Metabolic abnormalities in women who have had gestational diabetes mellitus (GDM) one year previously

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Metabolic abnormalities in women who have had gestational diabetes mellitus (GDM) one year previously

Thesis submitted in fulfilment of the requirement for the award of the degree of Doctor of Philosophy

By:
Shahin Yarahmadi
(MD)

1996
Dedicated to:

Imam Mahdi, who will rise and make the world a paradise
My dear husband, Habib,
My lovely sons, Mohammad, Reza, and Saeed,
and my devoted parents.
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Abbreviations

GDM = Gestational diabetes mellitus
OGTT = Oral glucose tolerance test
C = Cholesterol
FC = Free cholesterol
CE = Cholesteryl ester
TG = Triglyceride
VLDL = Very-low-density lipoprotein
IDL = Intermediate-density lipoprotein
LDL = Low-density lipoprotein
HDL = High-density lipoprotein
NEFA = Non-esterified fatty acid
Lp(a) = Lipoprotein (a)
CETP = Cholesteryl ester transfer protein
HTG = Hypertriglyceridemia
LPL = Lipoprotein lipase
HPL = Hepatic lipase
SHBG = Sex hormone binding globulin
CVD = Coronary vascular disease
PVD = Peripheral vascular disease
SUMMARY

Subjects who have had gestational diabetes mellitus (GDM) are, by definition, not hyperglycemic after pregnancy and do not usually receive the benefit of detailed follow-up of their metabolic status. We examined the metabolic status of 65 women who had had a diagnosis of GDM and 65 women who had had a normal non-diabetic pregnancy (control women) approximately one year after delivery. We hypothesised that the women who had had GDM would on the whole have more abdominal obesity than control women, that they would be relatively hyperinsulinemic and hyperglycemic, and that they would have a characteristic dyslipidemia on fasting (high VLDL, IDL and LDL and low HDL concentrations, high plasma non-esterified fatty acid (NEFA) concentration and cholesteryl ester transfer protein (CETP) activity in plasma, more small LDL and HDL particles). It was expected that these women would have been more likely to have a family history of NIDDM. We hypothesised also that the women who had had GDM would have a high plasma testosterone and low plasma sex hormone binding globulin (SHBG) concentration, would have higher blood pressure, and be more likely to have microalbuminuria than control women. Many of these predictions were borne out.

The study shows that the population of women who have had GDM are overall far from normal one year after the end of pregnancy. They are likely to have a set of abnormalities which our current knowledge suggests are precursors of the insulin resistance syndrome such as obesity, a positive family of diabetes mellitus, dyslipidemia, hyperinsulinemia, hyperandrogenicity, raised blood pressure, and microalbuminuria.
Many of these abnormalities are found in a subgroup of GDM subjects who appear to have developed the insulin resistance syndrome.
CHAPTER ONE

INTRODUCTION

1.1 Background

Gestational diabetes mellitus (GDM) is a problem of global significance (Boyd et al, 1991). GDM is a heterogenous clinical problem with phenotypic and genotypic variety (Freinkel et al, 1985). GDM is common in obese women and in those over 30 years old (Freinkel et al, 1985). There are geographical, ethnic, and racial differences in the prevalence of GDM.

The pathogenesis of GDM is not fully understood, but the syndrome is similar to non-insulin-dependent diabetes mellitus (NIDDM). In the GDM patients, the insulin response to glucose intake is delayed, while the basal plasma insulin level is normal or elevated (Kuhl et al, 1985).

In the development of GDM, like NIDDM, at least three factors may play important roles. They are as follows: a genetic tendency to the disease, an impaired pancreatic β-cell function, and a decreased insulin action in insulin-sensitive tissues including adipose tissue, skeletal muscle, and the liver (Horton, 1991).
Maternal obstetric complications of GDM include hydramnios, pregnancy-induced hypertension, chronic hypertension, pyelonephritis, and delivery by caesarean section (Goldman et al, 1991).

The Third International Workshop-Conference on GDM noted that the neonates of GDM mothers are at increased risk of macrosomia, neonatal hypoglycaemia, hypokalaemia, polycythemia, and hyperbilirubinemia (Metzger and The Organizing Committee, 1991). Macrosomia can cause birth trauma to both mother and neonate and it is thought that it may increase the risk of obesity and diabetes mellitus in later life in the offspring of mothers with GDM (Hod et al, 1991).

Mestman et al (1972) have reported that 30-50% of women with prior GDM develop diabetes mellitus during 3-5 years after delivery and the proportion is 60-100% after 12-18 years (Mestman, 1987).

1.2 Etiology of Gestational Diabetes Mellitus

O'Sullivan (1978) reported that up to 60% of women who had had GDM eventually developed diabetes. Oats et al (1988) have reported that 9.1% of women who had had GDM were found to be diabetic and 16.9% had impaired glucose tolerance using the WHO criteria at intervals from 1-15 years following diagnosis in Australia.

The etiology of GDM is poorly understood. The predisposing causes are probably the same as for NIDDM, clearly precipitated by the metabolic and hormonal changes of pregnancy (Brinkman III, 1987).

Glucose tolerance normally deteriorates in human pregnancy. It is known that plasma levels of the anti-insulin hormones rise in pregnancy (Peterson et al, 1991). The major
insulin antagonists of pregnancy are estrogen, prolactin, progesterone, cortisol, and human placental lactogen. These are at their highest concentration in the blood at 24-28 weeks of gestation, at which time GDM most commonly occurs (Peterson et al, 1991).

Several studies have suggested that the diabetic effect of pregnancy is not due to reduced secretion of insulin or disproportional secretion of pro-insulin or glucagon, nor is it due to an increased insulin degradation (Kuhl, 1991). The diabetogenicity of pregnancy is associated with induction of pronounced peripheral resistance to insulin (Kuhl, 1991, Buchanan, 1991).

The resistance is of a similar magnitude in normal pregnant women and those with GDM, and it does not seem to be caused by significant alterations in insulin receptor binding to target tissue (Kuhl, 1991). The insulin resistance of the whole body is increased to about three times that seen in the non-pregnant state. It is thought to be due to post-insulin receptor events and is probably caused by the cellular effects of increased plasma levels of one or more of the pregnancy-associated hormones and free cortisol (Kuhl, 1991). Evidence indicates that the insulin resistance occurs predominantly in skeletal muscle tissue.

Most normal pregnant women are able to counteract the peripheral insulin resistance by a significant increase in their basal and nutrient-stimulated insulin secretion. However, a few (2-3%) of the women are not able to produce a sufficiently large increase in insulin secretion and hence cannot overcome the peripheral insulin resistance. Thus, they become glucose intolerant and fulfil the diagnostic criteria for GDM (Kuhl, 1991).
Recent studies suggest that a high percentage of pregnant women who screen positive for glucose intolerance have serological evidence of an autoimmune response against the pancreatic islets, in spite of the state of relative immune tolerance during pregnancy. These data suggest that autoimmune phenomena may play a role in GDM and that the presence of islet cell antibodies can predict insulin-requiring gestational diabetes (McEvoy et al, 1991). It might be an explanation for those GDM subjects who will develop IDDM but the finding requires confirmation.

1.3 Pathogenesis of NIDDM

By definition, diabetes mellitus is characterized by a rise in glycemia above normal concentrations which is a consequence of an inability of body tissue to dispose of glucose, an excess production of glucose by the liver or both (Felber et al, 1993). Non-insulin-dependent diabetes mellitus (NIDDM) is thought to be caused by an imbalance between insulin sensitivity and insulin secretion (DeFronzo et al, 1992).

1.3.1 Impaired glucose tolerance

Glucose tolerance, as estimated by the oral glucose tolerance test (OGTT), depends on complex iteration among several physiological processes, including first-phase and second phase insulin secretion, insulin removal, insulin sensitivity, and the tendency of high glucose concentration (independent of insulin) to increase glucose utilisation (Martin et al, 1992).

Subjects with insulin resistance may be euglycemic maintained by hypersecretion of insulin, with hyperinsulinemia both in the fasting and postprandial states (Beck-Nielsen et al, 1990).
The risk for subsequent glucose intolerance or diabetes in women with previous GDM is related to pre-pregnancy body mass index (BMI) and fasting plasma glucose during pregnancy. Subjects who are fatter before pregnancy and whose plasma glucose concentrations are higher during pregnancy are more likely to develop glucose intolerance or diabetes after delivery (Coustan et al, 1993).

Effendic et al have reported that 60% of normal weight women with previous GDM had borderline or decreased oral glucose tolerance 6-36 months postpartum which could be explained either by low insulin response or by the combination of a low insulin sensitivity and low insulin response (Effendic et al, 1987).

1.3.2 Insulin Resistance

Insulin resistance may be defined as a state (of a cell, tissue, system or body) in which more than normal amounts of insulin are required to draw out a quantitatively normal response (Berson and Yalow, 1970). It should be emphasised that the level of glycemia may be within the normal range in a person with insulin resistance as a result of compensatory hyperinsulinemia (Flier, 1993).

Insulin regulates blood glucose by inhibiting hepatic glucose synthesis and by stimulating glucose utilization in skeletal muscle. Both insulin actions are impaired in IGT and NIDDM patients (Mitakou et al, 1992).
1.4 **The Cause of NIDDM Development**

Several investigations including prospective studies have suggested that increased insulin resistance precedes the development of IGT and NIDDM (Bennett, 1990, Haffner et al, 1990a, Skarfors et al, 1991, Martin et al, 1992).

The progression from normal glucose tolerance to IGT to NIDDM with mild fasting hyperglycemia (< 6.7-7.8 mmol/L) is characterized by progressive hyperinsulinemia (DeFronzo et al, 1992).

Most evidence indicates that insulin resistance is the major factor which initiates the sequence of events resulting in NIDDM. While the pancreas carries a sufficiently high insulin secretory response to degree of insulin resistance, glucose tolerance remains normal to mildly impaired. However, then the β-cell starts to fail, glucose tolerance impairs rapidly and overt diabetes mellitus follows (DeFronzo et al, 1992). Most studies have found a reduction in β-cell mass in NIDDM patients (Kloppel et al, 1985, Clark et al, 1988).

Genetic or familial factors contribute to the pathogenesis of NIDDM (Bennett, 1990, Martin et al, 1992, Haffner et al, 1988).

‘The thrifty phenotype’ hypothesis is another proposed explanation for β-cell failure in the pathogenesis of NIDDM. This hypothesis proposed that β-cell failure is due to intra-uterine and early postnatal malnutrition in fetus and newborn respectively (Hales and Barker, 1992). Malnutrition during intra-uterine period of life may lead to poor fetal development of the pancreas, particularly β-cells, which consequently are not capable to compensate sufficiently for insulin resistance later in life (Hales and Barker, 1992).
1.4.1 The Development of Insulin Resistance

Sedentary life-style is associated with obesity and physical inactivity, and these are causes of insulin resistance (Yki-Jarvinen, 1994). The distribution of fat within the body is an important factor in developing insulin resistance in individuals. Upper body (android) obesity is more common in NIDDM patients than in equally obese non-diabetic men and women (Bjorntorp, 1988). Helmrich et al (1991) have reported that physical activity even without weight loss plays an important role in the prevention of NIDDM, even in genetically susceptible people.

Furthermore, epidemiological studies have suggested that a high intake of dietary fat (Tsunehara et al, 1990, Marshall et al, 1991), an excessive consumption of refined carbohydrates particularly sucrose (Baird, 1972, Freskens and Kromhout, 1989), and a low dietary fibre intake (Truswell, 1975) play roles in the etiology of NIDDM.

1.4.2 The Development of Impaired Insulin Secretion

Several hypothesis have been proposed to account for the defect in insulin secretion in NIDDM.

Several studies have suggested that chronic hyperglycemia is deleterious to insulin secretion and also persuades insulin resistance. It may eventually result in progressive loss of β-cell function and in the NIDDM state (Yki-Jarvinen, 1992).

Amyloid deposition has been found frequently in NIDDM patents and amylin was shown to be its precursor (Clark, 1989, Nishi et al, 1990). Recently, amylin was suggested as a contributor to the defect in insulin secretion in isolated rat pancreatic islets (Ohsawa et
al, 1989). Galanin is the newest hormone that has been proposed to be involved in impaired insulin secretion in NIDDM (Dunning and Taborsky, 1988).

1.5 Significance of the Thesis

The identification of subjects at risk for diabetes mellitus has been a goal for physicians and scientists (Stern and Haffner, 1986, Nelson et al, 1988). The hope is that identification may lead to action which may help to prevent new cases or at least delay the onset of disease. Furthermore, early detection of diabetes in subjects who have the disease will enable the physician to initiate early treatment well before the onset of long term complications such as atherosclerosis.

GDM is a strong predictor of glucose intolerance or NIDDM later in the life of the mother (O'Sullivan, 1989, Persson et al, 1991). Several studies have suggested that normoglycemic obese and non-obese women with previous GDM exhibit abnormalities of insulin response to glucose administration and evidence of increased insulin resistance (Ward et al, 1985, Catalano et al, 1986, Effendic et al, 1987).

The factors responsible for development of impaired glucose tolerance and NIDDM in subjects with previous GDM are unclear (Persson et al, 1991). Effendic et al (1987) have reported a significantly decreased early insulin response and insulin sensitivity in women of normal weight who previously had GDM and who have had normal and abnormal oral glucose tolerance test (OGTT) postpartum. Abnormal glucose tolerance arising in women who have had GDM within the first postpartum year has been reported by several researchers (Metzger et al, 1985, Catalano et al, 1986).
Although several studies have shown that women have a severe risk of developing diabetes mellitus if they have had GDM, these have been few studies of metabolic abnormalities in a population of women who have had GDM.

A strategy to prevent or delay the development of NIDDM in women with previous GDM has the potential to save millions of health care dollars and to prevent substantial suffering caused by the disease (Gregory et al, 1993).

This study is of a group of such women one year after delivery, compared with a control group.

It is a population-based study, which should form the basis for a later longitudinal study.

### 1.6 Aims of the Study

The aim of this study is to test the following hypotheses:

1. We hypothesise that women who have had GDM are more likely to have known or postulated risk factors for NIDDM, namely age, a maternal history of diabetes mellitus and central obesity than controls.

2. Women who have had GDM are more likely (than controls) to have developed a high blood concentration of non-esterified fatty acids (NEFA) in the context of a disordered lipid metabolism. These are thought to be factors which predict the early development of NIDDM. Dyslipidemia includes moderate hypercholesterolemia, hypertriglyceridemia, and a low HDL-cholesterol concentration. Furthermore, correlates of dyslipidemia include higher plasma concentrations of apolipoprotein B, VLDL, and IDL, and lower
apolipoprotein A-I concentrations. Other correlates include denser and smaller LDL and HDL particles and increased cholesteryl ester transfer protein (CETP) activity in plasma.

3. We hypothesise that women with previous GDM are more likely (than controls) to have accelerated insulin resistance (evidenced by fasting hyperinsulinemia).

4. We hypothesise that women with previous GDM are more likely (than controls) to have developed elements of the insulin resistance syndrome (obesity, associated with hyperandrogenesity, dyslipidemia, hyperinsulinemia, high diurnal plasma glucose excursion, high blood pressure).

5. We hypothesise that women with previous GDM are more likely (than controls) to have high dietary intake of saturated fat and lack of exercise.

6. We hypothesise that women with previous GDM are more likely (than controls) to have microalbuminuria, suggesting organ damage associated with diabetes.
CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Diabetes mellitus is the most common metabolic disorder in humans, affecting about 2-5% of the general population worldwide. The National Diabetes Data Group (1970) and the WHO Expert Committee on Diabetes Mellitus (1980) classified the heterogenous syndrome diabetes mellitus according to its etiopathology (Table 2-1), but other classifications persist (Weiss, 1988).

Table 2-1 Classification of diabetes mellitus

| Type I: insulin dependent diabetes mellitus (IDDM) |
| Type II: non-insulin-dependent diabetes mellitus (NIDDM) |
| Obese |
| Lean |
| Secondary diabetes mellitus |
| Impaired glucose tolerance |
| Obese |
| Lean |
| Gestational diabetes mellitus |

Non-insulin-dependent diabetes mellitus (NIDDM) has an insidious onset and may exist for years without diagnosis. A number of risk factors have been recognized, including hyperglycemia, hyperinsulinemia, and other metabolic abnormalities obesity, physical inactivity, dietary factors (a high dietary fat intake, a lack of dietary fibre, and an
excessive consumption of sucrose and other refined carbohydrates), and low socio-economic status, (Knowler et al, 1988).


2.2 Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is the most common metabolic disorder in pregnancy. About 90% of all pregnant diabetic patients have GDM, and, IDDM and NIDDM together account for the remaining 10% (Hagay et al, 1992).

It is defined as carbohydrate intolerance of variable severity with onset or first recognition during pregnancy (Hunter et al, 1990). The onset is usually recognized in the latter part of the second and early part of the third trimester (24-36 weeks) (Brinkman III, 1987).

The incidence of GDM has been reported to be 2-13% of all pregnancies (Hadden, 1980, Sepe et al, 1985).

The majority of women who develop GDM will return to normal glucose tolerance during the puerperium. However, a significant number of these women, variously estimated to be between 30%-60% will progress to type II (NIDDM), within 5-10 years (Brinkman III, 1987).

Beischer et al have studied women living in Australia and reported that there are significant increases in the incidence of the more severe grades of GDM in women born in the Mediterranean region, Asia, the Indian subcontinent, Egypt, and Arabic countries.
The incidence of GDM increased significantly with time in all racial groups, rising from 3.3% during 1979-1983 to 7.5% during 1984-1988 (Beischer et al, 1991).

GDM is considered to be associated with increased perinatal morbidity and mortality which may be reduced by adequate diagnosis and management (Backx et al, 1989). In addition, women who develop GDM have an increased risk of permanent diabetes which increases with time of follow-up (Martin, 1991).

2.2.1 Screening

Currently, most obstetricians screen all of their pregnant women for gestational diabetes. The simplest screening test for GDM is the taking of a history. Historic risk factors such as the presence of a family history of diabetes, a previous perinatal loss, or the previous birth of a macrosomic baby clearly identify individuals at increased risk for GDM in their current pregnancies. Unfortunately, the taking of a history is a relatively insensitive screening test, identifying only half of women who proceed to develop GDM (Coustan, 1993).

The likelihood of GDM is increased with advancing maternal age (Coustan et al, 1989).

Additionally, as hormonally produced insulin resistance in pregnancy is apparent by the end of the second trimester, testing is best performed between 26-30 weeks’ gestation.

Renal glycosuria is common in pregnancy and is hard to evaluate. However, women with unrecognized pre-existing diabetes may become pregnant and the presence of glycosuria on routine testing in the first 12 weeks of pregnancy may then be significant. Such women would be regarded as having pre-existing diabetes, not GDM (Martin, 1991).
A modified oral glucose tolerance test (OGTT) is used to screening for GDM. A 75 g oral glucose load is given to all pregnant women at between 26-30 weeks’ gestation in the non-fasting state.

The plasma glucose level is then measured one hour later and women who ‘screen positive’ should have a 75g OGTT before 30 weeks’ gestation (Martin, 1991). For a 75g glucose load the cut-off plasma glucose value at 1 hour is 8.0 mmol/L and for 50g it is 7.8 mmol/L (Martin, 1991).

These recommendations must not prevent the recognition and testing for diabetes at any stage of pregnancy if suggestive clinical features are present (Martin, 1991).

Serum fructosamine and glycosylated haemoglobin (HbA1c) give similar clinical information, and may be used interchangeably for the metabolic control of diabetes mellitus patients. Neither have diagnostic value as screening test for GDM. It may be because fructosamine originates mainly from the nonenzymatic glycation of albumin, so, its concentration depends therefore not only on the concentration of glucose but also on albumin (Vermes et al, 1989).

2.2.2 Diagnostic Criteria

Oral glucose tolerance tests have been used for diagnosing GDM for many years (Hunter et al, 1990). The Procedure for the diagnosis of GDM is shown in Table 2-2.

2.2.3 Medical Management

The goal of medical management of GDM is the prevention of perinatal mortality and morbidity (Coustan, 1993). Several treatment strategies have been proposed: diet,
exercise, pharmacologic intervention using oral hypoglycemic agents, and insulin therapy (Langer, 1993).

Table 2-2 Procedure for diagnosis of GDM (Martin, 1991).

<table>
<thead>
<tr>
<th>Indication</th>
<th>Optimal gestation (weeks)</th>
<th>Test performed</th>
<th>Diagnostic criteria-plasma glucose level (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical suspicion of GDM</td>
<td>any time</td>
<td>75 g OGTT (fasting)</td>
<td>0 hours ≥ 5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 hours ≥ 8.0</td>
</tr>
<tr>
<td>Screening</td>
<td>26-28</td>
<td>50 g glucose load (non-fasting)</td>
<td>1 hour ≥ 7.8</td>
</tr>
<tr>
<td>Confirmation of diagnosis after positive screening test</td>
<td>26-30</td>
<td>75 g OGTT (fasting)</td>
<td>0 hours ≥ 5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 hours ≥ 8.0</td>
</tr>
</tbody>
</table>

The majority of GDM patients can be managed by diet alone (Hoolysworth et al, 1992) or exercise and diet (Horton, 1991), and a small percentage may require insulin to achieve euglycemia (Brinkman III, 1987). These patients are not prone to develop ketosis (Hoolysworth et al, 1992).

2.2.4 Diet Therapy

The diet therapy mainly improves the body’s sensitivity to insulin. Diet therapy may include caloric restriction. It is currently recommended that diet should include 50-55% carbohydrate, mainly complex carbohydrate with an emphasis on reduced intake of simple sugars because of their high absorption rate. Fat consumption should be limited to 20-30%, with decreased saturated fats and cholesterol and increased polyunsaturated fats. The diet should include a modest 20-30% protein. This diet approach will usually
result in a 0.83-1.1 mmol / L decline in maternal glucose level (Langer, 1993). Diet program must provide a caloric intake sufficient to avoid ketonuria (30 kcal / kg in women 80-12% ideal body weight) (Peterson et al, 1991). In addition, even a small weight loss will improve maternal glucose levels (Langer, 1993).

2.2.5 Exercise

GDM patients should be encouraged to walk 2-3 miles an hour for 20-30 minutes, 4 days a week or engage in any other equivalent activity that they enjoy (Langer, 1993). Cardiovascular conditioning exercise for 20 minutes three times per week has also been shown to be useful in lowering both fasting and post-prandial glucose levels (Mulford et al, 1993). Exercise has a major effect on glucose metabolism and reduction in lipid levels (Peterson et al, 1991).

2.2.6 Oral Hypoglycemic Agents

Oral hypoglycemic agents play a limited role in diabetes in pregnancy. The main objection to their use in pregnancy is the fear of congenital anomalies or fetal compromise through direct stimulation of the fetal pancreas (Langer, 1993). Langer et al recently have reported that second-generation oral hypoglycemic agents (glyburide) do not cross the placenta. It suggests that second-generation hypoglycemic agents may play a role in the management of type II and GDM in the future (Langer, 1993).

2.2.7 Insulin Therapy

If diet and exercise do not achieve the requisite glucose levels (glucose levels < 4.4 mmol/L or < 7.8 mmol/L 1 hour after meals), insulin therapy based on body weight and

2.3 Complications of GDM

The rationale for treatment of GDM is to prevent diabetic complications. Poor glycemic control in early pregnancy is associated with an increased risk of major congenital abnormalities in the fetus (Gregory et al, 1992). In managing the pregnant diabetic patients, two patients are being treated, the mother and fetus (Lieb, 1987). Studies both in vivo and in vitro using animal models have provided data suggesting that hyperglycemia, hyperketonemia, and reduced gluolysis are, under certain conditions and circumstances, all potentially teratogenic, particularly when they occur in the earliest stages of pregnancy (Baird, 1986).

Most neonatal complications such as macrosomia, hypoglycemia, hyperbilirubinemia, hypokalaemia, polycythemia, major congenital anomalies (Hod et al, 1991), intra-uterine death, prematurity, and respiratory distress syndrome are readily avoided if GDM diagnosed and treated early and promptly (Lieb, 1987).

The adult female offspring of GDM mothers may exhibit impaired glucose tolerance and develop GDM when they become pregnant (Van Assche et al, 1991).

Obstetric management centers around surveillance to detect potential fetal compromise and the establishment of the proper time for delivery. Other goals are the diagnosis of fetal macrosomia and the avoidance of shoulder dystocia. Sudden fetal death, most likely is related to suboptimal metabolic control, is well documented in diabetic
pregnancies, but is less likely in GDM (Coustan, 1993). Antepartum fetal surveillance should begin at 28 weeks gestation (Landon et al, 1985).

Goldman et al reported that polyhydramnios, pre-term labor, and pyelonephritis were not more frequent in GDM, but hypertension without proteinuria and pre-eclampsia were more frequent in GDM patients. Despite this, cesarean delivery was more common in GDM (Goldman et al, 1991).

2.4 Long-term Follow-up

Women with history of GDM are at increased risk for future development of NIDDM. Follow-up studies have estimated that 30-50% of these women develop diabetes during the first 3-5 years after a pregnancy complicated by GDM, which after 12-18 years the proportion is 60-100% (Gregory et al, 1993). For this reason, the American Diabetes Association (ADA) has recommended that at the first postpartum visit, subjects who have had GDM should have a standard 75g 2-hours OGTT.

The high prevalence of subsequent diabetes may serve to make women with previous GDM an ideal group on which to attempt interventions designed to prevent the development of diabetes in the future (Coustan, 1993).

Persson et al speculate that an elevated proinsulin / insulin ratio in women with previous GDM could be a marker for later development of NIDDM (Persson et al, 1991). The usefulness of this measurement in follow-up is unclear.
2.5 Metabolic Changes in Normal Pregnancy and GDM

In normal pregnancy insulin secretion increases throughout gestation whereas peripheral insulin sensitivity is decreased. Fasting plasma glucose levels are decreased by approximately 10% during the first trimester. Maternal amino acid levels are also reduced.

On the other hand, plasma lipids are increased. As gestation advances, progressively increasing concentrations of insulin antagonistic hormones are secreted by the placenta (Reece et al, 1994). In addition, the fasting insulin/glucagon ratio is increased in both normal and gestational diabetic pregnancy (Reece et al, 1994). Plasma total cholesterol, LDL-cholesterol, and triglyceride concentrations are higher in pregnancy compared with non pregnancy (Loke et al, 1991).

GDM patients exhibit an increased insulin response to oral glucose and amino acids during pregnancy when compared with the postpartum period (Kuhl et al, 1985). Fasting insulin concentration, plasma glucose, and free fatty acids are higher in obese GDM women compared with normal non-obese pregnant women, whereas 3-hydroxybutyrate levels are similar (Carpenter, 1993). Non-obese GDM patients have fasting insulin levels similar to non-diabetic pregnancies. Fasting plasma glucagon is probably not increased in GDM.

Lipids and amino acids levels are significantly elevated in GDM subjects compared with normal control subjects (Carpenter, 1993). The postpartum fall in insulin sensitivity measured by the euglycemic clamp technique is also more marked in GDM than in normal pregnancy (Carpenter, 1993).
2.6 Metabolic Abnormalities in Women With Previous GDM

Considerable research has been done on GDM subjects during pregnancy in the last two decades.

Subjects with histories of GDM, particularly those with fasting hyperglycemia, are at high risk for developing subsequent overt diabetes mellitus (Metzger et al, 1985, Kjos et al, 1990).

Several follow-up studies have been conducted on the populations with previous GDM. Those indicated a high prevalence of overt diabetes mellitus years after the onset of GDM (Table 2-3) (Mestman, 1988).

Table 2-3 Follow-up studies on the GDM population (Mestman, 1988)

<table>
<thead>
<tr>
<th>Author</th>
<th>Total subjects</th>
<th>Follow-up period (years)</th>
<th>Overt DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>O'Sullivan (1984)</td>
<td>615</td>
<td>22-28</td>
<td>25%</td>
</tr>
<tr>
<td>Stowers et al (1985)</td>
<td>112</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>Metzger et al (1985)</td>
<td>113</td>
<td>1</td>
<td>38%</td>
</tr>
<tr>
<td>Mestman et al (1972)</td>
<td>232</td>
<td>5</td>
<td>23%</td>
</tr>
</tbody>
</table>

On the other hand, Kjos et al (1991) have reported that lipid abnormalities are uncommon at 36 months postpartum in women with previous GDM and manifest predominantly as a mild elevation in the plasma TG level.

Our current understanding of the development of metabolic disorders in women with previous GDM until they develop diabetes mellitus is incomplete.
2.7 **Lipid and Lipoprotein Metabolism**

GDM is a potentially serious disease. Evidence from autopsy, clinical, and epidemiological studies indicates that atherosclerosis more readily becomes advanced in diabetic than in non-diabetic subjects (Stout, 1992). A group of 89 women were studied 12-18 years after the diagnosis of GDM. The results showed that the incidence of overt diabetes was 65.2%. 8.6% of the subjects who developed overt diabetes mellitus had had a cerebral vascular accident, 6.8% had suffered a myocardial infarct, and 3.4% were on dialysis therapy (Mestman, 1988).

Lipid and lipoprotein abnormalities have been implicated in the pathogenesis of atherosclerosis in both diabetic and non-diabetic individuals (Assmann et al, 1988, Stern et al, 1989). Kjos et al (1991) have studied women with recent GDM for a period of 36 months after delivery. They have reported that plasma TG level was elevated and HDL-C was decreased in women with previous GDM at 3-11 months after delivery but not thereafter (Kjos et al, 1991).

These findings prompted us to examine the lipid and lipoprotein levels of women with recent GDM, to determine whether they have lipid and lipoprotein profiles indicative of increased risk for atherosclerosis in particular hypercholesterolemia and dyslipoproteinemia (characterized by hypertriglyceridemia and low plasma HDL-C concentrations).

### 2.7.1 Endogenous and Exogenous Pathways of Lipoprotein Metabolism

Dietary lipids are transported from the intestine to the liver via the exogenous pathway (Figure 2-1), and lipids synthesised by the liver are transported from the liver to
peripheral tissues via the endogenous pathway (Figure 2-2) (Trimble and McDowell, 1992).

Dietary cholesterol, mostly as cholesteryl ester, is absorbed in the intestine. Esters are hydrolyzed by cholesterol esterase to release free cholesterol and fatty acids. TG are hydrolyzed by pancreatic and intestinal lipase and absorbed mainly as free fatty acids and monoglycerides. TG, cholesterol, and apolipoproteins are assembled into chylomicrons, lipoprotein particles which are secreted into the lymph and enter plasma thorough the thoracic duct. Chylomicrons are normally found post-prandially in plasma (Dominiczak, 1994).

![Figure 2-1 Exogenous pathway of lipoprotein metabolism](Trimble and McDowell, 1992).

![Figure 2-2 Endogenous pathway of lipoprotein metabolism](Trimble and McDowell, 1992)
The exogenous pathway of lipoprotein metabolism (Figure 4-1) begins with secretion of chylomicrons by the intestine following absorption of dietary fat. Lipoprotein lipase (LPL) acts on chylomicron particles to produce chylomicron remnants. Chylomicron remnants are removed from plasma by the liver (Trimble and McDowell, 1992). The nature of the receptor is unclear (Trimble and McDowell, 1992).

The endogenous pathway of lipoprotein metabolism (Figure 4-2) begins with hepatic secretion of the triglyceride-rich lipoprotein (VLDL) into the plasma. This apoB-100 containing lipoprotein is converted to IDL and subsequently to LDL in the plasma compartment.

In the process, the particles' core is hydrolysed by lipolytic enzymes (Trimble and McDowell, 1992). Both of the major plasma lipolytic enzymes, lipoprotein lipase and hepatic lipase (HLP) have been implicated in the metabolism of apoB100 containing particles. It seems that the affinity of LPL is higher for large triglyceride-rich lipoprotein particles than for smaller remnants, whereas HLP, which expresses both triglyceride lipase and phospholipase activities, seems to have higher affinity for smaller VLDL and IDL particles (Nicoll and Lewis, 1980).

CETP also is involved in these processes. LPL hydrolyses TG and CETP exchanges TG for cholesteryl ester from HDL.

Subsequently, LDL is cleared from plasma by the LDL receptor. Chemically modified LDL may be cleared by other pathways such as the scavenger receptor on macrophages. Brown and Goldstein (1990) have suggested that the macrophage scavenger pathway may be involved in the formation of foam cells and initiation of the atherosclerosis process.
2.7.2 Cholesterol

Numerous epidemiological and genetic studies have shown that hyperlipoproteinemia, in particular hypercholesterolemia, is one of the principal risk factors for coronary heart disease (Douste-Blazy and Kloer, 1989).

2.7.3 Triglyceride

Primary and secondary hypertriglyceridemias (HTG) are the commonest lipid transport disorders found in patients obesity and diabetes mellitus (Eisenberg, 1987).

In obese individuals with upper body obesity appear to have a higher lipolytic activity adipocytes compared with obese subjects with peripheral obesity (fat distribution in the hips, buttocks, and thighs), resulting in a greater flux of FFAs toward the liver (Kissebah and Peiris, 1989). High FFA concentrations have been reported to decrease the hepatic extraction of insulin, resulting in systemic hyperinsulinemia (Kissebah and Peiris, 1989). HTG in obesity is associated with increase in the hepatic production of VLDL (Howard et al, 1987).

Hepatic lipase is an enzyme that contains triglyceride lipase, monoglyceride lipase, and phospholipase activity. Hepatic lipase catalyzes the degradation HDL particles, more specifically HDL$_2$ (Tikkanen, 1989). Despres et al (1990 b) have suggested that LPL activity is decreased and hepatic triglyceride lipase activity is increased in abdominal obesity. Low LPL activity results in the reduction in the catabolic rate of TG-rich lipoproteins and high hepatic triglyceride lipase activity causes a reduction in plasma HDL$_2$ concentration.
Thus, hypertriglyceridemia and a low concentration of HDL-C, particularly HDL₂, are common in abdominal obesity (Despres et al, 1990 a, Kissebah and Peiris, 1989, Despres, 1991).

The apo CIII gene is a part of a cluster with apo A-I and apo A-IV on chromosome 11, and variation in this cluster has been implicated in hypertriglyceridemia. Apo CIII has been suggested as candidate for a rate limiting mediator in the control of plasma TG levels since apo CIII inhibits LPL (Talmud, 1993). Whether other factors such as diet, alcohol consumption, obesity, smoking, exercise and oral contraceptive use significantly aggravate hypertriglyceridemia (NIH Consensus Conference, 1993) via increasing apo CIII activity is uncertain.

In western countries, plasma TG concentrations usually are inversely related to plasma HDL-C (Marmot, 1993). The PROCAM study has shown that plasma TG and LDL-C are independent predictors of coronary heart disease (CHD) incidence, but the predictive effect of TG for the development of CHD is observed only where the LDL-C/ HDL-C ratio is high (greater than 5) (Marmot, 1993).

The association of hypertriglyceridemia with premature atherosclerosis may not be due to the direct effects of the TG itself, but may be associated with changes in the concentration and composition of lipoproteins such as IDL, LDL and HDL.

HTG may result in an alteration in CETP activity which has an important role in lipid transport (Deckelbaum et al, 1984). The presence of an increased mass of circulating VLDL and chylomicrons promotes plasma lipid transfer activity with loss of cholesteryl ester from HDL and production of TG-enriched HDL particles. This TG-enriched HDL
particle is susceptible to lipolysis by hepatic lipase resulting in further HDL degradation and the generation of smaller HDL₃ particles (Tikkanen, 1989).

Postprandial hypertriglyceridemia may be more important than the high fasting TG levels, but little is known about this at present (NIH Consensus Conference, 1993).

2.7.4 Non-esterified Fatty Acids (NEFA)

In human, ~ 200 g of NEFAs (often termed free fatty acids) are mobilized from adipose tissue each day and transported in the circulation at concentrations ~ 100-1000 fold higher than their monomer solubility limit (Cistola and Small, 1991). They principally consist of oleic (about 38%), palmitic (28%), stearic (15%) and linoleic (9%) acids (Fruchart et al, 1989).

Solubilization and transport is mediated primarily by serum albumin. Albumin prevents the combination of fatty acids into liquid-crystalline or crystalline aggregates at neutral pH (Cistola et al, 1988) and supplies a source of fatty acid for lipid synthesis and energy production (Cistola and Small, 1991).

Under normal circumstances, approximately 99% of the circulating NEFAs are bond to serum albumin. However, cellular membrane and plasma lipoproteins have a high affinity for NEFAs and under some circumstances (eg high NEFA concentration in plasma) they may compete with albumin for NEFA binding (Cistola and Small, 1991).

In NIDDM patients, the action of insulin in both suppressing hepatic glucose output (HGO) and stimulating peripheral glucose uptake into skeletal muscle is impaired, and the combination of these defects is the principal cause of postprandial hyperglycaemia (Walker et al, 1993). In addition, the action of insulin in suppressing adipose tissue
lipolysis and increasing re-esterification is impaired, leading to increased plasma NEFA levels and rates of lipid oxidation in the insulin-stimulated state in both obese and non-obese patients with NIDDM (Walker et al, 1993).

Elevated plasma NEFA concentration in NIDDM may contribute to insulin insensitivity (Walker et al, 1993). Studies on rat liver have shown that NEFA stimulates gluconeogenesis (Williamson et al, 1966). An increased NEFA concentration in plasma diminishes the suppressive action of insulin on hepatic glucose output (HGO) and also decreases peripheral insulin-mediated glucose disposal in healthy man (Lee et al, 1988).

Glucocorticoid induced insulin resistance in normal rats has been shown to be due to excessive NEFA oxidation; possibly via an increase in the glucose-fatty acid cycle ultimately inhibiting glucose transport, or via decreased glycogen synthesis, or by a direct effect on glucose transporter translocation or activity (Guillaume-Genil et al, 1993).

2.7.5 VLDL

VLDL is the primary lipoprotein product of the liver and the carrier of endogenous triglycerides. Most of the TG in the plasma is carried in VLDL, whereas LDL carries most of the cholesterol (Chappell and Spector, 1991).

Because of the essential role played by VLDL in lipoprotein metabolism and substrate flux and the possibility that VLDL may (indirectly) induce atherosclerosis (Howard et al, 1987), its concentration was measured in this study.

2.7.6 IDL

Epidemiological studies in humans suggest that plasma concentration of IDL or remnant lipoproteins are predictors of the severity or progression of atherosclerosis.
Nordestgaard and Tybjaery-Hassan (1992) have suggested that IDL or remnant lipoprotein concentrations were better predictors of the extent of atherosclerosis than were LDL or VLDL. Cholesterol-fed animals also demonstrate accumulation of IDL and rapid development of atherosclerosis (Nordestgaard and Lewis, 1991).

Analysis of human plasma by gradient gel electrophoresis (GGE) has shown the IDL fraction from normal subjects consist of two major subspecies with particle diameters in the range of 27.5 to 30.0 nm. The larger, usually predominant, subspecies has been designated IDL-1, and the smaller, IDL-2 (Musliner et al, 1986).

The IDL subfractions differ in their cholesterol enrichment, with IDL-2 being more cholesterol enriched than IDL-1. The plasma triglyceride level influences the predominant IDL subfraction present in the circulation. With higher plasma TG concentrations, IDL-1 is predominant and may facilitate lipid transfer by CETP action to a greater extent. Greater CETP activity is thought to result in the formation of smaller and denser LDL particles (Krauss, 1987).

2.7.7 LDL

*In vivo* studies of the metabolism of human VLDL and IDL subfractions in a rat model system have suggested pathways for production of two of the major LDL subclasses, LDL-I and LDL-II. Small VLDL and the larger IDL species (IDL-1) are specifically converted by lipolysis in the absence of CETP activity to particles of size and density similar to LDL-II, while IDL-2 appears to be a precursor of larger, more buoyant LDL-I particle. However, it is not known how the two major, discrete IDL size subspecies originate (Musliner et al, 1991).
Several studies in animals and in humans have shown that the preferential substrates of hepatic triglyceride lipase are chylomicrons and VLDL remnants (Nicoll and Lewis, 1980). The decreased hepatic triglyceride lipase activity may result in the accumulation of IDL and the TG-enrichment of LDL (Robert et al, 1989).

Austin et al (1988) identified two distinct human phenotypes (denoted A and B) by non-denaturing gradient gel electrophoretic analysis of plasma LDL. Phenotype A was characterized by predominance of large, buoyant LDL particles, and phenotype B by a major peak of small, dense LDL particles. Phenotype B appeared to be inherited as a single-gene trait with a dominant mode of inheritance (Austin et al, 1988), although later studies revealed a more complex picture.

Pattern A is defined as an LDL subclass pattern with the major gradient gel peak at a particle diameter of 25.5 nm or greater and the presence of a secondary peak of smaller LDL particles. Pattern B has the major peak at a particle diameter of less than 25.5 nm, with skewing of the curve toward larger particle diameters (Austin et al, 1988).

Later the same researchers reported that LDL subclass phenotypes are related to other lipoproteins and also apolipoproteins (Austin et al, 1990). Phenotype B is positively related to plasma TG and apoB levels, and VLDL and IDL mass. In addition, there is a negative correlation between phenotype B and HDL-C, HDL₂ mass, and plasma apoA-I concentration (Austin et al, 1990).

LDL subclass phenotype B is related to relatively increased plasma TG, apoB, VLDL, and IDL concentrations, decreased HDL and plasma apoA-I levels, and increased risk of coronary artery disease in comparison with subjects with LDL subclass phenotype A (Feingold et al, 1992). It is possible, therefore, that the initial observation by Austin et al
suggesting a single gene trait with dominant inheritance may need to be revised, in the light of these more complex associations.

2.7.8 HDL

HDL appears to be associated with ‘reverse cholesterol transport’, the delivery of peripheral tissue cholesterol back to the liver for recycling or for excretion from the body (Barter, 1993).

HDL appears to protect against the development of premature coronary atherosclerosis probably through the reverse cholesterol transport process (Miller and Miller, 1975). The Framingham Study has provided evidence that HDL-C is inversely related to the development of a myocardial infarction in both men and women (Miller and LaRosa, 1991). Elevated HDL levels have been shown to lead to regression of atherosclerosis in animal models (Johnson, et al, 1991).

In addition, Schmitz and Williamson (1991) suggested that HDL metabolism plays a major role in cell membrane protection. Another interesting reason for the anti-atherogenic properties of HDL may be that HDL prevents the oxidation of LDL (Tribble and Krauss, 1993).

HDL₃ is the major substrate for the esterification of plasma cholesterol by the enzymes CETP and LCAT, and therefore plays an important role in the transport of cholesterol through the plasma (Barter et al, 1985). The reason for the increased substrate reactivity of smaller HDL₃ particles with LCAT is not clear.

Several hypotheses have been suggested, for instance that the phospholipid / FC and apoA-I / apoA-II ratios in same way affect substrate reactivity (Fielding et al, 1972).
The increasing amount of CE in particles causes enlargement and enlargement may reduce their ability to act as substrate for LCAT (Fielding et al., 1972).

2.7.9 Lp(a)

Lp(a) was first reported in human plasma as a genetic variant of LDL by Berg in 1963 (Berg, 1963). Like LDL, CE is the major constituent of Lp(a), but the particle contains more TG than LDL (Fless et al., 1986). Lp(a) particles contain two proteins, apo(a) and apoB, linked together by a single di-sulfide bond (Gaubatz et al., 1983). Apo(a) is thought to be synthesized mainly in the liver (Tomlinson et al., 1989).

Elevated plasma Lp(a) concentration is a risk factor for coronary artery disease (CAD) (Loscalzo, 1990) and peripheral vascular disease (PVD) (Widmann and Sumpio, 1993). James et al. (1995) have suggested that Lp(a) concentrations greater than 30 mg/dl are independently correlated with vascular disease in IDDM and NIDDM patients.

Apo(a) seems to be little affected by age, sex, and diet (Nestel et al., 1993), although a recent study has reported that the apo(a) level was increased 25% by the trans fatty acid, elaidic acid in the diet (Nestel et al., 1993). Haffner et al. (1992) reported that Lp(a) levels were significantly higher in diabetic patients who had higher total and LDL cholesterol levels.

Apo(a) levels are higher in both IDDM (Jenkins et al., 1991) and NIDDM (Jenkins et al., 1992) patients with microalbuminuria and may contribute to their tendency to macrovascular disease and early mortality. Apo(a) is elevated in active diabetic retinopathy (Maioli et al., 1993) and renal failure (Black and Wilcken, 1992). Whether elevated Lp(a) is a consequence or a cause of diabetic microvascular disease need further study (Jenkins et al., 1992).
The physiological functions of apo(a) or Lp(a) are not clear. The structural homology of Lp(a) with LDL and plasminogen suggests that Lp(a) may be involved in both atheroma formation and thrombosis which are most important mechanisms in the pathogenesis of atherosclerosis (Campbell et al, 1992). Lp(a) may promote thrombosis through interference with plasminogen activation, but it does not interfere with thrombolytic therapy during acute myocardial infarction (Simons, 1993).

### 2.7.10 CETP

Human plasma contains a lipid transfer protein commonly called cholesteryl ester transfer protein (CETP). CETP facilitates the transfer of cholesteryl ester, triglyceride, and phospholipid among lipoproteins (Albers et al, 1990). The major function of CETP in human plasma is believed to be the transfer of LCAT-derived cholesteryl ester to lipoprotein acceptors, LDL, VLDL, or lipoprotein remnants. During this process, the acceptor lipoproteins donate triglyceride and phospholipid to HDL. Thus, CETP play a key role in the 'reverse cholesterol transport' pathway (Albers et al, 1990).

HDL cholesteryl ester also can be delivered to the liver by other mechanisms such as selective uptake of CE from HDL without particle endocytosis, and uptake of CE mediated by apoE on the hepatocyte membrane (McPherson, 1993).

A diet high in cholesterol content increases the plasma CETP concentration in man (Martin et al, 1991). Marcel et al (1990) reported that the levels of CETP are 25% higher in women compared with men.

CETP is synthesized in a number of tissues, mainly in the liver and adipose tissue (McPherson, 1993).
2.7.11 Apolipoproteins A-I and B

In patients with coronary heart disease, total plasma apoB levels is often significantly elevated in the presence or absence of a reduction of total plasma apoA-I (Douste-Blazy and Kloer, 1989).

Many studies have reported that plasma apolipoprotein A-I, the major protein of HDL, is a good negative predictor for coronary heart disease (Fruchart, 1990).

These are at least two main lipoprotein subclasses containing apo A-I: those which contain, as the main protein components apoA-I and apoA-II, designated Lp A-I:A-II, and those contain apoA-I but not apoA-II, designated Lp A-I (Fruchart, 1990). These subclasses can be separated immunochemically.

Fruchart et al recently reported that cholesterol efflux from cells is mediated by Lp A-I and not by Lp A-I:A-II, and that the lower HDL concentrations in coronary artery disease were linked with lower Lp A-I levels, while Lp A-I:A-II was unchanged (Fruchart et al, 1990).

