METABOLIC AND HORMONAL STUDIES IN SOUTH AFRICAN WOMEN OF INDIAN AND AFRICAN ORIGIN

Rita Waisberg

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy

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DECLARATION

I, Rita Waisberg, do hereby declare that this thesis is my own work. It is being submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg, South Africa. Any assistance that I received is stated in the acknowledgements. This work has not previously been submitted for any degree or examination at this, or any other, University. I certify that the protocol has been approved by the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg (Appendix 1; clearance certificate protocol number M970618).

Signed by: ………………………………

　　　　　　Rita Waisberg

On this the …6………day of…November…….2009

DEDICATION

This thesis is dedicated to a loving memory of my late husband, Peter, for his love and immense devotion. He was a constant source of inspiration in my life. His early and sudden departure left a big gap in our lives and he will be always remembered as a high spiritual and intelligent soul.

CONFERENCE PRESENTATIONS/POSTERS

Title: Insulin Secretion and Proinsulin-Processing in two Groups of South African Diabetics (poster). Presented at the 39th Society of Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) Congress, April 2003 Drakensberg, South Africa.

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Title: Comparison of Dietary Intake in Two South African Ethnic Groups (poster). Presented at the 43- rd SEMDSA, 13th NOFSA, April, 2008, Cape Town, South Africa.

ABSTRACT

Introduction: The data published by the Medical Research Council of South Africa demonstrated that cardiovascular disease and diabetes mellitus are the second and tenth leading causes of death in South Africa, respectively (Bradshaw *et al.*,2003). The prevalence of obesity is higher in the African than Indian population (Puoane *et al.,* 2002), whereas cardiovascular diseases (CVD) and diabetes are more common in the latter population (Omar *et al.,* 1994, Joffe *et al.,* 1994*).* Diabetes and hypertension has been related with abdominal obesity in a number of studies conducted in the African and mixed-ancestry communities of the Western Cape (Steyn *et al.,* 1996, Levitt *et al.,* 1993). The reason for the high prevalence of obesity in the African population is not known however it is known that the aetiology of obesity involves both environmental and genetic factors (Grundy, 2004).

Objective: The main aim of this project was to ascertain the role of metabolic, hormonal, anthropometric and environmental factors in the pathogenesis of obesity-related disorders in two South African ethnic groups namely Indian and African women. These populations were chosen because of the wide differences in risk factors for the development of CVD and diabetes reported in these groups.

Subjects and methods: Plasma and serum samples were taken during a 5-hour OGTT from 20 lean, 20 obese, 20 obese type 2 diabetic patients, and 10 overweight women of African and Indian origin, i.e. 140 subjects in total. All participants were recruited from an urban population of women residing in the Greater Johannesburg area. Serum insulin, C-peptide, proinsulin and adipokines were measured using ELISA kits. Fasting plasma glucose, serum cholesterol, HDL-cholesterol and triglycerides levels were measured on the ROCHE MODULAR System. Insulin resistance was calculated using HOMA. Visceral and subcutaneous fat areas were measured using a 5-level CT-scan. Nutrient intake was assessed using a validated quantified food frequency questionnaire. Socio-economic status was estimated from the level of education and the number of selected household amenities. The data collected from the project was analysed by using SAS System for Windows Release 8.02 SAS Institute Inc., Cary, NC, USA 1999-2001.

Results: Results from the study presented in the table below indicate that Indian females were more insulin resistant and had a worse atherogenic lipid profile than African females (statistically higher LDL and triglycerides levels). The greater visceral fat mass in the Indian subjects appears to be associated with triglycerides and correlated with insulin resistance (r=0.554, p<0.05). This effect was not observed in Africans. African females had a higher proportion of their energy intake as carbohydrates than Indians (49.3% and. 45.2%, respectively, p<0.05), whereas Indians had a higher proportion of their total energy intake as fat than Africans (34.0% and 29.9%, respectively, p<0.05). The level of educational attainment and possession of household amenities was lower in the African than Indian group, but this did not significantly influence any of the anthropometric variables.

Conclusions: Visceral fat accumulation was greater in diabetic and lean Indian subjects than in diabetic and lean African groups, which may explain their higher risk for obesity-related disorders at lower BMI. Significantly higher HOMA levels in obese Indians and significantly lower proinsulin/insulin ratio in lean and obese Indian women compared to lean and obese African women suggests that lean and obese Indians have better beta-cell proinsulin-processing efficiency than Africans, probably due to the higher secretory load imposed on beta cells by the higher level of insulin resistance in the Indian subjects. Triglycerides, one of the major components in the diagnostic criteria of metabolic syndrome, were significantly different in the obese group (higher in Indians) and this may lead to the higher prevalence of CVD in the Indian population. Interethnic differences for leptin levels were observed in the lean group of women with higher levels in the Indian subjects. When all non-diabetic subjects were combined serum leptin levels were significantly higher in Indian than African subjects. This is an intriguing result, since obesity is more common in the African than Indian populations of South Africa. Caloric intake was higher in lean African than Indian females. However, the hypothesis that lower leptin levels in lean African females may lead to higher dietary intake and thus an increased prevalence of obesity in this group must be evaluated in a longitudinal study of leptin levels and weight gain. The impact of lower socio-economic status in African than Indian population is not clear; however data from the literature does demonstrate a negative correlation of obesity prevalence with education and income.

