*POSTER SESSION 1*

## SOMATOTYPE AMONG MALAYSIAN WOMEN NETBALL PLAYERS

*Soh Kim Geok[1], Ruby Husain[2], Mohd. Nor' Che Noh[2] & Zaliha Omar[2]*
*[1]Faculty of Education, Universiti Putra Malaysia, Selangor; [2]Universiti Malaya*

The aim of the study is to determine the somatotype and anthropometric measurements among Malaysian national womenteam of netball players according to their age. The subjects in this studyare divided into four categories according to their age, which are: above 21 year old, under 21, 19 and 17 years old. Their somatotype measurements were measured by using Health and Carter somatotype measurements (Fox, Bowers and Foss[1], 1993). Besides the age factor, this study was also conducted to determine the somatype and anthropometric measurement among Malaysian national women teamsof netball players according to their playing positions. The playing position in netball game are attacker, centre and defender.

Results showed that there were no significant difference in the somatotype among the netball players in different groups and playing position categories. The average somatotype measurements of these netball players is 4.71 - 2.97 - 2.98. This result showed that Malaysian national women netball team players' had a ecto-endomorphy somatotype (Sheldon[2],1949).

Results of anthropometric test showed that there was significant differences in the netball players' height in different playing position categories. The reported F value is F=11.40 (p>0.01). The taller netball players are those who had to play near the goal post. Results for other anthropometric test were found to be not significant.

*POSTER SESSION 1*

## LEVELS OF SERUM SOLUBLE INTERLEUKIN-2 RECEPTOR IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

*Azizah MR*, Kuak SH*, Kong NCT**, Normaznah Y*, Rahim MN#*
**Biotechnology Centre, Institute for Medical Research, Kuala Lumpur **Department of Medicine, Hospital Universiti Kebangsaan Malaysia, Cheras #Department of Bio-Medicine, Faculty of Allied Sciences, Universiti Kebangsaan Malaysia*

Elevated serum levels of IL2R have been demonstrated in patients with diseases characterized by activation of the immune system including rheumatic conditions such as systemic lupus erythematosus (SLE) and rheumatoid arthritis. Elevated levels have been found to correlate with SLE disease activity. In this study we determined the levels of serum soluble interleukin 2 receptor (sIL2R) in 134 patients with SLE (122 females and 12 males, mean age: 34 years) using the technique of enzyme-linked immunosorbent assay (ELISA). This was to assess whethere there was any relationship between serum levels of sIL2R and disease activity, and their clinical and serological parameters. The clinical activity was evaluated by the Lupus Activity Index. Levels of sIL2R correlated significantly with disease activity ($r=0.2261$, $p<0.05$). We found a significant increase of sIL2R in the SLE group compared to controls (299 ± 63pg/ml (mean ± SEM) vs 103 ± 5pg/ml, $p<0.05$) and the active compared to the inactive groups (318.3 ± 71 vs 162.4 ± 63pg/m,, $p<0.05$). However sIL2R levels were not significantly higher in the inactive group compared to normal individuals. Levels of sIL2R were found to correlate significantly with clinical manifestations; fatigue, central nervous system, renal and pulmonary involvements but did not correlate with anti ds DNA antibodies and levels of C3 and C4. We did not observe any significant differences in sIL2R levels between patients receiving steroids and those not on steroids. These findings suggest that serum soluble receptor of IL2 may represent a new potentially useful serological marker to monitor disease activity in patients with SLE.

*POSTER SESSION 1*

## THE EFFECT OF IODINE SUPPLEMENTATION ON THYROID VOLUME, URINE IODINE AND PERFORMANCE MENTAL AMONG ABORIGINES CHILDREN IN HULU SELANGOR DISTRICT

*Noor Hayati, A.R., Zaleha, M.I., Neni E.J. & Osman A.*
*Department of Community Health, Faculty of Medicine, Universiti Kebangsaan Malaysia.*