2.8 Carbohydrate Metabolism

2.8.1 Factors Influencing Rates of Glucose Uptake

There are three major sources of plasma glucose (Figure 2-3), diet and hepatic gluconeogenesis and glycogenolysis (Owen et al, 1969).

Several factors influence the rate of glucose uptake. These are: plasma glucose level, free fatty acids, muscular work, and hormonal effects. A high concentration of available
NEFA will result in a decrease in the rate of glucose uptake by skeletal and cardiac muscle at any given level of insulin (Randel et al, 1963).

2.8.2 Insulin

Insulin is secreted by the pancreas. Insulin acts in two major ways: (1) it has a key role in the metabolism of carbohydrate, lipid, and proteins, and (2) it has growth-promoting effects on DNA synthesis, cell division, and cell differentiation (Vander et al, 1994 c).

Insulin and glucagon play a major role in the regulation of glucose homeostasis. Insulin stimulates anabolic processes and favours energy storage, whereas glucagon stimulates catabolic processes, which consume energy substrates. Thus, their effects on glyconeogenesis, glycogenolysis and gluconeogenesis are opposed, and their activities in relation to these multi-enzyme systems are complex (Freychet, 1990).

The major sites of action of these hormones are the liver, adipose tissue, and skeletal muscles. Insulin and glucagon act via specific membrane receptors in their target cells (Freychet, 1990).

2.8.3 Glucose Tolerance

Glucose tolerance, as estimated by the oral glucose tolerance test (OGTT), depends
on complex iteration among several physiological processes, including first-phase and second phase insulin secretion, insulin removal, insulin sensitivity, and the tendency of high glucose concentration (independent of insulin) to increase glucose utilisation (Martin et al, 1992). Subjects with insulin resistance may be euglycemic with normal blood glucose concentrations, maintained by hypersecretion of insulin. In these case,
hyperinsulinemia may be noted both in the fasting and postprandial states (Beck-Nielsen et al, 1990).

The risk of subsequent glucose intolerance or diabetes in women with previous GDM is related to pre-pregnancy body mass index (BMI) and fasting plasma glucose during pregnancy (as well as current BMI). Subjects who are fatter before pregnancy and whose plasma glucose concentrations are higher during pregnancy are more likely to develop glucose intolerance or diabetes during pregnancy and/or after delivery (Coustan et al, 1993).

Effendic et al have reported that 60% of normal weight women with previous GDM had borderline or decreased oral glucose tolerance 6-36 months postpartum which could be explained either by low insulin response or by the combination of a low insulin sensitivity and low insulin response (Effendic et al, 1987).

2.8.4 Insulin Resistance

Insulin resistance may be defined as a state (of a cell, tissue, system or body) in which more than normal amounts of insulin are required to draw out a quantitatively normal response (Berson and Yalow, 1970). It should be emphasised that the level of glycemia may be within the normal range in a person with insulin resistance as a result of compensatory hyperinsulinemia (Flier, 1993).

During last two decades, several possible mechanism have been suggested for the pathogenesis of insulin resistance.

The classification of insulin resistance:
(1) Circulating anti-insulin antibodies and excess insulin degrading enzymes have been suggested, but those have not been shown to be important causes.

(2) A primary (ie. genetically determined) defects in the ability of insulin to signal its target cells (ie. receptor mutations) is again not an important cause.

(3) The major category of mechanisms for insulin resistance involves post-receptor mechanisms. These include the mediation of local or circulating factors on target cells to increase resistance to one or more pathway of insulin action. This category may include primary genetic defects and cellular consequence of metabolic changes (Flier, 1993).

2.8.5 Insulin Resistance Syndrome

Insulin resistance is a common and multifaceted syndrome which occurs with high frequency in the general population. Much attention has recently been focused on the insulin resistance syndrome, also referred to as ‘syndrome X’. The syndrome of insulin resistance may include a cluster of several common metabolic disorders: hyperinsulinemia, obesity, NIDDM, hypertension, dyslipidemia, and as a complication of these, atherosclerotic vascular disease (DeFronzo and Ferrannini, 1991). Central obesity also has been added to the cluster (Zammet, 1989).

The evidence suggests that hyperinsulinemia plays a major role in the development of the features of syndrome X. While hyperinsulinemia appears to be crucial for the development of syndrome X, it must usually develop secondary to a decrease in insulin sensitivity. In terms of etiology, therefore, a decrease in insulin sensitivity may be the primary event in syndrome X (Walker and Alberti, 1993).
Insulin sensitivity has been shown to vary considerably in the normal population and be influenced by many different factors such as dietary composition and intake, physical activity, and endocrine status (Walker and Alberti, 1993).

Studies have suggested that genetic factors also may play role in the development of insulin insensitivity and hence syndrome X. Studies on the Pima Indian have shown that insulin resistance in this population may be due to an important genetic effect related to the fatty acid binding protein gene on chromosome 4 (Prochazka et al, 1992). In addition, studies on persons with a positive family history of diabetes in first degree relatives have suggested that these persons have insulin resistance which probably has a genetic basis (Eriksson et al, 1989).

Thus, it seems the likely sequence is that a combination of both environmental and genetic factors influence the overall insulin resistance state, then hyperinsulinemia occurs, and then phenotypic features of syndrome X occur (Walker and Alberti, 1993).

NIDDM is the disease classically associated with insulin resistance (Seely and Olefsky, 1993). Impaired glucose tolerance is a common clinical feature in all types of insulin resistance syndrome and hyperinsulinemia occurs in compensation for this defect in affected subjects (Moller and O'Rahilly, 1993).

### 2.8.6 HbA1c

Haemoglobin can combine non-enzymatically with glucose, and the haemoglobin conjugate, haemoglobin A1c, can be measured in blood. It increases in concentration roughly in proportion to the degree of sustained hyperglycaemia and is used clinically as a tool to indicate the degree of control of the hyperglycaemia in people with diabetes (Steinberg, 1990).
Other plasma proteins and a number of tissue proteins are also glycated in diabetes. Whether such glycation of proteins contributes to clinical complications is still not clear (Steinberg, 1990).

The Framingham Heart Study has reported that the plasma HbA1c levels are significantly above normal in non-diabetic women with an early cardiovascular event (Singer et al, 1992).

Giugliano et al (1992) also reported a significant correlation between HbA1c and classic risk factors of cardiovascular disease such as hypertension, and cholesterol in diabetic patients.

2.9 Raised Blood Pressure

Hypertension is defined as a chronically elevated systemic arterial pressure. The dividing line between normal blood pressure and hypertension is an arbitrary one, set at approximately 140/90 mm Hg.

Hypertension may be due to increased total peripheral resistance or increase in cardiac output or both. Increased total peripheral resistance may be the result of increased arteriolar constriction (Vander et al, 1994 b). About 95% of people with hypertension have essential hypertension (primary hypertension) in that the cause of hypertension is unknown (Ross, 1990).

2.9.1 Essential Hypertension and Insulin Resistance

The association between NIDDM, obesity, and essential hypertension has been known for many years (Kannel and McGhee, 1979, reporting data from the Framingham Heart Study).
Others have linked high blood pressure to insulin resistance. Modan et al reported that postprandial insulin concentrations were higher in both treated and untreated hypertensive patients than in normotensive controls. This finding was independent of the presence of obesity and diabetes (Modan et al, 1985). Parillo et al reported greater serum insulin and glucose levels 60 to 180 minutes after a glucose tolerance test in hypertensive patients than in controls (Parillo et al, 1988).

The CARDIA study reported that high fasting insulin concentration was associated with elevation in both systolic and diastolic blood pressure in 5115 participants, aged 18-30 years, of different sex, race, and education levels. The strength of these associations decreased (but the association were still significant) after adjustment for age, sex, race, BMI, alcohol consumption, smoking, physical activity, and exercise (Manolio et al, 1990).

Studies on hypertensive subjects using the euglycemic hyperinsulinemic clamp and minimal model techniques indicated that the increased insulin levels are a compensatory response to impaired insulin-stimulated glucose uptake (Ferrannini et al, 1987, Pollare et al, 1990).

Istfan et al (1992) have reported that obese hypertensive subjects had greater insulin resistance compared with obese non-hypertensive subjects. It is thought that the relationship between primary hypertension and insulin resistance is independent of obesity (Pollare et al, 1990).

The association between insulin resistance and high blood pressure has not been determined in all populations. Insulin level was correlated with blood pressure in young Caucasians but not in a black American population or in the Pima Indians (Saad et al,
Pima Indians have a higher prevalence of insulin resistance and hyperinsulinemia than other American (non-Indian American) populations but not a higher prevalence of hypertension (Saad et al, 1991).

It should be emphasized that the relationship between hyperinsulinemia and high blood pressure is not found in renal disease. Hypertension secondary to renal disease is not an insulin resistant state and is not associated with hyperinsulinemia (Marigliano et al, 1990).

An abnormal lipoprotein profile has been demonstrated in patients with essential hypertension similar to the dyslipidemia of diabetes (hypertriglyceridemia and a low level of HDL-c) (Shieh et al, 1987), suggesting that a number of patients with essential hypertension, in fact, have hypertension associated with the insulin resistance syndrome.

It is not known whether hyperinsulinemia is a cause of high blood pressure or is secondary to high blood pressure, or if both hyperinsulinemia and high blood pressure result from other factors. Shen et al reported that the reduction of blood pressure in hypertensive subjects is not related to normalisation of the plasma insulin levels (Shen et al, 1988), suggesting that hyperinsulinemia may be a causal factor for high blood pressure, or both may be caused by another factor or factors.

2.10 Microalbuminuria

Qualitative and quantitative analyses of urinary protein excretion are used to diagnose renal disease. A urinary albumin excretion rate (UAER) of 200 µg / min termed microalbuminuria (Jensen et al, 1993).
The UAER is increased in variety of renal and urinary tract diseases. UAER also appears to be elevated after prolonged upright posture and vigorous muscular exercise (Waller et al, 1989).

2.10.1 Pathophysiology of Microalbuminuria

Healthy adults normally excrete small amounts (less than 150 mg / day) of protein in the urine including mainly albumin and Tamm-Horsfall protein. Excretion of greater than 150 mg / day is termed proteinuria (Wilson, 1992).

Increased protein excretion may happen whenever there is an increased filtered load secondary to an increase in glomerular permeability (glomerular proteinuria), an increase in the plasma concentration of a protein (overflow proteinuria), or an increase in glomerular filtration rate (GFR).

Proteinuria will also result from a decrease in the tubular absorption of normally absorbed proteins (tubular or low molecular weight proteinuria) or an increase in protein secretion or non-specific addition of protein to the fluid in the urinary tract such as increase in secretory IgA during inflammation (urogenic or secretory proteinuria) (Beetham and Cattell, 1993).

Albuminuria associated with diabetes is usually a consequence of increased glomerular permeability. Diabetic nephropathy evidenced by hypertension, macroalbuminuria, and renal dysfunction, develops in about 40% of IDDM patients approximately 10-20 years after the onset of disease (Harris et al, 1987, Selby et al, 1990) and also in 25% of NIDDM patients 5-10 years after their diagnosis (Selby et al, 1990).
The pathogenesis of diabetic nephropathy is not completely clear. The relative contributions of hypertension, dietary protein, glucose control, dyslipidemia and other factors in the initiation and continuation of renal injury are not fully known. Strict blood pressure control and protein restriction are important treatment strategies in both early and established diabetic kidney disease (Carella et al, 1994). More than 25% of all cases of end-stage renal disease in the United States are due to diabetes mellitus (Krolewski et al, 1985).


The Pittsburgh Epidemiology of Diabetes Complications Study (PEDCS) suggested that glycemic control, age or duration of IDDM, dyslipidemia, and possibly hypertension all may contribute to the development of microalbuminuria. In addition, PEDCS proposed that the adverse cardiovascular risk profile exist in subjects with overt nephropathy may begin to develop even before the discovery of microalbuminuria (Coonroo et al, 1993).

2.11 Obesity and Androgen Status

2.11.1 Obesity

Obesity is a major problem for developed nations, affecting approximately 10-50% or more of the adult population (Bray, 1985). A useful measure of obesity is the body mass index (BMI), which is the weight in kilograms divided by the height in meters squared.
Obesity is associated with premature mortality, morbidity, and social disadvantage (Pi-Sunyer, 1991).

The reference range for BMI is 20 to 25 kg/m$^2$ for men and 19 to 24 kg/m$^2$ for women. Above 25 kg/m$^2$ for men and 24 kg/m$^2$ for women is considered 'overweight' and above 30 kg/m$^2$ for both men and women regarded as 'obese' (Egger, 1992). According data from the National Heart Foundation Risk Factor Prevalence Survey in Australia (No 3, 1989), approximately 48% of men and 34% of women aged 20-69 years have a BMI more than 25 kg/m$^2$ (Calvert, 1991).

The health risks of obesity increase with its severity and reach significance at a weight > 20% above optimal weight in individuals. Mortality risk begins to increase when the BMI exceeds 30 in adults (Redmond, 1991).

2.11.1.1 Classification

Obesity can be classified into categories. The most common classification divides subjects into two categories: upper body (android) and lower body (gynoid) obesity (Kissebah et al, 1989). Subjects with upper body obesity present a higher waist / hip ratio (WHR), increased deposition of mesenteric or visceral adipose tissue, hyperinsulinemia, with greater insulin resistance, and dyslipidemia in comparison with subjects with lower body obesity (Kissebah et al, 1989).

Abdominal obesity may be calculated by subscapular / triceps skinfold ratio (Centrality Index) or waist / hip ratio (WHR). WHR has been suggested as an independent risk factor for several diseases (ie diabetes mellitus, hypertension, cardiac events stroke, dyslipidemia, varicose veins, and arthritis), and also for mortality. WHR of more than 0.9 in males and 0.8 in females should be taken as representing increased risk (Egger,
Kalkhoff et al (1983) suggested that women with WHR = 0.83-0.99 should be considered to have upper body segment obesity and women with WHR = 0.50-0.73 should be considered to have lower body segment obesity.

Recently, Fujioka et al (1987) have proposed that there are two different types of obesity with respect to metabolic and clinical features: one is the 'visceral type', characterised by marked fat accumulation in the abdominal cavity and an association with metabolic alterations such as glucose intolerance, hyperlipidemia, and the other one is the 'subcutaneous type' in which fat accumulates predominantly in the subcutis and is rarely associated with metabolic disorders.

Both men and women with abdominal subcutaneous or visceral obesity tend to have large fat cells, increased LPL activity, increased lipolysis, and low antilipolytic effect of insulin.

High plasma sex steroid, glucocorticoid, catecholamine, and growth hormone concentrations are associated with the amount of visceral fat, and the association may be somewhat different in men and women (Bouchard, 1994).

2.11.1.2 Pathophysiology of Obesity

The causes of obesity are multiple. Energy expenditure is an important factor in determining weight gain or loss. Daily energy expenditure can be divided into three major components: basal metabolic rate, which represents 50-70% of daily energy expenditure, the thermic effect of food which represents about 10%, and the energy cost of physical activity which represents 20-40% of daily energy expenditure (Ravussin and Swinburn, 1992).
Dietary factors play an important role in the regulation of body weight. Diets high in fat, sugar, or both are the most effective promoter of obesity in animal investigations (Sclafani, 1986, Lucas and Sclafani, 1990).

In addition, the endocrine system plays an important role in the etiology and maintenance of obesity. Abnormalities in insulin secretion and function are probably the most important factors in metabolic changes in the obese subjects (Heber, 1994).

2.11.1.3 The Role of Obesity in the Pathogenesis of Diabetes Mellitus

The relationship between obesity and insulin resistance and NIDDM is very complex. Most patients with NIDDM are obese. Fujioka et al (1987) have reported that the fasting plasma glucose and area under the plasma glucose concentration curve after oral glucose loading (plasma glucose area) were significantly greater in obese than non-obese patients.

Subjects with excess intra-abdominal (visceral) fat have more insulin resistance related to obesity (Bjorntorp, 1989). Abdominal obesity is considered as a risk factor for the development of insulin resistance and its metabolically related abnormalities (Bjorntorp, 1990).

2.11.2 Androgen Status

Sex-hormone-binding globulin (SHBG) is a steroid-binding globulin which binds to oestradiol and testosterone. In normal women, about 65% of the circulating testosterone is tightly bound to SHBG, while most of the remaining hormone is loosely bound to albumin. Only 1-2% of the total testosterone is free. It is generally accepted that
testosterone bound to SHBG is not readily available to intracellular androgen receptors in target tissues and thus has little biologic activity (Goldfien and Monroe, 1994).

Women with overall or central obesity exhibit a lower plasma SHBG (Lindstedt et al, 1991, Haffner et al, 1991). SHBG shows a much higher affinity to testosterone than oestradiol and therefore any changes in SHBG concentrations cause a greater change in the free testosterone fraction than the free oestradiol fraction (Reed et al, 1993).

Some studies have reported that low plasma levels of SHBG and central obesity are correlated with higher plasma concentrations of free testosterone (Haffner et al, 1989). Thus, a low plasma concentration of SHBG results in hyperandrogenicity in women (Rosenfield, 1975).

Preziosi et al (1993) found a significant and negative relationship between SHBG and insulin level in healthy pre-menopausal and post-menopausal women. This relationship was independent of age, BMI, subscapular skinfold, and fasting and 2-h plasma glucose.

Studies have shown that excess sex steroid and glucocorticoid concentrations in the body may be associated with regional fat distribution (upper body segment) and related metabolism disorders and have suggested that this relationship may be causal (Kissebah et al, 1989). Hyperandrogenicity may decrease insulin sensitivity in pre-menopausal women (Evans et al, 1983).

Tikkanen and Nikkila (1987) have reported that sex steroids demonstrate significant effects on lipoprotein metabolism. Steroids with androgenic activity increase hepatic triglyceride lipase activity and decrease plasma HDL2-C levels. This phenomenon was also reported in obese women with a high abdominal adiposity (Despers et al, 1989).
2.12 Haemostatic Disorders


2.12.1 Fibrinogen

The Framingham Study has reported that fibrinogen levels independently are associated with cardiovascular disease and increase with age in both women and men (Kannel et al, 1987, Wilhelmsen et al, 1984).

Plasma fibrinogen concentration seems to be related to hyperlipoproteinemia, hypertension, smoking, and several life style factors such as dietary habits (Koenig and Ernst, 1992). Several studies have reported that the fatty acid composition of the dietary lipids is an important factor in the athero-thrombotic process (Berg-Schmidt et al, 1990, Grundy and Denke, 1990). Cigolini et al (1994) examined the fatty acid composition of adipose tissue as an objective index of dietary fat intake in healthy men and they found no correlation between fibrinogen level and adipose tissue fatty acids.

Baxter et al (1988) suggested hyperfibrinogenemia may be an important risk factor for peripheral arterial disease in men. Cigolini et al (1994) suggested that hyperfibrinogenemia may be considered as an integral part of syndrome X and that it may contribute along with impaired fibrinolytic activity to a 'procoagulant' state.
The exact mechanism underlying hyperfibrinogenemia and risk of CHD is not clear. There are several hypotheses. Hyperfibrinogenemia can adversely influence platelet aggregability and plasma viscosity, and thus predispose (perhaps) to thrombosis. In addition, atherosclerosis can cause hyperfibrinogenemia as fibrinogen is an acute-phase protein (Heinrich et al, 1994).

2.12.2 Factor VII

Factor VII is a vitamin K-dependent glycoprotein with a molecular weight of 50,000 dalton composed of a single polypeptide chain, synthesized in the liver. The physiologically important activator of factor VII is factor Xa which, in the presence of calcium and phospholipids, rapidly converts factor VII to factor VIIa. In the presence of thromboplastin, factor VIIa activates factor IX and factor X very rapidly. The coagulant pathway proceeds, ending in the production of thrombin (Vander et al, 1994 d). Figure 9-1 shows the classical view of the intrinsic and extrinsic pathways of the clotting cascade in humans (Vander et al, 1994 d).

Factor VII levels increase with age in both men and women, and may be higher in women. Factor VII levels increase in late pregnancy, with use of oral contraceptives, obesity, and in post-menopausal women (Bloom and Thomas, 1987, Poller et al, 1990).

Factor VII coagulant activity is also increased in hyperlipidemia (Miller et al, 1985), diabetes (Fuller et al, 1979), and with high dietary fat intake (Miller et al, 1986).

High levels of factor VIIc have been shown to be associated with ischaemic heart disease in men (Meade et al, 1986, Wilhelmsen et al, 1984). Increased factor VII levels have been reported in NIDDM patients with microalbuminuria and cardiovascular disease (Stehouwer et al, 1992).
Ceriello et al (1988) suggested that the plasma factor VII levels may be directly raised by high blood glucose in diabetic and normal subjects. On the other hand, Schernthaner et al (1989) showed that an altered haemostatic status (high plasma fibrinogen and factor VII) may not be completely corrected by better glycaemic control.
2.12.3 Von Willebrand Factor (vWF)

vWF is a complex glycoprotein synthesized by vascular endothelium (Bloom et al, 1973) and megakaryocytes (Nachman et al, 1977).

The initial response to blood-vessel damage is vasoconstriction and this is facilitated by formation of a platelet plug, then blood coagulation in the damaged blood vessels. vWF, secreted by the endothelial cells, is necessary for platelet aggregation (Vander et al, 1994d).

High plasma levels of vWF, indicating endothelial cell dysfunction, have been found in diabetic patients with retinopathy (Stehouwer et al, 1992, Porta et al, 1987, Coller et al, 1978). In addition, Silveira et al (1992) have suggested that abnormal degradation of vWF occurs in kidneys in IDDM patients with and without nephropathy.

The role of vWF in the pathogenesis of diabetic nephropathy is not clear. It is probably a marker of endothelial dysfunction, without being directly involved in the pathogenesis of diabetic nephropathy. Alternatively, vWF may stimulate adhesion of platelets to exposed subendothelium in the kidney (Stehouwer et al, 1991).

Cross-sectional studies have suggested that raised plasma factor VIII and vWF level is associated with clinical coronary artery disease (Sugrue et al, 1985). However, Myreng et al (1986) and Hamsten et al (1986) have reported no correlation between plasma vWF level and either clinical or angiographical findings of coronary artery disease.

Chetty et al (1988) found that race is a major determinant of vWF and factor VIII. Black people in the USA have higher factor VIII and vWF levels than whites.
Numerous studies have suggested that coagulation disorders in diabetic patients, which seem to be caused by hyperglycaemia (Ceriello, 1993), give rise to a thrombotic tendency.

High levels of plasma fibrinogen (Coller et al, 1978, Ganda and Arkin, 1992), factor VII (Ceriello et al, 1988), factor VIII, vWF (Osterman and Va-deLoo, 1986), factor X antigen and low activity of factor X in plasma (Ceriello et al, 1990) have been reported in diabetic patients and thought to be due to hyperglycaemia.

Figure 2-5 shows the pathway linking hyperglycaemia and a tendency to thrombosis in diabetic patients. Hyperglycaemia may influence thrombosis by two mechanisms; either by a mechanism connected with non-enzymatic glycation or by an increased oxidative reaction (Ceriello, 1993).

Figure 2-5 Pathway linking hyperglycaemia and thrombosis in diabetes mellitus. 
(+ = increased and − = decreased, Adapted from Ceriello, 1993).
2.13 Dietary Habits and Other Lifestyle Factors

2.13.1 Dietary Habits

Recently, there has been much interest in elucidating the underlying links between NIDDM and genetics, obesity, nutrition, physical activity, and other factors. Diet plays a significant role in determining plasma concentrations of TG, cholesterol, TG-rich lipoproteins, and also HDL-C.

The relationship between lipids and lipoproteins and atherosclerosis also encourages investigators to pay more attention to dietary factors related to disease.

2.13.1.1 Dietary Fat and Blood Lipids and Lipoproteins

The type and amount of fat in the diet influences blood lipid and lipoprotein concentrations. Keys et al (1965) have shown that dietary polyunsaturated fatty acids decrease and saturated fatty acids increase total plasma cholesterol concentrations. However, the mono-unsaturated oleic acid and saturated stearic acid are neutral. Many researchers have reported that monounsaturated fatty acids have no significant influence on serum cholesterol concentrations (Thomson et al, 1967, McGandy and Hegsted, 1975).

Grundy and Denke (1990) recently reported that only saturated fatty acids with chain lengths of 12, 14, and 16 carbon atoms in the diet increase total plasma cholesterol levels mainly by elevating the LDL levels, and the other fatty acids (such as stearic acid) have less effect on plasma LDL levels. Both polyunsaturated and monounsaturated fat reduce LDL levels when replacing saturated fat (Grundy and Denke, 1990).
The plasma TG concentrations change with diet. Postprandially, the plasma TG concentration increases due to the appearance of chylomicrons and their remnants in plasma. The concentration of TG depends on both the amount and the type of fat in the diet. Saturated fat in the diet produces a longer postprandial plasma triglyceride than polyunsaturated fat. The type and amount of fat in the diet also influence the fasting levels of TG, mainly by increasing the concentrations of VLDL (Norum, 1992).

The fasting TG concentration in plasma decreases when monounsaturated fat is substituted for carbohydrates (Grundy, 1986). Polyunsaturated fatty acids (n-6 type) also decrease the fasting TG concentrations in plasma (Chait et al, 1974).

Diet affects LPL activity. In the fasting state the LPL activity increases in muscle, however, in the postprandial state, the activity increases in adipose tissue. The activity of LPL seems to be mediated by insulin and the insulin sensitivity of the muscle and adipose cells (Kiens et al, 1989).

Dietary unsaturated fatty acids tend to cause larger chylomicrons than saturated fatty acids intake (Ockner et al, 1969 and Ockner and Jones, 1970). Larger chylomicrons are cleared from plasma faster than smaller chylomicrons.

Saturated fatty acids in the diet increase the plasma VLDL concentrations. However, dietary fish oil decreases the plasma VLDL concentration resulting a small effect on plasma cholesterol (Harris, 1989, Sullivan et al, 1986). The main effect of marine fish oil on plasma lipids is a reduction in plasma TG level (Sanders, 1987, Schmidt et al, 1993). The proposed mechanisms for the VLDL-lowering effect of fish oil might be due to reduced plasma FFA concentrations resulting from decreased peripheral lipolysis of TG (Singer et al, 1990), increased oxidation of fatty acids in the liver, or because saturated
and monosaturated fatty acids stimulate VLDL synthesis and n-3 polyunsaturated fatty acids do not have this effect (Harris et al, 1990, Dixon et al, 1991). In addition dietary marine fish oil tends to increase HDL-C (Schmidt et al, 1993).

LDL concentration is increased by using saturated fats in the diet, probably through effects on hepatic LDL receptors (a decrease in LDL receptor activity and thus a reduction in the LDL catabolism rate), but the mechanism is not clear (Norum, 1992). Dietary fatty acids do not appear to alter the rate of plasma cholesterol synthesis. Alteration in the plasma cholesterol concentrations may be due to an increase in the output of faecal neutral sterols and of faecal bile acids (Durrington, 1995).

On the other hand, saturated fat in the diet tends to produce smaller VLDL particles than polyunsaturated fat. It seems that smaller VLDL particles are more prone to convert to LDL particles than large VLDL particles are. This may be a possible mechanism underlying increased LDL levels by using saturated fat (Norum, 1992).

Bonanome et al (1992) have found that a diet rich in monounsaturated fatty acids (MUFA) compared with polyunsaturated fatty acids increases the resistance of plasma LDL to oxidative modification, independent of their content of antioxidant. This effect might lower the atherogenicity of these lipoproteins.

Thus, a low fat, monounsaturated fatty acid-rich diet is as effective as a low fat, polyunsaturated fatty acid rich diet in lowering total and LDL-C, but both also lower HDL-C concentrations (Wahrburg et al, 1992). Wahrburg et al (1992) suggested that the monounsaturated fatty acid-rich diet may be more advantageous than the polyunsaturated fatty acid-rich diet because it does not lower plasma apoA-I concentration as much as the polyunsaturated fatty acid rich diet.
Because of the anti-atherogenic effect of HDL-C, the effect of dietary fat on HDL-C has been investigated intensively. Diets low in fat cause both low plasma HDL and LDL concentrations, however a diet high in saturated fat results in high HDL and LDL concentrations. Dietary polyunsaturated fat, but not monounsaturated fat, leads to lower HDL-C levels (Grundy and Denke, 1990).

Animal studies have suggested that a diet rich in cholesterol and saturated fat (an atherogenic diet) enhances the plasma CETP activity (Stein et al, 1990, Quinet et al, 1990). Increased CETP activity may result in decreased levels of HDL-C and increased levels of VLDL-C and LDL-C (Grundy and Denke, 1990).

Trans-isomers of fatty acids, formed by the partial hydrogenation of vegetable oils to produce margarine and vegetable shortening, increase the LDL-C / HDL-C ratio. As a result, consumption of partially hydrogenated vegetable oils may contribute to the occurrence of coronary heart disease (Willett et al, 1993).

2.13.1.2 Dietary Cholesterol and Blood Lipids and Lipoproteins

Fungwe et al (1992) have reported that increasing dietary cholesterol intake increase the concentrations of TG and cholesteryl ester in liver and plasma in rat. This is accompanied by reduced fatty acid oxidation and increased incorporation of exogenous fatty acid into hepatic TG and increased secretion of VLDL (Fungwe et al, 1992).

Interestingly, Fungwe et al (1994) have recently suggested that dietary cholesterol stimulates the biosynthesis of FFA, while the addition of TG to the diet stimulates production of cholesterol.
Dietary cholesterol intake causes elevation in the plasma total cholesterol and LDL-C (Erickson et al, 1964, Hegsted et al, 1965). Studies have shown that the serum cholesterol response to dietary cholesterol tends to plateau at high cholesterol intakes (Keys, 1984, Hegsted, 1986).

Riccardi et al (1987) suggested that the most effective dietary change to reduce plasma cholesterol concentrations in human is to decrease the consumption of saturated fat and cholesterol.

2.13.1.3 Dietary Carbohydrate and Blood Lipids and Lipoproteins

A high carbohydrate diet increases plasma TG concentration but not that of cholesterol. The effects of dietary carbohydrates depend on the type of carbohydrate. Intake of sucrose or fructose within the range usually found in the population does not have any elevating effect on plasma triglycerides in most normal and diabetic subjects (Truswell, 1994). While the plasma TG levels increase, HDL-C levels decrease (Gonen et al, 1981, Brinton et al, 1990).

In human, increased fasting TG levels are observed with very high carbohydrate intake, > 35% of energy from sucrose or > 20% of energy from fructose, more commonly in men and in conjunction with saturated fat intake (Truswell, 1994).

The Lipid Research Clinics Study Prevalence Programme reported that there is a positive correlation between dietary cholesterol intake and the serum HDL-C in school-age white boys (Glueck et al, 1982). However, another study reported that serum HDL-C concentration was relatively unaffected by increases in dietary cholesterol (Mahley et al, 1978).
2.13.1.4 Dietary Fibre and Blood Lipids and Lipoproteins

Wheat bran and cellulose (insoluble polysaccharides) do not decrease plasma cholesterol concentration. In contrast, pectin, guar gum, oat bran, psyllium husk, bean (legumes), fruit and vegetables decrease plasma cholesterol and LDL-C concentrations (Schneeman and Tietyen, 1994, Riccardi and Rivellese, 1991). There is a negative correlation between plasma total cholesterol concentrations and dietary fibre intake (Kirby et al, 1981).

In general, evidence that fibre in fruits and vegetables has some hypocholesterolemic effect is better than for most of the cereals so far studied. One aim of a lipid-lowering diet must be to decrease saturated fat consumption and increase fibre intake (Durrington, 1995). Therefore, in practice, the consumption of legumes, vegetables, and fruit should be encouraged (Riccardi and Rivellese, 1991).

The mechanisms by which dietary fibre acts as a hypoglycemic and hypolipidemic agent are not clear. Several mechanisms have been proposed including increasing the faecal excretion of bile acids and decreasing the rate of lipid absorption in the large intestine (Schneeman and Tietyen, 1994).

The ability of dietary fibre to delay food digestion and nutrient absorption clearly has an important effect on lipid and carbohydrate metabolism (Riccardi and Rivellese, 1991).

2.13.1.5 Effect of Dietary Factors on Carbohydrate Metabolism and Insulin Resistance

Diet is an environmental factor that has changed in those populations with changing prevalence of non-insulin (type II) diabetes (Kawate et al, 1979), suggesting that diet is a causative factor for diabetes.
The San Luis Valley Diabetes Study has suggested that high fat / low-carbohydrate diets are correlated with the onset of NIDDM in humans (Marshall et al, 1991). Thus, fat consumption significantly predicts NIDDM risk in subjects with IGT after controlling for obesity and markers of glucose metabolism (Marshall et al, 1994).

Several hypotheses have been proposed on the mechanisms relating high fat intake to the etiology of NIDDM. A diet rich in fat causes obesity (Dreon et al, 1988, Romieu et al, 1988) and increased body fat and altered fat distribution (Storlien et al, 1986). These abnormalities lead to altered glucose metabolism (Leibel et al, 1989). Impaired fat oxidation and / or glycogen storage accompanying a diet rich in fat may increase FFA concentration in blood and lead to insulin resistance (Randle, 1989).

Another possible explanation for the correlation between fat consumption and NIDDM can be impaired insulin action due to altered cell membrane composition and (possibly) altered signal transduction across the cell membrane (Olefsky et al, 1988, Storlien et al, 1989).

Animal studies have revealed that a high fat diet leads to the induction of insulin resistance and hyperinsulinemia (Storlien et al, 1986). In addition, The Normative Aging Study has suggested that a diet high in saturated fatty acids is an independent factor for both fasting and postprandial insulin concentrations (Parker et al, 1993). Diets with high saturated fat content decrease insulin response and insulin resistance in isolated rat adipocytes (Van Amelsvoort et al, 1988, Chattaway et al, 1990).

Dietary treatment of diabetes remains a controversial area and the ideal diet remains to be defined. The specific diet and approach chosen will depend on the patient, the degree
of obesity and the extent of metabolic compensations and complications present (Griver and Henry, 1994).

The American Diabetes Association (1987) has recommended a the suitable diet for diabetic patients should provide 55-60% of energy as carbohydrates and < 30% as fat. The high amount of complex carbohydrates may cause an increase in insulin sensitivity. Smith (1994) suggested the diet for diabetic people should contain food with low glycemic index and be high in dietary fibre, particularly soluble fibres. Diet containing food with low glycemic index and high in dietary fibre appears to increase insulin sensitivity and may well reduce the problem of increased TG and decreased HDL-C levels.

2.13.2 Alcohol Consumption

Ethanol is a source of energy and it also has potent pharmacologic and biochemical properties that may influence other aspect of nutrition and metabolism (Mitchell, 1990).

Ethanol is metabolized in the liver to form acetaldehyde, which in turn is oxidized to acetate. Acetaldehyde (in excess) may cause liver damage by decreasing mitochondrial functions. In heavy alcohol consumers, alcohol becomes a preferred fuel for the liver and displaces fat as a source of energy. Oxidation of fatty acids is decreased and the production of TG is increased. If this processes continues a fatty liver may develop (Lieber and Pignon, 1989).

2.13.2.1 The Effect of Alcohol Ingestion on Lipids and Lipoproteins

The interaction of ethanol with lipid metabolism is complex. Alcohol intake may affect all lipid components of the plasma.
A number of studies have reported that alcohol consumption increases plasma TG levels (Ostrander et al, 1971, Castelli et al, 1977). The consumption of enough ethanol to give a blood concentration over 1 g/L and mild intoxication results in a rise in TG concentration in the plasma of normal subjects (Lieber and Pignon, 1989). Ethanol consumption leads to an increase in VLDL synthesis and limitation of alcohol may markedly decrease plasma TG concentrations (Ginsberg et al, 1974, NIH Consensus Conference, 1984).

There is a positive correlation between alcohol intake and HDL-C levels in population studies (Moore and Pearson, 1986). Several studies have found alcohol consumption may lead to increase in both HDL2 (the putative atherogenic protective fraction) and HDL3 (Lieber, 1987), however other researchers have reported that only the HDL3 fraction is increased (Haskell et al, 1984). ApoA-I and apoA-II are increased by alcohol (Camargo et al, 1985). Studies have reported that alcohol-related HDL-C elevation may be due to increased hepatic apoA-I synthesis (Taskinen et al, 1987, Okamoto et al, 1988).

Sillanaukee et al (1993) have reported that moderate to heavy intake of alcohol had no effect on HDL2 but increased the HDL3 fraction. The mechanism of elevated VLDL, HDL2, and HDL3 levels after ethanol intake was described by Lieber (1987). Overproduction of VLDL particles by the liver occurs, resulting from increased activities of synthetic enzymes and ready availability of precursors such as TG, cholesterol, and apolipoproteins. At the same time, HDL3 synthesis will increase in association with moderate alcohol intake. The degradation of VLDL and chylomicrons by LPL is associated with conversion of HDL3 to HDL2. As a result, an increased synthesis of
VLDL by the liver and increased LPL activity will cause increased HDL₂ levels (Lieber, 1987).

A reduction in LDL-C level may be seen in alcohol drinkers (Taskinen et al, 1982). The modification of LDL-apoB by acetaldehyde (the first metabolite of ethanol) increases the catabolic rate of LDL particles in the rat, resulting in lower LDL-C levels (Savolainen et al, 1987, Kesaniemi et al, 1987). It can be an explanation for decreased LDL-C concentrations in alcohol users.

2.13.2.2 The Effect of Alcohol Ingestion on Insulin Sensitivity

Women drinking a moderate amount of alcohol had lower plasma insulin concentrations and higher HDL-C levels than non-drinking women (Razay et al, 1992).

In addition, Facchini et al (1994) reported that light to moderate alcohol intake is correlated with increased insulin-mediated glucose uptake, lower plasma glucose and insulin levels in response to oral glucose, and a higher HDL-C level. The changes in insulin and glucose metabolism may contribute to the lower risk of coronary heart disease described in light to moderate drinkers.

2.14 Smoking

Smoking may be an important contributors to both glucose level and diabetes risk (Bjorntorp, 1988, Pederson, 1989). Smoking is a risk factor for ischaemic heart disease (IHD) (Meade et al, 1987).
2.14.1 Effects of Smoking on Lipoprotein Metabolism

Epidemiological studies have shown that smoking is associated with a number of abnormalities in plasma lipoproteins particularly decreased HDL-C (Criqui et al, 1980, Wilson et al, 1983). The correlation between smoking and decreased HDL-C was also confirmed by using multivariate analysis (Craig et al, 1989, Maede et al, 1991, Muscat et al, 1991). However, some researchers rejected the independent effect of smoking on HDL-C after correction for lifestyle factors (lack of exercise, obesity, alcohol intake, etc) (Wallentin and Sundin, 1985, Manttari et al, 1991).

Plasma HDL-C rises within 2-8 weeks to the normal range after smoking cessation (Fortman et al, 1986, Stamford et al, 1986, Moffatt, 1988). Some investigators consider it uncertain whether increased HDL-C level is due to stopping smoking or dietary changes (Quensel et al, 1989, Maida and Howlett, 1990). Quensel et al (1989) believe that the changes in HDL result from increased dietary fat and carbohydrate intake after stopping smoking.

Smokers have been reported to have lower plasma concentration of HDL₂ (Shennan et al, 1985), HDL₃ (Haffner et al, 1985, Moriguchi et al, 1990), and HDL₂ and HDL₃ (Robinson et al, 1987).

Craig et al (1989) also have found that apo A-I concentration is low in smokers. In addition, plasma levels of TG (Simons et al, 1984), VLDL (Freedman et al, 1986) and LDL-C have been reported to be higher in smokers compared with non-smokers (Craig et al, 1989, Freedman et al, 1986, Muscat et al, 1991).

Dullaart et al (1991) have reported that the CETP activity is elevated in IDDM patients who smoke. The concentrations of tissue LPL are elevated in smokers and this seems to

2.14.2 Smoking and Diabetes Mellitus

Cigarette smoking favours the development and progression of diabetic nephropathy. Several large prospective cohort studies have reported that the relative risk for all-cause mortality is approximately twice as high for smoking compared to non-smoking diabetic patients. Stopping smoking is probably the most cost-effective risk factor intervention for diabetic patients (Muhlhauser, 1994).

2.14.3 Smoking and Fibrinogen Levels

Data from several studies have suggested that the plasma levels of fibrinogen are higher in smokers than non-smokers (Balleisen et al, 1985, Kannel et al, 1987) and this may be one reason why smokers are more prone to ischaemic heart disease (Meade et al, 1987).

2.15 Physical Activity

Sedentary lifestyle is related to increased coronary atherosclerosis and physical activity decreases the risk of coronary atherosclerosis (Paffenbarger et al, 1986).

Physical activity tend to be associated with lower body fat, lower blood pressure, and beneficial effects on glucose, insulin, lipids, and lipoproteins (Bush, 1991).

2.15.1 Effects of Physical Activity on Lipids and Lipoproteins

It is well accepted that physical activity improves lipoprotein metabolism (Huttunen et al, 1979, Berg and Keul, 1985).

Exercise programs with moderate intensity seem to modify the effects of a hypocaloric, fat-restricted diet on HDL-C (Taylor and Ward, 1993).

2.15.2 Effects of Physical Activity on Carbohydrate Metabolism

In low to moderate intensity exercise, rapid adjustment in the supply of energy is the result of a fall in plasma insulin, and steady or increased glucagon production to maintain euglycemia. Hepatic glucose synthesis in moderate exercise is regulated mainly by the glucagon / insulin ratio (Purdon et al, 1993).

In contrast, intense exercise is related to a marked stimulation of glucose synthesis with resulting hyperglycemia, marked increases in plasma catecholamines, and moderate hyperglucagonemia. The hyperglycemia increases in recovery and is accompanied by hyperinsulinemia (Purdon et al, 1993).

King et al (1988) have reported that the reversal of increased insulin action that occurs within a few days after stopping exercise is due to a decrease in insulin sensitivity, not to a decrease in insulin secretion.
2.15.3 Therapeutic Exercise for GDM Patients

Besides diet and insulin therapy, exercise is important in the treatment of people with diabetes (Wasserman and Vranic, 1987).

Exercise is known to increased glucose utilization by several mechanisms, and regular exercise has been recommended since early time as an addition therapy (Bung et al, 1993).

Bung et al (1993) suggested that in the absence of ominous fetal heart rate changes or significant alterations in uterine activity following the exercise sessions or diabetes-related peri- and neonatal morbidity, regular physical activity appears to be a safe therapeutic method for the fetus of GDM mothers.

2.16 Obstetrics and Family Medical History

Subjects with GDM may present more frequent obstetric complications, affected both baby and mother (Beydoun, 1987). They also are more likely to have a positive family history of diabetes mellitus (Henry and Beischer, 1991).

2.16.1 Obstetrics History

Obstetric complications are more likely to occur in diabetic pregnancies and may increase both mortality and morbidity (Beydoun, 1987).

Women with GDM exhibit more frequent and severe obstetric complications. Cousins (1987) reported that subjects with GDM had a higher frequency of maternal complications such as polyhydramnios (5.3%), preeclampsia (10%), chronic hypertension
(9.9%), pyelonephritis (4%), and delivery by cesarean section (20.4%) than women with non-GDM pregnancies.

The fetus of the diabetic mother runs an increased risk of major congenital malformations, complications related to macrosomia, intra-uterine growth retardation, and biochemical abnormalities resulting from fetal hyperinsulinemia (Barret, 1987). The Third International Workshop Conference on GDM (1991) reported that the infant of the mother with GDM is also more likely to develop these morbidities.

Fetal macrosomia is a potentially dangerous complication of diabetic pregnancy, may be caused by hyperinsulinemia, a response to fetal hyperglycaemia following maternal hyperglycaemia. This and other complications can be prevented by strict control of maternal blood glucose levels, particularly in late pregnancy (Barret, 1987).

2.16.2 Oral Contraceptive

The most widely used contraceptive method is the combined-type oral contraceptive containing an estrogen and a progesterone steroid. Oral contraceptive intake causes alterations in glucose, lipid and protein metabolism (Molsted-Pedresen et al, 1991).

2.16.2.1 Effects of Oral Contraceptive on Lipids and Lipoproteins

Estrogen increases LDL receptor activity, so increasing the rate of clearance of both LDL and remnant particles (Knopp et al, 1993).

Estrogen also increases the amount of apoA-I in HDL particles by increasing synthesis and / or decreasing the catabolism of apoA-I, resulting in an increased number of HDL particles and increased plasma HDL-C levels including the HDL\textsubscript{2} subfraction (Knopp et al, 1993). It also increases TG and VLDL-C levels. The mechanisms by which these
changes occur are not clear but probably related to the effect on estrogen in reducing hepatic triglyceride lipase activity and inhibiting HDL catabolism (Miller and LaRosa, 1991).

The progestin component of oral contraceptives reduces glucose tolerance, increases insulin resistance, and tends to increase cholesterol concentration (Kjos et al, 1990).

Dyslipidemia characterised by elevated LDL and VLDL and also reduction of HDL particles has been reported during use of the combination-type oral contraceptive. This would increase the risk of atherosclerosis complications (Molsted-Pedersen et al, 1991).

2.16.2.2 Effects of Oral Contraceptive on Carbohydrate Metabolism

Exogenous oestrogen has an antagonistic effect to insulin (Spelsberg and Manson, 1993).

A combination of estrogens and progesterones has been reported to decrease insulin sensitivity at the cellular level in peripheral tissue (Skouby et al, 1987).

Eschwege et al (1990) reported that women who are using currently oral contraceptives have higher plasma glucose and insulin levels after a standard glucose challenge.

Rimm et al (1992) have found only a slightly increased in risk of NIDDM among women who have used oral contraceptives compared with those who have never used oral contraceptive. However, another study showed no increased risk of NIDDM in post-menopausal oral contraceptive users (past or present) in comparison to those who had never used oral contraceptives (Manson et al, 1992).
2.16.3 Family Medical History

A family history of diabetes and cardiovascular disease is often found in subjects who develop diabetes and cardiovascular disease.

Studies have suggested that genetic factors play a major role in the development of NIDDM (Newman et al, 1987, Fajan, 1989).

In the rare cases single genes alone can lead to NIDDM, but in the most cases it seems that NIDDM is results from combined defects of β-cell function and insulin action, either of which can in principle have a genetic or environmental basis (McCarthy and Hitman, 1993).

2.16.3.1 Family History of Diabetes Mellitus

Several studies on populations at high risk for developing NIDDM have showed that history of diabetes in a first degree relative is a powerful predictive factor for the development of diabetes and impaired glucose tolerance preceding NIDDM is more prevalent in non-diabetic offspring of diabetic patients than in control subjects without a positive family history of diabetes (Leslie et al, 1986, Haffner et al, 1988, Eriksson et al, 1989).

Warram et al (1990) have reported that decreased glucose clearance may be demonstrated one or two decades before NIDDM is diagnosed. This decreased glucose clearance is accompanied by compensatory hyperinsulinemia, suggesting that the primary defect in development of NIDDM is in the peripheral tissue response to insulin and glucose.
2.16.3.2 **Family History of Cardiovascular Disease (CVD)**

Bell et al (1990) have reported that black women with GDM have a higher incidence of family history of CVD.