Groups / Variables	LI (N=20)	OBI (N=20)	DI (N=20)	LA (N=20)	OBA (N=20)	DA (N=20)
Age (years)	44.0±1.7	47.0±1.9	52.0±1.2 [a, c]	42.0±1.4	43.0±1.2	47.6±1.5 [c]
BMI (kg/m^2)	22.9±0.4	32.9±0.6 [b]	34.8±1.0	22.4±0.4	34.4±0.7 [b]	36.2±1.0
Visceral Fat (cm^2)	51.3±6.2 [a]	96.7±7.8 [b]	117.5±9.9 [a]	30.3±2.9	81.4±6.4 [b]	93.9±6.2
HOMA - IR	2.23±0.27	4.28±0.52 [a, b]	7.54±0.74 [c]	1.87±0.28	2.83±0.51	6.56±1.26 [c]
Proinsulin/ insulin ratio	0.03±0.01 [a]	0.04±0.01 [a]	0.11±0.02 [c]	0.08±0.02	0.09±0.01	0.09±0.01
LDL (mmol/l)	3.24±0.17 [a]	3.30±0.18	3.10±0.22	2.58±0.17	3.12±0.18 [b]	2.98±0.2
Triglycerides (mmol/l)	1.05±0.11 [b]	1.44±0.11 [a]	2.11±0.36	0.79±0.1 [b]	1.09±0.12	1.41±0.11
TNF-α (pg/ml)	2.97±0.96 [a]	2.50 ±0.83	2.26 ±0.42 [a]	6.51 ±1.40	4.03 ±1.28	7.06±1.38
Leptin (ng/ml)	29.5±2.66 [a]	50.8±2.09 [b]	43.6±2.07 [c]	13.8±2.51	51.3±2.11 [b]	40.6±2.49 [c]
Education	3.35±0.15 [a]	2.70±0.16 [b]	2.40±0.13	2.35±0.17	2.70±0.15	2.10±0.21 [c]
Household amenities	3.85±0.08 [a]	3.80±0.09 [a]	3.70±0.11 [a]	3.00±0.16	3.35±0.15	3.15±0.18
Dietary energy intake (kJ) *	7496±468 [a]	7839±420	7423±386	10151±545	9125±557	9050±702

Data shown as Mean values ± SEM; adjusted for age; * excluding low energy reporters

[a] p<0.05 Indian vs. African; [b] p<0.05 Obese vs. Lean; [c] p<0.05 Diabetic vs. Obese

Abbreviations: LI- lean Indian; OBI-obese Indian; DI-diabetic Indian; LA-lean African; OBA-obese African; DA-diabetic African

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LIST OF ACRONYMS AND ABBREVIATIONS

A	Absorbance
Ach	Acetylcholine
ACE	American College of Endocrinology
ACOD	Acyl-CoA oxidase
ACTH	Adrenocorticotrophin
Acyl CS	Acyl-CoA synthetase
ADA	American Diabetes Association
ADP	Adenosine diphosphate
AE	acridinium ester
AHA	American Heart Association
AIDS	Autoimmune Deficiency Syndrome
Akt	protein kinase B
AMP	Adenosine Monophosphate
ANOVA	Analysis of Variance
Arc	arcuate
Arg	arginine
ARIC	Atherosclerosis Risk in Communities
ASCN/AIN	American Society for Clinical Nutrition and American Institute of Nutrition
ASP	Acyl Stimulating Protein
ATP III	Adult Treatment Panel III
ATP	Adenosine Triphosphate
AUC	Area under the Curve
BMI	Body Mass Index
BMR	Basal Metabolic Rate
BSA	Bovine Serum Albumin
c AMP	cyclic Adenosine Monophosphate
C	Celsius

c	curie
Ca	Calcium
CAD	Coronary Artery Disease
CART	Cocaine and Amphetamine Regulated Transcript
CC	Corpus Callosum
CCA	Common Carotid Artery
CCK	Cholecystokinin
CCX	Cerebral Cortex
CHD	Coronary Heart Disease
Cl	Chlorine
cm	Centimetre
CNS	Central Nervous System
CO_2	Carbon Dioxide
CoA	Coenzyme A
COOH	Carboxylic group
CPT-1	Carnitine Palmitoyl Transferase-1
CRH	Corticotropin Releasing Hormone
CT	Computerised Tomography
CV	Coefficient of Variation
CVD	Cardiovascular Disease
D	Deuterium
d	light path length
D_2O	Deuterium oxide
DA	Diabetic African
DAG	Diacylglycerol
db	leptin receptor gene mutation in mice
DBP	Diastolic blood pressure
dF	degree of Freedom
DI	Diabetic Indian
dl	decilitre
DMN	dorsomedial nucleus

DXA	Dual energy X-ray absorption
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
F	F factor
fa	Leptin receptor gene mutation in rats
FASEB	Federation of American Societies for Experimental Biology
FAT TOT	Fat total
FFA	Free Fatty Acid
FFM	Fat Free Mass
FIB TOT	Fibre total
FOV	Field of View
FSH	Follicle Stimulating Hormone
g	gram
GDP	Guanine Diphosphate
GFAT	Glutamine Fructose-6-phosphate Amidotransferase
GHRH	Growth hormone releasing hormone
GIP	Glucose Dependent Insulinotropic Peptide
GIT	Gastrointestinal Tract
GLP-1	Glucagon-like Peptide
GLUT 2 and 4	Glucose Transporters
GnRH	Gonadotropin-releasing Hormone
GOD	Glucose Oxidase
Gp	G-protein subtype
GPT	Glutamate Pyruvate Transaminase
GRB2	Growth Factor Bound Protein
GSIS	Glucose Stimulated Insulin Secretion Pathway
GTP	Guanine Triphosphate
H	Hydrogen
H_2O	Water
H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulphuric Acid