Iodine deficiency disorders is mainlycaused by insufficient levels of iodine in the environment and inadequate intake of iodine from food. The study was conducted to determine the prevalence of goiter among Aborigines living in the district of Hulu Selangor. Four villages were selected as the study areas, they wer Kampugn Kuala Kerling, Kampung Bukit Manchong, Kampung Pertak and Kampung Gerachi. Populations in the first three villages were given one dose of oral iodised oil in the form of capsules at the beginning of the studywhilst population inthe latter village were given iodised eggs daily for12 months. Goiter size was measured using portable ultrasound (Toshiba Sonolayer) and mean urinary iodine levels were determined using the alkaline-ashing method. Villagers between 4-12 years old were taken as subjects. It was found that the mean values of thyroid volume before intervention for Pertak, Gerachi, Kuala Kerling and Bukit Manchong were 10.2 ± 3.3 ml (range: 5.7-17.9 ml), 9.7 ± 3.8 ml (range: 2.4-20.4 ml). 8.9 ± 5.0 ml (range 2.9-19.0 ml) and 8.1 ± 4.0 ml (range 2.4 - 20.4 ml) respectively and the result after 12 months of intervention were 4.9 ± 2.9 ml (range: 1.3-12.1 ml), 6.7 ± 4.7 ml (range 2.1 - 18.6 ml), 4.2 ± 1.3 ml (range: 2.6 -6.7 ml) and 4.2 ± 2.3 ml (range 1.7-18.6 ml) respectively. There was a significant different in mean values of thyroid volume before andafter the intervention. Baseline mean urinary iodine for Pertak, Gerachi, Kuala Kerling and Bukit Manchong were 2.96 ± 1.27 mg/dl (range 0.15-5.04 mg/dl), 1.28 ± 0.70 mg/dl (range: 0.04-3.40 mg/dl), 1.68 ± 0.82 mg/dl (range: 0.26-4.11 mg/dl) and 2.04 ± 1.54 mg/dl (range: 0.01-9.26 mg/dl) respectively, whilst mean urinary iodine after intervention were 3.35 ± 1.22 ug/dl (range;1.98 ± 0.58 mg/dl (range: ), 2.90 + 1.07 mg/dl (range: ) and 3.07 + 1.40 mg/dl (range: ) respectively. The median score

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**Title** CHARACTERIZATION OF LOW DENSITY LIPOPROTEIN SUBFRACTION PROFILE AND APO E GENOTYPE AMONG DIABETIC PATIENTS

**Authors** Shahrul Bariyah Sahul Hamid, Faridah Abdul Rashid and Wan Mohamad Wan Bebakar*

**Institution** Dept. of Chemical Pathology and Medicine*, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

**Abstract** The present study aimed to examine the association between diabetes mellitus type 2 with low density lipoprotein particle size distribution and the influence of apolipoprotein E genotype in altering lipid profile. A total of 35 subjects with diabetes mellitus type 2 who were overweight and without any drug treatment were enrolled in this study. Results obtained were compared with that of 30 normal control subjects. Plain blood samples were taken after an overnight fast of 10-12 hours. Serum biochemical analysis was done using automated enzymatic methods (Hitachi 912) for the determination of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and glucose concentration. HDL cholesterol was performed after chemical precipitation. LDL cholesterol was calculated if triglycerides was less than 4.5 mmol/L. Otherwise, direct LDL cholesterol estimation was done. LDL subfraction area under curve percentage (% AUC) was determined by using non-denaturing 2-16 % polyacrylamide gel electrophoresis. APOE gene analysis was by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The lipid profil test results showed that male diabetics had higher triglycerides and LDL cholesterol whereas female diabetics had higher total cholesterol, triglycerides, HDL cholesterol, VLDL cholesterol and glucose. There was a positive and significant correlation between triglycerides and AUC of LDL 4. Triglycerides also correlated positively and significantly with glucose. This indicates that hypertriglyceridaemia could possibly lead to the formation of small LDL particles. The possibility of step-wise conversion of bigger LDL into smaller LDL was studied by looking into the correlation between the LDL subfractions. The AUC of LDL 1 correlated negatively with the AUC of LDL 3. Diabetics generally were found to have higher AUC values for the smaller LDL particles which comprise of LDL 3, LDL 4, and LDL 5. The study on APOE gene showed that ε3 and ε4 diabetics had elevated total cholesterol and glucose. Diabetics with the ε2 allele did not have any significant difference with the ε3 and ε4 subjects when the triglycerides concentration was compared with that of the 3 allele carrier. Allele frequency obtained for diabetics was ε2 (0.143), ε3 (0.714) and ε4 (0.143). The frequency distribution obtained was similar to the findings from the study on diabetic mellitus type 2 subjects by Boemi *et. al* (1995). Frequency comparison with the normal control showed that the ε2 allele frequency was higher in diabetics. Overweight ε4/3 diabetics portrayed the worst atherogenic profile with the highest triglycerides, LDL cholesterol and total cholesterol, and the lowest HDL cholesterol. We therefore conclude that a decrease in LDL size with high propensity for small LDL 4 therefore confers additional atherosclerotic risk to overweight individuals with diabetes mellitus type 2, especially those with ε4/3 genotype.