Diabetic patients have an increased chance to develop ischaemic heart disease (Barrett-Conner et al, 1985) and black women have a 70% greater risk of CVD and mortality than white women (Johnson et al, 1986). However, the relationship of CVD and development of GDM is unclear.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Introduction

This chapter introduces the data collection techniques, levels of measurements, and statistical techniques for analyzing data.

3.2 Subjects

This is a cross-sectional study. The study was performed on 65 women who had had GDM in their last pregnancy (the GDM group), studied approximately one year after delivery, and 65 women who gave birth at the same time as the women with GDM and who did not have GDM (the control group).

All women who have had GDM diagnosed and treated in the Illawarra Health Service (IAHS) in June 1992-January 1993 (80 subjects) were contacted and were offered a check which was centred on those factors thought to increase the chance of developing diabetes mellitus. Of the GDM group, 65 agreed to join the study (81%).

A control group of 90 individuals was chosen at random from the list of women presenting for labour at the same time as the patients with GDM. Of women from the control group, 65 also agreed to join to the study (72%).

Subjects received a full description of the study and gave informed consent. All subjects received a detailed information sheet and consent form were signed by all participants.
Those enrolled in the study were examined at the Outpatient Clinics, Illawarra Regional Hospital (Wollongong Campus), at the Medical Research Unit (MRU), and for 4 subjects at their homes by the investigator. This study was approved by the University of Wollongong Human Research Ethics Committee (HR 92/213).

3.3 Experimental design

Subjects were examined after an overnight (~12 h) fast. All subjects received a clinical examination and were interviewed before blood and urine sampling.

3.3.1 Demographic information

Demographic information was obtained including age, country of birth, details of obstetric and medical history and family history of diabetes mellitus, coronary heart disease, other inherited disease, peripheral vascular disease (PVD) and kidney disorders, smoking status, alcohol consumption, medication and oral contraceptive use (Appendix I).

3.3.2 Blood pressure measurement

Blood pressure measurement was done after 5 minutes rest in the sitting position, using a standard desk mercurial sphygmomanometer. Two blood pressure measurements were obtained and the mean was accepted as the blood pressure. Systolic blood pressure was defined by the appearance of sound, and diastolic blood pressure by the disappearance of sound.

Mean arterial pressure (MAP) was calculated by adding the sum of two thirds of the diastolic pressure and one third of the systolic pressure (Konen et al, 1993).
Chapter Three

Materials and Methods

The pulse pressure was calculated as systolic blood pressure minus diastolic blood pressure (Vander et al, 1994 a).

3.3.3 Blood sample taking

A blood sample (40 mL) was obtained from the left antecubital vein of seated subjects after approximately 5 minutes rest in the sitting position after blood pressure measurement.

A tourniquet was used but was released before collection of the blood sample to avoid artifactual increases in the concentration of plasma proteins and lipids.

Blood samples were divided into several types of tubes: (1) tubes containing di-potassium EDTA (1 mg/mL), (2) 3 mL tubes containing 0.3 mL sodium citrate solution (109 mM, 3.2%), and (3) plastic tubes containing gel (SARSTEDT Momovette, Serum Gel S/7.5 mL)) for separation of serum.

All blood samples were mixed thoroughly and cooled immediately (by placing in fridge or ice box).

The plasma was separated from the blood samples collected in the EDTA tubes by centrifugation (2300 rpm for 20 minutes) and stored at 4°C for lipid studies. Other blood samples (citrated plasma and serum) had analyses performed in the Pathology Laboratories, Illawarra Regional Hospital.

Remaining plasma or serum samples were frozen at -85°C, in 2 mL snap-top plastic tubes within a few hours for further study.
Some tests were carried out on fresh plasma and the others on frozen plasma (after approximately 5-6 months at -85 °C) as listed in Table-3-1.

**Table 3-1 Laboratory tests in this study**

<table>
<thead>
<tr>
<th>Fresh plasma or urine</th>
<th>Frozen plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, TG, apoA-I, and apoB in plasma</td>
<td>NEFA in plasma</td>
</tr>
<tr>
<td>VLDL isolation (Cholesterol, FC, TG, apoB, measurement)</td>
<td>Factor VII</td>
</tr>
<tr>
<td>IDL isolation (Cholesterol, FC, TG, apoB, measurement)</td>
<td>von Willebrand factor (vWF)</td>
</tr>
<tr>
<td>HDL isolation (Cholesterol and apoA-I measurement)</td>
<td>Lipoprotein (a)</td>
</tr>
<tr>
<td>HbA1-c</td>
<td>CETP activity %</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen</td>
<td></td>
</tr>
<tr>
<td>Albumin in urine</td>
<td></td>
</tr>
</tbody>
</table>

TC = total cholesterol, TG = triglycerides, apo = apolipoprotein, NEFA = non-esterified fatty acids, CETP = cholesterol ester transfer protein.

### 3.4 Lipid Study

EDTA plasma was used for lipid studies. Blood was cooled rapidly, briefly stored at 4°C, and standard lipid laboratory procedures were initiated within 0.5-2 hours after venipuncture.

#### 3.4.1 Lipids and apolipoproteins in plasma

Total cholesterol, free (non-esterified) cholesterol (FC), and triglycerides were measured on a Cobas-Fara automated centrifugal analyzer (Roche Centrifugal Analyzer, France).
Total cholesterol concentrations in plasma and different lipoproteins were measured by CHOD-PAP method (a standard enzymatic colorimetric method) based on Siedel et al, 1983 and Kattermann et al 1984) (Appendix II) with reagents supplied by Boehringer Mannheim, Germany (QC materials: Precinorm L, Cat No: L 781827 and Precipath Cat No: L 128574). Cholesterol was determined by enzymatic hydrolysis with subsequent determination of the liberated cholesterol (Test principle is presented in Appendix II) by colorimetry. The reaction product was read optically at 546 nm and compared to a known standard curve. Six reference points (254, 508, 1016, 2032, 4065, and 8130 μmol/L) and 2 quality controls, one low (range 3635-5585 μmol/L) and one high (7350-11250 μmol/L) for total cholesterol (Roche Control Serum Lipid Cat No 2023624) were used.

Boehringer-Mannheim Diagnostic kits for lipid standards, calibrators, and controls are traceable to Centers for Disease Control (CDC) (Myers et al, 1994).

As reference ranges 750-1070 μmol/L (calibrator or standard) for free cholesterol (FC), the Boehringer Mannheim standard (Preciset Cat No: 125512) was used.

TG concentrations in plasma and lipoproteins were measured by Triglyceride GPO-PAP (a standard enzymatic colorimetric method) based on Wahlefeld (1974) and Trinder (1969) (Appendix II) with reagents supplied by Boehringer Mannheim, Germany (QC materials: Precinorm L, Cat No: L 781827 and Precipath Cat No: L 128574). Enzymatic hydrolysis of triglycerides was determined with subsequent determination of the liberated glycerol by colorimetry. The reaction product was read optically at 500 nm and compared to a known triglyceride standard curve.

**Quality Control Values:**
- Precinorm L (1650 μmol/L)
- Precipath L (4650 μmol/L)
ApoB, and apoA-I concentrations were measured in fresh plasma on a Cobas-Fara by Apolipoprotein B, T Antiserum (standard immunoturbidimetric method) (Roche Diagnostics, France) (Appendix II). Turbidity of the antigen-antibody reaction was measured at 340 nm. As quality control (QC) for apo B and apo A-I, the apolipoprotein T Control (art. 0730696, Roche Diagnostics, France) was used.

Reference Values:

<table>
<thead>
<tr>
<th></th>
<th>ApoA-I</th>
<th>ApoB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1.15-1.90 g/L</td>
<td>0.70-1.60 g/L</td>
</tr>
<tr>
<td>Female</td>
<td>1.15-2.20 g/L</td>
<td>0.60-1.50 g/L</td>
</tr>
</tbody>
</table>

Lipoprotein (a) concentration in plasma was measured by an immunoturbidimetric method based on Levine et al (1992) by using SPQ TM kit, SPQ Calibration / Control: includes Standards 1-5 and Controls Levels 1 and 2, (INCSTAR Corportion, Stillwater, Minnesota, USA) on a Cobas-Fara analyzer. The immunoturbidimetric method is based on the formation of insoluble antigen-antibody complexes, light scattering is measured, following an 10 minutes incubation of sample and reagents, compared to a known standard curve at 340 nm.

A calibration curve is produced by a series of standards with known concentrations of lipoprotein (a) and using the instrument’s data reduction capability or manually plotting the change in absorbance versus concentration.

Reference Values:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Level 1</td>
<td>19 mg/dL</td>
</tr>
<tr>
<td>Control Level 2</td>
<td>46 mg/dL</td>
</tr>
</tbody>
</table>
3.4.2 Non-esterified fatty acids (NEFA)

Concentrations of non-esterified fatty acids in plasma were measured by the Wako enzymatic method with reagent from Wako Pure Chemical Industries, Osaka, Japan.

Oleic acid 1.0 mM was used as the NEFA standard solution (Wako Pure Chemical Industries, Ltd, Japan).

The method relies upon the acylation of co-enzyme A (Co-A) by the fatty acids in the presence of added acyl Co-A synthetase. The acyl Co-A is oxidized by added acyl Co-A oxidase with generation of hydrogen peroxide. Then, hydrogen peroxide in the presence of peroxidase permits the oxidative condensation of 3-methyl-N-β hydroxyethylene aniline (MEHA). MEHA condenses with 4 amino antipyrine to form a purple colored product which can be measured colorimetrically at 550 nm.

Reference Values: Plasma NEFA Concentration

0.20-1.08 mEq/L

3.4.3 Isolation of VLDL and IDL

VLDL and IDL were isolated from fresh plasma by sequential ultracentrifugation after progressively raising the solvent density according to Havel et al (1955). KBr solutions were made in 100 ml of deionized water and contained 0.02% sodium azide.

1.0 mL normal saline (0.15M NaCl, density 1.006 g/mL) was added to 4 mL of fresh plasma in a quick-seal polyallomer ultracentrifuge tube (100.4, Beckman Instruments Inc, USA).

Normal saline was added very carefully on top of plasma to avoid mixing (a syringe was used) and then the tube was sealed.
A Beckman TL-100 tabletop ultracentrifuge with a fixed angle, TLA-100.4 rotor (Beckman, USA) was used. The sample was subjected to ultracentrifugation for 25 minutes, 100,000 rpm, at 4°C. The VLDL fraction was isolated as the supernatant by tube slicing.

Crystalline KBr was used to raise the solvent density. The amount of KBr to be added was calculated by the following formula (Hatch and Lees, 1968):

$$KBr \ (g) = \frac{V_i \ (d_f - d_i)}{1 - (v \times d_i)}$$

- $V_i$ = initial volume (mL)
- $d_f$ = density final (g/mL)
- $d_i$ = density initial (g/mL 3-2)
- $v$ = value from Table 3-2

<table>
<thead>
<tr>
<th>Final density of KBr solution (g/ml)</th>
<th>$v$</th>
<th>KBr (g) added to 100 ml distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 1.019</td>
<td>0.2875</td>
<td>2.68722</td>
</tr>
<tr>
<td>2. 1.055</td>
<td>0.2923</td>
<td>7.9523</td>
</tr>
<tr>
<td>3. 1.063</td>
<td>0.2936</td>
<td>9.1582</td>
</tr>
<tr>
<td>4. 1.07</td>
<td>0.2943</td>
<td>10.2175</td>
</tr>
<tr>
<td>5. 1.09</td>
<td>0.2966</td>
<td>13.2997</td>
</tr>
<tr>
<td>6. 1.10</td>
<td>0.2977</td>
<td>14.8692</td>
</tr>
<tr>
<td>7. 1.12</td>
<td>0.2993</td>
<td>18.0509</td>
</tr>
<tr>
<td>8. 1.13</td>
<td>0.3003</td>
<td>19.6772</td>
</tr>
<tr>
<td>9. 1.14</td>
<td>0.3042</td>
<td>21.4325</td>
</tr>
<tr>
<td>10. 1.125</td>
<td>0.2997</td>
<td>18.8583</td>
</tr>
<tr>
<td>11. 1.21</td>
<td>0.3052</td>
<td>33.2959</td>
</tr>
<tr>
<td>12. 1.25</td>
<td>0.3084</td>
<td>40.6834</td>
</tr>
<tr>
<td>13. 1.27</td>
<td>0.3096</td>
<td>44.4951</td>
</tr>
</tbody>
</table>

Table 3-2 Required solid KBr for making different density solutions in 100 mL distilled (de-ionized) water (Hatch and Lees, 1968)
The infranatant was collected in a cylinder and the volume was measured. The sample was then adjusted to a density of 1.19 g/mL by adding solid KBr, according to the formula in Table 3-2. After adjustment, the sample was poured into a quick-seal centrifuge tube and filled by KBr solution density 1.019 g/mL. The IDL fraction was isolated as the supernatant after centrifugation at 65,000 rpm for 16 hours at 4°C. The infranatant was frozen immediately at -85°C.

Estimation of total cholesterol (TC), triglycerides (TG), and free cholesterol (FC) concentrations were carried out on fresh VLDL and the IDL fraction.

Cholesterol ester (CE) was calculated in both VLDL and IDL as the difference between total cholesterol and free (non-esterified) cholesterol. Apo B concentration was measured immunoturbidimetrically.

### 3.4.4 Isolation of HDL

ApoB-containing lipoproteins were precipitated with dextran sulfate-Mg\textsuperscript{2+} (Warnick et al, 1982) and HDL-C and HDL-apoA-I concentrations were measured in the supernatant. The procedure for isolation of HDL from plasma lipoproteins is described in detail in Appendix II.

### 3.4.5 Isolation of all lipoproteins for determination of HDL and LDL particle sizes

Plasma samples were adjusted to density 1.25 g/mL for isolation of all lipoproteins.

0.5 mL. fresh plasma was added to 0.1985 g KBr in a TL-100.1 Beckman centrifuge tube. It was mixed well and subjected to ultracentrifugation in a TLA-100.1 rotor at 100,000 rpm for 16 hours at 4°C.
After ultracentrifugation, 50μL (yellow-top layer) was recovered and collected in a snap-top plastic tube and stored at 4°C for electrophoresis of lipoproteins. It was diluted 1:4 with TBS buffer (Appendix II) for electrophoresis.

Electrophoresis was carried out on a Micrograd II Electrophoresis Apparatus (Gradipore Ltd Australia) using non-denaturing gradient gels (3-30% and 3-13%, Gradipore Ltd Australia) and a 24 slot comb. 3-30% gel and 3-13% gels were used for identification of HDL and LDL sub-populations, respectively.

3.4.6 Determination of HDL particle size by electrophoresis

HDL sub-populations were identified by electrophoresis using non-denaturing 3-30% polyacrylamide gradient gels. Samples were applied in a volume of 10μL containing 4 parts by volume of the density = 1.25 g/mL fraction and 1 part of a solution consisting of sucrose and bromphenyl blue (Appendix II).

A reference protein mixture was used for calibrating particle size radius. Each gel contained the reference protein mixture in 3 lanes and 6 samples (Figure 3-1). The reference protein mixture used for calibrating particle size radius included thyroglobulin (8.5 nm radius), apo-ferritin (6.10 nm radius), lactate dehydrogenase (4.08 nm radius), and bovine serum albumin (3.55 nm radius) (High molecular weight (HMW) Calibration Kit, Pharmacia LKB, Biotechnology AB, Uppsala).
Samples were electrophoresed at 170 V (constant voltage), 10°C for 17 hours in a buffer containing 90 mM Tris base, 80 mM boric acid, 3 mM Na₂EDTA and 3 mM sodium azide (pH = 8.35).

Gels were fixed in 10% sulfosalicylic acid (Appendix II) for 0.5 hour immediately after electrophoresis. After fixation, gels were stained for 3 hours in Coomassie Brilliant Blue dye (R-250) (Appendix II) and then destained in 5% acetic acid (Appendix II). Gels were rotated during fixing, staining, and destaining procedures. Each gel was then put in a small plastic bag with a few mL of 0.02% sodium azide solution (Appendix II) and sealed.

Stained gradient gels were scanned with an Ultrascan XL Enhanced Laser Densitometer (LKB Bromma Sweden) at wavelength 663 nm. The migration distances (peak R_f values) of the reference proteins of known radius were measured and a standard curve of particle radius (nm) against R_f was constructed. The particle radius of the samples was determined by reference to the standard curve.

The software package (LKB 2400 Gel Scan XL, Bromma) determined the peaks, and calculated position (mm), and relevant area under the curve as a % of the total % for each protein.

Gradient gel electrophoresis resolves human HDL into five subfractions. Two of the subfractions (HDL_{2b} and HDL_{2a}) correspond to HDL_2 and three of the subfractions (HDL_{3a}, HDL_{3b}, and HDL_{3c}) correspond to HDL_3 isolated by ultracentrifugation (Converse and Skinner, 1992). HDL sub-populations were identified according to the size ranges suggested by Nichols et al (1987).
3.4.7 Determination of LDL particle size by electrophoresis

The procedure for identifying LDL particle size by electrophoresis is identical with that for HDL, except that 3-13% non-denaturing polyacrylamide was used and calibration was performed using 19 nm (radius) calibrated latex microspheres (carboxylated latex beads, Duke Scientific Palo Alto, CA) as well as the HMW calibration kit (Pharmacia LKB, Biotechnology AB, Uppsala, Sweden) for construction of the standard curve. The calibration mixture was applied at the beginning of gels and after loading three samples (Figure 3-2).

![Figure 3-2 Pattern of samples loading in 3-13% gel for identifying LDL particles](image)

Fixation, staining and destaining procedures were the same as with 3-30% gels and the same equipment was used for scanning.

3.4.8 HDL radiolabelled in the cholesteryl ester moiety

Radiolabelled HDL₃ was prepared in the density range of 1.13-1.21 g/mL by using tritium [³H] cholesterol ester as described by Albers and Tollefson (1984) (procedure in Appendix II). One preparation of radiolabelled HDL₃ was used for all CETP activity assays.

3.4.9 Cholesteryl ester transfer protein activity (CETP activity)

CETP activity was measured in plasma (thawed after storage at -85°C for 6 months) by using the Abbey and Nestel method (1993). The assays were done in duplicate. All assays contained as quality controls a positive control (a plasma sample from a normal
healthy person) and a control for the estimation of non-specific transfer (not mediated by CETP).

The CETP assay was done three times with and without (5,5-dithiobis [2-nitrobenzoic acid]) but the results were not statistically different. As a result DTNB was not used routinely for this assay. The procedure is presented in Appendix II.

3.4.10 LDL-C Calculation

LDL-C was calculated according the modified Friedewald equation.

The modified Friedewald equation:

\[
LDL-C \text{ (mmol/L)} = \text{Total cholesterol (mmol/L)} - \text{HDL-C (mmol/L)} - \frac{\text{TG (mmol/L)}}{2.18}
\]

TG is an estimate of the concentration of cholesterol in VLDL particles in fasting plasma. This is based on the assumption that all the plasma TG is associated with VLDL and that the molar ratio of TG to cholesterol in VLDL is 2.18:1 (Samman and Truswell, 1993).

There are three restrictions in the use of the method presented by Friedewald et al for estimating plasma LDL concentrations.

Firstly, it is not applicable to the fed state, when the ratio of TG to cholesterol in VLDL and chylomicrons is likely to be higher, so that the standardised molar ratio of TG to cholesterol in VLDL is not 2.18:1, and if the formula is used in these circumstances, it results in overestimation of VLDL-C and hence an underestimation of the LDL-C concentration. All participating subjects in this study were in the fasting state.
Secondly, the technique gives erroneously high results in patients with type III hyperlipoproteinemia, because the Friedewald equation assumes that IDL-C is negligible. The lipid results did not suggest type III hyperproteinemia in any subjects.

Thirdly, LDL-C can not be accurately estimated when the plasma triglyceride concentration is more than 4.5 mmol/L (Samman and Truswell, 1993). None of the subjects had a plasma triglyceride concentration more than 4.5 mmol/L.

3.4.11 LDL-apoB Calculation

LDL-apoB values were calculated according to this formula:

\[ \text{LDL-apoB} = \text{total apoB} - (\text{VLDL-apoB} + \text{IDL-apoB}). \]

3.5 Tests related to carbohydrate metabolism

Fasting blood glucose, fasting blood insulin and HbA1c were measured by standard procedures described as follow:

3.5.1 Fasting blood glucose (FBG)

FBG was determined by a colorimetric method based on Trinder, 1969 and Curme et al, 1978 on an Ektachem 700 XR analyzer (Kodak, NY, USA) using the Kodak Ektachem Clinical Chemistry Slide (GLU).

The Kodak Ektachem Clinical Chemistry Slide (GLU) is a dry, multilayered analytical element coated on a clear polyester support. Kodak Ektachem Calibrators 1, 2, and 3 were used as calibrator. Kodac Ektachem Control was also used (Range for serum = 1.11-34.69 mmol/L).
A 10 μL drop of patient blood is deposited on the slide. It diffuses into the underlying reagent layer. The oxidation of glucose in the sample is catalyzed by glucose oxidase to form hydrogen peroxide and gluconate.

This reaction is followed by an oxidative reaction catalyzed by peroxidase in the presence of chromogens (4-aminoantipyrine + 1,7-dihydroxynaphthalene) to produce a red dye. The intensity of the red dye is measured by reflected light (wavelength = 540 nm) (Kodak Ektachem Clinical Chemistry Products' manual, 1992).

**Expected Values in Serum:**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (18-44 yr)</td>
<td>4.2-6.1 mmol/L</td>
</tr>
<tr>
<td>Female (18-64 yr)</td>
<td>3.6-5.8 mmol/L</td>
</tr>
</tbody>
</table>

### 3.5.2 Fasting blood insulin (FBI)

FBI was measured by microparticle enzyme immunoassay (MEIA) based on Travis 1980 method on an Abbot IMX analyzer (Abbott Diagnostics, Chicago, USA). In the IMX insulin assay, an antibody-insulin complex is formed by incubating the patient’s serum sample with coated microparticles. Then the anti-insulin:alkaline phosphatase conjugate is added to the antibody-insulin complex and binds to it. The substrate (4-methylumbelliferyl phosphate) is added to the complex and the end product of the reaction (the fluorescent product) is measured by the MEIA optical assembly (Abbott Diagnostic, 1994). The IMX insulin assay shows no cross-reactivity with proinsulin (Abbott Diagnostic, 1994).

The IMX Insulin Calibrators contain insulin (human) prepared in buffer at six different concentrations (0.000, 0.003, 0.010, 0.030, 0.100, and 0.300 mU/mL).
The IMX Insulin Controls contain insulin (human) prepared in buffer to yield the following concentration ranges.

<table>
<thead>
<tr>
<th>Bottle</th>
<th>Insulin concentration (mU/mL)</th>
<th>Range (mU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>0.008</td>
<td>0.006-0.010</td>
</tr>
<tr>
<td>M</td>
<td>0.040</td>
<td>0.032-0.048</td>
</tr>
<tr>
<td>H</td>
<td>0.120</td>
<td>0.096-0.144</td>
</tr>
</tbody>
</table>

3.5.3 HbA1c

HbA1c was determined by high performance liquid chromatography (HPLC), on a Pharmacia Mono S HR 5/5 column (Pharmacia LKB Technology, Almeda, CA) (Bio-Rad Haemoglobin A1c Micro-column Test Instruction Manual, 1990).

Lyphocheck Glycated Haemoglobin BioRad (Cat No C-555) level 1 and 2 were used as quality control material.

**Expected levels**

- > 10% Poor degree of glucose control
- 9-10% Fair " " "
- 8-9% Good " " "
- 7-8% Excellent " " "
- < 6% (4.2-5.9 %) Non-diabetic level

3.6 Haematological Tests

3.6.1 Fibrinogen

Fibrinogen was determined in fresh citrated plasma (one part citrate solution, 0.106 mol/L sodium citrate + nine parts blood) by the Corriveau and Fritsma method (1988).
Principle of test: In the presence of a high concentration of thrombin, the thrombin clotting time is inversely proportional to the fibrinogen concentration of plasma.

The reference range for fibrinogen is 2.0-4.0 mg/L at all ages, including full-term and premature newborn.

ARP or Uniplasmatrol Normal is used as control plasma. Calibration: CSL Human Thrombin (dried) 2500 U is mixed with 5 mL of distilled water to give a resulting concentration of 500 U/mL. 4 mL Owerns buffer is added to 1 mL of thrombin 500 U/mL to make a final concentration of thrombin of 100 U/mL. Six different dilutions (1:5, 1:7.5, 1:10, 1:15, 1:20, and 1:30) are assayed directly on the MLA and the corresponding thrombin times used in the construction of a standard curve.

3.6.2 Factor VII

Factor VII activity assay was performed on the MLA 1000C automated coagulation analyzer (Baxter Diagnostics, MLA 1000C) on citrated plasma. This assay is based on Seegers and Walz, 1986, Bajaj et al, 1981. Factor VII activity was measured by a clotting method utilizing the prothrombin time test (Baxter Diagnostic, MLA 1000C operator's manual, USA, 1988).

Immunoadsorbed Factor VII Deficient Plasma (human) with different concentrations (1:10, 1:20, ..., 1:160, 1:320) was used in the construction of a standard curve. Factor Assay Reference Plasma (Cat No B4234-30) and Ci-Trol Coagulation Control Level 1 (Cat No B4224-10) also were used. The result was calculated as % of activity. Factor VII activities over 100% is considered as higher than normal.
Note: There are several different methods available to evaluate plasma factor VII level such as factor VII coagulant activity (measurement of factor VII activity via a clotting method utilising the prothrombin time test), activated factor VII (factor VIIa) (quantitation of activated factor VII levels in plasma by using a tissue factor mutant selectively deficient in promoting factor VII activation), and factor VII antigen (factor VIIAg) (Baxter Diagnostic, Manual, 1988, Morrissey et al, 1993, respectively). We used factor VII coagulant activity method, because this method is commonly used in the region pathology laboratories. This method is traceable to Centers of Disease Control (CDC).

3.6.3 Von Willebrand Factor (vWF) antigen

vWF antigen was measured in frozen citrated plasma by the ELISA method based on Cejka (1982) using Dakopatts rabbit's anti-human von Willebrand Factor (Cat No. A082). Dakopatts rabbit anti-human von Willebrand Factor conjugated with horseradish peroxidase (Cat No. P 226) supplied by Bio Scientific Pty Ltd was used in the construction of a standard curve.

Principle of method: Dilutions of patients and pool reference plasma are incubated in microwells that have been coated with antibody to human vWF antigen raised in rabbits. The vWF antigen in the diluted samples and reference plasma is bound by the immobilized antibody. The microwells are then washed and a second antibody to human vWF antigen conjugated to horseradish peroxidase is added and the samples incubated.

A peroxidase substrate (TMB) was added after post-incubation washing. The substrate developed colour in the presence of conjugated enzyme. The absorbency of each well was read at 450 nm using the MR 5000 microtitre plate reader.
The vWF antigen concentration of the unknown patient sample is determined by reading off the standard curve the value corresponding to each patient's absorbance.

3.7 *Hormonal Tests*

3.7.1 *Total testosterone*

Total testosterone was determined in fresh serum using a solid phase $^{125}\text{I}$ radioimmunoassay based on testosterone-specific antibody immobilized to the wall of a polypropylene tube (Coat-A-Count, Total testosterone, Diagnostic Products Corporation Los Angeles, CA).

$^{125}\text{I}$-labelled testosterone competes for a fixed time with testosterone in the patient sample for antibody sites. The tube is then decanted, to separate bound from free, and counted in a gamma counter. The amount of testosterone present in the patient sample is determined from a calibration curve (DPC manual, 1995).

Human serum-based calibrators (TTC3-8) having testosterone concentrations ranging from 0.2-16 ng/mL (which is equivalent to 0.7-55 nmol/L or 20-1600 ng/dL) were used. These were provided by Diagnostic Products Corporation (DPC).

**Reference Values:** 4-1600 ng/dL = 0.7-55 nmol/L.

3.7.2 *Sex hormone binding globulin (SHBG)*

SHBG was measured in fresh serum using the Farmos SHBG IRMA test procedure based on the principles of a non-competitive 'liquid-phase' immunoradiometric assay Sex Hormone Binding Globulin [$^{125}\text{I}$] Immunoradiometric Assay Kit (Farmos Group Ltd, Oulunsalo, Finland).
Chapter Three

Materials and Methods

SHBG Standards (A-F) contain 6.25, 12.5, 25, 50, 100, and 200 nmol SHBG/L (Farmos Group Ltd, Finland) were used. SHBG standard was made from human pregnancy serum diluted in bovine serum.

Two SHBG controls (low and high) were used and expected values are as follows:

<table>
<thead>
<tr>
<th>Reference Values:</th>
<th>Quality Control L (Low)</th>
<th>Quality Control H (High)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.0-12.2 nmol/L</td>
<td>81.0-106 nmol/L</td>
</tr>
</tbody>
</table>

3.8 Albumin in Urine

The amount of albumin in urine was measured by rate nephelometry (micro ALBUMIN (MA), Reagent, P/N 441450) on a Beckman Array 360 analyzer. The method measures the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antigen-antibody reaction (Beckman MA micro ALBUMIN manual, 1990).

It is recommended that pooled urine or commercially available nephelometric quality control material with known concentrations of albumin be included in each set of determinations. In this test, pooled urine with known concentration of albumin was used.

Expected Value for Albumin in Urine: < 20 mg/dl

3.9 Anthropometry

All anthropometric measurements were carried out in accordance with the WHO standards (WHO, 1987).
Subjects stood clad in underclothes for the anthropometric measurements. The following measurements were made: height against a fixed scale, weight using a calibrated digital balance, hip circumference at the widest point of the hip by a new fabric tape measure, and waist circumference at the level of the umbilicus without compression of the skin by a fabric tape measure.

All skinfolds were measured on the right side of the body using a Harpendon calliper. Those were made at four sites on all subjects, at the biceps, triceps, subscapular, and suprailiac areas (Durnin and Wormersley, 1974).

Biceps skinfold was measured above the center of the cubital fossa, at the midpoint of the biceps muscle.

Triceps skinfold was measured halfway between the inferior border of the acromion process and the tip of the olecranon process and in line with the point on the elbow and the acromion process.

Suprailiac skinfold was measured above the superior iliac crest in the midaxillary line.

Subscapular skinfold was measured just below the tip of the scapula.

<table>
<thead>
<tr>
<th>Table 3-3 Body fat % calculation (Siri, 1956)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age bracket (years):</td>
</tr>
<tr>
<td>20-29 D = 1159.9-71.7 Log (X1)</td>
</tr>
<tr>
<td>30-39 D = 1142.3-63.2 Log (X1)</td>
</tr>
<tr>
<td>40-49 D = 1133.3-61.2 Log (X1)</td>
</tr>
<tr>
<td>where: D = body density</td>
</tr>
<tr>
<td>X1 = sum of biceps, triceps, subscapular, and suprailiac skinfold thicknesses</td>
</tr>
<tr>
<td>Body fat percentage was determined according to the formula:</td>
</tr>
<tr>
<td>% Body Fat = [(4950/D)-4.5] × 100</td>
</tr>
</tbody>
</table>
Body mass index (BMI) as a measure of body adiposity was calculated as BMI = weight (kg)/ height (m)$^2$ (Quetelet, 1869).

The ratio of waist to hip circumferences (W/H ratio) was calculated as a measure of central or abdominal adiposity and the skinfold ratio (subscapular / triceps ratio) was employed as a measure of upper-body fat (Cassano et al, 1992).

Body fat % was calculated by using Siri’s equation (1956).

### 3.10 The Dietary and Health Questionnaire Booklet

The booklet was explained completely to subjects. The subjects was asked to fill it carefully (at home) and return it by post as soon as possible.

Dietary intake was assessed using a self-administered, semi-quantitative food frequency questionnaire. This questionnaire has been used frequently in previous dietary intake studies in Australia and has been shown to have high repeatability (Baghurst et al, 1988).

This questionnaire was open-ended both with respect to the foods included and the usual serve size of the respondent. Subjects were instructed to indicate how often they usually have each of the foods and beverage (ie never, rarely, or so many times per day, per week, and per month). For most of the foods listed in the questionnaire, standard serve sizes were assumed (Baghurst et al, 1988).

In addition to these food frequency questions, there were a number of questions dealing with their health, family medical history, current medication, their dietary changes, physical activity, smoking, age, occupation status, income, country of birth, and marital status (Appendix III).
Daily nutrient intake was calculated using a modified version of the Frequan computer program (a computerized dietary analysis system for use with diet diaries or food frequency questionnaires, CSIRO Division of human Nutrition, Adelaide) (Baghurst and Record, 1984).

**% energy intake Calculation for different macronutrients:**

* % Energy intake from total carbohydrate intake =

\[
\frac{\text{Total carbohydrate (g) } \times 16 \times 100}{\text{Total energy intake (KJ)}}
\]

* Energy intake from total fat intake =

\[
\frac{\text{Total fat intake (g) } \times 37 \times 100}{\text{Total energy intake (KJ)}}
\]

* Energy intake from protein intake =

\[
\frac{\text{Protein (g) } \times 17 \times 100}{\text{Total energy intake (KJ)}}
\]

### 3.11 Data collection - validity

Every study in medicine must involve an interaction between the observer and the patient or object being observed. Measurement bias is a factor that every researcher must be wary of (Daly et al, 1991). To avoid this problem, the study protocol was piloted twice on volunteers by the researcher and her colleagues. Quality control was checked for each run of tests.

Data were extracted from demographic forms and all dietary questionnaires were coded by the researcher. The coded dietary questionnaires were sent to CSIRO and were analyzed by staff at the Division of Human Nutrition, CSIRO, Adelaide.
Then all results were entered on a spreadsheet associated with the JMP statistical package (SAS Institute Inc) on a Macintosh Quadra 650 computer. The data were checked several times to detect any mistakes and then statistical analysis was carried out.

### 3.12 Statistical Analysis

Statistical analysis were performed using the 'JMP' statistical package (SAS Institute Inc, NC, USA, Version 3). All statistical comparisons were two tailed. Variables were tested for normality (a Gaussian distribution) using the Shapiro-Wilk W test (because $n \leq 2000$). Several variables were not distributed normally and data transformation, commonly to the logarithm, was used to reduce skewness.

A significant cut-off level for the p value of 0.05 was used for all statistical analysis. The 0.05 level means that an obtained result that is significant at the 0.05 level should occur by chance only 5 times in 100 trials. The 0.05 level corresponds fairly well to two standard deviations from the mean of a normal probability distribution.

Student’s t-test (unpaired) was employed to assess the significance of differences in group means. The data were presented as mean ± standard deviation.

The chi-square (Pearson) test was used in this research for determining the relationship between nominal variables.

Cross-correlation used to determine the possible correlations between variables.

Simple and multiple regression analysis is a method of analyzing the variability of a dependent variable on one or more independent variables. These statistical techniques were used in this study.
The advantage of multiple regression over other techniques used is that it shows the combined effects of a set of independent variables and the separate effects of each independent variable controlling for the others.

The $\beta$ coefficient of each independent variable and the coefficient of multiple determination (denoted by $r^2$), which are two statistics that often accompany the report of the multiple regression, are used in this research for describing the relationships between the response variable ($Y$) and independent factors ($Xs$) in different models.

We chose nine variables (age, fasting insulin concentration, BMI, family history of diabetes mellitus, use of an oral contraceptive pill, smoking, fat and alcohol intake, and exercise) as independent variables for multiple regression analysis in this study. Subjects as a whole were entered in the models and identified as a variable named groups (coded as $1 = GDM$ subjects and $0 = control$ subject).

Nine different models were determined for BMI, fasting insulin, total cholesterol, total triglyceride, and NEFA concentrations, CETP activity (%) in plasma, serum SHBG concentration, systolic blood pressure, and the concentration of albumin in urine. The result is presented in Table 4-9 in Chapter four.

In multiple regression analysis, there is a nominal variable ($X =$ independent variable) for an interval variable ($Y =$ response variable) model. The model translates this specification into a linear model as follows. The nominal variables define a sequence of 'dummy' variables, which have only values 1, 0, and -1. For example, suppose you want to fit the response $Y$ to a nominal variable group with three levels. The linear model is written

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \epsilon_i$$
The first dummy variable is labeled group [1-3] in the report, which denotes that group = 1 contributes a value 1 and group = 3 contributes a value -1 to the dummy variable.

\[ x_{1i} = \begin{cases} 
1 & \text{if group} = 1 \\
0 & \text{if group} = 2 \\
-1 & \text{if group} = 3 
\end{cases} \]

The second dummy variable is labeled group [2-3] in the report and is given values

\[ x_{2i} = \begin{cases} 
0 & \text{if group} = 1 \\
1 & \text{if group} = 2 \\
-1 & \text{if group} = 3 
\end{cases} \]

The last level does not need a dummy variable because in this model its level is found by subtracting all the other parameters. Therefore, the coefficients sum to one across all the levels.

The estimates of the means for the three levels in terms of this parameterization are

\[ \mu_1 = \beta_0 + \beta_1 \]
\[ \mu_2 = \beta_0 + \beta_2 \]
\[ \mu_3 = \beta_0 - \beta_1 - \beta_2 \]

Solving for \( \beta \) yields

\[ \beta_0 = (\mu_1 + \mu_2 + \mu_3) / 3 = \mu \ (\text{the average over levels}) \]
\[ \beta_1 = \mu_1 - \mu \]
\[ \beta_2 = \mu_2 - \mu \]
\[ \beta_3 - \beta_1 - \beta_2 = \mu_3 - \mu \]

Thus, if the indicator coding is each level minus the previous level, the parameter coding is each level minus the mean of all levels (JMP Package Manual, 1994).
Each model will be fully described in their own sections.

Subgroups determination:

We determined several subgroups. We used a cut-off point of fasting insulin concentration > 11, BMI > 25 kg/m² for overweight persons, NEFA > 0.6 mmol/L, and family history of diabetes mellitus as positive (+) and negative (−) to determine the subgroups.

Subjects who had plasma NEFA levels > 0.6 mmol/L, positive family history of diabetes, and BMI > 25 kg/m² determined as subgroup (A).

Subjects who had plasma NEFA levels > 0.6 mmol/L, positive family history of diabetes, and BMI ≤ 25 kg/m² determined as subgroup (B).

The GDM group with fasting plasma insulin < 10 IU/L were distinguished from those with fasting insulin > 10 IU/L and named as hyperinsulinemic subgroup or subgroup C.

Each subgroup were compared with the residual GDM group and residual control group using Student’s t test.
CHAPTER FOUR

RESULTS

4.1 Comparison of Study and Control Populations

The overall response rate was 81.25% in the GDM group and 72.22% in the control group (Table 4-1). The uncorrected chi square test indicated that there is no significant difference between the response rate in the GDM and the control groups (Table 4-1).

Table 4-1 Response per groups

<table>
<thead>
<tr>
<th></th>
<th>GDM</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>Responded</td>
<td>65</td>
<td>65</td>
</tr>
</tbody>
</table>

\[ X^2 = 6.26 \]

\[ p = 0.61 \]

4.1.1 Demographic Data of the Study

The results showed there is no significant difference in age between the GDM and the control groups (Table 4-2 and Figure 4-1).

Subjects who had had GDM were more likely to have a positive history of diabetes in their first degree relatives (mother, father, sister, and brother) (Table 4-2). The frequency of familial diabetes mellitus in the maternal side (17%) was not significantly more than paternal side (18.5%) between the GDM subjects (Table 4-3).

The GDM individuals also were more likely to have a positive history of cardiovascular disease (CVD) in their first degree relatives (Table 4-2).
One GDM subject had had five abortions', when she was excluded from the data, no significant differences were found in parity, number of deliveries, number of abortions, and history of stillbirth between GDM and controls (Table 4-2).

### Table 4-2 Demographic Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number</th>
<th>GDMs</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean</td>
<td>31.52</td>
<td>31.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Std Dev</td>
<td>4.95</td>
<td>4.22</td>
<td></td>
</tr>
<tr>
<td>Family history of DM</td>
<td>No history</td>
<td>21 (32.3%)</td>
<td>43 (66.2%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>First degree relative</td>
<td>25 (38.5%)</td>
<td>8 (12.3%)</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>Second degree relative</td>
<td>19 (29.2%)</td>
<td>14 (21.5%)</td>
<td></td>
</tr>
<tr>
<td>Family history of CVD</td>
<td>No history</td>
<td>38 (58.5%)</td>
<td>42 (64.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>First degree relative</td>
<td>24 (36.9%)</td>
<td>10 (15.4%)</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>Second degree relative</td>
<td>3 (4.6%)</td>
<td>13 (20%)</td>
<td></td>
</tr>
<tr>
<td>Obstetrics history</td>
<td>Parity</td>
<td>2.35 ± 1.6</td>
<td>2.71 ± 1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Delivery</td>
<td>1.95 ± 1.15</td>
<td>2.32 ± 1.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abortion</td>
<td>0.23 ± 0.72</td>
<td>0.32 ± 0.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stillbirth</td>
<td>0.08 ± 0.21</td>
<td>0.05 ± 0.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Last baby weight</td>
<td>3410 ± 567</td>
<td>3304 ± 514</td>
<td></td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td>Mean</td>
<td>24.81</td>
<td>22.31</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>Std Dev</td>
<td>5.20</td>
<td>2.83</td>
<td></td>
</tr>
<tr>
<td>Current BMI</td>
<td>Mean</td>
<td>26.05</td>
<td>22.75</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>Std Dev</td>
<td>5.50</td>
<td>2.90</td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>Mean</td>
<td>0.85</td>
<td>0.82</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Std Dev</td>
<td>0.007</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Body Fat %</td>
<td>Mean</td>
<td>38.8</td>
<td>35.1</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>Std Dev</td>
<td>4.81</td>
<td>3.73</td>
<td></td>
</tr>
</tbody>
</table>

Std Dev = Standard deviation, BMI = Body mass index, WHR = Waist /Hip Ratio, * = p ≤ 0.05, ** = p ≤ 0.01, blank space = No significant.
The mean birth-weight was slightly greater in babies of GDM subjects than in babies of control subjects but the difference was not significant (Table 4-2 and Figure 4-2).

Women who had had GDM were more likely to be fatter, both pre-pregnancy and one year after delivery, than controls. The GDM subjects have also a significantly greater WHR and Body fat % than controls (Table 4-2, Figures 4-3, 4-4, 4-5, 4-6).

Figure 4-1 Means comparison of age in GDM and control subjects (the difference between means is not significant)

Figure 4-2 Means comparison of baby birth-weight between GDM and controls (the difference between means is not significant)
Figure 4-3 Means comparison of pre-pregnancy BMI in GDM and control subjects

(the difference between means is significant)

Figure 4-4 Means comparison of BMI in GDM and control subjects (the difference between means is significant)
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Figure 4-5 Means comparison of WHR in GDM and control subjects

(the difference between means is significant)

Figure 4-6 Means comparison of body fat % in GDM and control subjects

(the difference between means is significant)
Table 4-3 Frequencies of family history of diabetes mellitus in the GDM and control group

<table>
<thead>
<tr>
<th>Frequency of DM in relatives</th>
<th>GDM</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No positive family history</td>
<td>21 (32.3%)</td>
<td>43 (66.2%)</td>
</tr>
<tr>
<td>Mother</td>
<td>11 (16.9%)</td>
<td>2 (3.1%)</td>
</tr>
<tr>
<td>Father</td>
<td>12 (18.5%)</td>
<td>3 (4.6%)</td>
</tr>
<tr>
<td>Sister</td>
<td>2 (3.1%)</td>
<td>2 (3.1%)</td>
</tr>
<tr>
<td>Brother</td>
<td>0 (0.0%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>Grandmother (mother side)</td>
<td>11 (16.9%)</td>
<td>2 (3.1%)</td>
</tr>
<tr>
<td>Grandmother (father side)</td>
<td>0 (0.0%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>Grandfather (mother side)</td>
<td>1 (1.5%)</td>
<td>2 (3.1%)</td>
</tr>
<tr>
<td>Grandfather (father side)</td>
<td>2 (3.1%)</td>
<td>2 (3.1%)</td>
</tr>
<tr>
<td>Uncle (mother side)</td>
<td>2 (3.1%)</td>
<td>2 (3.1%)</td>
</tr>
<tr>
<td>Uncle (father side)</td>
<td>0 (0.0%)</td>
<td>2 (3.1%)</td>
</tr>
<tr>
<td>Aunt (mother side)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Aunt (father side)</td>
<td>1 (1.5%)</td>
<td>1 (3.1%)</td>
</tr>
<tr>
<td>Cousin (mother side)</td>
<td>0 (0.0%)</td>
<td>2 (3.1%)</td>
</tr>
<tr>
<td>Cousin (father side)</td>
<td>2 (3.1%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

4.1.2 Physiological Data of the Study

Plasma cholesterol concentrations were significantly higher in the GDM group than in the control group (Table 4-4 and Figure 4-7). The GDM subjects had significantly higher plasma TG levels than controls (Table 4-4 and Figure 4-8).

The plasma apoB levels were significantly higher in the GDM group than in the control group (Table 4-4 and Figure 4-9), but there was no significant difference between the GDM and control group means of plasma apo A-I concentrations (Table 4-4).
Plasma NEFA concentration after an overnight fast was significantly higher in the GDM subjects than in the control subjects (Table 4-4 and Figure 4-10).

Characteristics of VLDL in subjects outlined in Table 4-4. VLDL-TG concentration was significantly higher in the GDM group than in control group. Difference between means for other aspects of VLDL composition were not significant between the two groups (Table 4-4 and Figure 4-11).

Characteristics of the IDL fraction are outlined in Table 4-4. IDL-C, IDL-FC, and IDL-TG (but not IDL-CE and IDL-apoB concentrations) were significantly higher in the GDM group than in control group (Table 4-4 and Figure 4-12).

Calculated LDL-C levels were significantly higher in the GDM subjects than in controls (Table 4-4 and Figure 4-13). Calculated LDL-apoB was significantly greater in GDM subjects than in control subjects (Table 4-4 and Figure 4-14).

LDL-I particle sizes were smaller in GDM group than in the control group (Table 4-4 and Figure 4-15). LDL-II and LDL-III also were smaller in the GDM group than in the control group but not significantly smaller (Table 4-4).

There is no difference between plasma HDL-C level in GDM subjects and in controls (Table 4-4). HDL-apoA-I also was not significantly lower in the GDM than in control group (Table 4-4). HDL$_2$ area % / HDL$_3$ area % ratio (using electrophoresis scans) did not show any significant difference between the GDM and the control groups (Table 4-4).

HDL$_2$ and HDL$_3$ sub-population components (HDL$_2$b, HDL$_2$a, HDL$_3$a, HDL$_3$b, and HDL$_3$c) were compared between two groups (GDM and controls) using unpaired
Student's $t$ test. HDL$_{3b}$ and HDL$_{3c}$ were significantly smaller in GDM subjects than in controls but not other sub-population components (Table 4-4, and Figures 4-16, 4-17).