HbA1c	Glycated haemoglobin
HBr	Hydrogen Bromide
HCG	Human Chorionic Gonadotrophin
HCl	Hydrochloric Acid
HCO_3^-	Bicarbonate anion
HDL	High Density Lipoprotein
hGH	Human Growth Hormone
HGO	Hepatic Glucose Output
HI	Hippocampus
HOMA	Homeostasis Model Assessment
HPA	Hypothalamus Pituitary Adrenal Axis
HPL	Human Placental Lactogen
HPLC	High Performance Liquid Chromatography
HU	Hounsefield units
IAPP	Islet Amyloid Polypeptide
ICA	Internal Carotid Artery
IDL	Intermediate Density Lipoprotein
IGF-1	Insulin-like Growth Factor
IHD	Ischaemic Heart Disease
IL	Interleukin
IMT	Intimal Medial wall Thickness
IOTF	International Obesity Task Force
IP_3	Inositol 1, 4, 5-triphosphate
IR	Insulin resistance
IRAS	Insulin Resistance Atherosclerosis Study
IRS 1-4	Insulin Receptor Substrates
IRS	Insulin Resistance Syndrome
JNK	Janus Kinase
K	Potassium
kD	kilo Dalton
kg	Kilograms

KH_2PO_4	Potassium Dihydrogen Orthophosphate
kHz	Kilo Hertz
kJ	Kilojoules
K_m	Michaelis-Menten constant
kv	Kilo volts
L	Litre
LA	Lean African
LBM	Lean body mass
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
lh	Lateral hypothalamus
LH	Luteinizing Hormone
LI	Lean Indian
LPL	Lipoprotein Lipase
Lys	Lysine
m	Meter
M	Moles per litre
M_3	muscarinic receptor
mA	milli amperes
MAP	Mitogen Activated Protein
MBq	Mega Becquerel
MCH	Melanocyte Concentrating Hormone
ME	Median Eminence
mIu/ml	milli international unit per millilitre
MJ	Mega Joule
ml	millilitre
mm	millimetre
mM	millimoles per litre
mm/Hg	millimeters of mercury
MMWR	Morbidity Mortality Weekly Report
MODY	Maturity Onset Diabetes of Youth

MRI	Magnetic Resonance Imaging
mRNA	messenger Ribonucleic Acid
MSH	Melanocyte Stimulating Hormone
MUFA	Mono-unsaturated Fatty Acid
mv	millivolt
MW	Molecular Weight
Na	Sodium
Na_2CO_3	Sodium carbonate
Na_2HPO_4	Disodium Hydrogen Orthophosphate
NAD	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide (reduced)
NaH_2PO_4	Sodium Dihydrogen Orthophosphate
$NaHCO_3$	Sodium Bicarbonate
NCEP	National Cholesterol Education Program
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIDDK	National Institute of Diabetes and Digestive and Kidney Disease
NIDDM	Non-Insulin Dependent Diabetes Mellitus
nm	nanometer
nM	nanomoles per litre
NPY	Neuropeptide Y
NRC.FNB	National Research Council. Food and Nutrition Board
O_2	Oxygen
OBA	Obese African
OBI	Obese Indian
OGTT	Oral Glucose Tolerance Test
ohm	Units of electrical resistance (resistivity)
OWA	Overweight African
OWI	Overweight Indian
p	p level
PAI-1	Plasminogen Activator Inhibitor 1

PC 2 and (1/3)	Pro-Hormone Convertase
PCO_2	Carbon dioxide pressure
PEG	Polyethylene Glycol
PEG-C	Polyethyl Glycol Cholesterolase
pg	picogram
PGA	Phosphoglyceric Acid
PI-3 kinase	Phosphatidylinositol 3-kinase
PIP_2	Phosphatidylinositol 4,5-biphosphate
PKC	Protein Kinase C
pM	picomoles per litre
PMP	Paramagnetic Particles
POD	peroxidase
PPARγ	Peroxisome proliferator-activated receptor gamma
PUFA	Polyunsaturated Fatty Acid
PVN	Para Ventricular Nucleus
r	partial correlation coefficient
RAD	Ras-Associated with Diabetes
RER	Rough Endoplasmic Reticulum
RLUs	Relative light units
RMR	Resting Metabolic Rate
rpm	revolutions per minute
SATFA	Saturated fatty acid
SD	Standard Deviation
SEM	Standard Error Mean
SES	Socio-economic status
SFA	Saturated Fatty Acid
SH2	*src* homology 2
SHPTP2	Shuttle Protein
SSV	Single Strength Veronal buffer
TAG	Triacylglyceride
TBHB	2,4,6-tribromo-3-hydroxy benzoic acid

TCA	Tricarboxylic Acid
TG	Triglycerides
TGF-α	Transforming Growth Factor α
TGF-β	Transforming Growth Factor β
TNF-α	Tumour Necrosis Factor α
t-PA	Tissue Plasminogen Activator
Trans FA	Total trans- fatty acid
TRH	Thyrotrophin Releasing Hormone
TSH	Thyroid Stimulating Hormone
TUFA	Total Unsaturated Fatty Acid
Tyr	Tyrosine
U/L	Unit per litre
UDP	Uridine diphosphate
UK	United Kingdom
UKPDS	United Kingdom Prospective Diabetes Study
USA	United States of America
USSR	United Social Soviet Republic
UV	Ultra Violet
v	Volume
VLDL	Very Low Density Lipoprotein
WCC	White Cell Count
WHO	World Health Organisation
WHR	Waist to Hip Ratio
wt	Weight
z	Whole body impedance