There is no significant difference in the Lp(a) levels between GDM and control subjects (Table 4-4). Plasma CETP activity % (CETP.A %) was significantly higher in the GDM group versus the control group (Table 4-4 and Figure 4-18).

Table 4-4 Summarized data from lipoprotein metabolism related tests of study

<table>
<thead>
<tr>
<th>Variables</th>
<th>GDM</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Cholesterol (mmol/L)</td>
<td>4.94 ± 0.95</td>
<td>4.44 ± 0.88</td>
<td>**</td>
</tr>
<tr>
<td>Plasma TG (mmol/L)</td>
<td>1.07 ± 0.50</td>
<td>0.87 ± 0.39</td>
<td>*</td>
</tr>
<tr>
<td>Plasma apoB (g/L)</td>
<td>1.05 ± 0.24</td>
<td>0.88 ± 0.22</td>
<td>**</td>
</tr>
<tr>
<td>Plasma apo A-I (g/L)</td>
<td>1.50 ± 0.27</td>
<td>1.54 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>NEFA (mmol/L)</td>
<td>0.68 ± 0.22</td>
<td>0.54 ± 0.23</td>
<td>**</td>
</tr>
<tr>
<td>VLDL-C (μmol/L)</td>
<td>518.3 ± 30.6</td>
<td>489.4 ± 32.6</td>
<td></td>
</tr>
<tr>
<td>VLDL-TG (μmol/L)</td>
<td>446.8 ± 33.78</td>
<td>326.58 ± 33.77</td>
<td>*</td>
</tr>
<tr>
<td>VLDL-FC (μmol/L)</td>
<td>204.6 ± 16.2</td>
<td>184.0 ± 16.30</td>
<td></td>
</tr>
<tr>
<td>VLDL-CE (μmol/L)</td>
<td>333.6 ± 28.1</td>
<td>305.5 ± 28.2</td>
<td></td>
</tr>
<tr>
<td>VLDL-apoB (g/L)</td>
<td>0.136 ± 0.12</td>
<td>0.109 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>IDL-C (μmol/L)</td>
<td>91.82 ± 6.56</td>
<td>67.03 ± 6.51</td>
<td>**</td>
</tr>
<tr>
<td>IDL-TG (μmol/L)</td>
<td>130.06 ± 10.39</td>
<td>82.63 ± 10.39</td>
<td>**</td>
</tr>
<tr>
<td>IDL-FC (μmol/L)</td>
<td>42.02 ± 29.5</td>
<td>27.59 ± 20.1</td>
<td>**</td>
</tr>
<tr>
<td>IDL-CE (μmol/L)</td>
<td>49.81 ± 3.96</td>
<td>39.50 ± 3.93</td>
<td></td>
</tr>
<tr>
<td>IDL-apoB (g/L)</td>
<td>0.028 ± 0.012</td>
<td>0.025 ± 0.010</td>
<td></td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.32 ± 0.94</td>
<td>2.79 ± 0.75</td>
<td>**</td>
</tr>
<tr>
<td>LDL-apoB (g/L)</td>
<td>1.053 ± 0.236</td>
<td>0.882 ± 0.218</td>
<td>**</td>
</tr>
<tr>
<td>LDL-I size (nm)</td>
<td>30.7 ± 0.98</td>
<td>31.4 ± 0.99</td>
<td>*</td>
</tr>
<tr>
<td>LDL-II size (nm)</td>
<td>29.4 ± 0.99</td>
<td>29.7 ± 1.00</td>
<td></td>
</tr>
<tr>
<td>LDL-III size (nm)</td>
<td>28.2 ± 0.16</td>
<td>28.5 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>LDL-C / HDL-C Ratio</td>
<td>2.55 ± 0.12</td>
<td>2.06 ± 0.12</td>
<td>**</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.32 ± 0.04</td>
<td>1.38 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

TG = triglyceride, apo = apolipoprotein, * = $p \leq 0.05$, ** = $p \leq 0.01$, blank space = No significant.
Table 4-5 (continued)

<table>
<thead>
<tr>
<th>Variables</th>
<th>GDM</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-apoA-I (g/L)</td>
<td>1.39 ± 0.03</td>
<td>1.41 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>HDL₂ (%) / HDL₃ (%)</td>
<td>0.81 ± 0.09</td>
<td>0.97 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>HDL₂b size (nm)</td>
<td>10.91 ± 0.04</td>
<td>11.0 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>HDL₂a size (nm)</td>
<td>9.28 ± 0.04</td>
<td>9.34 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>HDL₃a size (nm)</td>
<td>8.46 ± 0.03</td>
<td>8.54 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>HDL₃b size (nm)</td>
<td>7.92 ± 0.01</td>
<td>7.98 ± 0.01</td>
<td>**</td>
</tr>
<tr>
<td>HDL₃c size (nm)</td>
<td>7.59 ± 0.02</td>
<td>7.66 ± 0.02</td>
<td>**</td>
</tr>
<tr>
<td>Plasma Lp (a) (mg/dl)</td>
<td>19.64 ± 16.8</td>
<td>23.71 ± 22.9</td>
<td></td>
</tr>
<tr>
<td>CETP activity (%)</td>
<td>19.51 ± 1.07</td>
<td>15.78 ± 1.06</td>
<td>*</td>
</tr>
</tbody>
</table>

Lp = lipoprotein, * = p ≤ 0.05, ** = p ≤ 0.01, blank space = No significant.

Figure 4-7 Means comparison for plasma cholesterol concentration between GDM and control groups (difference between means is significant, p = 0.0021)
Figure 4-8 Means comparison for plasma triglyceride concentration between GDM and control groups (difference between means is significant, $p = 0.0153$)

Figure 4-9 Comparison between mean values of plasma apoB concentrations in GDM and control groups (difference between means is significant, $p = 0.0000$)
Figure 4-10 Comparison between mean values of plasma NEFA concentrations in GDM and control groups (difference between means is significant, $p = 0.0013$)

Figure 4-11 Means comparison for VLDL composition levels between GDM and controls (there is only a significant difference between means of VLDL-TG)
Figure 4-12 Means comparison for IDL-C, IDL-TG, and IDL-FC concentrations between the GDM subjects and controls (means are significantly different).

Figure 4-13 Comparison of means of plasma LDL-C concentration between GDM and control subjects (there is a significant difference between means, $p = 0.0006$).
Figure 4-14 Comparison of means of LDL-apoB between GDM and control subjects
(there is a significant difference between means, $p = 0.0000$)

Figure 4-15 Comparison of means of LDL-I (size) between GDM and control subjects
(there is a significant difference between means, $p = 0.0006$)
Figure 4-16 Means comparison of $HDL_3b$ (diameter) between GDM and control groups

(there is a significant difference between means, $p = 0.0188$)

Figure 4-17 Means comparison of $HDL_3c$ (diameter) between GDM and control groups

(there is a significant difference between means, $p = 0.0028$)
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Figure 4-18 Means comparison for plasma CETP activity % between GDM and controls

(there is a significant difference between means, \( p = 0.0190 \))

The fasting blood glucose concentration was not significantly higher in GDM subjects than in controls (Table 4-5).

Fasting insulin level was significantly higher in GDM patients than in controls (Table 4-5 and Figure 4-19). In addition, the ratio of fasting insulin level / fasting glucose level (as a marker of insulin resistance) was significantly higher in GDM subjects (Table 4-5 and Figure 4-20).

Glycosylated haemoglobin (HbA1c) levels were similar in the GDM and control groups (Table 4-5).

The mean plasma fibrinogen concentration, factor VII coagulant activity (factor VIIc), and vonWillebrand factor (vWF) activity (%) were slightly higher in the GDM than in controls but did not differ significantly (Table 4-5).
The two groups (GDM and Controls) had significantly different plasma SHBG concentrations (Table 4-5 and Figure 4-21).

The GDM group with a lower concentration of SHBG had a slightly but not significantly higher plasma testosterone concentration than the control group (Table 4-5).

The testosterone / SHBG ratio was significantly greater in GDM than in control subjects (Table 4-5, Figure 4-22).

Both systolic and diastolic blood pressures were significantly higher in the GDM group than in the control subjects (Table 4-5 and Figures 4-23, 4-24). Mean arterial pressure (MAP) was significantly higher in GDM subjects than in controls (Table 4-5 and Figure 4-25). However, the pulse pressure was similar in GDM subjects and in controls (Table 4-5). The amount of albumin in urine (mg/L) was significantly greater in GDM subjects than in controls (Table 4-5 and Figure 4-26).
Table 4-5 Summarised data from other physiological tests of the study

<table>
<thead>
<tr>
<th>Variables</th>
<th>GDM</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.blood glucose (mmol/L)</td>
<td>4.59 ± 0.44</td>
<td>4.55 ± 0.40</td>
<td></td>
</tr>
<tr>
<td>Fasting blood insulin (IU/L)</td>
<td>7.53 ± 5.23</td>
<td>5.14 ± 1.96</td>
<td>**</td>
</tr>
<tr>
<td>Insulin / Glucose Ratio</td>
<td>1.20 ± 0.41</td>
<td>1.02 ± 0.27</td>
<td>**</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.30 ± 0.62</td>
<td>5.28 ± 0.50</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>3.11 ± 0.84</td>
<td>2.96 ± 0.73</td>
<td></td>
</tr>
<tr>
<td>Factor VII activity (%)</td>
<td>100.1 ± 19.5</td>
<td>93.95 ± 21.9</td>
<td></td>
</tr>
<tr>
<td>vWF (%)</td>
<td>80.38 ± 30.3</td>
<td>77.25 ± 27.31</td>
<td></td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>45.05 ± 29.02</td>
<td>57.69 ± 33.3</td>
<td>*</td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>1.04 ± 0.55</td>
<td>0.91 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>Testosterone / SHBG Ratio</td>
<td>0.034 ± 0.029</td>
<td>0.021 ± 0.015</td>
<td>**</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>110.15 ± 1.55</td>
<td>105.08 ± 1.55</td>
<td>*</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>76.67 ± 5.94</td>
<td>72.77 ± 5.45</td>
<td>**</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>87.23 ± 7.76</td>
<td>83.43 ± 7.01</td>
<td>**</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>34.35 ± 9.25</td>
<td>32.31 ± 9.32</td>
<td></td>
</tr>
<tr>
<td>Albumin in urine (mg/L) (Log)</td>
<td>1.10 ± 0.05</td>
<td>0.95 ± 0.05</td>
<td>*</td>
</tr>
</tbody>
</table>

F = fasting, MAP = main arterial pressure, * = p ≤ 0.05, ** = p ≤ 0.01, blank space = No significant.
Figure 4-20 Means comparison for insulin/glucose ratio between GDM and control groups (the difference between means is significant, $p = 0.0030$)

Figure 4-21 Means comparison for plasma SHBG level between GDM and control subjects (there is a significant difference between means, $p = 0.0239$)
Figure 4-22 Means comparison for Testosterone / SHBG ratio between GDM and control groups (the difference between means is significant, $p = 0.0021$)

Figure 4-23 Means comparison of systolic blood pressure in GDM subjects and controls (there is a significant difference between means, $p = 0.0225$)
Figure 4-24 Means comparison of diastolic blood pressure in GDM subjects and controls (there is a significant difference between means, \( p = 0.0026 \))

Figure 4-25 Means comparison of mean arterial pressure (MAP) in GDM subjects and controls (there is a significant difference between means \( p = 0.0045 \))
Figure 4-26 Means comparison for microalbuminuria between GDM and control subjects (there is a significant difference between means, $p = 0.0171$)

Table 4-6 shows the mean values of measured dietary factors in this study.

Poly-unsaturated fat and vitamin E intakes were significantly greater in GDM subjects diet than in controls (Figures 4-27, 4-28).

Other dietary variables were not significantly different between GDM and controls.

The mean of alcohol consumption was not statistically different between the GDM group and controls (Table 4-6).

4.1.3 Categorical Data of the Study

There was no significant difference in use of an oral contraceptive between the GDM and control groups (Table 4-7).

Smoking status was not significantly different between GDM and control subjects (Table 4-7).
There is no statistically significant difference between different measures of exercise (frequency, duration, and intensity) between the GDM and control groups (Table 4-7).

Table 4-6 Dietary data of the study

<table>
<thead>
<tr>
<th>Variables</th>
<th>GDM</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Energy Intake (KJ)</td>
<td>9568 ± 2622</td>
<td>9603 ± 3273</td>
<td></td>
</tr>
<tr>
<td>Total Dietary Fat (g)</td>
<td>89 ± 24</td>
<td>88 ± 35</td>
<td></td>
</tr>
<tr>
<td>% Energy Intake (Total fat)</td>
<td>35.4 ± 4.3</td>
<td>33.8 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>35.3 ± 11</td>
<td>38.4 ± 19</td>
<td></td>
</tr>
<tr>
<td>% Energy (Saturated fat)</td>
<td>14 ± 2.6</td>
<td>14.3 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Unsaturated Fat (g)</td>
<td>47 ± 14</td>
<td>44 ± 17</td>
<td></td>
</tr>
<tr>
<td>% Energy (Unsaturated fat)</td>
<td>18.5 ± 3</td>
<td>17 ± 3</td>
<td></td>
</tr>
<tr>
<td>Polyunsaturated Fat (g)</td>
<td>16.3 ± 8</td>
<td>14 ± 6</td>
<td>*</td>
</tr>
<tr>
<td>% Energy (Polyunsaturated fat)</td>
<td>6.4 ± 2.5</td>
<td>5.5 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Monounsaturated Fat (g)</td>
<td>30.2 ± 8.6</td>
<td>30.0 ± 13</td>
<td></td>
</tr>
<tr>
<td>% Energy (Monounsaturate fat)</td>
<td>12 ± 1.5</td>
<td>12 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Protein (g)</td>
<td>98 ± 31</td>
<td>101.98 ± 40</td>
<td></td>
</tr>
<tr>
<td>% Energy (Protein)</td>
<td>97.6 ± 2.9</td>
<td>100.9 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>Total Carbohydrate (g)</td>
<td>266.3 ± 74</td>
<td>275.2 ± 97</td>
<td></td>
</tr>
<tr>
<td>% Energy (total carbohydrate)</td>
<td>44.7 ± 5.6</td>
<td>45.4 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>Complex Carbohydrate (g)</td>
<td>130 ± 34</td>
<td>122 ± 38</td>
<td></td>
</tr>
<tr>
<td>% Energy (Comp. carbohydrate)</td>
<td>22.5 ± 4</td>
<td>21.6 ± 5</td>
<td></td>
</tr>
<tr>
<td>Sugars (g)</td>
<td>130 ± 47</td>
<td>138 ± 47</td>
<td></td>
</tr>
<tr>
<td>% Energy (Sugars)</td>
<td>22.5 ± 5</td>
<td>21.6 ± 4</td>
<td></td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>25 ± 7</td>
<td>25 ± 9</td>
<td></td>
</tr>
<tr>
<td>Water (g)</td>
<td>2486 ± 690</td>
<td>2306 ± 755</td>
<td></td>
</tr>
<tr>
<td>Retinol (mcg)</td>
<td>659 ± 522</td>
<td>555 ± 427</td>
<td></td>
</tr>
<tr>
<td>β-carotene (mcg)</td>
<td>3144 ± 1628</td>
<td>3443 ± 1772</td>
<td></td>
</tr>
<tr>
<td>Vitamin D (mcg)</td>
<td>2.63 ± 1.3</td>
<td>2.45 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>10.6 ± 4.38</td>
<td>9.0 ± 4.19</td>
<td>*</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>174 ± 78</td>
<td>172 ± 80</td>
<td></td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>1.45 ± 0.5</td>
<td>1.37 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>2.35 ± 0.7</td>
<td>2.34 ± 0.8</td>
<td></td>
</tr>
</tbody>
</table>

* = p ≤ 0.05, blank space = No significant.
### Table 4-6 (continued)

<table>
<thead>
<tr>
<th>Variables</th>
<th>GDM</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B-6 (mg)</td>
<td>1.88 ± 0.6</td>
<td>1.84 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Nicotinic Acid (mg)</td>
<td>21.9 ± 6.6</td>
<td>21.11 ± 6.1</td>
<td></td>
</tr>
<tr>
<td>Vitamin B-12 (mg)</td>
<td>4.4 ± 2.1</td>
<td>4.37 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Folic Acid (mcg)</td>
<td>253 ± 80</td>
<td>252 ± 10</td>
<td></td>
</tr>
<tr>
<td>Biotin (mcg)</td>
<td>22.55 ± 8</td>
<td>23.10 ± 10</td>
<td></td>
</tr>
<tr>
<td>Pantothenic Acid (mg)</td>
<td>6.6 ± 1.7</td>
<td>5.8 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>2865 ± 713</td>
<td>2657 ± 790</td>
<td></td>
</tr>
<tr>
<td>Chloride (mg)</td>
<td>4793 ± 1340</td>
<td>4641 ± 1735</td>
<td></td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>3582 ± 996</td>
<td>3906 ± 110</td>
<td></td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>331 ± 88</td>
<td>330 ± 95</td>
<td></td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1089 ± 376</td>
<td>1064 ± 404</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>1582 ± 521</td>
<td>1560 ± 609</td>
<td></td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>14 ± 4</td>
<td>14 ± 5</td>
<td></td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>13.4 ± 4.3</td>
<td>13.8 ± 6.1</td>
<td></td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>1.9 ± 0.6</td>
<td>2.0 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Sulphur (mg)</td>
<td>785 ± 287</td>
<td>826 ± 423</td>
<td></td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>2.06 ± 3.6</td>
<td>2.64 ± 3.5</td>
<td></td>
</tr>
</tbody>
</table>

Data is presented as mean ± standard deviation. There is no significant difference between two groups in the above dietary variables.
Figure 4-27 Means comparison for dietary polyunsaturated fat between GDM and controls (there is a significant difference between two groups, \( p = 0.0433 \))

Figure 4-28 Mean comparison for dietary vitamin E between GDM and controls (there is a significant difference between two groups, \( p = 0.0386 \))
Table 4-7 Summarized categorical data of the study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>GDMs</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral contraceptive</td>
<td>1 = Current use</td>
<td>28 (22%)</td>
<td>20 (15%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 = Former or Never use</td>
<td>37 (28%)</td>
<td>45 (35%)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>0 = Non smoker</td>
<td>38 (29%)</td>
<td>36 (28%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 = Smoker</td>
<td>10 (8%)</td>
<td>14 (11%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 = Ex-smoker</td>
<td>17 (13%)</td>
<td>15 (12%)</td>
<td></td>
</tr>
<tr>
<td>Freq of Exercise</td>
<td>0 = Not at all</td>
<td>18 (14%)</td>
<td>16 (12%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 = Less than once a week</td>
<td>17 (13%)</td>
<td>11 (9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 = Once or twice a week</td>
<td>14 (11%)</td>
<td>17 (13%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 = Three times a week</td>
<td>16 (12%)</td>
<td>20 (16%)</td>
<td></td>
</tr>
<tr>
<td>Dura of Exercise</td>
<td>0 = Not at all</td>
<td>18 (14%)</td>
<td>16 (12%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 = Less than 20 minutes</td>
<td>16 (12%)</td>
<td>12 (9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 = 20-30 minutes</td>
<td>19 (15%)</td>
<td>14 (11%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 = More than 30 minutes</td>
<td>12 (9%)</td>
<td>22 (17%)</td>
<td></td>
</tr>
<tr>
<td>Inten of Exercise</td>
<td>0 = No exercise</td>
<td>18 (14%)</td>
<td>16 (12%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 = None of exercise session</td>
<td>6 (5%)</td>
<td>4 (4%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 = Some of exercise session</td>
<td>30 (23%)</td>
<td>33 (26%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 = Most or all of exercise session</td>
<td>11 (11%)</td>
<td>10 (8%)</td>
<td></td>
</tr>
<tr>
<td>LDL Phenotype</td>
<td>A</td>
<td>44 (72%)</td>
<td>37 (62%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>17 (28%)</td>
<td>23 (38%)</td>
<td></td>
</tr>
</tbody>
</table>

Freq = frequency, Dura = duration, Inten = intensity. There is no significant difference between the GDM and control groups in the above variables.

4.1.4 Cross-correlation Between Different Variables

Table 4-8 shows correlation coefficients between different variables in the GDM group in this study. The underlined values have significant correlation (p ≤ 0.05).

Age showed only a negative and significant correlation with concentration of albumin in urine in the GDM group.
Total cholesterol concentration was positively and significantly correlated with plasma TG, apoA-I, apoB, LDL-C, VLDL-TG concentrations, and CETP activity in plasma. Plasma cholesterol was also positively and significantly correlated with factor VIIc, diastolic blood pressure, and markers of obesity (BMI, body fat %), but the correlation between plasma cholesterol concentration and WHR did not reach significance level. Total cholesterol level was also significantly and positively correlated with pre-pregnancy BMI. Plasma cholesterol concentration was not significantly related to plasma insulin level, fasting blood glucose, HbA1c, exercise, smoking, cholesterol intake or total fat intake (Table 4-8).

Fasting plasma insulin, apo B concentrations, factor VIIc, BMI, body fat %, and CETP activity in plasma were positively and significantly related to plasma TG concentration. Plasma TG level was negatively and significantly correlated with HDL-C and LDL1 particle size (Table 4-8).

Plasma apo A-I concentration was significantly related to plasma HDL-C, NEFA, SHBG concentrations and alcohol consumption. Plasma apoB level was positively and significantly related to LDL-C, IDL-C, and VLDL-TG concentrations and CETP activity in plasma as one might expected and negatively to HDL-C. Plasma apoB level also was positively correlated with fasting insulin level, factor VII, BMI, and body fat % (Table 4-8).

Plasma NEFA concentration was negatively and significantly correlated with blood pressure, alcohol intake, and amount of albumin in urine. Plasma NEFA concentration was also negatively correlated with CETP activity in plasma (Table 4-8).
HDL-C was negatively and significantly correlated with plasma TG, apoB, VLDL-TG, IDL-C concentration, and CETP activity in plasma. There is a positive and significant association between HDL-C level and alcohol consumption. HDL₃c was positively and significantly correlated with HbA1c (Table 4-8).

Data showed a relationship between LDL-C concentration and VLDL-TG level and CETP activity in plasma, as one might expect. LDL-C also is correlated with fasting plasma insulin level, BMI, and body fat %. There are several dietary factors which are negatively related to LDL-C. A negative linear relationship was between LDL-I (particle size) and a set of variables including plasma TG and apoB, VLDL-TG, IDL, insulin concentrations, BMI, and body fat %, however LDL-I had a positive correlation with HDL-C (Tables 4-8).

Statistical analysis showed that VLDL-TG was positively correlated with CETP activity in plasma, fasting plasma insulin concentration, BMI, and body fat %, while there was a negative correlation between VLDL-TG and LDL-I particle size (Table 4-8). IDL-C has positive correlation with CETP activity in plasma, BMI, and body fat. There was a positive correlation between IDL-C level and fasting plasma insulin level, but it was not significant (Table 4-8).

Plasma Lp (a) concentration was negatively and significantly correlated with concentration of albumin in urine (Table 4-8).

Plasma CETP.A % was related to total cholesterol, TG, apoA-I, apoB, VLDL-TG, IDL-C, LDL-C and NEFA concentrations. CETP activity in plasma was correlated positively and significantly with fasting insulin level, BMI, body fat. The relationship
between CETP activity in plasma and alcohol intake was negative and significant (Table 4-8).

There is a positive and significant association between fasting blood glucose level and factor VIIc. Fasting insulin concentration was positively and significantly with total cholesterol, TG, apoB, LDL-C, VLDL-TG, testosterone concentrations, CETP activity in plasma, factor VIIc, blood pressure, microalbuminuria, and obesity. There is a negative and significant association between plasma insulin level and LDL1 particle size (Table 4-8).

Plasma fibrinogen level was positively and significantly associated with obesity, diastolic blood pressure, and negatively and significantly with fiber intake. Plasma fibrinogen level also was correlated positively with IDL-C. There is a positive and significant correlation between factor VIIc and BMI. Factor VIIc was positively and significantly correlated with plasma cholesterol, TG, apoB, glucose, insulin concentrations. Von willebrand factor was positively and significantly associated with total fat intake (Table 4-8).

SHBG level in serum was negatively and significantly correlated with fasting apoA-I, insulin, testosterone concentrations and obesity. Serum testosterone concentration was positively and significantly associated with fasting insulin level, and obesity (Table 4-8).

Blood pressure was positively and significantly correlated with lipids and lipoprotein (total cholesterol, TG, NEFA, and LDL-C levels), insulin concentrations, and BMI (Table 4-8).
Markers of obesity (BMI, WHR, and body fat %) were significantly associated with plasma lipids and lipoproteins (plasma cholesterol, TG, apoB, VLDL-TG, IDL-C, and LDL-C levels), insulin, fibrinogen, factor VIIc, and testosterone concentrations, blood pressure, and CETP activity in plasma. Obesity also was negatively and significantly correlated with plasma SHBG, LDL particle size (Table 4-8).

The concentration of albumin in urine was significantly and positively correlated with plasma NEFA and insulin concentrations, however, The concentration of albumin in urine was significantly and negatively correlated with age (Table 4-8).

There is only a significant and positive correlation between baby weight and pre-pregnancy BMI (Table 4-8).

Alcohol consumption was positively and significantly associated with plasma apoA-I, NEFA, HDL-C concentration. The correlation between alcohol consumption and CETP activity in plasma was negative and significant (Table 4-8).

The amount of fibre in diet was negatively and significantly correlated with both systolic and diastolic blood pressure, plasma fibrinogen concentration, and BMI.

Dietary fibre intake was also correlated with plasma total cholesterol, apo B, and LDL-C concentrations, but the correlation did not reach the significance level (Table 4-8).

Dietary fat intake was not correlated with any of biochemical tests except vWF. Dietary fat intake and all other dietary factors were correlated with WHR, but not other variables (Table 4-8).
## Table 4-8 Cross-correlation between different variables in this study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age</th>
<th>TC</th>
<th>TG</th>
<th>Apo A-I</th>
<th>ApoB</th>
<th>NEFA</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.0592</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>0.1075</td>
<td>0.3388</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>0.0758</td>
<td>0.4416</td>
<td>-0.0294</td>
<td>0.2231</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Apo A-I</td>
<td>0.1428</td>
<td>0.8571</td>
<td>0.4769</td>
<td>0.2335</td>
<td>0.0278</td>
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<td>ApoB</td>
<td>0.1038</td>
<td>0.0367</td>
<td>0.1142</td>
<td>0.6166</td>
<td>-0.2512</td>
<td>0.1292</td>
<td></td>
</tr>
<tr>
<td>NEFA</td>
<td>0.1541</td>
<td>0.0597</td>
<td>-0.2996</td>
<td>0.8499</td>
<td>0.0392</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.0582</td>
<td>0.9983</td>
<td>0.3015</td>
<td>0.4398</td>
<td>0.0278</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-TG</td>
<td>0.1213</td>
<td>0.3242</td>
<td>0.7365</td>
<td>-0.0204</td>
<td>0.5042</td>
<td>-0.0860</td>
<td>0.0635</td>
</tr>
<tr>
<td>IDL-C</td>
<td>0.0700</td>
<td>0.1821</td>
<td>0.6313</td>
<td>0.0073</td>
<td>0.3395</td>
<td>-0.0700</td>
<td>-0.2762</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>0.1022</td>
<td>0.0781</td>
<td>0.1176</td>
<td>0.0120</td>
<td>0.0564</td>
<td>-0.1396</td>
<td>0.1206</td>
</tr>
<tr>
<td>CETP</td>
<td>0.0407</td>
<td>0.3997</td>
<td>0.4108</td>
<td>-0.2282</td>
<td>0.5930</td>
<td>-0.2372</td>
<td>-0.4782</td>
</tr>
<tr>
<td>HDL3b</td>
<td>0.0555</td>
<td>0.0197</td>
<td>-0.0149</td>
<td>0.2140</td>
<td>-0.0251</td>
<td>0.0023</td>
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<td>HDL3c</td>
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<td>-0.0078</td>
<td>0.0207</td>
<td>-0.1331</td>
<td>-0.0271</td>
<td>0.1729</td>
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<tr>
<td>HDL2/HDL3</td>
<td>0.0309</td>
<td>-0.0430</td>
<td>-0.0750</td>
<td>0.1864</td>
<td>-0.1365</td>
<td>0.0554</td>
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<td>LDLI</td>
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<td>-0.0410</td>
<td>-0.2109</td>
<td>0.1361</td>
<td>0.2475</td>
</tr>
<tr>
<td>FBG</td>
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<td>0.0702</td>
<td>0.0550</td>
<td>-0.0033</td>
<td>0.1705</td>
<td>0.1975</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.1315</td>
<td>0.2247</td>
<td>0.4143</td>
<td>-0.0936</td>
<td>0.2798</td>
<td>0.1070</td>
<td>-0.1288</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.0089</td>
<td>0.0939</td>
<td>0.0517</td>
<td>-0.0798</td>
<td>0.0457</td>
<td>-0.1080</td>
<td>0.0634</td>
</tr>
<tr>
<td>Fib</td>
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<td>0.1183</td>
<td>0.2160</td>
<td>0.0492</td>
<td>0.1647</td>
<td>0.0677</td>
<td>0.0564</td>
</tr>
<tr>
<td>FacVII</td>
<td>0.1259</td>
<td>0.2230</td>
<td>0.2346</td>
<td>0.0641</td>
<td>0.2692</td>
<td>-0.1794</td>
<td>0.0457</td>
</tr>
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<td>vWF</td>
<td>0.1119</td>
<td>-0.0691</td>
<td>0.1307</td>
<td>-0.0357</td>
<td>-0.0605</td>
<td>0.1274</td>
<td>-0.0345</td>
</tr>
<tr>
<td>SHBG</td>
<td>0.0116</td>
<td>-0.0231</td>
<td>-0.0749</td>
<td>0.3637</td>
<td>0.0398</td>
<td>0.0355</td>
<td>0.1614</td>
</tr>
<tr>
<td>Testos</td>
<td>0.0956</td>
<td>0.0816</td>
<td>0.0765</td>
<td>-0.1383</td>
<td>0.0004</td>
<td>0.0373</td>
<td>0.0327</td>
</tr>
<tr>
<td>SBP</td>
<td>0.1157</td>
<td>0.2218</td>
<td>0.2078</td>
<td>0.1253</td>
<td>0.2653</td>
<td>0.3467</td>
<td>0.026C</td>
</tr>
<tr>
<td>DBP</td>
<td>0.1448</td>
<td>0.2970</td>
<td>0.2106</td>
<td>0.1098</td>
<td>0.3470</td>
<td>0.3325</td>
<td>-0.004C</td>
</tr>
<tr>
<td>PBMI</td>
<td>0.1082</td>
<td>0.2823</td>
<td>0.2418</td>
<td>0.0340</td>
<td>0.3235</td>
<td>0.0383</td>
<td>0.1237</td>
</tr>
<tr>
<td>BMI</td>
<td>0.0895</td>
<td>0.3723</td>
<td>0.3808</td>
<td>-0.0164</td>
<td>0.4178</td>
<td>0.0094</td>
<td>-0.1982</td>
</tr>
<tr>
<td>WHR</td>
<td>0.1389</td>
<td>0.0933</td>
<td>0.1901</td>
<td>0.0190</td>
<td>0.0260</td>
<td>0.0588</td>
<td>0.0043</td>
</tr>
<tr>
<td>B.Fat%</td>
<td>0.0787</td>
<td>0.3479</td>
<td>0.3120</td>
<td>0.0738</td>
<td>0.3331</td>
<td>0.1052</td>
<td>0.148C</td>
</tr>
<tr>
<td>Albu</td>
<td>0.2380</td>
<td>0.1067</td>
<td>0.1432</td>
<td>0.0417</td>
<td>-0.0243</td>
<td>0.4049</td>
<td>-0.0792</td>
</tr>
<tr>
<td>Baby W (g)</td>
<td>0.0030</td>
<td>0.1338</td>
<td>0.0492</td>
<td>0.0396</td>
<td>0.0794</td>
<td>0.0959</td>
<td>0.0147</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.0607</td>
<td>0.0405</td>
<td>0.0868</td>
<td>0.3363</td>
<td>0.0103</td>
<td>0.2801</td>
<td>0.2556</td>
</tr>
<tr>
<td>Fiber</td>
<td>0.1425</td>
<td>-0.1655</td>
<td>-0.0424</td>
<td>-0.0664</td>
<td>-0.1759</td>
<td>-0.0148</td>
<td>0.0213</td>
</tr>
<tr>
<td>Na</td>
<td>0.0986</td>
<td>0.0889</td>
<td>0.0267</td>
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<td>0.1163</td>
<td>0.0180</td>
</tr>
<tr>
<td>Ener.In</td>
<td>0.0886</td>
<td>0.1581</td>
<td>-0.0743</td>
<td>0.0637</td>
<td>0.1964</td>
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<td>0.0050</td>
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<tr>
<td>Fat.In</td>
<td>0.1013</td>
<td>0.1160</td>
<td>0.0404</td>
<td>0.0142</td>
<td>0.1968</td>
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<td>0.0454</td>
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<td>Chol.In</td>
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<td>0.1372</td>
<td>0.0328</td>
<td>-0.0647</td>
<td>0.1818</td>
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<td>SFat</td>
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<td>-0.0590</td>
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<td>USFat</td>
<td>0.1584</td>
<td>0.0382</td>
<td>0.0725</td>
<td>0.0201</td>
<td>0.1279</td>
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<td>Carb.In</td>
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<td>0.0935</td>
<td>0.0950</td>
<td>0.1671</td>
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<td>0.0885</td>
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<td>Protein</td>
<td>0.0822</td>
<td>0.2204</td>
<td>0.0453</td>
<td>0.1500</td>
<td>-0.2593</td>
<td>-0.0487</td>
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</table>
Table 4-8 (Continued)

<table>
<thead>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LDLI</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>FBG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c</td>
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</tr>
<tr>
<td>Fib</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>FacVII</td>
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<td></td>
</tr>
<tr>
<td>vWF</td>
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</tr>
<tr>
<td>SHBG</td>
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</tr>
<tr>
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<td>SBP</td>
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<td></td>
</tr>
<tr>
<td>DBP</td>
<td></td>
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</tr>
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<td>PBM1</td>
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</tr>
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<td>BMI</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td></td>
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<tr>
<td>B.Fat%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baby W (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
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<tr>
<td>Fiber</td>
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<td></td>
</tr>
<tr>
<td>Na</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ener.In</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat.In</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chol.In</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFat</td>
<td>0.6621</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USFat</td>
<td></td>
<td>0.7877</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carb.In</td>
<td></td>
<td>0.4835</td>
<td>0.7273</td>
<td>0.7072</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td>0.7487</td>
<td>0.7907</td>
<td>0.7182</td>
<td>0.7206</td>
</tr>
</tbody>
</table>
Most of the correlations between plasma lipoprotein and lipoprotein constituents could be predicted with a knowledge of the physiology of plasma lipid and lipoprotein metabolism.

Note: Other correlations indicated that relationships could be further explored by two techniques, multiple regression analysis of a group of apparently related variables, and subgroup analysis (eg of a subgroup with particularly well-developed insulin resistance syndrome).

4.1.5 Multiple Regression Analysis

Multiple regression was used to investigate relationships between BMI, fasting insulin, total cholesterol and triglyceride, NEFA, and SHBG concentrations, CETP activity in plasma, systolic blood pressure, and microalbuminuria and their possible determinants.

Age, Groups (1= GDM group and 0 = control group), fasting plasma insulin concentration, BMI, family history of diabetes mellitus, use of a contraceptive pill, alcohol, fat, and protein intake, and exercise were chosen as independent variables in different multiple regression model. The results are presented in Table 4-9.

The multiple regression model for BMI showed that group (as 1 = GDM subjects and 0 = controls) and fasting insulin concentrations are significant determinants of BMI in this study (Table 4-9).

The multiple regression model for fasting insulin concentration indicated that BMI is the most important determinant in the variance of fasting insulin concentration in this investigation (Table 4-9).
Regression analysis showed that group (as 1 = GDM subjects and 0 = controls), BMI, family history of diabetes, and exercise are the significant determinants for the total cholesterol levels (Table 4-9).

**Table 4-9 Results from the multiple regression analyses**

<table>
<thead>
<tr>
<th></th>
<th>BMI</th>
<th>Insulin</th>
<th>TC</th>
<th>TG</th>
<th>NEFA</th>
<th>CETP</th>
<th>SIHGB</th>
<th>SBP</th>
<th>Albu</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>Adj R²</td>
<td>0.28</td>
<td>0.24</td>
<td>0.17</td>
<td>0.24</td>
<td>0.16</td>
<td>0.12</td>
<td>0.35</td>
<td>0.18</td>
<td>0.12</td>
</tr>
<tr>
<td>Intercept</td>
<td>25.33</td>
<td>-2.65</td>
<td>3.57</td>
<td>0.063</td>
<td>0.59</td>
<td>2.299</td>
<td>4.22</td>
<td>85.56</td>
<td>1.53</td>
</tr>
<tr>
<td>Age</td>
<td>-0.10</td>
<td>-0.02</td>
<td>-0.003</td>
<td>0.01</td>
<td>-0.002</td>
<td>-0.0003</td>
<td>0.01</td>
<td>0.01</td>
<td>-0.02*</td>
</tr>
<tr>
<td>Groups [0-1]</td>
<td>-1.03*</td>
<td>-0.66</td>
<td>-0.23*</td>
<td>-0.03</td>
<td>-0.07*</td>
<td>-0.10</td>
<td>0.13*</td>
<td>0.48</td>
<td>0.05*</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.48*</td>
<td>(-)</td>
<td>0.01</td>
<td>0.04*</td>
<td>0.01</td>
<td>0.03</td>
<td>-0.003</td>
<td>0.62*</td>
<td>0.03*</td>
</tr>
<tr>
<td>BMI</td>
<td>(-)</td>
<td>0.39*</td>
<td>0.05*</td>
<td>0.02</td>
<td>-0.004</td>
<td>0.01</td>
<td>-0.03*</td>
<td>0.73*</td>
<td>-0.01*</td>
</tr>
<tr>
<td>FH.DM [0-2]</td>
<td>0.08</td>
<td>0.55</td>
<td>0.14</td>
<td>0.02</td>
<td>-0.14</td>
<td>0.11</td>
<td>0.03</td>
<td>-0.36</td>
<td>-0.03</td>
</tr>
<tr>
<td>FH.DM [1-2]</td>
<td>-0.25</td>
<td>0.36</td>
<td>-0.22*</td>
<td>0.04</td>
<td>-0.0003</td>
<td>-0.07</td>
<td>-0.05</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>C.Pill [0-2]</td>
<td>-0.18</td>
<td>0.38</td>
<td>-0.12</td>
<td>0.14</td>
<td>-0.05</td>
<td>0.08</td>
<td>-0.23*</td>
<td>-3.41</td>
<td>-0.04</td>
</tr>
<tr>
<td>C.Pill [1-2]</td>
<td>-0.11</td>
<td>-0.19</td>
<td>0.15</td>
<td>-0.01</td>
<td>0.05</td>
<td>0.01</td>
<td>0.39*</td>
<td>2.34</td>
<td>0.09</td>
</tr>
<tr>
<td>Smoking [0-2]</td>
<td>-0.23</td>
<td>0.01</td>
<td>-0.01</td>
<td>0.013</td>
<td>0.02</td>
<td>0.08</td>
<td>-0.11</td>
<td>0.50</td>
<td>-0.03</td>
</tr>
<tr>
<td>Smoking [1-2]</td>
<td>-0.08</td>
<td>0.03</td>
<td>-0.06</td>
<td>0.05</td>
<td>0.05</td>
<td>-0.012</td>
<td>0.17*</td>
<td>0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.32</td>
<td>0.03</td>
<td>0.28</td>
<td>0.15</td>
<td>0.20*</td>
<td>-0.25*</td>
<td>0.15</td>
<td>5.21*</td>
<td>0.02</td>
</tr>
<tr>
<td>Fat in</td>
<td>-0.01</td>
<td>-0.001</td>
<td>-0.002</td>
<td>0.0001</td>
<td>0.001</td>
<td>-0.0004</td>
<td>-0.001</td>
<td>-0.03</td>
<td>0.001</td>
</tr>
<tr>
<td>Fre.Exer [0-3]</td>
<td>0.67</td>
<td>0.72</td>
<td>0.31*</td>
<td>-0.06</td>
<td>-0.05</td>
<td>0.13</td>
<td>0.08</td>
<td>0.41</td>
<td>0.02</td>
</tr>
<tr>
<td>Fre.Exer [1-3]</td>
<td>0.29</td>
<td>0.32</td>
<td>0.16</td>
<td>0.10</td>
<td>0.004</td>
<td>-0.06</td>
<td>0.02</td>
<td>-2.22</td>
<td>0.02</td>
</tr>
<tr>
<td>Fre.Exer [2-3]</td>
<td>0.02</td>
<td>-0.77</td>
<td>0.01</td>
<td>-0.06</td>
<td>0.02</td>
<td>0.07</td>
<td>-0.10</td>
<td>3.23</td>
<td>-0.05</td>
</tr>
</tbody>
</table>

The regression coefficients and their significant level of the relevant variables in the regression model are presented. N = number of observations, FH.DM = family history of diabetes mellitus, C.Pill = contraceptive pill, in = intake, Fre = frequency, Exer = exercise, Dur = duration, Int = intensity, (-) = not included in the model, albu = Albuminuria. “Note: the explanation of [ ] is in Chapter Three”.

In the final regression analysis, fasting insulin concentration was shown significantly associated with plasma TG concentration (Table 4-9).
Multiple regression analysis showed that group (as 1 = GDM subjects and 0 = controls) and alcohol consumption are significant independent variables in the plasma NEFA levels (Table 4-9).

CETP activity (%) in plasma was significantly associated with alcohol consumption in the multiple regression analysis (Table 4-9).

The multiple regression model for serum SHBG concentration indicated that group (as 1= the GDM subjects and 0 = controls), BMI, use of an oral contraceptive, and smoking are the significant determinants for serum SHBG model (Table 4-9).

Fasting insulin concentration, BMI, and alcohol consumption were significantly associated with systolic blood pressure in the multiple regression analysis (Table 4-9).

Regression analysis showed that age, group (as 1 = GDM subjects and 0 = controls), fasting insulin concentration, and BMI are the most important determinants of the amount of albumin in urine (Table 4-9).

4.2 Sub-population Analysis:

4.2.1 The Definition of Subgroups

The multiple regression models suggested that sub-populations with sub-clinical or overt disease may exist.

The populations (both GDM and controls) were categorised according to three factors: plasma NEFA concentration, family history of diabetes, and BMI. The frequencies are presented in Table 4-10).

In addition, another subgroup was determined according to fasting insulin concentration (hyperinsulinemic subgroup or subgroup C). Data suggested that a number of subjects...
in the GDM group might have the insulin resistance syndrome. The defining feature of the insulin resistance syndrome was taken to be fasting hyperinsulinemia. On initial analysis of fasting plasma insulin concentrations, it was clear that there was a group of GDM women who had a higher plasma insulin than the control group. The control group had a mean fasting plasma insulin 5.14 IU/L (range 1.1-9.8, 95% within range 1.36-9.54).

The GDM group with fasting plasma insulin < 10 IU/L were distinguished from those with fasting insulin > 10 IU/L. There were 65 women in the control group, 52 in the GDM group with fasting insulin <10 IU/L who were assessed not to have developed insulin resistance and 13 (ie 20% of the GDM group) who were assessed as having insulin resistance.

Table 4-10 Frequencies for different subgroups

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>GDM</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>h.NEFA, (+) FH, OW</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>h.NEFA, (+) FH</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>h.NEFA, OW</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>(+) FH, OW</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>h.NEFA</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>(+) FH</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>OW</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>None</td>
<td>5</td>
<td>26</td>
</tr>
</tbody>
</table>

h.NEFA = high plasma NEFA level, FH = family history, OW = overweight.

4.2.2 Subgroup (A) Results

Sub-group (A): Sub-population includes subjects who are overweight and have high plasma NEFA concentrations and a positive family history of diabetes.

There were no differences in age between the subgroup (A) and residual GDM group and controls (Table 4-11).
The subgroup (A) had slightly higher plasma cholesterol and apoB than residual GDM subjects, but significantly higher than controls (Table 4-11).

The subgroup (A) tended to have higher plasma TG, VLDL-TG, and IDL-C concentrations than the residual GDM or control groups. There was a tendency for the subgroup (A) to have a slightly higher LDL-C than the residual GDM group, and a significantly higher LDL-C than controls (Table 4-11).

All three subgroups were similar in the HDL-C concentrations. HDL particle sizes and the HDL$_2$/HDL$_3$ ratio were similar in the three groups (Table 4-11).

The subgroup (A) tended to have slightly smaller LDL-I than the residual GDM group and significantly smaller than the control group (Table 4-11).

The subgroup (A) also tended to have slightly higher CETP activity in plasma than the residual GDM group, but CETP activity in plasma was significantly higher in the subgroup (A) than controls (Table 4-11).

Fasting blood glucose means were similar in the three groups. However, fasting insulin levels were significantly higher in the subgroup (A) than other two groups (Table 4-11).

The mean systolic and diastolic blood pressures were slightly higher in subgroup (A) compared with the residual GDM subjects, and significantly higher in subgroup (A) than controls (Table 4-11).

The subgroup (A) showed a greater concentration of albumin in urine than both the residual GDM group and controls. The subgroup (A) had slightly greater amount of
albumin in their urine than the residual GDM group and significantly greater than the control group (Table 4-11).

The subgroup (A) tended to be fatter before they became pregnant (as described by pre-pregnancy BMI) than both the residual GDM and control groups (table 4-11).

Table 4-11 Comparison of subgroup (A) with the residual GDM population and controls (Continuous Data)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subgroup (A) (n = 14)</th>
<th>R.GDM (n = 51)</th>
<th>P₁</th>
<th>Controls (n = 65)</th>
<th>P₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>32.1 ± 6.1</td>
<td>31.4 ± 4.7</td>
<td>31.4 ± 4.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>5.2 ± 1.0</td>
<td>4.9 ± 0.9</td>
<td>4.4 ± 0.8</td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>TG (log)</td>
<td>1.06 ± 0.02</td>
<td>1.04 ± 0.02</td>
<td>**</td>
<td>1.04 ± 0.02</td>
<td>**</td>
</tr>
<tr>
<td>ApoB</td>
<td>1.12 ± 0.2</td>
<td>1.03 ± 0.3</td>
<td>0.88 ± 0.2</td>
<td>0.88 ± 0.2</td>
<td>**</td>
</tr>
<tr>
<td>VLDL-TG (log)</td>
<td>6.3 ± 0.6</td>
<td>5.8 ± 0.7</td>
<td>*</td>
<td>5.6 ± 0.7</td>
<td>**</td>
</tr>
<tr>
<td>IDL-C (log)</td>
<td>4.7 ± 0.5</td>
<td>4.2 ± 0.6</td>
<td>*</td>
<td>4.1 ± 0.6</td>
<td>**</td>
</tr>
<tr>
<td>HDL-C (log)</td>
<td>1.05 ± 0.01</td>
<td>1.05 ± 0.01</td>
<td>1.06 ± 0.02</td>
<td>1.06 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>LDL-C</td>
<td>3.5 ± 1.0</td>
<td>3.3 ± 0.9</td>
<td>2.8 ± 0.7</td>
<td>2.8 ± 0.7</td>
<td>**</td>
</tr>
<tr>
<td>HDL₃b Size</td>
<td>7.92 ± 0.10</td>
<td>7.92 ± 0.11</td>
<td>7.97 ± 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL₃c Size</td>
<td>7.62 ± 0.16</td>
<td>7.59 ± 0.16</td>
<td>7.65 ± 0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL₂ / HDL₃</td>
<td>0.85 ± 0.18</td>
<td>0.91 ± 0.19</td>
<td>0.92 ± 0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-I Size</td>
<td>30.34 ± 0.83</td>
<td>30.88 ± 1.06</td>
<td>31.39 ± 1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CETP.A (log)</td>
<td>3.0 ± 0.49</td>
<td>2.8 ± 0.60</td>
<td>2.6 ± 0.66</td>
<td>2.6 ± 0.66</td>
<td>*</td>
</tr>
<tr>
<td>FBG (log)</td>
<td>1.54 ± 0.08</td>
<td>1.50 ± 0.10</td>
<td>1.50 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>10.9 ± 6.5</td>
<td>6.6 ± 4.5</td>
<td>**</td>
<td>5.1 ± 2.0</td>
<td>**</td>
</tr>
<tr>
<td>SBP</td>
<td>114.5 ± 12.1</td>
<td>109.6 ± 12.7</td>
<td>105.5 ± 11.7</td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>DBP</td>
<td>76.9 ± 5.5</td>
<td>76.0 ± 6.0</td>
<td>73.2 ± 5.4</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Microalb (log)</td>
<td>1.16 ± 0.33</td>
<td>1.09 ± 0.37</td>
<td>0.9 ± 0.38</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>P.BMI</td>
<td>26.8 ± 5.2</td>
<td>24.1 ± 4.5</td>
<td>*</td>
<td>22.3 ± 2.8</td>
<td>**</td>
</tr>
<tr>
<td>Baby weight</td>
<td>3573 ± 547</td>
<td>3366 ± 569</td>
<td>3304 ± 513</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol in (log)</td>
<td>0.34 ± 0.40</td>
<td>0.28 ± 0.36</td>
<td>0.40 ± 0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium intake</td>
<td>7.95 ± 0.34</td>
<td>7.96 ± 0.26</td>
<td>7.87 ± 0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat intake</td>
<td>103.5 ± 57.5</td>
<td>93.14 ± 31.0</td>
<td>90.78 ± 28.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data presented as mean ± standard deviation. R.GDM = the residual GDM group, P₁ = p values of comparison of subgroup (A) vs the residual GDM group, P₂ = p values of comparison of subgroup vs controls, in = intake, log = Natural logarithm (base e), * = p ≤ 0.05, ** = p ≤ 0.01, and blank space in the P₁ and P₂ columns = No significant difference.
The babies of the subgroup (A) mothers tended to be slightly heavier than the babies of the other two groups. Fat, alcohol and sodium consumption were similar in three groups. (Table 4-11). There were no significant differences between use of an oral contraceptive, smoking, and exercise between the subgroup (A) and other two groups (Table 4-12).

Table 4-12 Comparison of subgroup (A) with rest of the GDM population and controls (Categorical Data)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subgroup (n = 14)</th>
<th>R.GDM (n = 51)</th>
<th>Controls (n = 65)</th>
<th>p₁</th>
<th>p₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.Pill Current use</td>
<td>7 (50%)</td>
<td>22 (43%)</td>
<td>21 (32%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F or N use</td>
<td>7 (50%)</td>
<td>29 (57%)</td>
<td>44 (68%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking Non-smoke</td>
<td>9 (64%)</td>
<td>29 (57%)</td>
<td>36 (55%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>0 (0%)</td>
<td>10 (20%)</td>
<td>14 (22%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex-smoke</td>
<td>5 (36%)</td>
<td>12 (24%)</td>
<td>15 (23%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fre.Exer 0</td>
<td>5 (36%)</td>
<td>13 (26%)</td>
<td>16 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3 (21%)</td>
<td>14 (27%)</td>
<td>12 (19%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 (14%)</td>
<td>12 (24%)</td>
<td>17 (26%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4 (29%)</td>
<td>12 (24%)</td>
<td>20 (31%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dur.Exer 0.</td>
<td>5 (36%)</td>
<td>13 (26%)</td>
<td>16 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>4 (29%)</td>
<td>12 (24%)</td>
<td>13 (20%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>3 (21%)</td>
<td>16 (31%)</td>
<td>14 (22%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>2 (14%)</td>
<td>10 (20%)</td>
<td>22 (34%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Int.Exer 0-</td>
<td>5 (36%)</td>
<td>13 (26%)</td>
<td>17 (26%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-</td>
<td>0 (0%)</td>
<td>6 (12%)</td>
<td>5 (8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-</td>
<td>7 (50%)</td>
<td>23 (45%)</td>
<td>33 (51%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-</td>
<td>2 (14%)</td>
<td>9 (18%)</td>
<td>10 (15%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R.GDM = rest of the GDM group, p₁ = p values of comparison of subgroup (A) versus rest of GDM group, p₂ = p values of comparison of subgroup versus controls, F = former, N = never, Smoke = smoker, 0 = Not at all, 1 = Less than once a week, 2 = Once or twice a week, 3 = Three times a week, 0. = Not at all, 1. = Less than 20 minutes, 2. = 20-30 minutes, 3. = More than 30 minutes, 0- = No exercise, 1- = None of exercise session, 2- = Some of exercise session, 3- = Most or all of exercise, and blank space in the p₁ and p₂ columns = No significant difference.
4.2.3 Subgroup (B) Results

Sub-group (B): Sub-population includes subjects who have high plasma NEFA concentrations and positive family history of diabetes.

There were no significant difference for age between subgroup (B) and other two groups (the residual GDM and control groups) (Table 4-13).

The subgroup (B) had slightly lower plasma cholesterol than the residual GDM group, but not significantly. Plasma cholesterol means were similar between the subgroup (B) and controls (Table 4-13).

The subgroup (B) had significantly lower plasma TG, VLDL-TG, and IDL-C concentration than the residual GDM group. Those three variables were similar between the subgroup (B) and controls (Table 4-13).

There was a tendency for the subgroup (B) to have a slightly lower LDL-C than the residual GDM group, and a similar mean of LDL-C concentration with controls (Table 4-13).

All three subgroup were similar in the HDL-C concentrations. HDL₃c particle sizes were significantly smaller in the subgroup (B) than the control group, but were not significantly smaller compared with the residual GDM subjects (Table 4-13).

The HDL₂ / HDL₃ ratio were similar in the three groups. LDL-I particle size also were similar between three groups (Table 4-13).

The subgroup (B) also tended to have slightly lower CETP activity in plasma then the residual GDM and control groups, but not significantly lower (Table 4-13).
Fasting blood glucose means were similar in the three groups. However, fasting insulin levels were significantly lower in the subgroup (B) than the residual GDM group, and slightly lower than the control group (Table 4-13).

The mean systolic and diastolic blood pressures were slightly higher in the subgroup (B) compared with the residual GDM, but the subgroup (B) tended to have significantly higher diastolic blood pressure than the control group (Table 4-13).

Subgroup (B) tended to have similar SHBG concentration with the control group and to have significantly higher SHBG concentration than the residual GDM group (Table 4-13).

Testosterone concentration were significantly lower in the subgroup (B) than in the residual GDM group, but there is no significant difference between testosterone concentrations in the subgroup (B) and controls (Table 4-13).

The subgroup (B) showed significantly greater concentration of albumin in urine than the controls, but the difference between means for concentration of albumin in urine between the subgroup (B) and the residual GDM were not significant (Table 4-13).

The subgroup (B) tended to be slimmer before they became pregnant (as described by pre-pregnancy BMI) than both the residual GDM and control groups. The difference between means of the residual GDM group and subgroup (B) was significant (Table 4-13).

The babies of the subgroup (B) mothers tended to be slightly lighter than the babies of the other two groups (Table 4-13).
Fat, alcohol and sodium consumption were similar in three groups. (Table 4-13).

Table 4-13 Comparison of subgroup (B) with the residual GDM population and residual controls (Continuous Data)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subgroup (B) (n = 15)</th>
<th>R.GDM (n = 50)</th>
<th>( P_1 )</th>
<th>R.Controls (n = 57)</th>
<th>( P_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>29.7 ± 4.4</td>
<td>32.1 ± 5.0</td>
<td></td>
<td>31.5 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>± 1.1</td>
<td>5.0 ± 0.9</td>
<td></td>
<td>4.4 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>0.84 ± 0.4</td>
<td>1.13 ± 0.5</td>
<td>*</td>
<td>0.87 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>ApoB</td>
<td>0.10 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td></td>
<td>0.88 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>VLDL-TG (log)</td>
<td>5.47 ± 0.7</td>
<td>6.01 ± 0.6</td>
<td>**</td>
<td>5.55 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>IDL-C (log)</td>
<td>3.9 ± 0.6</td>
<td>4.4 ± 0.60</td>
<td>**</td>
<td>4.1 ± 0.60</td>
<td></td>
</tr>
<tr>
<td>HDL-C (log)</td>
<td>1.06 ± 0.01</td>
<td>1.05 ± 0.01</td>
<td></td>
<td>1.06 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>LDL-C</td>
<td>3.11 ± 1.12</td>
<td>3.38 ± 0.88</td>
<td></td>
<td>2.79 ± 1.12</td>
<td></td>
</tr>
<tr>
<td>HDL(_3)b Size</td>
<td>7.92 ± 0.11</td>
<td>7.92 ± 0.11</td>
<td></td>
<td>7.97 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>HDL(_3)c Size</td>
<td>7.57 ± 0.14</td>
<td>7.60 ± 0.16</td>
<td></td>
<td>7.64 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>HDL(_2) / HDL(_3)</td>
<td>0.90 ± 0.15</td>
<td>0.89 ± 0.20</td>
<td></td>
<td>0.92 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>LDL-1 Size</td>
<td>30.9 ± 0.95</td>
<td>30.7 ± 1.06</td>
<td></td>
<td>31.4 ± 1.00</td>
<td></td>
</tr>
<tr>
<td>CETP.A (log)</td>
<td>2.48 ± 0.73</td>
<td>2.94 ± 0.49</td>
<td>**</td>
<td>2.58 ± 0.66</td>
<td></td>
</tr>
<tr>
<td>FBG (log)</td>
<td>1.50 ± 0.14</td>
<td>1.51 ± 0.07</td>
<td></td>
<td>1.52 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>4.51 ± 2.39</td>
<td>8.45 ± 5.52</td>
<td>**</td>
<td>5.15 ± 1.96</td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>111.1 ± 10.9</td>
<td>110.5 ± 13.2</td>
<td></td>
<td>105.5 ± 11.7</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>77.1 ± 4.7</td>
<td>75.9 ± 6.1</td>
<td></td>
<td>73.2 ± 5.4</td>
<td>*</td>
</tr>
<tr>
<td>SHBG (log)</td>
<td>3.92 ± 0.63</td>
<td>3.59 ± 0.54</td>
<td>*</td>
<td>3.92 ± 0.50</td>
<td></td>
</tr>
<tr>
<td>Testostero (log)</td>
<td>1.03 ± 0.02</td>
<td>1.05 ± 0.02</td>
<td>*</td>
<td>1.04 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Microalb (log)</td>
<td>1.19 ± 0.37</td>
<td>1.08 ± 0.36</td>
<td></td>
<td>0.95 ± 0.38</td>
<td>*</td>
</tr>
<tr>
<td>P.BMI</td>
<td>22.03 ± 2.36</td>
<td>25.64 ± 5.5</td>
<td>*</td>
<td>22.31 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>Current BMI</td>
<td>22.07 ± 2.20</td>
<td>27.15 ± 5.66</td>
<td>**</td>
<td>22.75 ± 2.95</td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>0.81 ± 0.05</td>
<td>0.86 ± 0.07</td>
<td>*</td>
<td>0.82 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Baby weight</td>
<td>3202 ± 530</td>
<td>3473 ± 567</td>
<td></td>
<td>3304 ± 514</td>
<td></td>
</tr>
<tr>
<td>Alcohol in (log)</td>
<td>0.24 ± 0.30</td>
<td>0.31 ± 0.38</td>
<td></td>
<td>0.40 ± 0.40</td>
<td></td>
</tr>
<tr>
<td>Sodium intake</td>
<td>8.07 ± 0.26</td>
<td>7.93 ± 0.28</td>
<td></td>
<td>7.87 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>Fat intake</td>
<td>92.82 ± 32.9</td>
<td>96.14 ± 39.6</td>
<td></td>
<td>90.78 ± 38.4</td>
<td></td>
</tr>
</tbody>
</table>

R = residual, Testostero = testosterone, Microalb = microalbuminuria, * = \( p \leq 0.05 \), ** = \( p \leq 0.01 \), blank space = No significant. Data presented as mean ± standard deviation. Most (60%) of subjects in the subgroup (B) were using oral contraceptive pill, at time of study, compared with other two groups, but only the difference between the subgroup (B) and controls was significant (Table 4-14), log = natural logarithm (base e).
Table 4-14 Comparison of subgroup (B) with the residual GDM population and residual controls (Categorical Data)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subgroup (n = 15)</th>
<th>R.GDM (n = 50)</th>
<th>R.Controls (n = 57)</th>
<th>( P_1 )</th>
<th>( P_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.Pill</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current use</td>
<td>9 (60%)</td>
<td>20 (40%)</td>
<td>16 (28%)</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>For N use</td>
<td>6 (40%)</td>
<td>30 (60%)</td>
<td>41 (72%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoke</td>
<td>7 (47%)</td>
<td>31 (62%)</td>
<td>29 (50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>6 (40%)</td>
<td>4 (8%)</td>
<td>*</td>
<td>14 (25%)</td>
<td></td>
</tr>
<tr>
<td>Ex-smoke</td>
<td>2 (13%)</td>
<td>15 (30%)</td>
<td>14 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fre.Exer</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (13%)</td>
<td>16 (32%)</td>
<td>14 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (7%)</td>
<td>16 (32%)</td>
<td>10 (18%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (47%)</td>
<td>7 (14%)</td>
<td>17 (30%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (33%)</td>
<td>11 (22%)</td>
<td>16 (28%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dur.Exer</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (13%)</td>
<td>16 (32%)</td>
<td>14 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (13%)</td>
<td>14 (28%)</td>
<td>11 (19%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 (33%)</td>
<td>14 (33%)</td>
<td>13 (23%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (33%)</td>
<td>6 (12%)</td>
<td>*</td>
<td>19 (33%)</td>
<td></td>
</tr>
<tr>
<td>Int.Exer</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (13%)</td>
<td>16 (32%)</td>
<td>15 (26%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 (0%)</td>
<td>6 (12%)</td>
<td>3 (5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 (60%)</td>
<td>21 (42%)</td>
<td>31 (54%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 (27%)</td>
<td>7 (14%)</td>
<td>8 (14%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R.GDM = rest of the GDM group, \( P_1 \) = p values of comparison of subgroup B versus rest of GDM group, \( P_2 \) = p values of comparison of subgroup B versus controls, F = former, N = never, Smoke = smoker, 0 = Not at all, 1 = Less than once a week, 2 = Once or twice a week, 3 = Three times a week, 0. = Not at all, 1. = Less than 20 minutes, 2. = 20-30 minutes, 3. = More than 30 minutes, 0- = No exercise, 1- = None of exercise session, 2- = Some of exercise session, 3- = Most or all of exercise, * = \( p \leq 0.05 \), and blank space in the \( P_1 \) and \( P_2 \) columns = No significant difference.

The subgroup (B) tended to smoke more than both other groups, but only the difference between subgroup (B) and the residual GDM group was statistically significant (Table 4-14).

The three components of exercise (frequency, duration, and intensity) were similar between the subgroup (B) and controls. However, the subgroup (B) tended to do more exercise than the residual GDM group (Table 4-14).
4.2.4 Hyperinsulinemic subgroup (Subgroup C) Results

There were no differences in age between the three groups.

The insulin resistant group had significantly higher plasma cholesterol level than the other two groups. The insulin resistant group tended to be mildly hypertriglyceridemic (range 0.8-2.8 mmol/L, median 1.4 mmol/L), and as a group, they had higher plasma TG values than residual GDM or control groups.

Plasma apoB, VLDL-TG, IDL-C, LDL-C, and NEFA were higher in insulin resistant group than other two groups.

There was a tendency for the insulin resistant group to have a slightly lower HDL-C than the other two groups, but this was not significant. CETP activity was significantly higher in the insulin resistant group than in other two groups.

HDL₃ particle sizes were similar in three groups, however LDLI particle size slightly smaller in the insulin resistant group than in the residual GDM group and significantly smaller than in controls (Table 4-15).

The mean systolic and diastolic blood pressures were significantly higher in the insulin resistant group than in the other two groups (Table 4-15).
Table 4-15 Comparison of subgroup (C) with rest of the GDM population and controls
(Continuous Data)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subgroup (n = 13)</th>
<th>R.GDM (n = 52)</th>
<th>P1</th>
<th>Controls (n = 65)</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>30.9 ± 7.1</td>
<td>31.7 ± 4.3</td>
<td></td>
<td>31.39 ± 4.22</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>5.4 ± 0.8</td>
<td>4.8 ± 1.0</td>
<td>*</td>
<td>4.39 ± 0.76</td>
<td>**</td>
</tr>
<tr>
<td>TG</td>
<td>1.06 ± 0.02</td>
<td>1.04 ± 0.02</td>
<td>**</td>
<td>1.03 ± 0.02</td>
<td>**</td>
</tr>
<tr>
<td>ApoB</td>
<td>1.23 ± 0.22</td>
<td>1.01 ± 0.22</td>
<td>**</td>
<td>0.88 ± 0.22</td>
<td>**</td>
</tr>
<tr>
<td>NEFA</td>
<td>1.029 ± 0.008</td>
<td>1.028 ± 0.01</td>
<td></td>
<td>1.023 ± 0.01</td>
<td>*</td>
</tr>
<tr>
<td>VLDL-TG (log)</td>
<td>6.54 ± 0.49</td>
<td>5.72 ± 0.61</td>
<td>**</td>
<td>5.55 ± 0.70</td>
<td>**</td>
</tr>
<tr>
<td>IDL-C (log)</td>
<td>4.50 ± 0.58</td>
<td>4.28 ± 0.64</td>
<td></td>
<td>4.05 ± 0.60</td>
<td>*</td>
</tr>
<tr>
<td>HDL-C (log)</td>
<td>1.05 ± 0.02</td>
<td>1.06 ± 0.02</td>
<td></td>
<td>1.06 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>LDL-C</td>
<td>3.78 ± 0.80</td>
<td>3.20 ± 0.95</td>
<td>*</td>
<td>2.79 ± 0.75</td>
<td>**</td>
</tr>
<tr>
<td>HDL₃b Size</td>
<td>7.93 ± 0.12</td>
<td>7.92 ± 0.10</td>
<td></td>
<td>7.97 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>HDL₃c Size</td>
<td>7.61 ± 0.19</td>
<td>7.59 ± 0.15</td>
<td></td>
<td>7.65 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>HDL₂ / HDL₃</td>
<td>0.86 ± 0.17</td>
<td>0.90 ± 0.19</td>
<td></td>
<td>0.92 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>LDL-I Size</td>
<td>30.43 ± 0.83</td>
<td>30.85 ± 1.06</td>
<td></td>
<td>31.39 ± 1.00</td>
<td>*</td>
</tr>
<tr>
<td>CETP.A (log)</td>
<td>3.22 ± 0.36</td>
<td>2.74 ± 0.60</td>
<td>**</td>
<td>2.58 ± 0.66</td>
<td>**</td>
</tr>
<tr>
<td>FBG (log)</td>
<td>1.54 ± 0.06</td>
<td>1.50 ± 0.10</td>
<td></td>
<td>1.52 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>121.5 ± 6.9</td>
<td>108.0 ± 12.3</td>
<td>**</td>
<td>105.5 ± 11.7</td>
<td>**</td>
</tr>
<tr>
<td>DBP</td>
<td>79.6 ± 5.2</td>
<td>75.4 ± 5.7</td>
<td>*</td>
<td>73.2 ± 5.4</td>
<td>**</td>
</tr>
<tr>
<td>SHBG (log)</td>
<td>3.47 ± 0.72</td>
<td>3.71 ± 0.53</td>
<td></td>
<td>3.92 ± 0.50</td>
<td>*</td>
</tr>
<tr>
<td>Testoste (log)</td>
<td>1.05 ± 0.03</td>
<td>1.04 ± 0.02</td>
<td></td>
<td>1.04 ± 0.01</td>
<td>**</td>
</tr>
<tr>
<td>Testoste / SHBG</td>
<td>0.31 ± 0.07</td>
<td>0.28 ± 0.04</td>
<td>*</td>
<td>0.27 ± 0.04</td>
<td>**</td>
</tr>
<tr>
<td>Microalb (log)</td>
<td>21.89 ± 10.6</td>
<td>17.02 ± 18.3</td>
<td></td>
<td>13.22 ± 14.2</td>
<td>*</td>
</tr>
<tr>
<td>P.BMI</td>
<td>31.06 ± 4.75</td>
<td>23.25 ± 4.02</td>
<td>**</td>
<td>22.31 ± 2.83</td>
<td>**</td>
</tr>
<tr>
<td>Current BMI</td>
<td>32.59 ± 4.53</td>
<td>24.32 ± 4.38</td>
<td>**</td>
<td>22.8 ± 2.95</td>
<td>**</td>
</tr>
<tr>
<td>Baby weight</td>
<td>3460 ± 488</td>
<td>3398 ± 589</td>
<td></td>
<td>3304 ± 514</td>
<td></td>
</tr>
<tr>
<td>Alcohol in (log)</td>
<td>0.24 ± 0.31</td>
<td>0.31 ± 0.38</td>
<td></td>
<td>0.40 ± 0.41</td>
<td></td>
</tr>
<tr>
<td>Sodium intake</td>
<td>7.93 ± 0.29</td>
<td>7.97 ± 0.28</td>
<td></td>
<td>7.87 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>Fat intake</td>
<td>96.41 ± 40.3</td>
<td>95.11 ± 37.7</td>
<td></td>
<td>90.78 ± 38.4</td>
<td></td>
</tr>
</tbody>
</table>

Testoste = testosterone, P.BMI = pre-pregnancy BMI, * = p ≤ 0.05, ** = p ≤ 0.01, blank space = No significant difference. Data presentes as mean ± standard deviation.
No study subject was hypertensive: in the insulin resistant group the highest systolic blood pressure was 130 mm Hg, in the residual GDM group it was 140 mm Hg and in the control group it was 130 mm Hg. The highest diastolic blood pressure in the insulin resistant and the residual GDM groups was 90 mm Hg and in the control group it was 80 mmHg. Fasting blood glucose levels were similar in three groups.

The group with insulin resistance tended to be fatter than either the control or the residual GDM group, with all being overweight (BMI > 25) or obese (BMI > 30) (Table 4-15). The minimum BMI in the insulin resistant group was 26.6, and 9 of the 13 in this group (about 70%) were obese. On the other hand, only one of the 65 control subjects was obese (BMI = 34) and 11 (17%) were overweight. Of the residual (non-insulin resistant) GDM group 16 (31%) were overweight and 3 (6%) were obese.

Pre-pregnancy BMI was significantly higher in the insulin resistant group than other two groups (Table 4-15).

The insulin resistant group show a greater amount of albumin in urine (Table 4-15).

Alcohol, sodium, and fat intake were not significantly different between three groups (Table 4-15).

There were no significant differences between use of an oral contraceptive, smoking, and exercise between the subgroup (C) and other two groups (Table 4-16).
Table 4-16 Comparison of subgroup (C) with the residual GDM population and controls
(Categorical Data)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subgroup (C) (n = 13)</th>
<th>R.GDM (n = 52)</th>
<th>P₁</th>
<th>Controls (n = 65)</th>
<th>P₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.Pill</td>
<td>Current use</td>
<td>7 (54%)</td>
<td>22 (42 %)</td>
<td>21 (32%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F or N use</td>
<td>6 (46%)</td>
<td>30 (58%)</td>
<td>44 (68%)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>Non-smoke</td>
<td>8 (62%)</td>
<td>29 (56%)</td>
<td>36 (55%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Smoker</td>
<td>0 (0.0%)</td>
<td>10 (19 %)</td>
<td>14 (22%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ex-smoke</td>
<td>6 (46%)</td>
<td>13 (25%)</td>
<td>15 (23%)</td>
<td></td>
</tr>
<tr>
<td>Fre.Exer</td>
<td>0</td>
<td>5 (39%)</td>
<td>13 (25%)</td>
<td>16 (25%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2 (15%)</td>
<td>14 (27%)</td>
<td>12 (19%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3 (23%)</td>
<td>13 (25%)</td>
<td>17 (26%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3 (23%)</td>
<td>12 (23%)</td>
<td>20 (31%)</td>
<td></td>
</tr>
<tr>
<td>Dur.Exer</td>
<td>0.</td>
<td>5 (39%)</td>
<td>13 (25%)</td>
<td>16 (25%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.</td>
<td>3 (23%)</td>
<td>13 (25%)</td>
<td>12 (19%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.</td>
<td>3 (23%)</td>
<td>16 (31%)</td>
<td>14 (22%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.</td>
<td>2 (15%)</td>
<td>10 (19%)</td>
<td>22 (34%)</td>
<td></td>
</tr>
<tr>
<td>Int.Exer</td>
<td>0-</td>
<td>5 (39%)</td>
<td>13 (25%)</td>
<td>17 (26%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-</td>
<td>1 (8%)</td>
<td>6 (12%)</td>
<td>5 (8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-</td>
<td>6 (46%)</td>
<td>23 (44%)</td>
<td>33 (51%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3-</td>
<td>1 (8%)</td>
<td>10 (19%)</td>
<td>10 (15%)</td>
<td></td>
</tr>
</tbody>
</table>

R.GDM = rest of the GDM group, P₁ = p values of comparison of subgroup C versus rest of GDM group, P₂ = p values of comparison of subgroup C versus controls, F = former, N = never, Smoke = smoker, 0 = Not at all, 1 = Less than once a week, 2 = Once or twice a week, 3 = Three times a week, 0. = Not at all, 1. = Less than 20 minutes, 2. = 20-30 minutes, 3. = More than 30 minutes, 0- = No exercise, 1- = None of exercise session, 2- = Some of exercise session, 3- = Most or all of exercise, blank space in the P₁ and P₂ columns = No significant difference.
CHAPTER FIVE

DISCUSSION

The objective of this chapter is to discuss the finding of the present study on women with previous GDM one year after delivery compared with controls.

In this study, age was not significantly different between the GDM and control groups. GDM is most common in women over 30 years old (Wiess, 1988). This discrepancy may be due to sample size of this study.

5.1 Family Medical History in Women with Previous GDM

GDM subjects gave a history of diabetes mellitus (n = 25, 38.5%) and / or CVD (n = 24, 36.9%) in their first degree relatives more often than in controls (n = 8, 12.3% and n = 10, 15.4%, respectively).

Haffner et al (1988) have suggested that fasting insulin concentrations are significantly higher in normoglycemic persons who have diabetic parents than in persons with no diabetic relatives. In addition, Eriksson et al (1988) have found that persons with positive family history of diabetes have decreased non-oxidative glucose disposal compared with persons with no family history of diabetes.

Weisser et al (1993) have reported that there is a correlation between insulin and blood pressure in subjects with a positive family history of diabetes or hypertension and this genetic influence may be related to insulin resistance.

Aerts et al (1990) have reported that 35% of patients with GDM are offspring of diabetic mothers versus only 5% of normoglycemic women, and GDM occurs more frequently in the offspring of diabetic mothers (35%) than in offspring of diabetic fathers (7%).
A history of diabetes on the mother's side has been reported to be a more important and frequent factor for developing diabetes in offspring than a history of diabetes in the father's side. Our finding are in agreement with these reports.

Several metabolic abnormalities may occur in adulthood including NIDDM and obesity in the offspring of mothers with GDM (Pettitt et al, 1988, Pettitt et al, 1987, respectively). Our finding in GDM subjects in this study is consistent with these findings.

Van Assche et al (1991) suggested that even mild diabetes mellitus induces an abnormal intra-uterine milieu that causes morphological and functional changes in fetal development with consequences for later life. Intra-uterine factors may be responsible for the transmission of diabetes mellitus from one generation to another. The incidence of NIDDM is much higher in the offspring of diabetic mothers than in normal mothers (Aerts et al, 1990).

GDM subjects had more frequently significant and positive family histories of CVD in comparison with control subjects. Bell et al (1990) previously reported a similar finding in GDM subjects.

Our findings also indicate that the GDM subjects are more likely to have a diabetic mother than controls and that the GDM subjects are more likely to have history of diabetes in the mother side in father side. The GDM subjects also are more likely to have a first degree relative with CVD.
5.2 Obstetrics History in Women with Previous GDM One Year after Delivery

Fetal risks accompanying diabetes in the mother include spontaneous abortion, prenatal death, and malformation (Crandall, 1987). In our study, there is a similar incidence of those and other complications in patient who have had GDM and controls.

This good outcome is possibly due to intensive diet management and insulin therapy to maintain euglycemia. Caesarean delivery was more common in GDM but not statistically different between two groups. Our finding is in agreement with similar findings by Goldman et al (1991).

GDM subjects did not have greater parity than controls; our findings in this agree with those of Molsted-Pedersen et al (1989).

Three GDM subjects had had GDM in previous gestations. They were obese, and they required insulin to achieve normoglycemia in their most recent and previous pregnancies. Gaudier et al (1992) reported that women with a history of GDM and BMI > 35 kg / m², if they required insulin therapy during pregnancy, were at increased risk (52%) of recurrence of GDM.

5.3 Oral Contraceptive Use in Women with Previous GDM One Year after Delivery

There is no significant difference in use of an oral contraceptive between the GDM and control groups.

In our study, current use of oral contraceptive was not significantly associated with plasma cholesterol, LDL-C, HDL-C, NEFA, and insulin concentrations. Our results are consistent with those of Kjos et al (1990) who reported that low-dose oral
contraceptives in women with prior GDM did not seem to have any adverse effect on carbohydrate or lipid metabolism.

Current oral contraceptive use was positively and significantly correlated with plasma cholesterol, apoB, and apo A-I concentrations. Lussier-Cacan et al (1991) reported that apoB and apoA-I both were higher during oral contraceptive therapy.

The serum concentration of SHBG is known to be increased by estrogen, tamoxifen, phenytoin, or thyroid hormone administration and by hyperthyroidism and cirrhosis. SHBG also is decreased by exogenous androgen, glucocorticoids or growth hormone and by hypothyroidism. Only one GDM subject was excluded from analysis, because she was taking replacement thyroxine (Braunstein, 1994). There is a positive and significant correlation between oral contraceptive pill and SHBG in our study. SHBG is synthesized in the liver, and its synthesis is stimulated by estrogens and inhibited by androgen (Goldfien and Monroe, 1994).

Normal and healthy women taking oral contraceptive pills have a slightly increased risk of cerebrovascular and cardiovascular disease, and the risk is higher for the diabetic patients (Rapkin, 1987).

5.4 Obesity in Women with Previous GDM One Year after Delivery

In our study, subjects who have had GDM have significantly greater WHR, BMI, and body fat % than control subjects. WHR seems to be the simplest and most reproducible measure of central obesity (Haffner et al, 1987) and it is strongly associated with visceral fat mass (Ashwell et al, 1985).
Factors affecting percent body fat include age, gender, level of energy balance, composition of the diet, level of physical activity, and a variety of social factors including smoking and alcohol intake (Bouchard et al, 1990). In this study, we examined these factors in GDM populations.

Metabolic disorders in obesity have been shown to be correlated with abdominal obesity (Kalkhoff et al, 1983, Larsson et al, 1984, Ohlson et al, 1985). In addition, Ashwell et al (1985) have found a strong correlation between the intra-abdominal fat:subcutaneous fat area ratio and WHR by using CT scanning at an umbilical level. Shimokata et al (1989) have reported that WHR is strongly affected by age and BMI. WHR has a positive correlation with age and BMI. Our data also indicates that WHR has a positive correlation with age and BMI, too.

Central obesity is positively associated with overall adiposity, particularly in females. In our study, GDM subjects also represent a higher BMI in company with higher WHR and body fat %.

Insulin resistance occurs in both NIDDM and obesity, and most NIDDM patients are obese. In fact, obesity precedes the development of NIDDM in more than 80% of cases in Pima Indians (Seely and Olefsky, 1993). In the present study, GDM subjects had higher BMI and higher insulin levels leading to dyslipidemia (Table 4-8).

Multiple regression analysis showed that fasting insulin concentration is the strongest determinant of BMI in the GDM subjects (Table 4-9).

WHR, BMI, and body fat % showed a positive and significant correlation with fasting plasma insulin level and insulin / glucose ratio. Baynes et al (1991) reported a similar result in both diabetic males and females. Hyperinsulinemia may develop through
progressive development of insulin resistance, presumably due to post-receptor site alterations (Lillioja and Bogardus, 1988, Olefsky et al, 1988).

Women are resistant to insulin action at an average body fat % of 37.7% (in GDM subjects mean was 38.79 %), while men develop an equivalent condition at an average body fat % of 23.9% (Plaisted and Istfan, 1994).

In mildly hyperinsulinemic obese subjects, only a decrease in insulin sensitivity exists. As the hyperinsulinemia worsen, a post-receptor defect also occurs. Furthermore, there is a direct association between degree of insulin resistance or hyperinsulinemia and the magnitude of the post binding defect (Seely and Olefsky, 1993).

Glucose oxidation is lower in obese subjects than normo-weight controls, but lipid oxidation is 30% higher in obese subjects than controls in the fasting state. Thus, there is a negative correlation between glucose oxidation and lipid oxidation which may be explained by the glucose-fatty acid cycle (Seely and Olefsky, 1993).

The association between insulin and hypertriglyceridemia has been shown in Figure 5-1. Insulin resistance causes increased insulin secretion, and increased flux of NEFA to the liver (where they stimulate TG synthesis), and increasing hyperinsulinemia (Durrington, 1995). Hyperinsulinemia, in turn, can be a cause of overproduction of hepatic TG and increased hepatic VLDL secretion, and as a result cause hypertriglyceridemia (Olefsky et al, 1974) (Figure 5-1).

GDM subjects in this investigation have a significantly higher plasma NEFA concentration than controls. NEFAs are thought to increase insulin resistance in muscle and liver and increase hepatic gluconeogenesis and lipoprotein synthesis and perhaps decrease hepatic removal of insulin (Bjorntorp, 1992). NEFAs are a determinant of
VLDL production. High NEFA levels may give rise to high plasma VLDL, LDL, apoB-100, and glucose concentrations, and perhaps secondarily, hypertension (Bjorntorp, 1992). All these variables have been shown to be raised when plasma NEFA is raised in our investigation. All these variables were significantly and positively related to obesity among the GDM group.

Figure 5-1 Alternative hypothesis to explain the association between insulin and hypertriglyceridemia (Data from Durrington, 1995 and Olefsky et al, 1974). TG = triglyceride, NEFA = non-esterified fatty acid.
Grundy and Barnett (1990) have suggested two basic mechanisms for the hypertriglyceridemia of obesity: overproduction of VLDL-TG and impaired lipolysis of the TG-rich lipoproteins. The high plasma TG concentrations in subjects (GDM) may be due to a reduction in VLDL catabolism, through the decreased plasma post-heparin LPL activity, and/or an increased VLDL synthesis (Despres et al, 1990b).

Stromblad and Bjorntorp (1986) have reported that high FFA concentrations in portal blood are associated with reduced hepatic insulin clearance in rat with dietary-induced obesity.

In addition, Svedberg et al (1990) have demonstrated that elevated FFA levels inhibit insulin binding and degradation in isolated rat hepatocytes. Thus, central obesity and high FFA concentrations may increase insulin resistance and thus hyperinsulinemia.

BMI showed a significant and negative correlation with LDL particle size in this study. The correlation of abdominal obesity with denser and smaller LDL was reported previously (Harris-Peeples et al, 1989). BMI and WHR were positively associated with LDL-C but it did not reach significant level.

HDL-C may be decreased in obese subjects, either through direct clearance of HDL-C from circulation by the adipose tissue or through separation of the lipid from its apo A-I or its cholesterol ester (Grundy and Barnett, 1990). In the present study, BMI was not significantly correlated to HDL-C but WHR was significantly and negatively associated with plasma HDL-C concentration.

CETP was a significantly and positively correlated with BMI and body fat % in the GDM group. Increased CETP activity is associated with increased lipid exchanged between VLDL and HDL in abdominal obesity (Despres et al, 1992).
There was a significant and positive correlation between BMI and systolic blood pressure, diastolic blood pressure, and mean arterial pressure. Previous studies have reported a similar correlation between obesity and both systolic and diastolic blood pressure in obese subjects (Van Gaal et al, 1988, Peiris et al, 1989).

Obesity is a strong predictor for the development of hypertension (Sowers et al, 1991). Although the mechanisms by which obesity cause hypertension are not fully understood, hypercaloric intake, hyperinsulinemia, insulin resistance, enhanced metabolic activity of visceral adipose tissue, enhanced sympathetic activity, and even the greater body mass present in obese patients are all thought to be involved mechanisms (Plaisted and Istfan, 1994).

### 5.5 Carbohydrate Metabolism in Women with Previous GDM One Year after Delivery

Data from a study on Nauruans indicated that subjects with higher post-load serum insulin levels, presumably indicative of insulin resistance, at baseline tended to develop IGT or diabetes over a six year period (Sicree et al, 1987). It is generally accepted that insulin resistance plays an important role in the pathogenesis of NIDDM (Alzaid and Rizza, 1993).

Reduced glucose clearance, accompanied by compensatory hyperinsulinemia, is present one or two decades before NIDDM is diagnosed in the offspring of diabetic parents. It indicates that the primary defect is in the peripheral tissue response to insulin and glucose, not in the pancreatic cell (Warram et al, 1990). The same authors suggested that a low glucose clearance rate and hyperinsulinemia are the best predictors of the
subsequent development of diabetes in the offspring of diabetic parents (Warram et al., 1990).

Several studies have showed that fasting hyperinsulinemia is a good predictor for the development of NIDDM in different populations such as in Nauruans (Sicree et al., 1987), Pima Indians (Knowler et al., 1990, Saad et al., 1988, Lillioja et al., 1988), Mexican Americans (Haffner et al., 1990 b), Swedish women (Lundgren et al., 1990), the offspring of diabetic parents in America (Warram et al., 1990), and French policemen (Charles et al., 1991). The cellular mechanisms involved in the alteration of insulin function are still unclear (Knowler et al., 1993). It may be applicable for GDM subjects and say that high fasting insulin level is a predictor of the development of diabetes mellitus in these population.

The current hypothesis is that as a result of chronic insulin resistance, compensatory hypersecretion of insulin develops to maintain glucose homeostasis and that eventually diabetes mellitus develops when pancreatic hypersecretion fails (Haffner et al., 1990 b).

The present study showed that 20% of the GDM subjects had developed insulin resistance (as defined by fasting hyperinsulinemia) one year post-partum. They were overweight or obese and they tended to have mild dyslipidemia. They also tended to have a higher blood pressure, lower SHBG concentration, and microalbuminuria.

BMI was the unique determinant factor in the insulin multiple regression model (Table 4-9).

There is considerable evidence to suggest that insulin resistance is caused (at least in part) by excess availability of fatty acids. Randle has suggested that elevated plasma FFA levels impair glucose metabolism. Increased uptake of free fatty acids and
subsequent oxidation inhibits glucose oxidation and glycogen synthesis (Randle et al, 1963).

Infusion of FFA into normal subjects using the insulin clamp technique to measure insulin resistance and indirect calorimetry showed that increased FFA concentrations may result in mild insulin resistance, reduction in glucose transport, and a reduction in both glucose oxidative and non-oxidative metabolism (Ferrannini et al, 1983, Yki-Jarvinen et al, 1991).

Haffner et al (1992) have demonstrated that increased fasting insulin concentrations predict a cluster of metabolic disorders including dyslipidemia, hypertension, and NIDDM, and other have previously shown that fasting insulin predicts NIDDM (Saad et al, 1988, Haffner et al, 1990 a, Charles et al, 1991, Zimmet et al, 1992).

Subjects who have GDM have an increased risk of developing NIDDM later in life and more than 50% of these women develop NIDDM within 25 years (O'Sullivan, 1975). Women with GDM who need insulin treatment during pregnancy have a higher risk of later development of diabetes mellitus than those who have been controlled by diet alone (Henry and Beischer, 1991).

In our study, 17% (11 cases) of GDM patients have received insulin for control their blood glucose levels during pregnancy and 83% (54 cases) have been treated only by diet to achieve an acceptable level of glucose. GDM subjects who received insulin treatment during pregnancy had a higher BMI before and after pregnancy and dyslipidemia (higher plasma cholesterol and LDL-C).

None of the GDM subjects including those with insulin resistance were sufficiently hyperglycaemic to elevate glycosylated haemoglobin (as % of blood haemoglobin).
As a result, the severity of insulin resistance appears to be predicted by factors such as antecedent glycemic control, level of fitness, age, obesity, body fat distribution, and hypertension (Alzaid and Rizza, 1993).

Our study showed that a subgroup of those women who have a history of GDM have hyperinsulinemia and mild hypertriglyceridemia. They tended to be fatter, to be dyslipidemic (higher plasma cholesterol and TG concentrations), to have a higher blood pressure, to have a greater amount of albumin in urine, and to have lower plasma SHBG concentrations, but not to be older.

Alterations in lipid metabolism associated with insulin resistance are outlined in Table 5-1 (Frayn, 1993). In the present study all these changes were found in the GDM group, particularly those with insulin resistance.

### Table 5-1 Alterations in lipid metabolism commonly associated with insulin resistance

<table>
<thead>
<tr>
<th>Alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated plasma TG concentration (particularly VLDL-TG)</td>
</tr>
<tr>
<td>Decreased plasma HDL-C concentration (particularly HDL$_2$-C)</td>
</tr>
<tr>
<td>Elevation NEFA</td>
</tr>
<tr>
<td>Small, dense LDL particles</td>
</tr>
</tbody>
</table>

#### 5.6 Lipoprotein Metabolism in Women with Previous GDM One Year after Delivery

Plasma cholesterol and apoB concentrations in the women with prior GDM tend to be significantly higher than in women with no history of GDM of the same age. Apo B level has been reported as a more effective marker for coronary artery disease than LDL or total cholesterol level (Levinson and Wagner, 1992). As shown in recent study, apo B level may be measuring the atherogenic potential of both VLDL and LDL (Grundy and Vega, 1992).
The plasma cholesterol concentration was not related to cholesterol intake and cholesterol intake was similar between two groups.

Elevated TG levels may result from overproduction or defective removal. The latter may be due to genetic or acquired deficiency of LPL or apoC-II (the activator of LPL), or an excess of apoC-III which inhibits apoC-II activity and β-VLDL uptake (Kashyap, 1991).

A clinically important aspect of HTG is that it signals the presence of other metabolic disorders (Vergani et al, 1990). In addition to HTG and low level of HDL, affected subjects frequently have one or more of the following: overweight, insulin resistance with fasting hyperinsulinemia and glucose intolerance, hypertension, and hyperuricemia. Different terms have been used to define this syndrome: ‘plurimetabolic syndrome’, ‘overweight-hyperlipidemic-hyperuricemic syndrome’, ‘glucose intolerance-obesity-hypertension condition’, and ‘symptom complex’ (Vergani et al, 1990). The name most used now is ‘insulin resistance syndrome’. The syndrome is strongly related to coronary heart disease (Vergani et al, 1990).

According to Zavaroni and Reaven, an increase in the plasma triglyceride concentration and a decrease in the plasma HDL-C concentration are the expected results of insulin resistance and hyperinsulinemia (Vergani et al, 1990).

In the current investigation, GDM subjects have HTG, but not a low level of HDL-C compared with controls. In these subjects HTG seems statistically to be related with hyperinsulinemia, high plasma NEFA concentration, NEFA, and smoking. Interestingly, BMI and alcohol consumption were not strong predictors in the subjects. This lack of correlation might be due to considerable homogeneity in the subjects.
Reaven and Greenfield have suggested that HTG associated with insulin resistance is related to plasma insulin concentration and/or free fatty acids (Reaven and Greenfield, 1981). In impaired glucose tolerance states, subjects exhibit insulin resistance and a compensatory hyperinsulinemia. The hyperinsulinemia causes an increased rate of synthesis and secretion of VLDL-TG and perhaps HTG due to impaired clearance capacity (Nikkila, 1984). On the other hand, high plasma FFA concentration derived from VLDL metabolism give rise to hypertriglyceridemia (Galton and Krone, 1991).

Genetic studies have suggested that the high concentration of apoB in circulation can be a strong coronary risk factor (Douste-Blazy and Kloer, 1989). In human, apoB100 is a protein constituent of VLDL, IDL, and LDL. Each of these lipoprotein particles contains but a single copy of apoB100 (Innerarity, 1990). In our study, the GDM subjects exhibited a higher level of plasma apoB and LDL-apoB concentrations than controls, and they also had slightly higher levels of VLDL-apoB and IDL-apoB than controls but not to an exact that was statistically significant.

Plasma FFA (NEFA) concentration is a function of rates of both input and removal from the plasma concentration. Inflow of FFA is governed by the rate of lipolysis in adipose tissue and also the release of FFA during the hydrolysis of circulating TG-rich particles, particularly in the postabsorptive state (Taskinen et al, 1985). The rate of FFA removal is determined by both esterification (or re-esterification) and lipid oxidation. It has been generally accepted that plasma FFA concentration is controlled mainly by FFA production (ie by the rate of lipolysis), whereas the efflux rate of FFA is secondary to changes in plasma FFA concentration. This concept implies that the removal of FFA from plasma is not controlled independently (Taskinen et al, 1985).
As is shown by simple regression, intensity of exercise is related positively to NEFA level. The activation of lipase and subsequent lipolysis and mobilization of FFA from adipose tissue is increased by the hormones epinephrine, norepinephrine, glucagon, and growth hormone. Because plasma concentrations of these hormones are increased during exercise, this mechanism for lipase activation provides the muscle with a supply of a potent energy substrate. Alterations in lipase activity may partly explain the enhanced utilization of fat observed with aerobic exercise training (McArdle et al, 1991 a).