LIST OF SYMBOLS

α	alpha
β	beta
ε	absorption coefficient
δ	del per mil
>	greater than
<	less than
λ	lambda
%	percentage
γ	gamma
4-AA	4-aminoantipyrene
Δ	difference between two values
μA	microamperes
μg	microgram
μM	micro moles per litre

A wise man should consider that health is the greatest of human blessings, and learn how by his own thought to derive benefit from his illnesses.

Hippocrates (460 BC -377 BC), Regimen in Health

CHAPTER 1- LITERATURE REVIEW

1.1 Hormones of the endocrine pancreas

The pancreas is a complex organ with both endocrine and exocrine components that are integrated in function, providing both the enzymes for digestion (the exocrine pancreas) and the hormones to regulate the metabolism of the digested nutrients (the endocrine pancreas). The endocrine pancreas, only 1%-2% of the pancreatic volume, is dispersed throughout the exocrine tissue as micro-organs called the islets of Langerhans. Each islet is comprised of mainly insulin-producing β cells (60%-80%) with ~ 5% somatostatin-producing δ cells and 15%-35% of either glucagon-producing α cells or pancreatic polypeptide-producing (PP) cells (Fig.1.1).
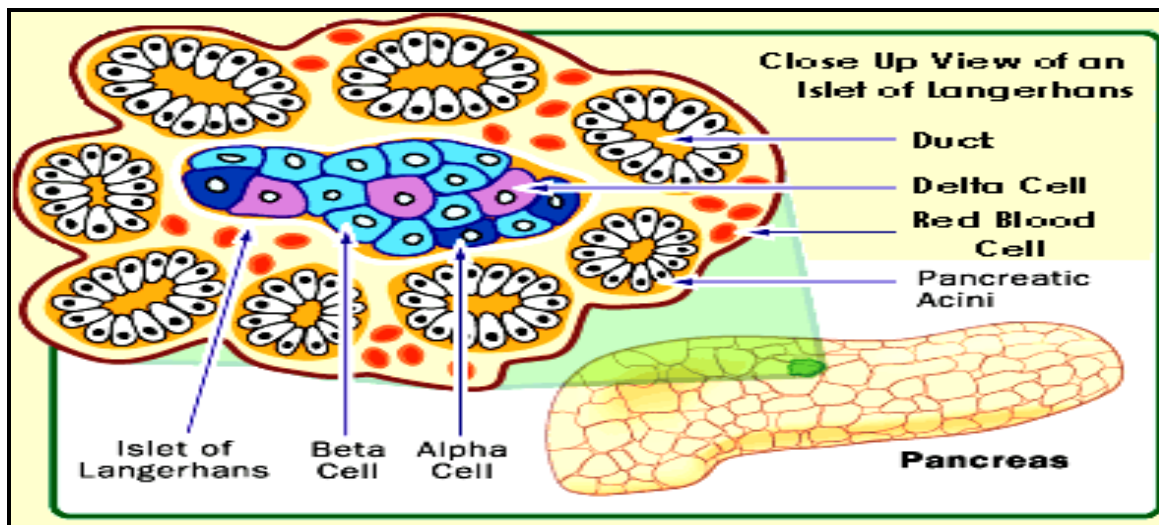
Figure 1.1 Physiologic anatomy of the pancreas (http://www.rajeun.net/aadiet.html)

1.1.1 Physiologic effects of glucagon, somatostatin and pancreatic polypeptide

When insulin was first crystallized, the preparations were found to contain a hyperglycaemic factor, which was later identified as the polypeptide hormone glucagon secreted by the alpha cells of the pancreatic islets with twenty-nine amino acid residues (MW 3,483). Unlike insulin, it consists of only a single polypeptide chain and contains no disulfide bonds (Montgomery *et al.,* 1977).

Glucagon is synthesized from a large precursor, proglucagon, in a tissue-specific manner in pancreatic alpha-cells and is also expressed within the intestinal tract, where it is processed not into glucagon, but to a family of glucagon-like peptides (enteroglucagon) (Young, 2005). Glucagon-like peptide 1 (GLP-1) constitutes of 30-amino acids and is involved in regulating secretion of insulin which is dependent on blood glucose levels. Glucagon-like peptide 1 (GLP-1) is created in the intestinal epithelial endocrine L-cells by differential processing of proglucagon, the gene that is expressed in these cells. GLP-1 also involved in inhibition of glucagon secretion, in that way contributing to limit postprandial glucose excursions. GLP-1 is immensely rapidly metabolized and inactivated by the enzyme dipeptidyl peptidase IV even before the hormone has left the gut, raising the possibility that the actions of GLP-1 are transmitted via sensory neurons in the intestine and the liver expressing the GLP-1 receptor (Holst, 2007).