The most substrates used by the heart for energy are glucose, fatty acids, and lactic acids. During exercise the myocardial metabolism of FFAs rises to approximately 70% of the total energy requirement (McArdle et al, 1991 a).

Emphasis has recently been placed on abnormalities not only in lipid and apolipoprotein concentrations but also in lipid and apoprotein composition of different lipoproteins in diabetic as well as in non-diabetic individuals (Steinberg et al, 1989).

Compared with non-diabetic people, diabetic patients have decreased clearance of both VLDL-apoB and VLDL-TG, and increased production of VLDL-TG. Obese subjects have increased production of both VLDL-apoB and VLDL-TG and more VLDL of normal composition (Howard et al, 1987). Study on VLDL turnover showed that production of both VLDL-apoB and VLDL-TG were significantly correlated with insulin concentrations, and the rates of clearance of both were significantly correlated with plasma glucose levels (Howard et al, 1987).

In GDM subjects, it may be said that clearance of both VLDL-TG and VLDL-apoB are decreased and also the production of VLDL-TG is increased. It explains significant elevation in the level of VLDL-TG and a slightly higher (but not significantly higher)
concentration of VLDL-apoB but not significant in the GDM group compared with controls. Saheki et al (1993) reported that VLDL from diabetic patients are a poor substrate for LPL compared with normal VLDL.

IDL particles are thought to be associated with increased atherosclerosis risk (Tatami et al, 1981, Steiner, 1981). This supposition is supported by the observation that particles with size and flotation similar to those of human cholesterol-enriched IDL have been associated with a predisposition to coronary atherosclerosis in the cholesterol-fed monkey and that elevated levels of IDL mass and IDL-C have been related to increased risk of coronary disease in human (Krauss, 1987).

On this basis, Fielding et al (1984) have suggested that the block to cholesteryl ester transfer to VLDL and LDL in NIDDM patients is mediated by their increased free cholesterol (FC) content and may be related to the increased risk of these patients for developing atherosclerosis.

In the GDM group as a whole, the plasma LDL-C and LDL-apoB concentrations were significantly greater in the GDM subjects than in controls.

Elevated levels of LDL-C might be secondary to either an increase in the synthesis rate of LDL-C or a decrease in the rate of its catabolism and clearance from plasma (Barter, 1994). A down-regulation of the B/E receptor may be involved in the decreased rate of LDL-C catabolism (Barter, 1994). On this basis, a defect in VLDL degradation possibly due to LPL activity disorders and an enhanced synthesis of VLDL from the liver can be a cause of HTG and combined HTG and hypercholesterolemia (Barter, 1994), and thus a greater level of LDL-C in plasma.
Deckelbaum et al have suggested that transformation of IDL and larger LDL to smaller, denser LDL subspecies can result from the combined effects of TG hydrolysis and lipid transfer (Musliner et al, 1991).

The factors that determine the size and composition of LDL are not fully known. Investigations have shown that genetic factors are involved in the size of LDL. Studies have demonstrated that environmental factors or other disease states may also influence the size and composition of IDL (Feingold et al, 1992). Firstly, age has a positive correlation with LDL phenotype B. Secondly, weight loss induced by exercise decreases the concentration of small, dense LDL, and thirdly, studies have recently found that in subjects with acquired immunodeficiency syndrome (AIDS) the prevalence of the LDL B phenotype B is increased 2.5 fold (Feingold et al, 1992).

Krauss and Burke (1982) suggested that the TG-enriched LDL found in the larger and less dense subfractions may give rise to smaller, dense LDL subspecies via lipolysis and loss of core TG and other lipids. However, in the smallest and most dense LDL subspecies, LDL-TG is increased, suggesting that another metabolic pathway is involved in their formation. Some of the smaller, denser subspecies may also conceivably be precursors of larger, less dense LDL subspecies formed as a result of transfers of lipid and protein components to and from these particles (Krauss and Burke, 1982).

McNamara et al (1987) have found that the presence of small LDL particle is associated with increased age, plasma cholesterol and TG concentrations, and lower HDL-C and apoA-I levels. On the other hand, Barakat et al (1990) have found that LDL particles are smaller and denser in patients with IGT and NIDDM. LDL size was correlated with plasma insulin concentrations independent of plasma TG level, age and BMI. In addition,
this does not necessarily testify a causal relationship and it is difficult to identify the mechanisms that govern the alterations in LDL particle size. It may be a link between hyperinsulinemia and atherosclerosis (Barakat et al, 1990).

Smaller LDL particle size is prevalent in subjects with IGT and NIDDM but not in normoglycemic obese subjects whose plasma insulin concentrations are normal. It seems the presence of small LDL may be related to the degree of resistance of tissues to insulin action. LPL also may play a role in the alteration in LDL size (Barakat et al, 1990), because adipose tissue and lipoprotein metabolism are associated with insulin (Eckel, 1989).

Feingold et al believe that whether HTG itself leads to the formation of small, dense LDL or whether those who inherit the LDL B phenotype are more susceptible to the development of HTG is unresolved (Feingold et al, 1992).

HDL consists of a heterogenous group of particles defined either by size or by their apolipoproteins (Silverman et al, 1993).

Data from this study suggests that HDL-C is not significantly lower in GDM subjects and also the HDL₂ %:HDL₃ % mass ratio (the HDL₂ area %:HDL₃ area % ratio measured after gel electrophoresis separate the HDL subfractions) is not increased, resulting in no evidence for impaired LCAT activity. Silverman et al suggested that transfer of CE via CETP, followed by lipolysis of TG by HPL, results in the conversion of HDL₂ into HDL₃ (Silverman et al, 1993).

It is thought both HDL₂ and HDL₃ are acceptors of phospholipid, FC, and apoCs from VLDL and chylomicrons, but the major acceptor particles are probably HDL₃. With
higher substrate concentrations the HDL$_3$ decreases and may be completely transformed to the density of HDL$_2$ (Nikkila et al, 1987).

Deckelbaum et al (19884) have reported that there is a positive correlation between plasma TG concentrations and TG content of HDL in normolipidemic and hypertriglyceridemic subjects.

TG-enriched HDL$_3$ particles show a positive relationship with posprandial lipemia and the evidence suggests that they serve also as substrate for hepatic lipase and are converted to particularly small, dense HDL$_3$ (Patsch et al, 1987).

Behr et al (1981) have reported that an immunologically mediated blockage of the effect of LPL (by influencing antibodies to LPL) in the chicken resulted in accumulation of TG-rich lipoproteins, a higher TG content of HDL, and the replacement of the larger HDL$_2$ by smaller, denser HDL$_3$.

Largost (1992) have suggested that CETP promotes a reduction in the size of HDL$_3$ when HDL$_3$ was incubated with purified CETP at 37°C for 24 hours. In addition, NEFAs have been reported to modulate the redistribution of particle size in HDL$_3$ by CETP even in the absence of any other lipoprotein classes (Largost and Barter, 1991).

In hypertriglyceridemia, the fractional catabolic rate (FCR) for apoA-I is increased, and low plasma concentrations of apoA-I and HDL-C are presumed secondary to the increased FCR (Tribble and Krauss, 1993). In present study, GDM subjects were moderately hypertriglyceridemic in comparison with controls and their HDL-C and HDL apoA-I levels were slightly lower than controls but not to a statistically significant extent.

In the GDM group Lp (a) concentration in plasma was not significantly different between GDM and control subjects. Plasma Lp (a) concentration was not associated
with plasma cholesterol, TG, VLDL-C, IDL-C, LDL-C, apoB, smoking, blood pressure, and dietary factors, these being other risk factors for atherosclerosis, but it was significantly correlated with plasma insulin concentration and markers of obesity in this study. Lp (a) also was associated with amount of albumin in urine (Table 4-8). It seems likely that obesity and hyperinsulinemia are associated with higher plasma Lp (a) concentrations, but the underlying mechanisms are not clear. Steinmetz et al (1993) reported that Lp(a) and atherosclerosis are associated, but the underlying mechanism is not clear.

In conclusion, it can be said that a number of metabolic and pathophysiological mechanisms may modulate the concentration of Lp (a) and that these are not yet fully understood.

In this investigation, CETP activity % was higher in the GDM group than in control group. Studies have suggested that increased CETP activity may promote atherogenesis by stimulating enhanced transfer of CE to LDL in patients with low plasma HDL concentrations (Sparks et al, 1991).

The CETP mediated transfer of cholesteryl esters from HDL to apoB-containing lipoproteins can be potentially atherogenic, particularly in circumstances when plasma concentrations of apoB-containing particles are elevated, a circumstance perhaps in which the cholesteryl ester-rich apoB-containing lipoproteins are more likely to transfer cholesterol to peripheral cells in the arterial wall (Albers et al, 1990).

The activity of CETP has been shown to be positively related to adiposity (Dullaart et al, 1994). The data in this study suggest that there is a positive relationship between higher CETP activity and markers of obesity, BMI and body fat %. In addition, CETP activity
showed a significant and negative correlation with exercise in the present study. It is possible that obese subjects may have greater plasma CETP activity because of their lack of physical activity, but that needs to be practically examined.

CETP plays a major role in modifying the composition of lipoproteins in the plasma compartment (Albers et al, 1990). For example, CETP facilitates the exchange of LDL cholesteryl ester for VLDL triglyceride. By this process, the TG-enriched LDL formed can then subsequently undergo lipolysis, leading to the formation of smaller, dense LDL (Albers et al, 1990).

McPherson et al (1991) have reported that plasma CETP concentrations are elevated in patients with hypercholesterolemia, sometimes in combination with HTG and hyperchylomicronemia. Correction of the abnormal lipoprotein phenotype by dietary therapy caused significant reductions in plasma CETP concentrations in patients with hypercholesterolemia (McPherson et al, 1991).

In light of the above information, it seems a number of the GDM group demonstrate a syndrome of dyslipidemia compared with controls. The dyslipidemic syndrome in the GDM subjects includes hypercholesterolemia, hypertriglyceridemia, higher plasma apo B and NEFA concentrations, alterations in VLDL, IDL, and LDL compositions (ie higher VLDL, IDL-C, IDL-C, LDL-C, and LDL-apoB in the GDM subjects compared with controls), greater CETP activity in plasma, and denser and smaller HDL and LDL particles.

Approximately 37% of the GDM group in the current study have the dyslipidemic syndrome. The results showed that subjects with dyslipidemia had hyperinsulinemia and
insulin / glucose ratio, greater BMI and body fat %, higher blood pressure (both systolic and diastolic), and lower plasma SHBG concentration.

Plasma cholesterol and LDL-C concentrations were positively associated with insulin levels in men but not in women (Haffner et al, 1988). Plasma insulin concentration has been reported to be associated with smaller and denser LDL particles which seem to be more atherogenic (Barakat et al, 1990). In this investigation, plasma insulin concentration and insulin / glucose ratio, as a marker of insulin resistance, were positively correlated to plasma cholesterol and LDL-C concentrations in GDM subjects.

Gary et al (1988) have reported that insulin-mediated glucose disposal is negatively correlated with fasting TG concentration as well as VLDL-C, VLDL-TG, and LDL-C / HDL-C ratio. Laakso et al (1990) have found insulin resistance is associated with low HDL-C and high total TG and VLDL-TG levels in subjects with normal and impaired glucose tolerance and NIDDM. In our investigation, the GDM subjects with hypertriglyceridemia had higher fasting plasma insulin concentrations and insulin / glucose ratio, and VLDL-TG levels, they also had slightly lower HDL-C levels but not to a statistically significant extent.

Over all, it seems that abnormalities founded in this study in LDL composition and size can be due to HTG and a high activity of CETP in plasma (GDM subjects represent higher plasma TG levels and CETP activity compared with controls). These changes may be due to insulin resistance.

In addition, LDL composition was altered ( higher LDL-C and LDL-apoB levels) in women who had had GDM in comparison with controls. LDL phenotype was not significantly different in women who had had GDM than control women.
As a result, based on the results of our study, it is possible to say that subjects with previous GDM exhibit a complex of lipid and lipoprotein disorders which can be risk factors for atherosclerosis. Subjects with history of GDM presented mild hypercholesterolemia, hypertriglyceridemia, higher apoB and NEFA levels, and greater CETP activity. Additionally, they had abnormal VLDL, IDL, and LDL compositions and they possessed denser and smaller LDL-I and HDL\textsubscript{3b} and HDL\textsubscript{3c} particles.

Subjects with dyslipidemia were overweight or obese and had hyperinsulinemia. It may be that the dyslipidemic syndrome in a number of the GDM subjects is secondary to insulin resistance.

Many studies recently have suggested that a profile of plasma apolipoprotein values may be useful in the evaluation of coronary heart disease risk, although the final goal should be to identify by means of their apolipoprotein components atherogenic and non-atherogenic lipoprotein particles (Douste-Blazy and Kloer, 1989).

Thus, abnormalities in lipid metabolism may be very important in GDM patients and should be considered carefully by those caring for them. Lipids and lipoproteins should be checked in following these patients up and action taken where appropriate.

5.7 Hyperandrogenicity in Women with Previous GDM One Year after Delivery

In the present study, GDM patients exhibited lower plasma SHBG levels than controls but the plasma levels of testosterone were not significantly elevated in GDM subjects. On the other hand, total testosterone / SHBG ratio (the free androgen index) was significantly higher in women with previous GDM than in women with history of a normal pregnancy (p = 0.0048).
GDM subjects demonstrated a significant negative correlation between plasma SHBG concentration and the plasma level of testosterone. Rosenfield (1975) reported the same linkage between low level of SHBG and higher level of free testosterone in pre-menopausal and post-menopausal women. It is considered a marker of hyperandrogenicity in women. A small increase in total testosterone in the blood (as exist in GDM subjects) accompanied by a decrease in the concentration of SHBG may result in a significant increase in circulating biologically active hormone (Goldfien and Monroe, 1994).

Studies have reported an inverse relationship between body weight and the plasma SHBG concentration (Demoore and Joosens 1970), BMI and upper body obesity in pre-menopausal women (Haffner et al, 1989). In our study, we found an inverse relationship between the plasma SHBG concentration and WHR, BMI, and body fat % in the GDM patients.

Evans et al (1983) reported that abdominal obesity causes low SHBG and high free testosterone levels in women. In addition, Kirschner et al (1990) demonstrated that sex hormone production and metabolism are different in women with upper body (abdominal) and lower body obesity. Women with upper body obesity exhibit higher androgen production rates, higher testosterone and estradiol (E2) levels and lower SHBG levels. However, women with lower body obesity make increased amounts of E2 from peripheral aromatization.

Use of an oral contraceptive was strongly related to the plasma SHBG concentration. Estrogen therapy is one of the factors which increased SHBG synthesis (Goldfien and Monroe, 1994). The mechanism of this effect will be discussed later in this Chapter.
Data from the present investigation showed that SHBG has significant and positive correlations with plasma apoA-I and HDL-apoA-I concentrations but the relationship between SHBG and HDL-C was not significant.

Previously, Haffner et al (1989) reported that SHBG is positively correlated with HDL-C in pre-menopausal women and Longcope et al (1990) found that SHBG is significantly related to HDL-C and apoA-I in women. The numbers of subjects in our study may not have been great enough to demonstrate statistical significance for the relation of SHBG and HDL-C.

Longcope et al (1990) suggested that the positive associations between SHBG and HDL-C, HDL₂, and apoA-I are related to the fact that these proteins are synthesized in the liver, and their syntheses may be stimulated through similar mechanisms.

Insulin is known as a regulator of SHBG production in vitro and in vivo (Peiris et al, 1993). Insulin is capable of suppressing SHBG production in a hepatic cell line and may also promote hepatic secretion of TG-rich VLDL (Longcope et al, 1990). It is possible therefore that hyperinsulinemia in the GDM patients decreases the production of SHBG and has an independent effect on lipoprotein synthesis.

Lindstedt et al (1991) have suggested that low plasma SHBG concentration is a strong independent risk factor for the development of NIDDM in women.

Whether the low plasma SHBG concentration or the high testosterone / SHBG ratio we have found in women who have had GDM can be regarded in any way as a causative factor for GDM is uncertain.
In conclusion, epidemiological studies have suggested that obesity is an independent risk factor for cardiovascular disease and related mortality (Bray and Gray, 1988 and Manson et al, 1990).

Most studies have shown that there is a positive correlation between obesity and prevalence of NIDDM (Ashley and Kannel, 1974, Knowler et al, 1981, Stern et al, 1983). Finally, it can be said that GDM patients are more likely to develop NIDDM than controls. In addition, hyperandrogenicity, as assessed by decreased SHBG concentrations, is an important independent risk factor for NIDDM in women (Haffner et al, 1993 b).

In general, obesity is a heterogenous condition. The regional fat distribution seems to be an important determinant of the metabolic complications that have been associated with obesity.

In this study, women with previous GDM exhibit higher WHR, BMI, and body fat % and several abnormalities in lipids and lipoproteins, hyperinsulinemia and insulin resistance, higher blood pressure, and a low level of SHBG.

5.8 Raised Blood Pressure in Women with Previous GDM One Year after Delivery

In this study, the GDM group exhibited higher systolic and diastolic blood pressure and higher MAP than the control group.

Blood pressure was significantly correlated with BMI, plasma insulin, and insulin resistance index (insulin / glucose ratio). It confirms previous findings by other researchers such as Sechi et al (1992). However, this is the first report of raised blood pressure in subjects who have had GDM which suggests that hyperinsulinemia due to
insulin resistance may be the possible underlying mechanism. Fasting insulin concentration, BMI, and alcohol consumption were the significant independent variables in multiple regression analysis for systolic blood pressure.

The pathogenic role of hyperinsulinemia is currently unclear. The insulin resistance-hyperinsulinemia-hypertension hypothesis was shown in Figure 4-2. Insulin has been found to play an important role in the regulation of sodium reabsorption in the distal tubules of the kidney (Skott et al, 1989, DeFronzo, 1981). Water is also reabsorbed with sodium (Feldt-Rasmussen et al, 1987). The mechanism could be that insulin activates sodium-potassium-adenosine triphosphatase activity in the distal tubules of the kidney (Skott et al, 1989) and thus elevates plasma levels of noradrenaline and hence the sympathetic tone (Feldt-Rasmussen et al, 1987). In the pathogenesis of hypertension, impaired vasoconstriction due to imbalance of vasoactive substances and / or raised cytosolicCa\(^{2+}\) and / or a structural vasculopathy is thought to be an important causative event. Insulin resistance in certain trans-membranous cation exchange systems may increase cytosolic Ca\(^{2+}\) (Weidmann et al, 1993).

Another possible mechanism for raised blood pressure in hyperinsulinemic subjects may be based on some alteration in their smooth muscle cells. Intracellular sodium content is increased in erythrocytes from hypertensive patients. If this also occurs in their smooth muscle cells, it may cause narrowing of the lumen of small arteries and therefore explain the peripheral resistance existed in hypertensive patients (Beck-Nielson et al, 1990). Figure 5-2 schematically shows the possible mechanisms for raised blood pressure in the insulin resistance state (Meehan et al, 1993).
Nevertheless, the role of insulin in the pathogenesis of hypertension is controversial. The relation between hyperinsulinemia and blood pressure is not invariable. It is possible that insulin play a role in concert with other factors in the development of essential hypertension (Meehan et al, 1993) or insulin resistance may be due to other factors such as obesity.

Grunfeld and colleagues have found that hyperinsulinemia precedes the onset of clinical hypertension in a population of genetically hypertension-prone subjects such as children of at least one essential hypertensive parent (Ferrari et al, 1991, Grunfeld et al, 1994).

In the present investigation, simple linear regression showed a significant and positive relationship between systolic blood pressure and insulin but not between diastolic blood pressure and insulin.
pressure and insulin. However, multiple linear regression indicated that BMI and the plasma NEFA level are two strong contributors of the variance of both systolic and diastolic blood pressure. As a result BMI and hyperlipidemia and perhaps other factors may cause insulin resistance and insulin resistance could be the precursor mechanisms for other factors in Figure 5-2

A possible explanation for finding BMI as a strong predictor of hypertension (both systolic and diastolic) can be found in the San Antonio Heart Study which showed that insulin was a risk factor for hypertension predominantly in a lean group, with increasing risk associated with rising plasma insulin level. However, no such association was seen in an obese group. Conceivably, hypertension is heterogeneous and insulin plays a role in its development only in lean individuals (Stern, 1983). In our study, GDM subjects had significantly greater BMI and body fat % than controls.

From the frequently noted relation between diabetes mellitus, obesity, and hypertension, the following pathogenic inter-relationships may be proposed (Figure 5-3) (Weidmann et al, 1993).

Several possible mechanisms may be involved in raised blood pressure in obesity, including an increased sympathetic tone, hyperaldosteronism, enhanced blood pressure sensitivity to salt intake, increased cardiac output due to hypervolemia, and also an increased in cystosolic free calcium (Ca^{2+}) and decreased magnesium (Mg^{2+}) levels (Weidmann et al, 1993 b).
Most studies of insulin resistance have concentrated on the regulation of glucose metabolism by insulin. A few researchers have reported impaired fatty acid regulation in the insulin resistance state (Fraze et al, 1986, Swislocki et al, 1987). Plasma NEFA and TG concentrations were higher and HDL-C lower in a hypertensive group than a normotensive group (Pollare et al, 1990).

Singer et al (1985) have reported that 24-hour metabolic profiles of patients with hypertension suggested that insulin, glucose, and NEFA concentrations were increased,
suggesting an impaired sensitivity to insulin for both glucose homeostasis and antilipolysis.

Furthermore, Swislocki and Tsuzuki have reported that the serum FFA (NEFA) level is elevated both in the fasting state and in response to feeding in the spontaneously hypertensive rat (SHR) and they have suggested that this interesting finding is another potential locus of insulin resistance in hypertension (Swislocki and Tsuzuki, 1993). It may be a model for the relationship between NEFA and blood pressure in the present study.

An important link exists between obesity, NIDDM, and hypertension. Hypertension is understood to contribute to the increased cardiovascular morbidity and mortality in patients with obesity and NIDDM (Daly and Landsberg, 1991).

Hypertension in obese individuals with or without NIDDM is clearly related to hyperinsulinemia and hyperinsulinemia excites sympathetic nervous system (SNS) activity. Both hyperinsulinemia and increased SNS activity cause decreased sodium excretion, thus elevating blood pressure (Daly and Landsberg, 1991). This is an possible explanation for raised blood pressure in women with previous GDM.

In conclusion, based on the present information, it may be said that there are several possible explanation for the relationship between insulin resistance or hyperinsulinemia and hypertension.

Firstly, insulin resistance and hypertension may be causally independent but have a common underlying mechanism, such as altered cellular electrolyte metabolism, increased vascular resistance or decreased capacity to vasodilate (especially in skeletal
muscles), increased sympathetic nervous system activity and, once it occurs, obesity (Weidmann et al, 1993). It may be an explanation for our finding.

Secondly, insulin resistance may occur as a secondary modification during the development of hypertension, not affecting its pathogenesis. Insulin resistance and / or hyperinsulinemia, regardless of their underlying mechanism and time of occurrence, may cause or worsen hypertension (Weidmann et al, 1993).

5.9 Microalbuminuria in Women with Previous GDM One Year after Delivery

GDM subjects exhibit a significant microalbuminuria compared to controls based upon spot urine.

Arterial hypertension causes increased UAER. The possible mechanism for this effect is thought an increase in hydrostatic pressure in the glomerular capillaries or a change in permeability in the glomerular basement membrane (Parving et al, 1974, Ljungman, 1990).

Several investigations have suggested that microalbuminuria is strongly related to increased cardiovascular disease risk (Haffner et al, 1990 a, Winocour et al, 1992) and predicts increased mortality rate (Yudkin et al, 1988, Damsgaard et al, 1990). Ross hypothesised that increased UAER is a marker of endothelial dysfunction, including increased trans-endothelial permeability of different macromolecules such as albumin (Ross, 1986). According to the 'response-to-injury' hypothesis, endothelial dysfunction is one of the early step in the atherogenesis process (Ross, 1986).

We found a negative correlation between microalbuminuria and age and it confirms the results reported by Watts et al (1988). The underlying mechanism is not clear.
Microalbuminuria was also related to fasting insulin concentration and insulin / glucose ratio (as a marker of insulin resistance) in women with previous GDM. This association between microalbuminuria and insulin resistance was reported previously by Nosadini et al (1992) in NIDDM or essential hypertension patients with microalbuminuria.

In this study, there is a positive and significant correlation between BMI and microalbuminuria in GDM subjects. Hasslacher et al (1993) also have reported a correlation between BMI and decline of kidney function in proteinuric NIDDM patients.

Subjects with microalbuminuria have higher arterial pressure than diabetic patients without microalbuminuria (Feldt-Rasmussen et al, 1982). We also found that microalbuminuric GDM patients have a higher systolic blood pressure than normoalbuminuric GDM patients.

Approximately 75% of IDDM patients with nephropathy are hypertensive and 25% of them could be normotensive. It is thought that serious morphologic injury might be independent of hypertension in IDDM patients (Sherman and Gosking, 1988, Mauer et al, 1992).

In animal models of IDDM, increased glomerular capillary pressure generally precedes the development of both systemic hypertension and microalbuminuria. There are no comparable data on NIDDM patients. Thus, increased arterial pressure may not be a causal factor but rather the process that provokes a different underlying process (Abott et al, 1994).

Recently, microalbuminuria was reported to be associated with hyperinsulinemia in non-diabetic subjects (Haffner et al, 1990 a). Our study showed an association between
microalbuminuria and increased fasting insulin level in subjects with past medical history of GDM.

The microalbuminuric GDM subjects had significantly elevated plasma cholesterol than normoalbuminuric subjects. Metacalf et al (1992) recently reported the same finding in non-diabetic subjects.

Moreover, the microalbuminuric group demonstrated higher plasma TG levels than the normoalbuminuric group, it confirming Woo’s report (Woo et al, 1992). In the present study, microalbuminuric GDM patients had significantly higher concentrations of VLDL-C and VLDL-TG than normoalbuminuric GDM patients.

Jones et al have reported higher concentration of VLDL-C in IDDM patients and they believe prolonged exposure to dyslipidemia and blood pressure and other risk factors may result the increased risk of coronary heart disease (Jones et al, 1989). The reasons for the apparent effect of hyperlipidemia on kidney function are not fully known.

Animal experiments indicate an increased uptake of lipoproteins by ‘mesangial’ and ‘epithelial’ cells of the glomerulus are a cause of the negative effect of hyperlipoproteinemia on kidney function (Wassermann et al, 1989, Kasiske et al, 1990, Grone et al, 1990, Raner et al, 1990). Secondary to increased uptake of lipoproteins by mesangial and epithelial cells, proliferation of mesangial cells and increased production of mesangial matrix occurs. This promotes the development of glomerulosclerosis (Hasslacher et al, 1993).

Several investigations were performed on humans to study the effect of treatment of hypercholesterolemia with HMG-CoA reductase inhibitors on proteinuria. Rabelink et al (1990) reported a decrease in protein secretion in nephrotic syndrome patients treated by
simvastatin. However, Hommel et al (1992) reported no difference in plasma lipoprotein and renal function during simvastatin treatment in diabetic nephropathy. Further experiments are necessary to clarify this.

Factor VII was significantly and positively related to microalbuminuria in the GDM group. A significant positive correlation was found between urinary albumin excretion rate and factor VII, by us and by Knobl et al (1993). It may suggest that microalbuminuria might be the expression of vascular endothelial damage associated with a hypofibrinolytic and hypercoagulative circumstance (Gruden et al, 1994).

In the present study, fasting insulin level presents a significantly contribution in a multiple regression model to the development of microalbuminuria in the GDM group. In this study, our finding is similar to that of Winocour et al (1992). Nosadini et al have suggested that extrahepatic defects in insulin sensitivity seem to precede the development of hypertension and microalbuminuria in NIDDM patients (Nosadini et al, 1994).

It may be hypothesised that the relationship between hyperinsulinemia as a causal factor and microalbuminuria indicate microalbuminuria as part of the pre-diabetic state. Subsequently, hyperinsulinemia and insulin resistance are the strong predictors of the development of NIDDM (Bogardus et al, 1987, Saad et al, 1988). Also, raised blood pressure and dyslipidemia precede the onset of diabetes (McPhillips et al, 1990, Medalie et al, 1975).

The exact mechanism involved in the role of insulin in microalbuminuria is not completely clear. Nestler et al have reported that transcapillary escape rate of albumin is increased in non-diabetic men in response to marked pharmacological hyperinsulinemia (Nestler et al, 1990). Another possible mechanism behind hyperinsulinemia and
microalbuminuria was suggested by Niskanen and Laakso (1993). They suggested that impaired insulin action or hyperinsulinemia at the kidney level could lead intraglomerular pathology and subsequent albuminuria.

Animal and human studies in renal disease other than diabetic nephropathy have shown that dietary sodium intake influences urinary albumin excretion rate (Donson et al, 1989, Tuck et al, 1990, Lax et al, 1992). Insulin influences renal sodium handling, which causes the sodium retention in diabetic patients (DeFronzo et al, 1992). Sodium intake did not show a significant correlation with microalbuminuria in this study.

The existence of microalbuminuria in non-diabetic subjects indicates that hyperglycaemia cannot be an essential condition for its development. The relationship between microalbuminuria and ischaemic heart disease in both diabetic and non-diabetic subjects propose that a similar, non-glucose related mechanism underlies this relationship in both groups, with the role of hyperglycaemia only to increase renal protein loss and thus progression of glomerulosclerosis to renal failure (Yudkin, 1993).

Haffner et al (1993 a) suggested that microalbuminuria may be a feature of the pre-diabetic state. This may also be the case in this population of women with history of GDM, but follow up studies will be required to see if this is the case.

Based on Stiegler’s suggestion, increased albuminuria may be a marker of early and accelerated atherosclerosis (Stiegler, 1993).

Microalbuminuria is correlated to a hypercoagulative and hypofibrinolytic state. Haemostatic dysfunctions could be a pathogenic link between microalbuminuria and cardiovascular disease (Gruden et al, 1994).
5.10 Coagulopathy in Women with Previous GDM One Year after Delivery

Numerous studies have suggested that coagulation disorders in diabetic patients, which seem to be caused by hyperglycaemia (Ceriello, 1993), give rise to a thrombotic tendency.

High levels of plasma fibrinogen (Coller et al, 1978, Ganda and Arkin, 1992), factor VII (Ceriello et al, 1988), factor VIII, vWF (Osterman and Va-deLoo, 1986), factor X antigen and low activity of factor X in plasma (Ceriello et al, 1990) have been reported in diabetic patients and thought to be due to hyperglycaemia.

The figure 5-5 shows the pathway linking hyperglycaemia and a tendency to thrombosis in diabetic patients. Hyperglycaemia may influence thrombosis by two mechanisms; either by a mechanism connected with non-enzymatic glycation or by an increased oxidative reaction (Ceriello, 1993).

Hyperfibrinogenemia was correlated with body fat % and BMI in the GDM group. A similar result was found by Cigolini et al (1994). In addition, dietary fibre intake showed an independent and negative correlation with fibrinogen in GDM subjects. The mechanism of this association is unclear.
Oral contraceptive use has been shown by Meade et al (1979) to be associated with raised plasma fibrinogen levels and fibrinolytic activity, whereas in our study, there was no significant correlation between oral contraceptives and fibrinogen.

Factor VIIc levels were related positively to BMI in GDM subjects. The same relationship was reported previously by Meade et al (1987) in subjects who had given up smoking. In addition, Factor VIIc also demonstrated significant correlations with lipid and lipoproteins such as plasma cholesterol, TG concentrations, VLDL-TG, IDL-C, and IDL-TG. The mechanism for these correlations is not clear.

In conclusion, in the present investigation, there were no significant haematologic abnormalities in the GDM subjects compared with controls.
Statistical analysis using simple regression indicates that abnormalities in the coagulation system are correlated with aspects of lipid and carbohydrate metabolisms in the GDM subjects.

5.11 Dietary Habits in Women with Previous GDM One Year after Delivery

GDM subjects had significantly higher PUFA and apoE intake in comparison with controls.

Mensink and Katan (1992) have proposed that dietary fatty acids are not the sole or even most important determinant of serum lipid concentrations. Age, genetic background, and degree of obesity are stronger determinants.

In our study, total energy and all macronutrients (fat, carbohydrates, and proteins) intake were associated with WHR as a marker of obesity particularly central obesity (p < 0.001 for total energy, fat and carbohydrate intake and p < 0.01 for protein intake). Studies indicated that weight gain resulting in obesity occurs when energy intake chronically exceeds energy expenditure (Jequier and Schutz, 1983, Woo et al, 1985, Prentice et al, 1986).

Dietary cholesterol intake did not represent an association with plasma cholesterol in this study. Several studies reported no linear relationship between dietary cholesterol intake and total cholesterol levels (Shekelle et al, 1981, Katan et al, 1988), whereas others report a positive correlation between those two (Lamon-Fava et al, 1994).

Our data demonstrated the negative correlation of dietary fibre intake and total cholesterol, apoB, LDL-C, blood pressure, BMI and body fat %. Rouse et al (1983) reported that both systolic and diastolic blood pressure were lower in a vegetarian
population than a meat eating population. The major differences in dietary intake between two groups were that the vegetarian population had a higher intake of polyunsaturated fat, fibre, potassium, magnesium, and calcium and less protein and saturated fat intake.

In addition, other investigators have reported a negative correlation between increased fibre from fruit and vegetables and blood pressure (Kestin et al, 1989). Our findings are consistent with this result.

Soluble dietary fibre such as pectin and oat-bran increases faecal bile-acid excretion and often the excretion of other faecal steroids, causing increased concentrations of hepatic lipoprotein B/E receptors. As a result, circulating cholesterol ester-rich lipoproteins are removed more rapidly (Williams et al, 1986), resulting in decreased plasma total cholesterol. This is an explanation for the negative correlation between fibre intake and total plasma cholesterol.

Dietary fibre intake was shown as an independent variable in simple regression analysis for BMI. The mechanism by which dietary fibre influences obesity are thought to be due to its physiological effects. Dietary fibre fills the stomach and provides a sense of satiety, normalizes intestinal transit time, delay gastric emptying and slow the rate of digestion and absorption of nutrients (Mahan and Arlin, 1992).

In addition, dietary fibre intake was negatively correlated with plasma fibrinogen level and factor VIIc. The mechanisms of this associations (if confirmed) are unclear.

Subjects with > 53% of energy intake as carbohydrates in their diet exhibited significantly lower HDL-C and apoA-I levels than did subjects with lower carbohydrate intake (Lamon-Fava et al, 1994). In the present study, subjects had ~ 45% of energy
intake as carbohydrate. We found a negative correlation between carbohydrate intake and plasma total cholesterol, apoB, LDL-C, and CETP activity in plasma in GDM subjects, and perhaps this was because of their relatively low carbohydrate and high fat intake.

There were positive correlations between all types of energy intake (fatty acids, protein, and carbohydrate) and markers of obesity. It is generally accepted that all sources of energy can be stored as adipose tissue in the body.

Micronutrients are the small quantities of vitamins and minerals that play highly specific roles in facilitating energy transfer. Vitamins contain no useful energy for the body, but generally serve as essential links to help regulate the chain of metabolic reactions that facilitate the release of energy bound in the food molecules and control the process of tissue synthesis (McArdle et al, 1991 c). The GDM subjects included significantly greater quantity of vitamin E in their diet. It is difficult to relate this finding to NIDDM and it may be a chance effect.

There was a suggestion that the intake of green leafy vegetables was lower in the insulin resistant group of people who had had GDM than in others. Whether this observation is a result of chance or whether it reflects reality is not clear. Nevertheless, it seems clear that those women who are overweight and who have insulin resistance are not following current dietary recommendations.

It may be said that the first step to improve the metabolic abnormalities in the GDM or NIDDM subjects who are overweight or obese is weight reduction. The weight loss is an effective tool in improving abnormalities in both carbohydrate and lipid metabolism in
those subjects. A high carbohydrate, low-fat diet such as the Mediterranean diet, is correlated with a reduced risk of CHD (Keys et al, 1986).

The reduction in risk of CHD probably is due to contribution in lipid and lipoprotein metabolism by these diets. A WHO study group has suggested that less than 30% of food energy should be provided by fat and no more than 10% of energy from saturated fat.

5.12 Alcohol Consumption in Women with Previous GDM One Year after Delivery

The significant correlation between alcohol intake and apo A-I and HDL-C levels has previously been observed by other researchers, but this is the first report in a GDM population.

Many studies have observed that alcohol use raised HDL-C both in males (Hubert et al, 1987, Contaldo et al, 1989, Linn et al, 1989) and in females (Linn et al, 1989, Hubert et al, 1987, Bush et al, 1988). The positive correlation between alcohol use and plasma apoA-I which we found is consistent with other investigators’ findings (Camargo et al, 1985, Moore et al, 1988). Alcohol-related HDL-C elevations may be due to increased hepatic apo A-I production (Okamoto et al, 1988).

The mechanism by which ethanol influences HDL concentration remains unknown.

The mechanisms underlying the effect of ethanol on FFA is complex. The effects may occur through several mechanisms.

(1) Increased mobilization of peripheral fat. These actions are depends on the dose of alcohol intake and the dietary factors.

(2) Decreased hepatic fatty acid oxidation.
(3) Increased hepatic fatty acid production.

(4) Increased formation of fatty acyl esters. A clinical study, using some makers of lipolysis, showed an increase of hepatic FFAs with progression of alcoholic liver injury. Usually the free fatty acids are bound to the fatty acyl binding protein (FABP) and this protein increase by ethanol use. Increased formation of fatty acyl esters tends to counteract the accumulation of free fatty acids (Lieber and Pignon, 1989).

5.13 Smoking in Women with Previous GDM One Year after Delivery

In the present study, smoking was not correlated with plasma HDL concentration. Subjects were not heavy smokers.

Current smoking was correlated with increased plasma TG, IDL-TG, IDL-FC, and NEFA concentrations in our investigation. Steiner et al (1987) reported that the men who currently smoked had higher levels of IDL-apoB and of IDL-TG in comparison with men who never smoked or had not smoked for at least 2 years.

It has previously been postulated that smoking may play a role in the development of atherosclerosis and it may be through elevated VLDL remnants levels due to smoking (Topping and Turner, 1975, Topping et al, 1977). Other investigators reported a correlation between TG and smoking (Simons et al, 1984, Cowan et al, 1985). Our findings support all previous findings.

Smoking was associated independently with plasma insulin levels (corrected for age, BMI, use of contraceptive pill, etc) in GDM subjects. The influence of smoking on insulin sensitivity was first studied by Helve et al (1986). They suggested that smoking has a negative effect on insulin sensitivity. Facchini et al (1992) have reported that non-
diabetic smokers are more insulin resistant, hyperinsulinemic, and dyslipidemic than non-diabetic non-smokers. Attval et al (1993) have found that smoking results in impaired insulin action mainly due to decreased peripheral glucose uptake. This results support the Facchini et al study.

Some studies have revealed that glycaemic control is worse among diabetic patients who smoke than diabetic patients who do not smoke (Lundman et al, 1990, Bott et al, 1994). Cigarette smoking has been identified as a risk factor for diabetic nephropathy (Muhlhauser et al, 1986, Stegmayr and Lithner, 1987).

Heavy smoking (a packet or more per day) increases the risk of diabetes (NIDDM) in women (Rimm et al, 1993), it has also been associated with higher HbA1c levels among subjects with normal glucose tolerance (Modan et al, 1988).

The mechanism for any changes in lipids and lipoproteins by smoking is uncertain.

5.14 Physical Activity in Women with Previous GDM One Year after Delivery

Physical activity was related to plasma insulin levels and WHR in this study. Regular physical activity improves glucose tolerance and may induces weight loss (Rauramaa, 1984) and increases insulin sensitivity (Soman et al, 1979). In addition, short periods of exercise can increase plasma glucose concentrations by increasing peripheral and splanchnic insulin sensitivity (Delvin et al, 1987).

Both cross-sectional and prospective studies have suggested an increase in the HDL-C following exercise training especially after aerobic exercise (McCropy et al, 1994). There is no significant correlation between HDL-C and exercise in our study. This
discrepancy might be due to a dose response relation between the amount of habitual exercise and HDL-C elevation (Lakka and Salonen, 1992). It may be said the GDM subjects should exercise more to achieve the beneficial metabolic effects of exercise, or it is due to sample size.

The significant correlations between exercise and CETP activity, plasma apoA-I, VLDL-TG, and NEFA may support the beneficial influence of exercise on lipoprotein metabolism

The significant correlation between exercise and CETP activity indicates that exercise is associated with an increase in reverse cholesterol transport (Gupta et al, 1993).

There was a significant and negative correlation between exercise and WHR. Horton and Geissler (1994) have found that habitual exercise leads to increases daily energy expenditure by 8-14% and increase lean body mass (and results loss of body fat and thus WHR). Alternatively, fat persons might not like to take exercise, resulting in increased obesity.

Long et al (1994) have suggested that weight loss in patients with clinically severe obesity prevents the progression of impaired glucose tolerance to NIDDM by > 30-fold.

Both frequency and intensity of exercise were related to systolic blood pressure in GDM subjects. Systolic blood pressure increases in proportion to oxygen consumption and cardiac output during physical activity, whereas diastolic blood pressure remains relatively unchanged or slightly increased. Following exercise blood pressure falls below pre-exercise levels, and may remain lower for several hours (McArdle et al, 1991 b).

Physical activity is an effective, non-dietary, non-pharmacological option for the management of mild hypertension. Physical activity produced average falls in blood
pressure of 11 mmHg systolic and 6 mmHg diastolic respectively in reported series of hypertensive patients subjected to various forms of isotonic exercise such as walking, running, cycling, swimming and cross-country skiing (Stokes, 1992).

The beneficial effects of physical activity on serum lipids seem to be mediated partially by a decreased serum insulin level and body adiposity (Lakka and Salonen, 1992).

In addition, regular physical activity is effective in preventing NIDDM, and the protective benefit is particularly pronounced in subjects at the highest risk for developing NIDDM (Helmrich et al, 1991).

Results from subgroups analysis showed that the GDM subjects who had positive family history of diabetes mellitus, high plasma NEFA concentrations, and were overweight or obese (BMI > 25 kg / m²) (subgroup A) had several metabolic abnormalities compared with the residual GDM group and controls.

Subgroup A of the GDM group (those who are overweight, have high plasma NEFA concentrations, and a positive family history of diabetes mellitus) tended to be hyperinsulinemic and hyperlipidemic (significantly higher plasma TG, VLDL-TG, and IDL-C concentrations) compared with the residual GDM group.

Subgroup A also tended to be more hyperinsulinemic, hyperlipidemic (significantly higher plasma cholesterol, TG, apoB, VLDL-TG, IDL-C, and LDL-C concentrations, and greater CETP activity in plasma) compared with controls.

The GDM subjects who had positive family history of diabetes and high plasma levels of NEFA but not overweight (BMI ≤ 25 kg / m²) (subgroup B) tended to have better metabolic status than the residual GDM group and similar to controls.
Subgroup B tended to have less insulin resistance (lower fasting insulin concentrations) than the residual GDM group but similar to the residual controls.

In addition, subgroup B tended to have significantly lower lipids and lipoprotein concentrations (plasma cholesterol, TG, VLDL-TG, and IDL-C concentrations) than the residual GDM group but similar to the residual controls.

The subgroup B also had significantly lower BMI and WHR than the residual GDM, but similar to the residual controls.

As a result, a comparison of subgroup A and B suggests that being overweight or obese is an important factor in developing metabolic disorders in women who have positive family history of diabetes mellitus.
CHAPTER SIX

CONCLUSION

6.1 Background

The purpose of our investigation was to test the hypothesis that certain factors are altered in patients who have been diagnosed with GDM compared with controls. Identification, treatment, and follow-up of women with GDM are very important because GDM subjects are at the risk of adverse maternal, fetal, and neonatal outcomes. Subjects who have had GDM are at a high risk of later development of NIDDM. The present study showed that the GDM subjects (ie subjects who had had GDM in their last pregnancy one year previously) were more overweight or obese than controls. The GDM subjects had a family history of diabetes mellitus in first and second degree of relatives, particularly in the maternal side. Obesity like NIDDM is commonly characterized by insulin resistance with increased plasma NEFA concentrations. Distribution of excessive fat on the upper body segment or abdomen, as measured by WHR is correlated with decreased insulin sensitivity and increased risk for developing diabetes (Park, 1993).

The links between insulin resistance and obesity might be multifactorial and bidirectional. Insulin resistance may precede the obesity or insulin resistance may be due to obesity (Park, 1993).
NEFA may have an important role in the metabolic influence of obesity. Plasma NEFA concentration is elevated and fatty acid turnover rate is increased in obesity, and these abnormalities are more pronounced in upper body obesity than lower body obesity (Jensen et al, 1989).

Increased NEFA concentrations result in increased fatty acid oxidation, and this may lead to decreased glucose oxidation and thus decreased glycogen synthetase activity. Decreased glycogen synthetase activity associates result in development of insulin resistance in both liver and muscle. Therefore, increased availability of NEFA is an important link between insulin resistance and obesity (Park, 1993).

Upper body obesity is associated with large fat cells which in turn tend to be insulin resistant. Fat localization (upper body or central) is related to increased activity of androgens, as manifest by lower SHBG and higher free testosterone. In turn, increased activity of androgens is also associated with increasing WHR, increasing plasma glucose and insulin concentrations in both fasting and in response to oral glucose challenge, and decreased insulin sensitivity in obese pre-menopausal women (Evans et al, 1983).

Low SHBG and high testosterone concentration are caused by abdominal obesity (Evans et al, 1983). In the present study, BMI was independently correlated with SHBG levels in multiple regression analysis. In addition, low serum SHBG level has been suggested as strong independent risk factor for the development of NIDDM in women (Lindstedt et al, 1991).

The GDM subjects were overweight or obese, had family history of diabetes, high plasma NEFA concentration, and low SHBG level. As a result, the GDM subjects were more likely to have hyperinsulinemia and insulin resistance. The GDM subjects exhibited higher plasma insulin levels than controls.
Evidence suggests that insulin resistance and hyperinsulinemia may continue for many years in a compensated state with normal glucose tolerance. It appears likely that many of the metabolic disorders associated with insulin resistance have their onset during this compensated state, long before glucose intolerance or frank diabetes.

Thus, insulin resistance may help explain the metabolic disorders often observed in obesity and NIDDM, including dyslipidemia, hypertension, and atherosclerosis (Williams et al, 1994). The present study suggests that insulin resistance secondary to obesity and a family history of diabetes may be cause of dyslipidemia, raised blood pressure, microalbuminuria, and impaired glucose tolerance and NIDDM in women with history of GDM (Figure 6-1).

![Figure 6-1 Hypothetical model based on the present study (DM = diabetes mellitus)]
6.2 Insulin Resistance, Dyslipidemia, and 'GDM'

Increased availability of FFA is an important link between insulin resistance and obesity. The possible mechanism for hyperlipoproteinemia and its related abnormalities found with insulin resistance might be that hyperlipoproteinemia may be caused by enhanced FFAs flux on the insulin-resistance liver, perhaps resulting from central obesity (Park, 1993).