Glucagon's action on glucose and ketone formation by hepatocytes is mediated by increase in cyclic-AMP-dependent protein kinase. It is most probably at this level the opposite effect of insulin upon glucagon-mediated events occurs. Cyclic-AMP-dependent protein kinase activity is low when glucagon secretion or action is blocked even in the absence of insulin, which could explain why marked glucose and ketone production is absent in bihormonal deficiency states (Unger, 1985). Glucagon stimulates hepatic glucose output and that leads to increases in glycemia. Glucagon is secreted from islets in a pulsatile fashion, and such pulsatile deliveries of glucagon are more beneficial in inducing hepatic glucose output in vitro, ex vivo, and in vivo (Jiang *et al.*, 2003). Diseases linked with excessively high or low secretion of glucagon are uncommon. Only in a case of cancers of alpha cells (glucagonomas) there is an excessive

glucagon secretion. Alpha pancreatic islet cell tumors, known as glucagonomas, and when they active they can produce a syndrome characterized by necrolytic migratory erythema, diabetes mellitus, weight loss, anaemia, glossitis, thromboembolism, neuropsychiatry disturbances and hyperglucagonemia (Carvajal *et al.*, 2002). Glucagon itself is responsible for most of the known signs and symptoms, and its induction of hypoaminoacidemia is belief to lead to necrolytic migratory erythema. Many patients are diagnosed very late in the clinical course for cure, but successful palliation of symptomatology can usually be accomplished with surgical and medical intervention (Chastain, 2001).Hyperglucagonemia plays an important role in initiating and maintaining hyperglycaemia when combined with delayed or deficient insulin secretion, as in the cases of type 1 and type 2 diabetes (Jiang *et al.*, 2003).

Somatostatin is another hormone recognized to inhibit glucagon secretion. The gene for somatostatin is on the long arm of chromosome 3. It codes for a 116-amino-acid peptide, preprosomatostatin, from whose carboxyl terminus is cleaved the hormone somatostatin, a 14-amino-acid cyclic polypeptide with a molecular weight of 1640. The name somatostatin comes from its ability to inhibit release of growth hormone (pituitary somatotropin) and was first isolated from the hypothalamus.

Almost every known stimulator of release of insulin from pancreatic beta cells also promotes somatostatin release from delta cells. This includes glucose, arginine, gastrointestinal hormones, and tolbutamide. The metabolic clearance of exogenously infused somatostatin in humans is extremely rapid; the half-life of the hormone is less than 3 minutes. Somatostatin acts in several ways to restrain the movement of nutrients from the intestinal tract into the circulation (Karam *et al.*,1991). In pharmacologic amounts, somatostatin significantly blunts the ketosis associated with acute insulin deficiency (Granner, 1990).

Pancreatic polypeptide (PP) is a 36-amino acid peptide (MW ~ 4200) is found in F cells located chiefly in islets in the posterior portion of the head of the pancreas. PP levels released rapidly into the circulation after ingestion of a meal and remain high for several hours (Adrian *et al.*,1976). In the brain, PP has a mainly orexigenic effect when administered directly (Asakawa *et al.*,1999). PP inhibits gastric emptying rate, gallbladder motility and exocrine pancreatic secretion in the gastrointestinal tract (Hazelwood,1993), and in contrast to its central effects, intraperitoneal administration of PP decreases food intake and increases energy expenditure (Asakawa *et al.*,1999). Continual administration of PP to obesity-prone animals

suppressed excessive weight gain (Asakawa *et al.*,2003), and mice who over express PP in the pancreas because of genetic manipulation are characterized by decreased food intake and lower body weight (Ueno *et al.*,1999).

1.1.2 Biosynthesis of insulin

A precursor molecule, preproinsulin, a long-chain peptide of molecular weight 11,500, is cleaved by microsomal enzymes to proinsulin (MW about 9000) almost immediately after synthesis and is formed by DNA/RNA-directed synthesis in the rough endoplasmic reticulum of pancreatic beta cells. Proinsulin is transported to the Golgi apparatus, where packaging into clathrin-coated secretory granules takes place. Maturation of the secretory granule is connected with loss of the clathrin coating and conversion of proinsulin into insulin and a smaller connecting peptide, or C-peptide. "Normal mature (uncoated) secretory granules contain insulin and C -peptide in equimolar amounts and only small quantities of proinsulin, a small portion of which consists of partially cleaved intermediates" (Karam *et al.*,1991).

1.1.3 Proinsulin processing

Proinsulin is a linear peptide of 86 amino acids. It contains within it the entire sequences of insulin and C-peptide and is the precursor of both of these molecules. Three intra-chain disulphide bridges within the insulin portion of the molecule maintain its tertiary structure *(*Kjems *et al.,* 1997*)*. The basis for the conversion of proinsulin, by enzymatic cleavage in the secretory granules of the β-cell, has been understood for some years. The two junctions of insulin and C-peptide are marked by pairs of basic amino acids that are target sequences for endopeptidases that cleave on the C-terminal side of the pair. Both pairs of amino acids are subsequently removed to leave C-peptide and insulin. This processing pathway is largely an ordered sequence rather than a random one (Halban,1994, Hutton,1994, Bailyes *et al.,*1994). Proinsulin is first cleaved at the 32-33 junctions by the proinsulin-converting enzyme (PC) 1, to give the cleaved molecule known as 32-33 split proinsulin (Fig.1.2). The newly exposed

amino acids at the C-terminal of insulin B-chain are rapidly excised by the exopeptidase carboxypeptidase H, producing des 31, 32 split proinsulin. A second endopeptidase, PC2, cleaves at the 65-66 junctions to produce mature insulin, and a further carboxypeptidase H (CPH) trimming of the cleaved peptide produces C-peptide. It seems that both PC1 and PC2 are largely specific for their substrates; proinsulin is a poor substrate for PC2, thus the 65-66 split and des 64, 65 split forms of proinsulin are probably not normally produced in significant quantities. Moreover, the speed of CPH processing is so rapid that normally neither split form survives long enough to be measurable, except as the des-split form (Kjems *et al.,*1997).