Most causes of insulin resistance including diet, central obesity, and physical activity result in increased flux of FFAs and (probably) glucose to the liver. Cambien et al (1987) reported that hyperinsulinemia insulin is associated with elevated VLDL-TG concentrations positively and decreased HDL-C concentration. This appears to be a consistent relationship between hyperinsulinemia and hypertriglyceridemia in healthy, normal-weight subjects (Zavaroni et al, 1989). The possible underlying mechanism is outlined in Figure 6-2.

Incubation of cell lines with VLDL causes insulin resistance due to either a decrease in insulin receptors or a post-binding defect (Hunt et al, 1989). Thus, it seems insulin resistance promotes dyslipidemia and dyslipidemia may induce insulin resistance.

Winocour et al (1992) have found that hyperinsulinemia is associated with a higher TG / apoB ratio and lower HDL-C / apoA-I ratio after adjustment for BMI in women. Despite a higher apoA-I and HDL synthetic rate in NIDDM patients than controls, plasma HDL-C is reduced in NIDDM patients because of an increased apoA-I and HDL fractional clearance rate (Golay et al, 1987).
LDL is synthesized from hepatic-derived VLDL. IDL particles are produced during the conversion of VLDL to LDL, and IDL particles are thought to be atherogenic (Nordestgaard and Tybjaerg-Hassan, 1992).

Impaired LPL activity can reduce VLDL removal and therefore indirectly influence the flux of material to HDL particles. Additionally, insulin resistance might inhibit CETP or LCAT activity. Alterations in the lipolytic cascade may cause more dense LDL particles (Howard, 1993).

From the results of this study, it may be concluded that the GDM subjects who have a positive family history of diabetes and also are overweight or obese exhibit hyperinsulinemia (as a compensatory response to the insulin resistance), resulting in dyslipidemia in this population.

Dyslipidemia in the GDM subjects is characterized by hypertriglyceridemia and high plasma concentrations of VLDL-TG, high plasma total cholesterol, IDL-C, and LDL-C levels, slightly lower HDL-C, higher CETP activity in plasma, and smaller and denser LDL and HDL₃ particles. There is abundant evidence that hyperinsulinemia and insulin resistance appear to be associated with frequency of atherosclerosis (Stout, 1993).
Figure 6-2 Insulin resistance and lipoprotein abnormalities

6.3 Insulin Resistance, Raised Blood Pressure, and 'GDM'

It has been recognized for many years that hypertension is common in people who are obese (Havlik et al, 1983, Modan et al, 1985) and those who have NIDDM (Modan et al, 1985, Turner, 1985). On the other hand, exercise (Maiorano et al, 1989) and weight loss (MacMahon et al, 1985) has been reported to be effective in lowering the blood pressure in obese and diabetic patients by improving insulin sensitivity.

DeFronzo (1982) has reported that improvement of insulin sensitivity, resulting in lower plasma insulin concentration is correlated with a reduction in both systolic and diastolic blood pressure in non-diabetic obese patients. Thus, it might be said that insulin resistance per se, or through compensated hyperinsulinemia, results in raised systolic and diastolic blood pressure (Manicardi et al, 1986).

Studies have proposed that hyperinsulinemia and / or insulin resistance assist sodium retention, which plays an important role in the development of hypertension. Sodium retention in turn, results in water reabsorption in the kidney tubules (Sharma et al, 1991, DeFronzo, 1981, Kolanowski, 1981).

Hyperinsulinemia may lead to the development of hypertension through activation of the sympathetic nervous system (O'Dea et al, 1982, Sowers et al, 1982).

The possible mechanisms underlying raised blood pressure in the GDM subjects are summarized in Figure 6-3.
6.4 Insulin Resistance, Microalbuminuria, and ‘GDM’

It is thought that there is link between microalbuminuria and insulin resistance. Angiotensin converting enzyme inhibitor agents such as captopril cause an increase in insulin sensitivity (Pollare et al, 1989) and use of these drugs decreases microalbuminuria irrespective of their antihypertensive effects (Parving et al, 1989).
Haffner et al (1990 a) have reported that there is an association between microalbuminuria and a number of cardiovascular risk factors such as hyperinsulinemia, hypertension, and hypertriglyceridemia in non-diabetic subjects.

Nestler et al (1990) have reported an increased trans-capillary escape of albumin in non-diabetic subjects. It may be a possible link between microalbuminuria and hyperinsulinemia. Insulin resistance and or hyperinsulinemia at kidney level may result in intraglomerular pathology and subsequent albuminuria (Niskanen and Laasko, (1993).

In our study, microalbuminuria was correlated with plasma insulin concentration.

6.5 Insulin Resistance, IGT, NIDDM, and ‘GDM’

The pathogenesis of NIDDM can be divided into four phases: genetic susceptibility, insulin resistance, IGT, and NIDDM itself (Bennett et al, 1988). Figure 6-4 demonstrates briefly the pathogenesis of NIDDM.

People who have had GDM with a positive family history of diabetes mellitus have a genetic or inherited susceptibility to NIDDM. In addition, they are significantly fatter and hyperinsulinemic than controls in the present investigation.

Defects in insulin action may be exist in some subjects years before the onset of overt diabetes mellitus. Impaired insulin action is present in individuals at risk for developing NIDDM (pre-diabetic), including relatives of NIDDM patients, people with impaired glucose tolerance, and in ethnic populations with a high prevalence of NIDDM such as Pima Indians (Alzaid and Rizza, 1993).

As a result, we may say that women with previous GDM are likely to develop NIDDM later and hyperinsulinemia is its hallmark.
Several lines of evidence suggest that those who develop overt NIDDM may at first manifest only insulin resistance. Acquired factors such as obesity and inactivity may be additive (Seely and Olefsky, 1993).

![Diagram of proposed etiology for the development of NIDDM](image)

*Figure 6-4 Proposed etiology for the development of NIDDM (adapted from Seely and Olefsky, 1993)*

Impaired glucose tolerance (IGT) is a phase occurring after the insulin resistance phase and before overt NIDDM (WHO, 1985). IGT subjects represent a greater degree of
insulin resistance compared with subjects with normal glucose tolerance. Among IGT subjects those with the greater insulin resistance are most likely to develop overt NIDDM (Bennett et al, 1988).

NIDDM in obese subjects seems to be the end result of a series of changes that occur over many years that culminate in the failure to maintain glucose homostasis (Bennett, 1988).

The proposed pathogenic mechanisms for NIDDM include a primary defect relating to the development of insulin resistance and a secondary defect relating to pancreatic insulin secretory failure (Bennett, 1988).

It seems NIDDM can be delayed or even prevented if somehow the pancreas could be protected from failure. It seems likely this may be achieved by diminishing insulin resistance. Weight loss in obese and overweight subjects, regular exercise, a healthy diet with low saturated fat and adequate fruit and vegetables, stopping smoking, and correction of the medical problems can reduce the degree of insulin resistance degree and may delay or prevent NIDDM in GDM subjects.

In conclusion, this study revealed that women with previous GDM are more likely to have a family history of diabetes mellitus and be fatter than controls, resulting in hyperinsulinemia. Obesity causes higher plasma NEFA concentration and hyperandrogenicity and these abnormalities may cause insulin resistance and hyperinsulinemia. The GDM subjects demonstrated a cluster of abnormalities related to insulin resistance and hyperinsulinemia including dyslipidemia (high cholesterol, TG, VLDL-TG, IDL-C, and LDL concentrations, smaller and denser LDL, and greater CETP activity in plasma), raised blood pressure, and microalbuminuria.
Recommendations for Future Research

This study has investigated the metabolic abnormalities in subjects with previous GDM (post-GDM) one year after delivery. In light of this study, there are several areas in which future research could be conducted:

The major areas of interest will be to determine the crucial factors determining the development of diabetes in these women who have demonstrated a predisposition to diabetes, and determining ways in which this slide into diabetes could be arrested.

Two particular study areas are suggested by the work described in this thesis:

* Subgroup analysis showed that being overweight appears to be a very important factor in the development of metabolic disorders in the GDM group. A study based on weight reduction in both post-GDM women and also in women who are at risk of development of GDM (women who have a positive family history of diabetes and high plasma NEFA concentrations) may show that the abnormalities associated with the insulin resistance syndrome can be reversed.

* Another intervention study may examine the effects of a low fat-high fibre diet and enough exercise in the post-GDM subjects. A low fat-high fibre diet (more fruit and vegetable consumption) and physical activity may induce more insulin sensitivity and thus reduce the risk of development of diabetes mellitus and cardiovascular disease in the GDM group.
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APPENDIX I

Demographic Information

FILE: ........................................ DATE: ..................................................

NAME: ........................................................................................................

ADDRESS: ................................................................................................

................................................ .......................................................

TELEPHONE NO: ....................................................................................

DATE OF BIRTH: .....................................................................................

Name and address of family / personal doctor:

...........................................................................................................

...........................................................................................................

Name and address of two relatives / friends (This will help us locate you if you move from the above address):

...........................................................................................................

...........................................................................................................

...........................................................................................................
Do you presently take any medications:

(please circle correct answer)

No

Yes

(If your answer is yes- please give the name of the medications and briefly give reasons for taking them)

Family history of diabetes, eg. parents, brothers, sisters, other relatives; and age of onset.

What treatment for diabetes mellitus were your relatives receiving? (please tick the relevant correct answer)

............ Diet alone

............ Diet and tablets

............ Diet and insulin
Obstetric History:

Number of pregnancy: .................................................................

Number of stillbirth: .................................................................

Number of abortion: .................................................................

Number of delivery: .................................................................

Date of birth of baby (and maturity- full-term or premature):

Weight of baby at birth: ............................................................

Were you on insulin at any stage of your pregnancy?

(please tick the correct answer)

No

Yes (if yes, please give dosage).................................................

Have you ever taken oral contraceptive pill?

No

Yes

For how long altogether have you taken the oral contraceptive pill?

less than 6 months .................................................................

between 6 months and 2 years ..............................................

between 2 and 5 years ..........................................................

between 5 and 10 years .........................................................

longer than 10 years .............................................................
Are you now taking the oral contraceptive pill?

Yes

No

How often do you usually drink alcohol?

I do not drink alcohol ..........................................
Less than once a week ..........................................
On 1 or 2 days a week ..........................................
On 3 or 4 days a week ..........................................
On 5 or 6 days a week ..........................................
Everyday.................................................................

On a day when you drink alcohol, how many drinks do you usually have?

1 or 2 drinks ..............................................................
3 or 4 drinks ................................................................
5 to 8 drinks .............................................................
9 to 12 drinks ............................................................
13 to 20 drinks ..........................................................
More than 20 drinks ...................................................

Have you ever smoked cigarettes, cigars or pipe regularly?

Yes

No

At what age did you start smoking regularly?

I started smoking regularly at .................. years of age.

Have you given up smoking?

yes, I gave up smoking in ...................( month / year).

No, I still smoke..........................................................
Appendix I
Demographic and Anthropometry Data

If you have given up smoking please answer the following questions:

Now much did you smoke?

I used to smoke .................. manufactured cigarettes a day

.................. grams ‘hand-rolled’ cigarette tobacco per week

.................. cigars per week

.................. grams pipe tobacco per week

**Anthropometric Measurements:**

Usual weight: .................. Present weight ..................

Height ..................

Waist measurement: 1. ..................

2. ..................

3. ..................

Hip measurement: 1. ..................

2. ..................

3. ..................

Waist / Hip ratio: ..................

Skinfold thickness: 1. 2. 3. Triceps

1. 2. 3. Biceps

1. 2. 3. Subscapular

1. 2. 3. Suprailiac

Blood pressure: 1. 2. 3.
Data Elements to be Collected from the patient's medical record and from the patient:

This part of the study will involve the collection retrospectively of data from the patient's medical records, and from the patient. Access to the patient's details will be sought after consent has been given by the patient and agreement negotiated with the Area Health Service.

Data to be collected:

Age:
Height:
Weight:
Waist to Hip Ratio:
Ethnicity:
Date of diagnosis:
Family history of diabetes:
Family history of cardiovascular disease:
Current dietary intake:
Glaucoma: present / not present
Diabetic retinopathy:
Evidence of peripheral vascular disease (PVD):
Evidence of renal disease (pyelonephritis, cystitis, etc):
History of cardiovascular disease in participant:
Blood glucose level:
APPENDIX II

(Chemical Solutions and Assays)

1. GGE buffer (Tris/Borate buffer)

(pH = 8.35)

Measure:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration (mili molar)</th>
<th>Amount in 4 litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trizma base(Tris)</td>
<td>90 mM</td>
<td>43.6 g</td>
</tr>
<tr>
<td>Boric acid</td>
<td>8 mM</td>
<td>19.79 g</td>
</tr>
<tr>
<td>EDTA Na₂</td>
<td>3 mM</td>
<td>4.47 g</td>
</tr>
<tr>
<td>NaN3</td>
<td>3 mM</td>
<td>0.78 g</td>
</tr>
</tbody>
</table>

Measure 4000 ml water in a big beaker and then add solid chemicals.

This buffer can be used 5-6 times for isolated lipoproteins from plasma. Buffer which has been used for whole plasma samples should not be used, subsequently, for density d=1.25 on 4-30 % gels due to albumin contamination.

2. Tris buffers

(a) Making 1 litre of 1 molar Tris buffer with pH = 7.4

Calculation:

\[ g = CVM \Rightarrow \text{Amount (mass)} = \text{Concentration} \times \text{Volume} \times \text{Molecular Mass} \]

\[ g = 1 \times 1 \times 121.1 \]

\[ g = 121.1 \text{ gram Tris} \]
This amount of Tris is dissolved in 1 litre distilled water in a flask.

Solution is put in beaker and pH is measured (should be pH = 7.4), if pH is higher than 7.4, 32% concentrated HCL solution should be added to reach the target pH (pH = 7.4).

This solution will be used as stock to make TBS buffer.

(b) TBS + 0.005% EDTA + 0.02% NaN₃ (for 5 l)

Measure:

43.83 g NaCL

0.25 g EDTA

1 g NaN₃

Mix the measured chemicals with 50 ml of Tris buffer

Measure twice 2 litter of distilled water in volumetric flask and last 1 litter measure in cylinder (but -50 ml of Tris) (950 ml of water).

This buffer can be used for dialysing and dilution.

2. Tracking dye

(Sucrose bromophenol blue solution)

Measure and mix:

4 gram of Sucrose (D-glucose)

0.001 gram of bromophenol blue

Add 10 ml of Tris borate buffer (TBS)

3. GGE stain (protein)

Measure 0.4 g page blue stain (brilliant blue)
Add 950 ml distilled water and stir 2 hours.

Then add 50 ml HClO₄ and stir again 2 hours.

Then filtrate and collect the solution in a labelled bottle.

4. Making 10% sulfosalicylic acid (SSA)

Mix 10 grams of SSA in 100 ml of distilled water in a volumetric flask and stir it. Then filtrate and storage at room temperature

(Note: 10% SSA solution can be reused. After each use it should be filtrated).

This solution is used as a fixer for gels in electrophoresis.

5. Making 5% acetic acid

Measure 950 ml of distilled water in a cylinder and then add 50 ml of acetic acid. very slowly.

6. HDL determination in plasma

Procedure:

1. Allow specimens and precipitation reagents to equilibrate to room temperature.

2. Using a manual pipette, transfer 1.0 ml aliquot of plasma into appropriately labelled eppendorf tubes.

3. Add 100µL of the combined of detrain sulfate-Mg²⁺ solution to each tube.

Immediately after addition of this reagent, mix the contents of each tube in sequence for at least 3 seconds, with a vortex-type mixer.
This amount of Tris is dissolved in 1 litre distilled water in a flask.

Solution is put in beaker and pH is measured (should be pH = 7.4), if pH is higher than 7.4, 32% concentrated HCL solution should be added to reach the target pH (pH = 7.4).

This solution will be used as stock to make TBS buffer.

(b) TBS + 0.005% EDTA + 0.02% NaN₃ (for 5 l)

Measure:

43.83 g NaCl
0.25 g EDTA
1 g NaN₃

Mix the measured chemicals with 50 ml of Tris buffer

Measure twice 2 litter of distilled water in volumetric flask and last 1 litter measure in cylinder (but -50 ml of Tris) (950 ml of water).

This buffer can be used for dialysing and dilution.

2. Tracking dye

(Sucrose bromophenol blue solution)

Measure and mix:

4 gram of Sucrose (D-glucose)
0.001 gram of bromophenol blue

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Procedure:

1. Allow specimens and precipitation reagents to equilibrate to room temperature.

2. Using a manual pipette, transfer 1.0 ml aliquot of plasma into appropriately labelled eppendorf tubes.

3. Add 100μL of the combined of detrain sulfate-Mg²⁺ solution to each tube. Immediately after addition of this reagent, mix the contents of each tube in sequence for at least 3 seconds, with a vortex-type mixer.
4. Allow the tubes to stand at room temperature for 10 minutes before sedimenting the insoluble lipoproteins by centrifugation.

5. Centrifuge tubes for 30 minutes with a refrigerated centrifuge (4°C, 1500×g) or with an unrefrigerated benchtop centrifuge at 1000 rpm for 15 minutes.

6. Remove tubes from the centrifuge and inspect supranatants for turbidity. Transfer the clear supernatants solution by a Pasture pipette to a second labelled vial for later compositional analysis.

*Note:* Any turbidity or cloudiness in the supranatant indicates incomplete sedimentation of VLDL, IDL, and LDL and consequent contamination and overestimation of HDL. This is usually observed in high triglycerides levels in samples.

7. Turbid supranatant can be conveniently cleared by one of the following methods:

   (a) Without separating the turbid supranatant from the precipitate, add to the separation tube 1.0 ml. of 0.15 mol/L NaCl solution and another 100μL of combined precipitant reagent.

   Mix thoroughly with a vortex-type mixer, then centrifuge as previously described.

   Obtain the clear supranatant for analysis. Supernatant cholesterol must be multiplied × 2 to correct for this dilution.

   (b) Alternatively, the turbidity can be removed by ultrafiltration with an 0.22 μm filter.
7. Procedure for HDL Radiolabelled in the cholestryl ester moiety

1. A volume of 50 ml human plasma (from a healthy volunteer) was adjusted to density of 1.13 g/ml with solid KBr and ultracentrifuged for 24 hours at 49,000 rpm in a TL-50.3 rotor at, at 4°C, by using a L8-70 Centrifuge (Beckman, USA).

2. The plasma fraction of d > 1.13 g/ml was recovered by tube slicing and dialyzed overnight against TBS (buffer was changed several times during dialyzing period).

3. A quantity of 200 µmol of [1α, 2α (n)-3H]-cholesterol (specific activity, 46.3 Ci /mmol) in plastic scintillation vial was evaporated to dryness under a stream of nitrogen for approximately 1 hour.

4. Dried radiolabelled cholesterol was then redissolved in 50 µl of ethanol.

5. The plasma fraction of 1.13 g/ml was added to the radiolabelled cholesterol solution under gentle stirring.

6. The mixture was incubated for 24 hours at 37°C in a shaking water bath to allow cholesterol esterification by the LCAT (lecithin:cholesterol acyltransferase) reaction.

7. After incubation period, the solution was measured and adjusted to density 1.13 g/ml with solid KBr and subjected to centrifugation, for 24 hours at 49000 rpm in a 50.3 rotor, at 4°C, by using a L8-70M centrifuge (Beckmann, USA). The aim of this last centrifugation was to remove any radiolabelled lipoproteins of density < 1.13 g/ml, possibly produced during the incubation period.
8. After slicing, infranatant was collected in cylinder and measured.

9. The measured plasma fraction was adjusted to density >1.21 g/ml with solid KBr and centrifugated for 40 hours at 49000 rpm in a TL-50.3 rotor at 4°C.

10. After slicing, supernatant was recovered as HDL₃ radiolabelled.

11. (Washing Spin): KBr solution density = 1.21 was added to HDL₃ radiolabelled sample and subjected to ultracentrifugation for 16 hours at 100000 rpm in a TLA-100.4 rotor, at 4°C, by using a tabletop TL-100 centrifuge (Beckmann, USA)

12. After slicing supranatant was dialyzed against TBS for 24 hours (buffer was changed several times).

13. Thin layer chromatography (TLC) was done on radiolabelled HDL₃. Approximately 95 % of total radioactivity was in cholesteryl ester (CE) as ascertained by (TLC).

8. **TLC determination of cholesterol ester (CE) in radiolabelled HDL₃ and LDL**

Prepare following solutions:

**1. Extraction solution**

160 ml isopropanol

40 ml n-heptane
4 ml 1 N (0.5 M) H$_2$SO$_4$

2. Developing solution for TLC plates

45 ml hexane
20 ml diethyl ether
1.5 ml methanol
1 ml acetic acid

The recovery of cholesterol ester is determined by adding a know amount of 14C-cholesteryl oleate to the extraction solution. Prepare stock solution by adding 10 ul 14C-cholesteryl oleate to 100 ml extraction solution. Prepare working solution by diluting stock solution 1:8 with extraction solution (ie. 0.5 ml stock + 3.5 ml extraction solution). From this, take 2.5 ml at next step.

**procedure:**

1. Add 5 µl radiolabelled HDL$_3$ or radiolabelled LDL to 495 µl TBS buffer (pH = 7.4).

2. Add 40 µl unlabelled HDL$_3$ or LDL (carrier lipoprotein)

3. Add 2.5 ml extraction solution (with 14C-cholesteryl oleate)

4. Cap and vortex

5. Stand at room temperature 20 minutes
6. Add 1.5 ml n-heptane and 1 ml water

7. Cap and vortex

8. Stand at room temperature for 15 minutes. Prepare tank and TLC plate

9. Transfer 800 µl of heptane (top) phase to a scintillation vial labelled ‘CE’ (cholesteryl ester)

10. Transfer 400 µl of heptane (top) phase to a scintillation vial labelled TC (total count)

11. Dry sample under N₂ gas

12. Add 10 ml scintillation fluid to TC vial

13. Add 200 µl chloroform to CE vial and dissolve dried lipid

14. Spot 100 µl of solution from CE vial onto silica gel TLC plate

15. Put the silica gel TLC plate in tank and develop until solvent is front about 2 cm from top of the silica gel TLC plate (CE runs with solvent front)

16. Remove the silica gel TLC plate from tank and dry in fume cupboard

17. Develop with iodine (few crystals)

18. Scrape CE band into scintillation vial. Add 10 ml scintillation fluid

19. Express CE counts as % of total ³H counts

20. Calculate recovery of 14C-cholesteryl oleate
CE vial count / TC vial count × 100 = X (% recovery of CE)

9. CETP activity % in plasma Procedure:

1. 100 µl plasma was mixed with 50 µl [³H] HDL3 (containing 2.5 nmol cholesterol. 
2.2 × 10⁵ counts/minute per 50 µl)

2. Incubate it for 3 hours at 37°C.

3. Place the tubes immediately on ice.

4. Precipitated the apo B containing lipoproteins with dextran sulfate-Mg²⁺ (Warnick et al, 1982)

The CETP assay procedure is summarized in Table 1

5. Supranatants (0.8 of total volume) were collected in plastic scintillation vials. A volume of 10 mL of scintillation fluid (Instagel, Pachard Instrument Company, IL, USA) was added to each vial and the radioactivity was assayed for 10 minute in a ‘Hewlett-Packard scintillation counter’.

The percentage of radioactivity transferred to apo B containing lipoproteins was calculated, as the difference between the total radioactivity of the assay and the radioactivity of the supranatant after precipitation of the VLDL, IDL, and LDL, with adjustment for non-specific transfer.
Table-1: Summerized CETP assay procedure

<table>
<thead>
<tr>
<th>Tube</th>
<th>TBS</th>
<th>HDL₃</th>
<th>plasma</th>
<th>Total volume</th>
<th>Incub Period</th>
<th>15 µl</th>
<th>Total volume</th>
<th>Recover volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2</td>
<td>100 µl</td>
<td>50 µl</td>
<td>-</td>
<td>150 µl</td>
<td>3.h</td>
<td>TBS</td>
<td>165 µl</td>
<td>132 µl</td>
</tr>
<tr>
<td>3,4</td>
<td>100 µl</td>
<td>50 µl</td>
<td>-</td>
<td>150 µl</td>
<td>3.h</td>
<td>Dextr sulfate</td>
<td>165 µl</td>
<td>132 µl</td>
</tr>
<tr>
<td>5,6</td>
<td>-</td>
<td>50 µl</td>
<td>100 µl (+cont)</td>
<td>150 µl</td>
<td>3.h</td>
<td>Dextr sulfate</td>
<td>165 µl</td>
<td>132 µl</td>
</tr>
<tr>
<td>7,8</td>
<td>-</td>
<td>50 µl</td>
<td>subject sample</td>
<td>150 µl</td>
<td>3.h</td>
<td>Dextr sulfate</td>
<td>165 µl</td>
<td>132 µl</td>
</tr>
<tr>
<td>9,10</td>
<td>-</td>
<td>50 µl</td>
<td>subject sample</td>
<td>150 µl</td>
<td>3.h</td>
<td>Dextr sulfate</td>
<td>165 µl</td>
<td>132 µl</td>
</tr>
</tbody>
</table>

Incub = Incubation, h = hours, Dextr = Dextran, (+ cont) = positive control, Recover = Recovery.
Appendix II Chemical Solutions and Assays

CHOL

Contents

<table>
<thead>
<tr>
<th>Preparation and stability of the reagent solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPR 1: Dissolve contents of one bottle 1 by adding 32 ml redist. water.</td>
</tr>
<tr>
<td>MPR 2: Dissolve contents of one bottle 1 by adding 100 ml redist. water.</td>
</tr>
<tr>
<td>MPR 3: Dissolve contents of one bottle 1 by adding 500 ml redist. water.</td>
</tr>
</tbody>
</table>

The reagent solution is ready to use after 10 minutes. Stable for four weeks at +2 to 8°C seven days at +15 to 25°C.

MPR 1 1442341 10 x 32 ml
MPR 2 1442350 10 x 100 ml
MPR 3 236691 4 x 500 ml

Cholesterol CHOD-PAP method

MPR 1
10

Preparation and stability of the reagent solution

Cholesterol

Method

Test principle

Cholesterol esters + H₂O ➞ cholesterol esterase ➞ cholesterol + RCOOH
cholesterol + O₂ ➞ cholesterol oxidase ➞ Δ⁴-cholestenone + H₂O₂
2 H₂O₂ + 4-aminophenazone + phenol ➞ POD ➞ 4-(p-benzoquinone-monooimin)-phenazone + 4 H₂O

Clinical interpretation according to the recommendations of the European Atherosclerosis Society:

<table>
<thead>
<tr>
<th>Lipid disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
</tr>
<tr>
<td>&lt; 200 mg/dl</td>
</tr>
<tr>
<td>&lt; 200 mg/dl</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Triglycerides</td>
</tr>
<tr>
<td>&lt; 200 mg/dl</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>&lt; 35 mg/dl</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>&lt; 350 mg/dl</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>&gt; 300 mg/dl</td>
</tr>
<tr>
<td>Yes</td>
</tr>
</tbody>
</table>

Values in mmol/L:

Cholesterol:
35 mg/dl = 0.9 mmol/l
200 mg/dl = 5.2 mmol/l
300 mg/dl = 7.8 mmol/l

Triglycerides:
200 mg/dl = 2.3 mmol/l

Sample material

Serum, heparinized plasma or EDTA plasma
Stable for six days at +4 to 25°C
four months at -20°C.

Procedure

Wavelength: Hg 546 nm (470-560 nm)
Spectrophotometer: 500 nm
Cuvette: 1 cm light path
Incubation temperature: 20-25°C or 37°C
Measure against reagent blank (RB).

Dilution threshold

1000 mg/dl (25.9 mmol/l)
At higher cholesterol concentrations, dilute 0.1 ml of sample material with 0.2 ml of 0.9% NaCl solution and repeat assay (result x 3).

Calculation of the concentration (c) of cholesterol in the sample:

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>mg/dl</th>
<th>mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg 546 nm</td>
<td>c = 853 x A₃₀₀</td>
<td></td>
</tr>
<tr>
<td>500 nm</td>
<td>c = 575 x A₃₀₀</td>
<td></td>
</tr>
</tbody>
</table>

Concentrations of the reagent solution

Tris buffer: 100 mmol/l; pH 7.7; Mg²⁺: 50 mmol/l; 4-amino-phenazone: 1 mmol/l; sodium cholate: 10 mmol/l; phenol: 6 mmol/l; 3,4-dichlorophenol: 4 mmol/l; fatty alcohol polyglycol ether: 0.3%; cholesterol esterase ≥ 0.4 U/ml; cholesterol oxidase ≥ 0.25 U/ml; peroxidase ≥ 0.2 U/ml

Quality control

Accuracy: Precinorm® U, Precinorm® L, Precipath® U, Precipath® L
Precision: Precinorm® UPX

Please note

If difficulties encountered in quality control point to inadequate linear response on the part of the photometer, or if measurements cannot be taken at Hg 546 nm or 500 nm, a calibration curve must be constructed using Preciset® Cholesterol (Cat.No. 125 512). The cholesterol values are read off the curve.

Bilirubin concentrations above 4 mg/dl interfere with the test (results up to 10% too low).

Hemoglobin concentrations up to 200 mg/dl do not affect the test.

Reference

1 Study Group, European Atherosclerosis Society.
Appendix II Chemical Solutions and Assays

Test-Combination

Free Cholesterol

CHOD-PAP method
Enzymatic colorimetric method
Cat. No 310328 for 2 x 90 ml

Method

Test principle
cholesterol $+ O_2 \rightarrow$ cholesterol oxidase $\rightarrow$ cholesterol-1,2-dihydroxyacetone + H$_2$O$\_2$
2 H$_2$O$_2$ + 4-aminophenazone + phenol + POD $\rightarrow$ 4-(p-benzoquinonemonoimino)phenazone + 4 H$_2$O

Normal values for free cholesterol:
22-30% of total cholesterol


Sample material
Serum, heparinized plasma or EDTA plasma

Reagents

<table>
<thead>
<tr>
<th>Contents</th>
<th>Initial concentrations of solutions:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Buffer (potassium phosphate)</td>
<td>0.4 mol/l, pH 7.7</td>
</tr>
<tr>
<td></td>
<td>20 mmol/l</td>
</tr>
<tr>
<td></td>
<td>methanol</td>
</tr>
<tr>
<td></td>
<td>1.85 mol/l</td>
</tr>
<tr>
<td>2 Buffer (potassium phosphate)</td>
<td>0.4 mol/l, pH 7.7</td>
</tr>
<tr>
<td></td>
<td>2 mmol/l</td>
</tr>
<tr>
<td></td>
<td>methanol</td>
</tr>
<tr>
<td></td>
<td>1.85 mol/l</td>
</tr>
<tr>
<td></td>
<td>hydroxypropyloxycylohexadecane</td>
</tr>
<tr>
<td></td>
<td>0.4%</td>
</tr>
<tr>
<td>3 Cholesterol oxidase</td>
<td>$\geq 12$ U/ml</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>$\geq 8$ U/ml</td>
</tr>
</tbody>
</table>

Quality control
For control of accuracy: Pectrip E.L., Precinorm L

Preparation and stability of solutions
1, 2 and 3 Use solutions undiluted.
Stable up to the expiry date specified when stored at +2 to +8°C.

4 Cholesterol reagent:
Pipette 0.5 ml of sol. 3 into bottle 2 and add the entire contents of one bottle 1, or make up stock solution according to the following table:

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>solution 1, ml</th>
<th>solution 2, ml</th>
<th>solution 3, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ca. 9</td>
<td>5</td>
<td>5</td>
<td>0.05</td>
</tr>
<tr>
<td>ca. 19</td>
<td>20</td>
<td>20</td>
<td>0.20</td>
</tr>
<tr>
<td>ca. 39</td>
<td>40</td>
<td>40</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Stable for two weeks at +2 to +8°C
Stable for two days at +15 to 25°C.

5 Reagent for sample blank:
(req'd only for assays on hemolytic or turbid sera)
Mix equal volumes of solutions 1 and 2 and store away from light
Stable for two weeks at +2 to +8°C
Stable for two days at +15 to 25°C.

Procedure A
Sample material: clear, non-hemolylic serum
Wavelength: Hg 546 nm (470-560 nm)
Spectrophotometer: 500 nm
Glass cuvette: 1 cm light path
Incubation temperature: 20-25°C or 37°C
Measure against reagent blank (RB)
One reagent blank is sufficient for each assay series.

One reagent blank is sufficient for each assay series.

Calculation

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>mg/100 ml</th>
<th>mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg 546 nm</td>
<td>$c = 855 \times \Delta A$</td>
<td>$c = 22.1 \times \Delta A$</td>
</tr>
<tr>
<td>500 nm</td>
<td>$c = 585 \times \Delta A$</td>
<td>$c = 15.1 \times \Delta A$</td>
</tr>
</tbody>
</table>

Please note
If difficulties encountered in quality control point to inadequate linear response on the part of the photometer, or if measurements cannot be taken at Hg 546 nm or 500 nm, a calibration curve must be constructed using Preciset Cholesterol (Cat.No. 125 512). The cholesterol values are read off the curve.
A slight yellow coloration of solution 2 does not affect the test.
Even low concentrations of ascorbic acid and ß-methyldopa in the specimen depress the free cholesterol level.

Solutions 1 and 2 contain methanol, which is poisonous. Do not swallow or inhale vapors. Avoid contact with skin. If solution comes into contact with skin or mucous membranes, flush immediately with large quantities of water. If solution comes into contact with eyes, immediately flush liberally with water and consult an ophthalmologist.

Boehringer Mannheim GmbH
Diagnostica
Triglycerides GPO-PAP

High performance Enzymatic colorimetric test

Perldochrom® Triglycerides GPO-PAP
Cat.No. 701 882 for 6 x 13 ml reagent
Cat.No. 701 904 for 15 x 32 ml reagent

Test-Combination Triglycerides GPO-PAP
Cat.No. 701 912 for 5 x 100 ml reagent

Additional reagent for measurement against standard:
Precimat® Glycerol, Cat.No. 166 588

Method
Enzymatic hydrolysis of triglycerides with subsequent determination of the liberated glycerol by colorimetry.

References:
- Nagale, U. (Boehringer Mannheim GmbH), personal communication.

Test principle...

Sample material
Serum, heparinized plasma or EDTA plasma

Reagents
1 Buffer solution
2 Reagent strips or lyophilisate

Reagent concentrations in solution
1 Tris buffer: 0.15 mol/l, pH 7.6; magnesium sulphate: 17.5 mmol/l; EDTA, disodium salt: 10 mmol/l; 4-chlorophenol: 3.5 mmol/l; sodium chloride: 0.15%; potassium hexacyanoferrate (II): 6 mmol/l; hydroxypropyl ether-β-alkanes: 0.12%
2 ATP ≥ 0.5 mmol/l; 4-aminophenazone: 0.35 mmol/l; lipase ≥ 3 U/ml; glycerol phosphate oxidase ≥ 2.5 U/ml; glycerol kinase ≥ 0.2 U/ml; peroxidase ≥ 0.15 U/ml

Quality control
For control of accuracy: Precinorm® U, Precinorm® L, Precipath® U, Precipath® L
For precision control: Precinorm® UPX

Preparation and stability of reagent solution
Perldochrom® Triglycerides GPO-PAP
Do not touch the reagent patches or surrounding area.
Immerse one reagent strip in one bottle of buffer solution and use to stir the bottle contents for ca. 10 sec. Allow to stand in buffer solution for 5 min; stir once again for ca. 10 sec, and then discard reagent strip.

Test-Combination Triglycerides GPO-PAP
Connect one bottle to one bottle 2 with one of the adapters provided in the kit and flush several times to ensure complete dissolution of the lyophilisate.
Reagent solution from different bottles may be pooled for large series.
Stable for two weeks at +2 to 8°C.
two days at +15 to 25°C.

Sample preparation
The sample can be stored up to three days at +4°C.
four months at —20°C.

Procedure
Wavelength: Hg 546 nm
Spectrophotometer: 500 nm
Cuvette: 1 cm light path
Incubation temperature: 20–25°C or 37°C
Measure against reagent blank. One reagent blank is sufficient for each series.

Pipette into test tubes:
serum or plasma reagent solution 2.00 ml
Mix, and incubate at 20–25°C for 10 min.
Read absorbance of sample against reagent blank within 60 min.

If incubating at 37°C (10 min), the absorbance of the standard must be determined once for each assay series using Precimat® Glycerol instead of serum or plasma. The absorbance of the sample and the standard must be read within 30 min.

Calculation via factor
Obtain the concentration (c) of triglycerides in the sample from the enclosed table of values or calculate as follows:

Calculation via standard
Calculate the concentration (c) of triglycerides as follows:

Additional reagent for measurement against standard: Precimat® Glycerol, Cat.No. 166 588

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Stable for two weeks at +2 to 8°C.
two days at +15 to 25°C.

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four months at —20°C.

Procedure
Wavelength: Hg 546 nm
Spectrophotometer: 500 nm
Cuvette: 1 cm light path
Incubation temperature: 20–25°C or 37°C
Measure against reagent blank. One reagent blank is sufficient for each series.

Pipette into test tubes:
serum or plasma reagent solution 2.00 ml
Mix, and incubate at 20–25°C for 10 min.
Read absorbance of sample against reagent blank within 60 min.

If incubating at 37°C (10 min), the absorbance of the standard must be determined once for each assay series using Precimat® Glycerol instead of serum or plasma. The absorbance of the sample and the standard must be read within 30 min.

Calculation via factor
Obtain the concentration (c) of triglycerides in the sample from the enclosed table of values or calculate as follows:

Calculation via standard
Calculate the concentration (c) of triglycerides as follows:
Apolipoprotein A-1
4 x 10.5 mL

Intended use
Unimate 3 APOA is an in vitro diagnostic reagent intended for the quantitative immunological determination of human apolipoprotein A-1 (Apo A-1) in serum.

Method
Immunoturbidimetric.

Principle
Human apolipoprotein A-1 forms a precipitate with a specific antiserum which is determined turbidimetrically (measurement of cloudiness) at 340 nm (fixed time method).

Specimen
Serum.
Samples should be fresh, stored for a maximum of 1 week at +2 to +8 °C or stored frozen once at -20 °C.

Reference values
Apolipoprotein A-1
Females 115–220 mg/dL (1.15–2.2 g/L)
Males 115–190 mg/dL (1.15–1.9 g/L)
Apolipoprotein B
Females 60–150 mg/dL (0.6–1.5 g/L)
Males 70–160 mg/dL (0.7–1.6 g/L)
Ratio Apolipoprotein B/Apolipoprotein A-1
Females 0.35–1.15
Males 0.45–1.25
For the IFCC Reference Preparation SP 1 (October 1992 WHO-IRP) for apolipoprotein A-1 and the IFCC Reference Preparation SP 3-07 for apolipoprotein B the following additional reference values apply:

Apolipoprotein A-1
Females 1.20–2.20 g/L
Males 1.10–2.00 g/L
Apolipoprotein B
Females 0.55–1.25 g/L
Males 0.55–1.35 g/L

Quality control
Apolipoproteins T Control, art. 07 3721 6 (US # 42391)

Kit contents
Reagents
Working reagent
The reagent R is ready to use. Warm up the reagent to room temperature prior to assay. The reagent once opened is stable for 4 weeks at +2 to +8 °C in the original bottle if it is immediately and tightly closed after use.
Additionally required (not delivered with the kit):
Apolipoproteins T Standard, art. 07 3720 8 (US # 42390)
Apolipoproteins T Control, art. 07 3721 6 (US # 42391)
NaCl solution 154 mmol/L (0.9%)

Procedure
For applications on COBAS® instruments please refer to the corresponding test instruction manuals.

Notes
Measuring range: 35–550 mg/dL (0.35–5.5 g/L).
The measuring range is determined by the batch specific apolipoprotein A-1 in the standard serum and the serial dilutions selected.
The reagent R contains < 0.1% sodium azide as preservative. Avoid swallowing and contact with skin or mucous membranes.

References
**UNI-KIT**

**Apolipoprotein B**

**T Antiserum**

Art. 07 2995 7 (4 x 10.5 ml)

Antiserum (rabbit) for the quantitative immunological determination of human apolipoprotein B in serum.

For in vitro diagnostic use

Also required:
- T Standard Apolipoproteins (Roche) (art. 07 3068.8)
- NaCl solution 154 mmol/l (0.9%)

### General information

**Method**

Immunoturbidimetric.

**Principle**

Human apolipoprotein B forms with a specific antiserum a precipitin which is determined turbidimetrically (measurement of cloudiness) by a fixed time method at 340 nm (or 334 or 365 nm).

**Sample**

Fresh serum or stored for a maximum of 1 week at +2 to +8 °C. Very turbid (lipemic) samples should be diluted prior assay.

**Package**

T Antiserum Apolipoprotein B 10.5 ml per bottle

Antiserum specific for human apolipoprotein B in phosphate buffer stabilized with sodium azide.

The bottles, when unopened, are stable at +2 to +8 °C until the expiry date stated on the labels.

**Reference values**

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo A-1</td>
<td>1.15-1.90 g/l (median 1.45 g/l)</td>
<td>1.15-2.0 (median 1.60 g/l)</td>
</tr>
<tr>
<td>Apo B</td>
<td>0.70-1.60 g/l (median 1.15 g/l)</td>
<td>0.60-1.50 (median 1.05 g/l)</td>
</tr>
<tr>
<td>Ratio Apo B/Apo A-1</td>
<td>0.45-1.25 (median 0.80)</td>
<td>0.35-1.15 (median 0.65)</td>
</tr>
</tbody>
</table>

**Quality control**

T Control Apolipoproteins (Roche) (art. 07 3069 6)

**Manual procedure**

**Preparations**

The T Antiserum Apolipoprotein B is ready to use. Prior assay warm up the reagent to room temperature. The reagent, once opened is stable 4 weeks at +2 to +8 °C. 1 week at +15 to +25 °C in the original bottle if it is immediately and tightly closed after use.

**Samples and controls**

Dilute the samples and controls with NaCl (0.9%) in ratio 1:21 (1:20).

**Main standard**

Reconstitute T Standard Apolipoproteins (Roche) with 0.5 ml of distilled water. Allow to stand at +15 to +25 °C for 30 minutes. Then gently swirl for 1 minute. The reconstituted standard is ready to use and is stable for 1 week at +2 to +8 °C.

**Standard serial dilutions**

Dilute the reconstituted T Standard Apolipoproteins (Roche) with NaCl solution in a geometric series, starting with 1:6 in order to have the following series: 1:6, 1:12, 1:24, 1:48, 1:96.

For diluting this pipetting schema can be used:

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>T Standard reconstituted</td>
<td>1:6</td>
</tr>
<tr>
<td>Dilution 1:6</td>
<td>1:12</td>
</tr>
<tr>
<td>Dilution 1:12</td>
<td>1:24</td>
</tr>
<tr>
<td>Dilution 1:24</td>
<td>1:48</td>
</tr>
<tr>
<td>NaCl 0.3%</td>
<td>500</td>
</tr>
<tr>
<td>NaCl 0.9%</td>
<td>300</td>
</tr>
<tr>
<td>NaCl 1.8%</td>
<td>300</td>
</tr>
</tbody>
</table>

The dilutions of the standard are stable during 1 week at +2 to +8 °C if stored in closed vials (risk of contamination).

**Bibliographie**

Diet and Health Booklet
ABOUT THIS STUDY

This survey is one of a series designed to describe the current health and nutrition status of groups of Australians. It forms part of a study on aspects of health which has been outlined in a covering letter to you.

In this booklet there are a number of questions which provide information on food habits and usual dietary intake. Such information is essential if we are to identify any nutrition problems and understand the relationships between diet and disease in the general population and in groups of people at special risk. You may be a member of the general public in good health or you may be a person with a health problem. Information from both groups of people is necessary.

All the information you give us will be treated in the strictest confidence. No personal details that can identify you will be revealed to anyone, and your name will not be attached to this information; instead, a code number will be used. If the study in which you are involved is one in which we may want to contact you some time in the future, we need to keep your name, address, contacts and code number securely on file, but separately from other information relating to you. All such identifying information which may be used to contact you in the future will be kept in a secure place by Professor Calvert.

Please answer all the sections carefully. You may have made arrangements to return the booklet to a research associate. If you have not made any such arrangement, please return the booklet to me as soon as possible.

Thank you for your help.

Dennis Calvert  
Professor of Medicine and Public Health  
Medical Research Unit  
Illawarra Regional Hospital (Wollongong Campus)  
Crown St  
WOLLONGONG NSW 2500

Telephone (042) 266594

Please ring me on this number if you have any problems. Subsequent enquiries may be directed to Ms Kim Roser, Secretary to the Human Ethics Committee of the Illawarra Area Health Service/University of Wollongong.
YOUR HEALTH

Q-1 In general, how has your health been in the last year? (Please circle one)

<table>
<thead>
<tr>
<th></th>
<th>Very Good</th>
<th>Good</th>
<th>Fair</th>
<th>Poor</th>
<th>Very Poor</th>
</tr>
</thead>
</table>

Q-2 How much of an effect do you think the following things have on the health of people like yourself? (Circle one for each)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Bad effect</th>
<th>Moderate</th>
<th>No effect</th>
<th>Don't know</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eating the wrong foods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air and chemical pollution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working in a dirty job, with dust or chemicals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking too much alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not having a good doctor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Having parents who have or had poor health</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not keeping weight under control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excess stress caused by work, lack of work or work at home</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of exercise or physical activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Problems within the family</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not living in a clean, safe house and neighbourhood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bad luck</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worries caused by not having enough money</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**YOUR HEALTH**

These are questions about your current health and that of close family or friends, as these factors may influence the way you eat.

**Q-3 (a) Have you or your spouse (if relevant) ever had any of the following conditions?**

(Circle YES or NO)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yourself</th>
<th>Your Spouse (husband/wife/partner etc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>High blood pressure</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>High blood cholesterol</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Heart problems (including angina)</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Bowel complaints</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>A malignant growth or cancer</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Arthritis</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Any other medical condition</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>(please describe)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Q-3 (b) Has any other close family member or friend (parent, child, sister, brother, close friend) ever suffered from any of the following? (Circle YES or NO for each and tells us what relation they are to you)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>What relation?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td></td>
</tr>
<tr>
<td>High blood pressure</td>
<td></td>
</tr>
<tr>
<td>High blood cholesterol</td>
<td></td>
</tr>
<tr>
<td>Heart problems (including angina)</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
</tr>
<tr>
<td>Bowel complaints</td>
<td></td>
</tr>
<tr>
<td>A malignant growth or cancer</td>
<td></td>
</tr>
<tr>
<td>Arthritis</td>
<td></td>
</tr>
<tr>
<td>Other condition (please specify)</td>
<td></td>
</tr>
</tbody>
</table>

**Q-4 Are you currently on any type of medication?** (Circle one number)

1. No
2. Yes
   - if Yes, what type(s) of medication?
YOUR EATING HABITS

This section is about the kinds of foods you usually eat. On the next few pages you will find lists of foods, separated by questions about your dietary habits.

Read through each list of foods and record about how often you usually eat these foods. We realise that your food intake may vary from time to time, so just try to give us the best overall picture of your diet that you can.

We are interested in YOUR diet, not that of someone else in your household.

THIS IS HOW TO ANSWER

We are going to ask you "About how often do you usually eat these foods?"
Use the following simple code to write your answer in the space next to each food.

If you NEVER have a food ........................................... write N
If you RARELY have a food (less than once a month) .... write R

If you usually eat a food

About once a MONTH ............................................. write 1M
About twice a MONTH ......................................... write 2M
About three times a MONTH ................................... write 3M

About once a WEEK ............................................. write 1W
About twice a WEEK .......................................... write 2W
About three times a WEEK .................................... write 3W
and so on .................................................................. (4W, 5W, 6W, etc)

About once a DAY ................................................ write 1D
About twice a DAY ............................................... write 2D
and so on .................................................................. (3D, 4D, 5D, etc)

Standard serve
Alongside each food there is a "standard serve" size. The "standard" serve is not necessarily a "normal" serve, it is simply there to help us measure food intake. If you usually eat more or less than the standard serve size for a particular food, please indicate on the COMMENTS line how much more, or less, is eaten at a time.

For example, if when you eat icecream you have one "scoop" instead of our "standard" serve of two "scoops", indicate how often icecream is eaten, and then write "one scoop only" on the comments line.

On the opposite page you will see some examples of how to fill out the questionnaire. Please read these carefully before you start to fill out the answers for your diet.
HOW TO ANSWER

<table>
<thead>
<tr>
<th>NEVER</th>
<th>RARELY</th>
<th>Times a MONTH</th>
<th>Times a WEEK</th>
<th>Times a DAY and so on</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>R</td>
<td>1 M</td>
<td>3 W</td>
<td>3 D</td>
</tr>
</tbody>
</table>

HERE ARE SOME EXAMPLES

<table>
<thead>
<tr>
<th>Food</th>
<th>Quantity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Custard</td>
<td>1/2 cup</td>
<td>3 W...</td>
</tr>
<tr>
<td>Boiled egg</td>
<td>1 egg</td>
<td>3 M... two eggs</td>
</tr>
<tr>
<td>Cucumber</td>
<td>3 slices (each 0.5 cm thick)</td>
<td>R...</td>
</tr>
<tr>
<td>Tea</td>
<td>1 cup</td>
<td>4 D...</td>
</tr>
<tr>
<td>Beetroot - canned</td>
<td>2 slices</td>
<td>2 M... one slice</td>
</tr>
</tbody>
</table>

The person above has, on average:

- A standard serve of custard three times a week
- Two boiled eggs three times a month
- Rarely eats cucumber
- Four cups of tea every day
- Half a standard serve (1 slice) of beetroot - canned, twice a month

We realise that some people have an exact idea of how often they eat particular foods, whilst others only have an approximate idea. Be as accurate as you can but do not spend too much time choosing your answers.

PLEASE GIVE AN ANSWER FOR EVERY FOOD.
### HOW TO ANSWER

<table>
<thead>
<tr>
<th>NEVER</th>
<th>RARELY</th>
<th>TIMES A MONTH</th>
<th>TIMES A WEEK</th>
<th>TIMES A DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>R</td>
<td>M</td>
<td>W</td>
<td>D</td>
</tr>
</tbody>
</table>

and so on and so on

### ABOUT HOW OFTEN DO YOU USUALLY EAT THESE FOODS?

#### CEREALS

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porridge/Oatmeal</td>
<td>1 cup (cooked)</td>
<td></td>
</tr>
<tr>
<td>Muesli</td>
<td>1/2 cup</td>
<td></td>
</tr>
<tr>
<td>Other breakfast cereal</td>
<td>1 cup</td>
<td></td>
</tr>
<tr>
<td>Plain bran (raw)</td>
<td>1 tablespoon</td>
<td></td>
</tr>
<tr>
<td>Wheatgerm</td>
<td>1 tablespoon</td>
<td></td>
</tr>
<tr>
<td>Bread roll (NOT hamburger buns)</td>
<td>1 roll</td>
<td></td>
</tr>
<tr>
<td>Fried rice</td>
<td>1 cup (cooked)</td>
<td></td>
</tr>
<tr>
<td>Boiled rice</td>
<td>1 cup (cooked)</td>
<td></td>
</tr>
<tr>
<td>Instant noodles (Maggi etc)</td>
<td>1 cup (cooked)</td>
<td></td>
</tr>
<tr>
<td>Other pasta (spaghetti, macaroni etc)</td>
<td>1 cup (cooked)</td>
<td></td>
</tr>
</tbody>
</table>

#### Q-1 How many slices of bread do you usually eat? Remember the bread in toast and sandwiches. If you do not eat bread, write 'none'.

<table>
<thead>
<tr>
<th>Slices/day</th>
<th>Slices/week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Q-2 What type of bread do you usually eat? (Circle the number beside one answer)

1. Wholemeal or mixed grain
2. White
3. About half the time wholemeal and half white
4. Other breads (eg rye, Hi-Fibre)
   (please specify type)
5. I do not eat bread

#### Q-3 Do you eat low-salt types of bread? (Circle one answer)

ALL or MOST OF THE TIME OCCASIONALLY RARELY/NEVER
Q-4 Which of the following do you usually spread on bread or crackers? (Circle one answer)

1. Butter
2. Polyunsaturated margarine
3. Table or cooking margarine
4. Reduced-fat margarine (eg Era)
5. Dripping/Lard
6. I don't use anything
7. I don't eat bread or crackers
8. Something else: please name.................................................................

Q-5 For whatever spread you use is it usually the regular variety or reduced-salt? (Circle one answer)

1. I usually use the regular variety
2. I usually use the reduced-salt variety

Q-6 What types of breakfast cereals do you most commonly eat?

Please name: ........................................................................................................

Q-7 If you eat muesli is it:

1. Homemade muesli
2. Pre-packaged muesli

Q-8 How many cups of milk do you usually add to breakfast cereal, porridge or muesli? (Circle the number closest to the amount you have)

1. None
2. About a half a cup
3. About one cup
4. About one and a half cups
5. About two cups or more (please state how much.............................................)

Q-9 What type of milk do you usually add to cereals, porridge or muesli? (eg whole milk, Skimmer, Rev, Tone, Trim, powdered skim, Shape, High-Low, goat's milk, condensed/evaporated milk etc)?

Type of milk added: ...........................................................................................

Q-10 How many teaspoons of sugar or honey do you usually add to cereal, porridge or muesli? (Note: 1 dessertspoon = 2 teaspoons)

Write the number of teaspoons you have here: .........................

Q-11 Do you add salt to your porridge? (Circle one number)

1. Yes
2. No
3. I don't eat porridge
### ABOUT HOW OFTEN DO YOU USUALLY EAT THESE FOODS?

#### CEREAL FOODS

<table>
<thead>
<tr>
<th>Food Description</th>
<th>Never</th>
<th>Rarely</th>
<th>Once a Month</th>
<th>Once a Week</th>
<th>Once a Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crumpet or Muffin</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Croissant</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit Loaf/Currant bread</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Sweet bun/Doughnut</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crispbread/Cracker</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salted biscuits</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain sweet biscuits</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fancy biscuits (eg choc-coated)</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cake</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Comments

- Crumpet or Muffin: ........................................
- Croissant: ........................................
- Fruit Loaf/Currant bread: 1 slice
- Sweet bun/Doughnut: ........................................
- Crispbread/Cracker: ........................................
- Salted biscuits: ........................................
- Plain sweet biscuits: ....................................
- Fancy biscuits (eg choc-coated): 2
- Cake: 1 small cake or 1 slice large cake
- Milk pudding (eg rice, sago): 1/2 cup
- Steamed sponge - suet: 1/4 small pudding

Q-1 What types of cake do you most commonly eat? (eg fruit cake, cheesecake, sponge cake, iced sponge, tea-cake etc)

Please name: ...................................................

Q-2 Are the cakes you most commonly eat: (Circle one answer)

1. Pre-made wrapped cakes
2. Frozen wrapped cakes (eg Sara-Lee)
3. Bought homestyle cakes (eg from bakery)
4. Homemade (packet mix)
5. Homemade (own ingredients)
6. I do not eat cake
**HOW TO ANSWER**

<table>
<thead>
<tr>
<th>NEVER</th>
<th>RARELY</th>
<th>Times a MONTH</th>
<th>Times a WEEK</th>
<th>Times a DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>R</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>M</td>
<td>W</td>
<td>D</td>
</tr>
<tr>
<td>and so on</td>
<td>and so on</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**HOW OFTEN DO YOU USUALLY HAVE THESE DRINKS?**

**BEVERAGES**

- Sustagen (made with powder) 1 cup
- Sustagen Gold small carton (300 ml)
- Carton of other flavoured milk (eg Iced coffee, strawberry etc) small carton (300 ml)
- Cocoa 1 cup
- Drinking Chocolate/Milo/Quik etc 1 cup
- Akta-Vite 1 cup
- Glass of milk (as such) 1 glass
- Milk shake/Thick shake regular size
- Tea 1 cup
- Herbal tea 1 cup
- Instant coffee 1 cup
- Ground coffee (eg filter/drip) 1 cup
- Decaffeinated coffee 1 cup
- Coffee substitute (eg Caro) 1 cup

**COMMENTS**

Q-1 *Do you have milk:*  
(Circle one for each)  
- in your tea? YES NO DON'T DRINK TEA  
- in your coffee? YES NO DON'T DRINK COFFEE  
- in your coffee substitute? YES NO DON'T DRINK COFFEE SUBSTITUTE
Q-2 Do you make your cocoa/chocolate/Milo/Akta-Vite with: (Circle one number)

1. Mostly milk?
2. Mostly water?
3. About half and half?
4. I do not drink these drinks.

Q-3 What type of milk do you usually add to tea/coffee/cocoa/chocolate etc? (Please state the type of milk used eg. whole milk, Skimmer, Rev, Trim, Tone, powdered skim, Shape, High-Low, goat's milk, condensed milk, evaporated milk etc).

Type of milk added: ........................................................................................................................................................................................................................................................................................................

Q-4 How many teaspoons of sugar/honey do you usually have in each cup of:

(Circle one number for each drink)

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tea?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee substitute?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocoa?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milo/Quik/Chocolate?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Q-5 When you are choosing the type of milk that you use how important are the following factors? (Circle one number for each)

<table>
<thead>
<tr>
<th></th>
<th>NOT SO IMPORTANT</th>
<th>VERY IMPORTANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Price</td>
<td>1</td>
<td>2 3 4 5 6 7</td>
</tr>
<tr>
<td>Taste</td>
<td>1</td>
<td>2 3 4 5 6 7</td>
</tr>
<tr>
<td>Healthiness</td>
<td>1</td>
<td>2 3 4 5 6 7</td>
</tr>
<tr>
<td>Convenience of storage</td>
<td>1</td>
<td>2 3 4 5 6 7</td>
</tr>
<tr>
<td>Weight (eg easy to carry)</td>
<td>1</td>
<td>2 3 4 5 6 7</td>
</tr>
</tbody>
</table>
### HOW TO ANSWER

<table>
<thead>
<tr>
<th>NEVER</th>
<th>RARELY</th>
<th>TIMES A MONTH</th>
<th>TIMES A WEEK</th>
<th>TIMES A DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>R</td>
<td>M</td>
<td>W</td>
<td>D</td>
</tr>
</tbody>
</table>

### HOW OFTEN DO YOU USUALLY EAT THESE FOODS?

#### DAIRY PRODUCTS and EGGS

<table>
<thead>
<tr>
<th>Food Description</th>
<th>Amount</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese</td>
<td>30 grams (1 slice)</td>
<td></td>
</tr>
<tr>
<td>Low-fat Collage Cheese</td>
<td>100 gm (1/2 carton)</td>
<td></td>
</tr>
<tr>
<td>Cream</td>
<td>1 tablespoon</td>
<td></td>
</tr>
<tr>
<td>Yoghurt</td>
<td>200 gm (1 carton)</td>
<td></td>
</tr>
<tr>
<td>Icecream (from a tub)</td>
<td>2 scoops (SUMMER)</td>
<td></td>
</tr>
<tr>
<td>Icecream desserts (eg Symphony, Vlenella)</td>
<td>1 serving (SUMMER)</td>
<td></td>
</tr>
<tr>
<td>Icecream (on a stick/cone)</td>
<td>1 icecream (SUMMER)</td>
<td></td>
</tr>
<tr>
<td>Vanilla</td>
<td>1 cone (SUMMER)</td>
<td></td>
</tr>
<tr>
<td>Ice Block/Icy Pole</td>
<td>1 (SUMMER)</td>
<td></td>
</tr>
<tr>
<td>Custard</td>
<td>1/2 cup</td>
<td></td>
</tr>
<tr>
<td>Fried egg</td>
<td>1 egg</td>
<td></td>
</tr>
<tr>
<td>Boiled egg</td>
<td>1 egg</td>
<td></td>
</tr>
<tr>
<td>Omelette/Scrambled eggs</td>
<td>2 eggs</td>
<td></td>
</tr>
</tbody>
</table>
Q-1 When you eat cheese, do you have the reduced-salt varieties? (Circle one number)
1 Always or nearly always
2 Sometimes
3 Rarely or never
4 I do not eat cheese

Q-2 When you eat cheese, do you have the reduced-fat varieties? (Circle one number)
1 Always or nearly always
2 Sometimes
3 Rarely or never
4 I do not eat cheese

Q-3 When you eat yoghurt which type is it? (Circle one number)
1 Plain (eg not fat-reduced)
2 Plain, low-fat
3 Fruit flavoured (not fat-reduced)
4 Fruit flavoured, low-fat
5 Frozen yoghurt
6 I do not eat yoghurt

Q-4 When you eat ice-cream, diet-ice or similar is it usually? (Circle one number)
1 Low calorie
2 Regular ice-cream
3 Other (please state)

Q-5 In recent years have you made any changes to your intake of the following foods?
(Circle one for each food)

<table>
<thead>
<tr>
<th>Food</th>
<th>I am having</th>
<th>I am having</th>
<th>I am having</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>MORE</td>
<td>LESS</td>
<td>the SAME</td>
</tr>
<tr>
<td>Cheese</td>
<td>MORE</td>
<td>LESS</td>
<td>the SAME</td>
</tr>
<tr>
<td>Eggs</td>
<td>MORE</td>
<td>LESS</td>
<td>the SAME</td>
</tr>
<tr>
<td>Coffee</td>
<td>MORE</td>
<td>LESS</td>
<td>the SAME</td>
</tr>
<tr>
<td>Tea</td>
<td>MORE</td>
<td>LESS</td>
<td>the SAME</td>
</tr>
</tbody>
</table>
## HOW TO ANSWER

<table>
<thead>
<tr>
<th>NEVER</th>
<th>RARELY</th>
<th>TIMES A MONTH</th>
<th>TIMES A WEEK</th>
<th>TIMES A DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>R</td>
<td>M</td>
<td>W</td>
<td>D</td>
</tr>
</tbody>
</table>

ABOUT HOW OFTEN DO YOU USUALLY EAT THESE FOODS?

### MEATS

<table>
<thead>
<tr>
<th>Food</th>
<th>Quantity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steak (eaten as such)</td>
<td>1 medium</td>
<td></td>
</tr>
<tr>
<td>Pork chop</td>
<td>1 chop</td>
<td></td>
</tr>
<tr>
<td>Lamb chop</td>
<td>2 chops</td>
<td></td>
</tr>
<tr>
<td>Roast pork/Pork fillet</td>
<td>2 slices</td>
<td></td>
</tr>
<tr>
<td>Roast beef/veal</td>
<td>2 slices</td>
<td></td>
</tr>
<tr>
<td>Roast lamb</td>
<td>2 slices</td>
<td></td>
</tr>
<tr>
<td>Sausages</td>
<td>2 thick or 3 thin</td>
<td></td>
</tr>
<tr>
<td>Frankfurters/Saveloys</td>
<td>2 thick or 3 thin</td>
<td></td>
</tr>
<tr>
<td>Bacon</td>
<td>2 rashers</td>
<td></td>
</tr>
<tr>
<td>Ham</td>
<td>3 thin or 2 thick slices</td>
<td></td>
</tr>
<tr>
<td>Luncheon meat/Fritz/Devon/Windsor etc</td>
<td>3 slices (1 cm thick)</td>
<td></td>
</tr>
<tr>
<td>Continental sausage (Salami/Mettwurst/Cabanossi)</td>
<td>3 slices</td>
<td></td>
</tr>
<tr>
<td>Pate/Liver paste</td>
<td>1 tablespoon</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1/2 liver (150 gm)</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>2 kidneys</td>
<td></td>
</tr>
<tr>
<td>Brains</td>
<td>1/2 cup</td>
<td></td>
</tr>
<tr>
<td>Pureed meat dishes (canned/bottled)</td>
<td>1/2 cup</td>
<td></td>
</tr>
</tbody>
</table>
### ABOUT HOW OFTEN DO YOU USUALLY EAT THESE FOODS?

<table>
<thead>
<tr>
<th>MIXED DISHES</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamburger WITH bun</td>
<td></td>
</tr>
<tr>
<td>1 medium</td>
<td></td>
</tr>
<tr>
<td>Hamburger patty (WITHOUT bun)</td>
<td></td>
</tr>
<tr>
<td>1 medium</td>
<td></td>
</tr>
<tr>
<td>Pizza (frozen)</td>
<td></td>
</tr>
<tr>
<td>1 mini or 1/4 large pizza</td>
<td></td>
</tr>
<tr>
<td>Pizza (homemade or take-away)</td>
<td></td>
</tr>
<tr>
<td>1/2 small or 1/4 large pizza</td>
<td></td>
</tr>
<tr>
<td>Sausage roll</td>
<td></td>
</tr>
<tr>
<td>1 large, 2 small</td>
<td></td>
</tr>
<tr>
<td>Meat pie</td>
<td></td>
</tr>
<tr>
<td>1 pie</td>
<td></td>
</tr>
<tr>
<td>Meat pie (home made)</td>
<td></td>
</tr>
<tr>
<td>1 indiv. pie OR 1 slice large pie</td>
<td></td>
</tr>
<tr>
<td>Pastie</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Crumbed veal (schnitzel)</td>
<td></td>
</tr>
<tr>
<td>1 large piece</td>
<td></td>
</tr>
<tr>
<td>Stew/Casserole/Curry/Goulash (with meat or chicken)</td>
<td></td>
</tr>
<tr>
<td>1 cup</td>
<td></td>
</tr>
<tr>
<td>Stew/Casserole/Curry/Goulash (without meat or chicken)</td>
<td></td>
</tr>
<tr>
<td>1 cup</td>
<td></td>
</tr>
<tr>
<td>Chinese meat &amp; veg dish</td>
<td></td>
</tr>
<tr>
<td>1 cup</td>
<td></td>
</tr>
<tr>
<td>Savoury pies/pastries (eg quiche)</td>
<td></td>
</tr>
<tr>
<td>1 indiv. pie OR 1 slice large pie</td>
<td></td>
</tr>
<tr>
<td>Mince meat (eaten as such)</td>
<td></td>
</tr>
<tr>
<td>1 cup</td>
<td></td>
</tr>
<tr>
<td>Mince meat dishes (eg shepherds pie)</td>
<td></td>
</tr>
<tr>
<td>1 piece (6x8x4cm)</td>
<td></td>
</tr>
<tr>
<td>Spicy mince added to pastas (eg spag. sauce)</td>
<td></td>
</tr>
<tr>
<td>1/2 cup mince</td>
<td></td>
</tr>
</tbody>
</table>
## HOW TO ANSWER

<table>
<thead>
<tr>
<th>NEVER</th>
<th>RARELY</th>
<th>Times a MONTH</th>
<th>Times a WEEK</th>
<th>Times a DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>R</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

**ABOUT HOW OFTEN DO YOU USUALLY EAT THESE FOODS?**

**CHICKEN, FISH and SEAFOOD -**

<table>
<thead>
<tr>
<th>Food Description</th>
<th>Usually Cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roast/Barbecue chicken</td>
<td>2 slices breast or 1 drumstick/2 wings</td>
</tr>
<tr>
<td>Boiled chicken</td>
<td>as above</td>
</tr>
<tr>
<td>Crumbed, fried chicken</td>
<td>4 small pieces</td>
</tr>
<tr>
<td>Chicken/Flsh nuggets</td>
<td>6 nuggets</td>
</tr>
<tr>
<td>Fish fried</td>
<td>1 piece</td>
</tr>
<tr>
<td>Fish without batter (steamed/grilled/bolled)</td>
<td>1 piece</td>
</tr>
<tr>
<td>Canned fish (luna, salmon etc)</td>
<td>1/3 cup</td>
</tr>
<tr>
<td>Fish fingers</td>
<td>3-4 fingers</td>
</tr>
<tr>
<td>Seafood (prawns, crab, lobster etc)</td>
<td>1/2 cup</td>
</tr>
<tr>
<td>Mornay dishes</td>
<td>1 cup</td>
</tr>
</tbody>
</table>

**COMMENTS**

**Q-1** If you eat the following meals, how are they usually cooked? (Circle one for each food)

<table>
<thead>
<tr>
<th>Meal</th>
<th>Usually Cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steak</td>
<td>FRIED</td>
</tr>
<tr>
<td>Chops</td>
<td>FRIED</td>
</tr>
<tr>
<td>Sausages</td>
<td>FRIED</td>
</tr>
<tr>
<td>Bacon</td>
<td>FRIED</td>
</tr>
</tbody>
</table>

**Q-2** When you eat meat with fat on it, do you eat: (Circle one number)

1. All of the fat
2. Most of the fat
3. About half of the fat
4. Little or none of the fat
5. I do not eat meat
Q-3 Do you take the skin off chicken? (Circle one number)

1. Always or nearly always
2. Sometimes (about half the time or less)
3. Rarely (less than a quarter of the time)
4. Never
5. I do not eat chicken

Q-4 If you eat fried fish, in which of the following is it usually coated? (Circle one number)

1. Batter
2. Breadcrumbs
3. Flour
4. Other coating; please name...........................................................................
5. Fried without coating

Q-5 When you eat fish coated in batter, crumbs etc how often is it: (Circle one (or each)

Coated at home .................. ALWAYS  SOMETIMES  RARELY  NEVER
Pre-packed, frozen cooked at home .................. ALWAYS  SOMETIMES  RARELY  NEVER
Bought ready cooked from fish shop .................. ALWAYS  SOMETIMES  RARELY  NEVER

Q-6 How often do you eat complete main meals bought from the freezer section at the shops? (eg Lean Cuisine, "TV" dinners) (Circle one number)

1. Daily
2. About weekly
3. About once a fortnight
4. About monthly or less
5. Never

Q-7 How often do you eat "meals in a can" (eg, Irish Stew, ready to heat and eat meals) (Circle one number)

1. Daily
2. About weekly
3. About once a fortnight
4. About monthly or less
5. Never

Q-8 How often do you eat canned meals such as Camp Pie, corned beef, tinned ham etc (Circle one number)

1. Daily
2. About weekly
3. About once a fortnight
4. About monthly or less
5. Never
<table>
<thead>
<tr>
<th></th>
<th>NEVER</th>
<th>RARELY</th>
<th>Times a MONTH</th>
<th>Times a WEEK</th>
<th>Times a DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>R</td>
<td>1 M</td>
<td>2 W</td>
<td>3 D</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABOUT HOW OFTEN DO YOU USUALLY EAT THESE FOODS?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CANNED and DRIED VEGETABLES**

<table>
<thead>
<tr>
<th>Food</th>
<th>Quantity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato - canned</td>
<td>2-3 small</td>
<td></td>
</tr>
<tr>
<td>Potato - packet (powdered)</td>
<td>1/3 cup</td>
<td></td>
</tr>
<tr>
<td>Potato salad</td>
<td>1/3 cup</td>
<td></td>
</tr>
<tr>
<td>Carrots - canned</td>
<td>1/3 cup</td>
<td></td>
</tr>
<tr>
<td>Beetroot - canned</td>
<td>2 slices</td>
<td></td>
</tr>
<tr>
<td>Green beans - canned</td>
<td>1/3 cup</td>
<td></td>
</tr>
<tr>
<td>Haricot, Lima beans - canned</td>
<td>1/3 cup</td>
<td></td>
</tr>
<tr>
<td>Baked beans in tomato sauce</td>
<td>1/3 cup</td>
<td></td>
</tr>
<tr>
<td>Green peas - canned</td>
<td>1/3 cup</td>
<td></td>
</tr>
<tr>
<td>Lentils - dried/canned</td>
<td>1/3 cup</td>
<td></td>
</tr>
<tr>
<td>Zucchini salad</td>
<td>1/3 cup</td>
<td></td>
</tr>
<tr>
<td>Sweetcorn - canned (including creamed corn)</td>
<td>1/3 cup</td>
<td></td>
</tr>
<tr>
<td>Mushrooms - canned</td>
<td>6-7 small ones</td>
<td></td>
</tr>
<tr>
<td>Mushrooms - canned in sauce</td>
<td>1/3 cup</td>
<td></td>
</tr>
<tr>
<td>Olives</td>
<td>3 medium</td>
<td></td>
</tr>
<tr>
<td>Gherkins/Pickled onions</td>
<td>3 pieces</td>
<td></td>
</tr>
<tr>
<td>Pureed vegetables (canned/bottled)</td>
<td>1/3 cup</td>
<td></td>
</tr>
</tbody>
</table>
The following list of foods contains some vegetables that may be eaten much more frequently at some times of the year than others (eg. in the warmer or cooler weather). Please fill in how often each food is eaten in BOTH the warmer months of the year (SUMMER) and the cooler months (WINTER).

For example—If you usually have:

- A standard serve of peas about twice a week during the warmer months of the year and about every day during the cooler months;

and:

- Two medium potatoes (roasted) a week throughout the year;

You would write......

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Summer</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green peas</td>
<td>1 cup</td>
<td>2 W</td>
<td>1 D</td>
</tr>
<tr>
<td>Potato - roasted</td>
<td>1 medium</td>
<td>1 W</td>
<td>1 W two potatoes</td>
</tr>
</tbody>
</table>

ABOUT HOW OFTEN DO YOU USUALLY EAT THESE FOODS?

**SEASONAL VEGETABLES**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Summer</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato - fresh &amp; mashed (with milk)</td>
<td>1/3 cup</td>
<td>........</td>
<td>........</td>
</tr>
<tr>
<td>Potato - fresh, boiled</td>
<td>1 medium</td>
<td>........</td>
<td>........</td>
</tr>
<tr>
<td>Potato - roasted</td>
<td>1 medium</td>
<td>........</td>
<td>........</td>
</tr>
<tr>
<td>French Fries/Hot chips</td>
<td>17-18 chips</td>
<td>........</td>
<td>........</td>
</tr>
<tr>
<td>Potato Gems/ Pommes Noisettes</td>
<td>about 5</td>
<td>........</td>
<td>........</td>
</tr>
<tr>
<td>Carrots (fresh/frozen)</td>
<td>1/3 cup</td>
<td>........</td>
<td>........</td>
</tr>
<tr>
<td>Turnip, Swede (fresh/frozen)</td>
<td>1/3 cup</td>
<td>........</td>
<td>........</td>
</tr>
<tr>
<td>Broad beans (fresh/frozen)</td>
<td>1/2 cup</td>
<td>........</td>
<td>........</td>
</tr>
<tr>
<td>Green beans (fresh/frozen)</td>
<td>1/3 cup</td>
<td>........</td>
<td>........</td>
</tr>
<tr>
<td>SEASONAL VEGETABLES (continued)</td>
<td>Summer</td>
<td>Winter</td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>--------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>Green peas (fresh/frozen)</td>
<td>1/3 cup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cabbage</td>
<td>1/3 cup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brussels sprouts (fresh/frozen)</td>
<td>5-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silver beet/Spinach (fresh/frozen)</td>
<td>1/3 cup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broccoli (fresh/frozen)</td>
<td>1/3 cup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cauliflower (fresh/frozen)</td>
<td>1/2 cup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pumpkin</td>
<td>1/3 cup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweetcorn (fresh/frozen)</td>
<td>1 small cob</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zucchini (courgettes)</td>
<td>1 medium sized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onion - fried</td>
<td>1/4 cup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onion (raw, baked, boiled) (fresh/frozen)</td>
<td>1 medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato - fresh</td>
<td>1 medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato - grilled/fried</td>
<td>1/2 medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>2 small leaves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cucumber</td>
<td>3 slices (each 0.5 cm thick)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coleslaw</td>
<td>1/2 cup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celery (fresh/frozen)</td>
<td>one 15 cm stick</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capsicum (Green Pepper) (fresh/frozen)</td>
<td>2 strips (each 0.5 cm thick)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mushrooms - fresh</td>
<td>6-7 small ones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprouted bean shoots</td>
<td>1/3 cup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fried mixed vegetables (eg stir fried)</td>
<td>1/2 cup</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Q-1 When you use canned vegetables, are they reduced-salt varieties? (Circle one number)

1. Always or nearly always
2. Sometimes
3. Never or rarely
4. Only for some vegetables (please state which)

Q-2 Is salt added to the cooking water when boiling the following foods? (Circle one for each food)

Vegetables | USUALLY | SOMETIMES | NEVER
Pasta and rice | USUALLY | SOMETIMES | NEVER

Q-3 If salt is added to the cooking water when boiling foods, is the water:

1. Lightly salted
2. Medium salted
3. Heavily salted
4. Salting is highly varied
5. Salt is not added to cooking water

Q-4 How often do you add salt to meals after they are cooked? (Circle one number)

1. Rarely or never
2. Sometimes
3. Always or nearly always

Q-5 When you add salt at the table, how much do you usually add? (Circle one number)

1. A light sprinkle
2. A medium sprinkle
3. A heavy sprinkle
4. Salting is highly varied
5. I do not add salt at the table

Q-6 When your vegetables are cooked which of the following methods is the one most commonly used? (Circle one number)

1. Boiled in a little water
2. Boiled in a lot of water
3. Steamed
4. Cooked in a pressure cooker
5. Microwaved
6. Stir-fried
Q-7

1 Vegetable oils (olive, sunflower etc) 4 Dripping/lard/meat juices
2 Cooking or table margarine 5 Polyunsaturated margarine
3 Butter 6 Nothing

From the list above write which type of fat/oil is most commonly used:

(a) When roasting/frying meats/lush.................................................................
(b) When roasting/frying vegetables.................................................................
(c) On vegetables when served (eg butter on peas)............................................

Q-8 Is butter or margarine added to your potatoes when they are mashed? (Circle one number)

1 Yes, always
2 Yes, occasionally
3 Never

HOW TO ANSWER

NEVER RARELY Times a MONTH Times a WEEK Times a DAY
N R M W D
1 2 3 1 2 1 2 1
and so on and so on

HOW OFTEN DO YOU USUALLY EAT THESE FOODS?

FRUIT

Orange, Mandarin, Grapefruit 1 medium
Apple, Pear - fresh/baked 1 medium
Banana 1 medium
Fresh fruit salad 1 cup
Dried fruit (apple/apricot etc) 4-5 pieces
Raisins, sultanas or currants 1/3 cup
Fruit in syrup or stewed (including fruit salads) 1/2 cup
Fruit canned in water (low-cal) (including fruit salads) 1/2 cup
Fruit pie or pastry or fritters 1 small pie or 1 slice large

COMMENTS

...
## HOW TO ANSWER

<table>
<thead>
<tr>
<th>NEVER</th>
<th>RARELY</th>
<th>Times a Month</th>
<th>Times a Week</th>
<th>Times a Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>R</td>
<td>M</td>
<td>W</td>
<td>D</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

The fruits listed below are only available for a short time during the year. Therefore we only want you to record how often you have them when they are IN SEASON.

## HOW OFTEN DO YOU EAT THESE FOODS WHEN THEY ARE IN SEASON?

### SEASONAL FRUITS

<table>
<thead>
<tr>
<th>Fruit Type</th>
<th>Size/Serve</th>
<th>Comments</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berries - fresh/frozen</td>
<td>3/4 cup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melon (not watermelon)</td>
<td>1 large slice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peach - fresh</td>
<td>1 medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plum - fresh</td>
<td>3-4 plums</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nectarine - fresh</td>
<td>1 medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apricot - fresh</td>
<td>3 apricots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grapes - fresh</td>
<td>about 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pineapple - fresh</td>
<td>1 slice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avocado</td>
<td>1/2 an avocado</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please list here, along with your standard serve size, any other fruit that you eat (eg mango, pureed, canned/bottled fruits):

- [Comments]

## HOW OFTEN DO YOU USUALLY EAT THESE FOODS?

### NUTS and SNACKS

<table>
<thead>
<tr>
<th>Snack Type</th>
<th>Size/Serve</th>
<th>Comments</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato crisps, Twists etc</td>
<td>1 small bag or 14-15 pieces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanuts (fresh)</td>
<td>9-10 nuts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuts - salted &amp; cooked</td>
<td>9-10 nuts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other unsalted nuts (fresh walnuts/almonds etc)</td>
<td>5-6 nuts</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
HOW TO ANSWER

<table>
<thead>
<tr>
<th>NEVER</th>
<th>RARELY</th>
<th>Times a MONTH</th>
<th>Times a WEEK</th>
<th>Times a DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>R</td>
<td>1 M</td>
<td>2 W</td>
<td>3 D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and so on</td>
<td>and so on</td>
<td></td>
</tr>
</tbody>
</table>

ABOUT HOW OFTEN DO YOU USUALLY EAT THESE FOODS?

**SOUPS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
<th>Winter</th>
<th>Fall</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canned soup (eaten as such)</td>
<td>1 cup</td>
<td>(WINTER)</td>
<td>(SUMMER)</td>
<td></td>
</tr>
<tr>
<td>Packet soup (eaten as such)</td>
<td>1 cup</td>
<td>(WINTER)</td>
<td>(SUMMER)</td>
<td></td>
</tr>
<tr>
<td>Homemade soup (eaten as such)</td>
<td>1 cup</td>
<td>(WINTER)</td>
<td>(SUMMER)</td>
<td></td>
</tr>
</tbody>
</table>

Write an example of the type of soup you most often eat (eg canned tomato; homemade pea and ham)

.......................... ................................................

Below we have listed a number of products that may be used as ingredients when you are cooking (for example you might add these foods to casseroles, stews etc). Please fill in how often you would use these foods as ingredients in cooking.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canned or Packet soup mixes</td>
<td></td>
</tr>
<tr>
<td>Bottled sauces (eg soy sauce, tomato sauce)</td>
<td></td>
</tr>
<tr>
<td>Gravy mixes/powders</td>
<td></td>
</tr>
<tr>
<td>Stock cubes/powders</td>
<td></td>
</tr>
<tr>
<td>Canned tomatoes</td>
<td></td>
</tr>
<tr>
<td>Tomato pastes/purees</td>
<td></td>
</tr>
<tr>
<td>Other pureed foods (eg apple, baby foods)</td>
<td></td>
</tr>
<tr>
<td>Evaporated milk</td>
<td></td>
</tr>
<tr>
<td>Condensed milk</td>
<td>23</td>
</tr>
</tbody>
</table>
### HOW TO ANSWER

<table>
<thead>
<tr>
<th></th>
<th>NEVER</th>
<th>RARELY</th>
<th>Times a MONTH</th>
<th>Times a WEEK</th>
<th>Times a DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>R</td>
<td>M</td>
<td>W</td>
<td>D</td>
</tr>
</tbody>
</table>

#### ABOUT HOW OFTEN DO YOU USUALLY EAT THESE FOODS?

**CONFECTIONERY, JAMS and SAUCES**

- **Chocolate**: 1 small bar (50 grams)
- **Chocolate covered bar (eg Mars/Bounty)**: 1 bar
- **Individually wrapped lollies; toffees**: 4-5 lollies
- **Packet lollies (eg Lifesavers/Polos)**: 1 small packet
- **Muesli bar/Health bar**: 1 bar
- **Honey, jam, marmalade**: 1 tablespoon
- **Vegemite, marmite etc**: 1/2 teaspoon
- **Thick sauces (tomato/HP etc)**: 1 tablespoon
- **Polyunsaturated Mayonnaise/ Salad Cream**: 1 tablespoon
- **Regular Mayonnaise/ Salad Cream**: 1 tablespoon
- **Low calorie salad dressings**: 1 tablespoon
- **Polyunsaturated salad dressings**: 1 tablespoon

**COMMENTS**
### HOW TO ANSWER

<table>
<thead>
<tr>
<th>NEVER</th>
<th>RARELY</th>
<th>TIMES A MONTH</th>
<th>TIMES A WEEK</th>
<th>TIMES A DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>R</td>
<td>1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
</tr>
</tbody>
</table>

and so on and so on

### ABOUT HOW OFTEN DO YOU USUALLY HAVE THESE FOODS?

#### BEVERAGES

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Container Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass of cordial</td>
<td>medium glass</td>
<td></td>
</tr>
<tr>
<td>Glass of cola (eg Coca-Cola)</td>
<td>medium glass</td>
<td></td>
</tr>
<tr>
<td>Glass of fizzy drink</td>
<td>medium glass</td>
<td></td>
</tr>
<tr>
<td>Includes mineral water with juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass of low-calorie fizzy drink</td>
<td>medium glass</td>
<td></td>
</tr>
<tr>
<td>Fruit drink (eg Fruit Box)</td>
<td>1 carton (250 ml)</td>
<td></td>
</tr>
<tr>
<td>Pure fruit Juice</td>
<td>medium glass</td>
<td></td>
</tr>
<tr>
<td>Vegetable Juice</td>
<td>small glass</td>
<td></td>
</tr>
<tr>
<td>Water/Spring water</td>
<td>medium glass</td>
<td></td>
</tr>
<tr>
<td>Mineral water</td>
<td>medium glass</td>
<td></td>
</tr>
<tr>
<td>Low-alcohol beer</td>
<td>medium glass 230 mls</td>
<td></td>
</tr>
<tr>
<td>Beer</td>
<td>medium glass 230 mls</td>
<td></td>
</tr>
<tr>
<td>Alcoholic cider</td>
<td>medium glass 230 mls</td>
<td></td>
</tr>
<tr>
<td>Wine</td>
<td>1 wine glass</td>
<td></td>
</tr>
<tr>
<td>Wine Cooler</td>
<td>1 wine glass</td>
<td></td>
</tr>
<tr>
<td>Sherry/Port/Liqueur</td>
<td>1 standard serve</td>
<td></td>
</tr>
<tr>
<td>Spirits (whisky, brandy etc)</td>
<td>1 nip</td>
<td></td>
</tr>
</tbody>
</table>

#### COMMENTS
If you have any other foods or drinks that we have not mentioned, at least once a month, please write them down here and tell us how often you have them using the same response scale as before (eg 1D, 3M etc).

### HOW TO ANSWER

<table>
<thead>
<tr>
<th></th>
<th>NEVER</th>
<th>RARELY</th>
<th>Times a MONTH</th>
<th>Times a WEEK</th>
<th>Times a DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>R</td>
<td>1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
</tr>
</tbody>
</table>

and so on and so on

### FOODS AND DRINKS I CONSUME THAT HAVE NOT BEEN MENTIONED:

<table>
<thead>
<tr>
<th>Name of Food</th>
<th>Your usual serve size</th>
<th>How often do you eat it?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### VITAMIN AND MINERAL SUPPLEMENTS

If you take any vitamins or minerals, or any other dietary supplements, such as fibre tablets, lecithin, kelp, yeast, 'slimming' products, etc, please fill in the table below. (Check the label on the box or bottle if you are unsure of some of the answers).

<table>
<thead>
<tr>
<th>BRAND (e.g. Nyal)</th>
<th>NAME OF PRODUCT (e.g. vitamin C pill)</th>
<th>SIZE OF DOSE (e.g. 250 mg)</th>
<th>NUMBER OF DOSES (e.g. 2 per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**SHOPPING**

**Q-1** In your household, who does most of the shopping? (Circle two if necessary)

1. Self
2. Spouse/Partner
3. Someone else (specify who)

---

**Q-2** How much do you feel that your eating habits are influenced by:

<table>
<thead>
<tr>
<th>Not Relevant</th>
<th>Major Influence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lack of money</td>
</tr>
<tr>
<td></td>
<td>Transport problems</td>
</tr>
<tr>
<td></td>
<td>Availability of foods in shops</td>
</tr>
<tr>
<td></td>
<td>Nutritional value of food</td>
</tr>
<tr>
<td></td>
<td>Physical disabilities</td>
</tr>
<tr>
<td></td>
<td>Some other factor (please state)</td>
</tr>
</tbody>
</table>

---

**Q-3** Do you ever pay attention to the nutrients or ingredients listed on food labels? (Circle one number)

1. No
2. Yes
3. Never noticed them

---

**Q-4** When you look at the information on food labels, do you ever use it to avoid a particular ingredient or nutrient, or avoid getting too much of it? (Circle one number)

1. I don't look at food label information
2. No
3. Yes - If so, what do you try to avoid? (Please be specific)

---

**Q-5** When you look at the nutrients or ingredients listed on food labels, do you use this information to compare different brands? (Circle one number)

1. I don't look at food label information
2. I don't use it to compare brands
3. I sometimes use it to compare brands
4. I often use it to compare brands
Q-6 When you are choosing between brands of similar items (e.g., two types of high fibre breakfast cereal or two varieties of low-salt margarine), how important would these factors be to you? (Circle one number for each)

<table>
<thead>
<tr>
<th>Factor</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Price</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taste</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, salt or sugar content</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Additives or preservatives</td>
<td></td>
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<tr>
<td>Convenience of packaging</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Endorsement by health authorities</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Health claims by the manufacturer</td>
<td></td>
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</tr>
</tbody>
</table>

Q-7 (a) What do you think are the advantages, if any, of canned foods?

________________________________________________________

________________________________________________________

Q-7 (b) What do you think are the disadvantages, if any, of canned foods?

________________________________________________________

________________________________________________________

Q-8 (a) What do you think are the advantages, if any, of frozen foods?

________________________________________________________

________________________________________________________

Q-8 (b) What do you think are the disadvantages, if any, of frozen foods?

________________________________________________________

________________________________________________________
Q-1 How would you rate the nutritional value of the following? (Circle one for each food)

<table>
<thead>
<tr>
<th>Food</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh fish</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Fresh carrots</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Frozen fruits</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Complete frozen meals</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Canned fish</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Canned peas</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Frozen mixed vegetables</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Canned meats</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Frozen pizza</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Fresh peas</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Frozen carrots</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Canned fruit</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Frozen fish</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Frozen peas</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Takeaway pizza</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Fresh red meat</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Frozen meats</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Complete canned meals</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Canned carrots</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Fresh fruit</td>
<td>2 3 4 5</td>
</tr>
<tr>
<td>Canned mixed vegetables</td>
<td>2 3 4 5</td>
</tr>
</tbody>
</table>
DIETARY CHANGES

Q-1 Have you tried to make any of the following changes to your diet in the last few years? (Circle one answer for each)

Increase fibre intake  YES  NO
Reduce fat intake  YES  NO
Reduce sugar intake  YES  NO
Increase calcium intake  YES  NO
Reduce salt intake  YES  NO
Eat less food in general  YES  NO
Something else  YES  NO

(please describe............................................................................................................................)

Q-2 If you have NOT TRIED to make changes state, in your own words, why not?

__________________________________________________________________________________________

__________________________________________________________________________________________

Q-3 If you have TRIED to make dietary changes, either successfully or unsuccessfully, in the last few years, what were the major difficulties you encountered?

__________________________________________________________________________________________

__________________________________________________________________________________________

__________________________________________________________________________________________

Q-4 If you have TRIED to make changes:

In your own words, what specific changes have you tried to make to the foods you eat or your cooking methods etc?

__________________________________________________________________________________________

__________________________________________________________________________________________

__________________________________________________________________________________________

Why have you made these changes?

__________________________________________________________________________________________

__________________________________________________________________________________________
Q-1  What is your current height? _____feet______inches OR _____centimetres

What is your current weight? _____stones_____pounds OR _____kilograms

Q-2  About how often do you exercise? (Please circle one number)

1  Less than once a week
2  Once or twice a week
3  Three times a week or more
4  NOT AT ALL—go to Question 3 below

Q-2(a) About how long do you exercise for each time? (Circle one number)

1  Less than 20 minutes
2  20-30 minutes
3  More than 30 minutes

Q-2(b) How much of the exercise you do makes you puff and sweat? (Circle one number)

1  None of it
2  Some of it
3  Most or all of it

Q-3 (a)  Have you ever smoked on a regular basis? (Please circle one number)

1  YES
2  NO

Q-3 (b) Do you currently smoke cigarettes, pipes or cigars? (Please circle, and write in amount)

1  YES, I smoke___________cigs/day

2  YES, I smoke___________cigars/day

3  YES, I smoke___________pipes/day

4  NO, I don’t smoke at all
Please answer these questions accurately so that we can examine general relationships between lifestyles and diet. Let us reassure you again that this information will be kept completely confidential.

Q-1 Your sex: (Circle one)
1 Male
2 Female

Q-2 Your present age: _______ YEARS

Q-3 What is your usual occupation?
   Even if you are retired or otherwise not currently employed, please state what your occupation usually would be. (Be specific)

Q-4 Are you (circle number(s))
1 Employed full-time
2 Employed part-time
3 Not employed outside the home
4 Student
5 Retired
6 Unemployed

Q-5 If you live with a spouse or partner, what is his/her usual occupation? (Be specific)

Q-6 Is your spouse/partner (circle number(s))
1 Employed full-time
2 Employed part-time
3 Not employed outside the home
4 Student
5 Retired
6 Unemployed

Q-8 Which one of these best describes your situation? (Circle one number)
1 Living with spouse or partner and dependent children
2 Living with spouse or partner
3 Living alone with dependent children
4 Living alone
5 Living with my family (eg mother, father, brother, sister)
6 Share house or flat with others
7 Living with my child's family (eg daughter or son's family)
8 Retirement home
9 Another situation; please describe:

Q-9 If you were born overseas, for how long have you lived in Australia?

Q-10 What is the current level of income (before tax) in your household? (circle number next to range)
1 nil - $7,000 per year
2 $7,001 - $15,000 per year
3 $15,001 - $25,000 per year
4 $25,001 - $40,000 per year
5 $40,001 and over per year
6 I do not wish to answer this question
At this stage, we ask you to please go back through the questionnaire and check that you have answered all questions.

YOUR COMMENTS

Thank you very much for your help. We realise that you had to give up some of your time to answer our questions. We would like you to know that we appreciate this help.

We believe that the results from this survey will be useful in determining what people eat, and in the future, possible relationships between diet and disease.

If you have any further comments to make about the design of this questionnaire please feel free to write them down here. We might be able to incorporate your suggestions in our future work.

Thank you again for your help.