It is known that PC1 activity, but not that of PC2, increases in parallel with proinsulin concentration as the pancreas responds to glucose. Consequently 32-33 split and des 31, 32 split forms increase in concentration as PC2 becomes rate-limiting (Hutton,1994). The pancreas produces a small amount of proinsulin that escapes cleavage and secreted intact into the bloodstream, along with insulin and C- peptide. Because the liver does not remove proinsulin, it has a half-life 3-4 times that of insulin. This allows proinsulin to accumulate in the blood, where it accounts for 12-20% of the circulating immunoreactive "insulin" in the basal state in humans. Human proinsulin has about 7-8% of the biologic activity of insulin. The kidney is the principal site of proinsulin degradation (Karam *et al.,*1991).
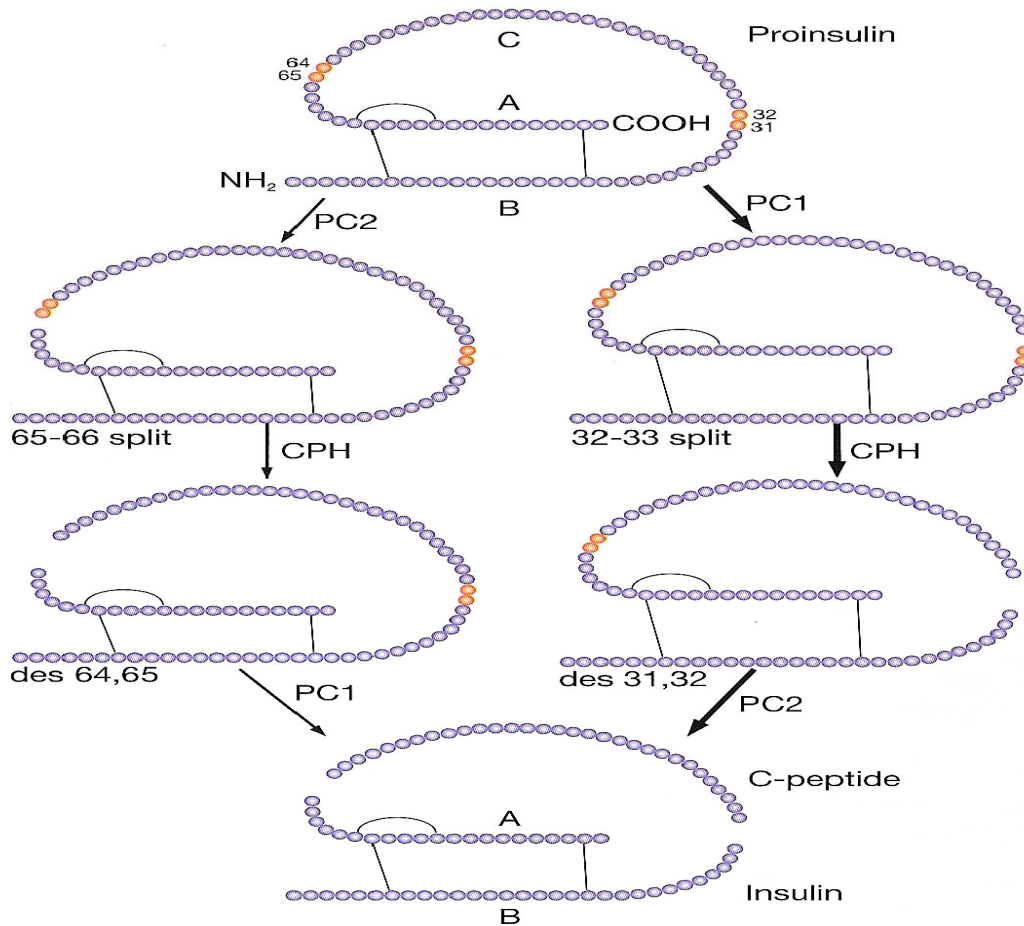
Figure 1.2 Proinsulin processing (Kjems *et al.*,1997)

1.1.4 C-peptide release

C-peptide is a small polypeptide of 31 amino acids (MW 3020 D) and is considered biologically inert. C-peptide plays a role in linking A and B-chains of insulin in the proinsulin molecule When the granule contents are released by exocytosis C-peptide and insulin are secreted in equimolar quantities from the pancreatic β-cells (Kjems *et al.,*1997). C-peptide is released into the portal vein, and passes through the liver almost unextracted (5-8% is

extracted). C-peptide has 2 to 5 times longer half-life than insulin therefore higher levels of C-peptide stay in the peripheral circulation and these levels vary less than insulin. Consequently plasma C-peptide levels may reflect pancreatic insulin secretion more reliably than the level of insulin itself. Most C-peptide not removed by the liver is degraded, or excreted by the kidney into the urine where it remains stable and can be quantified (Haibach *et al.,* 1987).

1.1.5 Insulin structure and metabolic effects

Insulin belongs to a family of structurally related regulatory proteins. Insulin-like growth factors and relaxin also included in this group. Molecular weight of insulin is 5808 and it consists of two chains linked by two disulphide bonds. One A-chain is comprises of 21 amino acids and the B-chain of 30 amino acids, with disulphide bonds located at positions A7-B7 and A20-B19. The A-chain also has an internal disulphide bond bridging A6 and A11.

There are minor differences in the amino acid sequence of the molecule from species to species. These are generally not sufficient to affect the biological activity but are enough to make the insulin antigenic. Glucose is the primary signal that stimulates insulin secretion by the β-cells of the pancreatic islets. Insulin is secreted directly into the portal venous circulation and the half-life of the hormone in the circulation, in humans is about five minutes. Almost all tissues have the ability to metabolize insulin, but over 80% of secreted insulin is normally degraded in the liver and kidneys (Kjems *et al.,*1997).

The main effects of insulin are to decrease plasma glucose and free fatty acid concentrations. Insulin also has a stimulatory effect on protein synthesis. The circulating insulin facilitates the diffusion of glucose into muscle cells and adipocytes, enhancing glucose utilization in these tissues. There appears to be a membrane barrier to glucose uptake in muscle and adipose tissue that insulin helps to overcome by increasing levels of glucose transporter 4 (GLUT4) in the plasma membrane. Hepatocytes, on the other hand, are freely permeable to glucose, and there is no need for enhancement of glucose entry into the liver. However, insulin does have a profound influence on hepatic glucose metabolism by inducing the synthesis of a specific glucokinase (Montgomery *et al.,*1977).

In addition to its role in peripheral metabolism, insulin may influence central regulation of energy balance (Schwartz *et al.,*1992). Fasting and glucose-stimulated circulating insulin levels are comparatively stable during infancy and childhood, and increase during puberty owe to decreased insulin sensitivity (Amiel *et al.,*1991). Glucose counter-regulatory hormones, such as glucagon, glucocorticoids, growth hormone and epinephrine, decrease insulin sensitivity and insulin action; insulin levels may increase during exogenous administration of these substances (Gerich, 1988, Rasmussen *et al.,* 1990).

1.1.6 Normal insulin secretion

Glucose is promptly taken up by the pancreatic β cell by the glucose transporter 2 (GLUT2), upon which it is phosphorylated by glucokinase, which is the rate-limiting step of β- cell glucose metabolism. Continue degradation leads to formation of pyruvate, which is then taken up in the mitochondria in which further metabolism leads to ATP formation. ATP is essential for the delivery of energy for the release of insulin, but it is also implicated in beta cell membrane depolarization. Increases in ADP/ATP ratio lead to activation of the sulphonylurea receptor 1 (SUR1) protein and subsequently lead to closure of the adjacent potassium channel (potassium inward rectifier ([KIR] 6.2 channel). The shutting of the potassium channels will change the membrane potential and open calcium channels, which triggers the release of preformed insulin-containing granules (Stumvoll *et al.,*2005).

1.1.7 Insulin receptor and mechanism of insulin action

The joining of insulin to a receptor on the surface of the target cell membrane indicates the start of insulin actions. The binding of these receptors in fat, liver, and muscle cells is related with the biologic response of these tissues to the hormone. The binding of these receptors with insulin is very rapid process with high specificity and with an affinity high enough to bind picomolar amounts (Karam *et al.,*1991).

The insulin receptor is a heterotetrameric protein includes two extracellular α subunits (MW 130,000) and two transmembrane β subunits (MW 90,000). The alpha subunits connected by a disulfide bonds to two transmembrane beta subunits. The binding of the ligand to the α subunit of insulin receptor accelerates the tyrosine kinase activity intrinsic to the β subunit of the receptor (Zhang, 2002).

Immense number of studies have indicated that the ability of the receptor to autophosphorylate and phosphorylate intracellular substrates is essential for its mediation of the complex cellular responses to insulin (Ellis *et al.*,1987, Kasuga *et al.*,1982, Rosen *et al.*,1983, Yu *et al.*,1984). The two α subunits equally take part in insulin binding and the kinase domains in the two β subunits are juxtaposed allowing autophosphorylation of tyrosine residues in the first step of insulin receptor activation (Luo *et al.*,1999, Ottensmeyer *et al.*,2000). The kinase domain go through conformational change upon autophosphorylation, providing a basis for activation of the kinase and binding of downstream signaling molecules (Hubbard,1997, Hubbard *et al.*,1994).

The insulin receptor additionally to tyrosine autophosphorylation is also subjected to ß-subunit serine/threonine phosphorylation, which attenuates receptor function. The chronic high levels of insulin that takes place as a result of insulin resistance could stimulate the relevant serine kinases, perhaps through the IGF-1 receptor, which can also be incited by high insulin levels. This kind of relation could release a mechanism for a vicious cycle of insulin-induced insulin resistance (Pessin *et al.*,2000). In the same way, counter-regulatory hormones and cytokines can trigger serine kinases, particularly protein kinase C (PKC), which has been involved in the development of peripheral insulin resistance. A number of PKC isoforms are chronically activated in human and rodent models of insulin resistance (Avignon *et al.*,1996, Considine *et al.*,1995b, Ishizuka *et al.*,1998). The insulin sensitivity and insulin receptor tyrosine kinase activity is improved by pharmacologic inhibition of PKC activity or reduction in PKC expression (Donnelly *et. al.,* 1998).

The insulin receptor, when is activated, phosphorylates a number of important proximal substrates on tyrosine, including members of the insulin receptor substrate family (IRS1/2/3/4), the Shc adapter protein isoforms, SIRP family members, Gab-1, Cbl, and APS. Tyrosine phosphorylation of the IRS proteins generates recognition sites for additional effector molecules containing Src homology 2 (SH2) domains. These contain the small adapter

proteins Grb2 and Nck, the SHP2 protein tyrosine phosphatase and, most significantly, the regulatory subunit of the type 1A phosphatidylinositol 3–kinase (PI 3-kinase) which is considerably important for insulin-stimulated glucose uptake via GLUT4 translocation (Czech *et al.*, 1999).

While PI 3-kinase activity is clearly necessary for insulin-stimulated glucose uptake, additional signals are also needed for the stimulation of GLUT4 translocation. Therefore, activation of PI 3-kinase by induction with IL-4 or by engagement of certain integrins does not induce GLUT4 translocation (Guilherme *et al.*,1998, Isakoff *et al.*,1995). However, two natural insulin receptor mutations that were fully proficient of activating PI 3-kinase failed to induce GLUT4 translocation and glucose uptake (Krook *et al.*,1997). "Glycogen synthase kinase 3 (GSK-3) is a cytoplasmic serine/theronine kinase that plays key roles in insulin signal transduction and metabolic regulation" (Dajani *et al.*,2001, Frame *et al.*,2001, Harwood, 2001).

In the insulin-signaling pathway, GSK-3 is active in the lack of insulin and it phosphorylates (and in that way inhibits) glycogen synthase and several other substrates. Insulin binding to the receptor activates a phosphorylation cascade, heading to inhibitory phosphorylation of GSK-3 by Akt. Therefore, insulin activates glycogen synthase by promoting its dephosphorylation through the inhibition of GSK-3 (Ryves *et al.*,2001). Additionally to regulating GSK-3 via Akt, insulin also accelerates compartmentalized activation of protein phosphatase 1 (PP1) in the complex containing glycogen particles, glycogen-targeting subunits and enzymes for glycogen synthesis and breakdown (Newgard *et al.*,2000).

1.1.8 Insulin and Lipid Metabolism

Insulin plays an important role in the co-ordination of whole-body lipid metabolism after the meal. In adipose tissue, insulin supports triacylglycerols (TAG) storage by exciting plasma lipoprotein uptake (Farese *et al.*,1991) and preventing TAG release as fatty acids in the plasma (Jensen *et al.*,1989a). Insulin affects hepatic lipid metabolism directly through its actions to modulate the expression of enzymes or secretory proteins [e.g. apolipoprotein B (apoB)]; and

indirectly by determining the rate of delivery of esterified or non-esterified fatty acids to the liver, through its antilipolytic effect on adipose tissue and its influence on the expression of lipoprotein lipase activity. Under normal conditions, the liver is regularly exposed, via the portal circulation, to major increases in insulin concentration in response to food intake (Zammit, 1996).

Work in human subjects has provided support for the hypothesis that the hepatic sensitivity to the inhibitory effect of insulin is a major determinant of lipemia, and, furthermore, in insulin-resistant subjects, that the decreased sensitivity to the inhibitory effect of insulin is an important contributory factor to the hypersecretion of very low density lipoprotein (VLDL) (Bourgeois *et al.*,1996, Bourgeois *et al.*,1995). Studies in humans have shown that intraportal infusion of insulin in humans results in a decrease in the secretion of VLDL-TAG (Vogelberg *et al.*,1980). Similarly, when humans are treated with insulin, the secretion of $apoB_{100}$-associated TAG is decreased. Although a large proportion of this effect can be attributed to the decrease in circulating NEFA concentrations due to the anti-lipolytic action of insulin, even when the concentration of NEFA was kept high, insulin still gave a decrease in hepatic VLDL secretion rate of about 30% (Lewis *et al.*,1995).

Insulin has an effect on the state of phosphorylation of hormone-sensitive lipase and lipolysis via a cAMP-dependent pathway, involving reduction of cAMP, and via a cAMP-independent pathway, that involves activation of a protein phosphatase activity that dephosphorylates both the regulatory and basal phosphorylation sites of hormone-sensitive lipase (Stralfors *et al.*,1989).

"Lipoprotein lipase is an endothelium-bound lipase that hydrolyses the triglyceride carried on lipoprotein particles to release glycerol and NEFA" (Goldberg,1996). In both the fasting and postprandial states diminished action of insulin on lipoprotein lipase results in decreased clearance of the triglyceride-rich lipoproteins, VLDL, and chylomicrons. This is a significant fundamental factor to hypertriglyceridaemia in type 2 diabetes (Valabhji *et al.*,2003). Glycerol is an essential intermediate of glucose and lipid metabolism by capacity of its ability to maintain glycogenesis in fundamental various systems, as well as serving as a precursor for the synthesis of triglycerides (TG) and other glycerolipids (Rognstad *et al.*,1974, Baba *et al.*,1995, McCabe,1995). While glycerol is a well recognized indicator of lipolysis and is a gluconeogenic precursor (Rognstad *et al.,* 1974), the relationship between glycerol and

glucose homeostasis is complicated and not yet completely explained (Gaudet *et al.*, 2000). Insulin is an important regulator of almost all aspects of adipocyte biology, and adipocytes are one of the most highly insulin-responsive cell types. Insulin enhances adipocyte triglyceride stores by a number of mechanisms that includes assisting the differentiation of preadipocytes to adipocytes, in mature adipocytes, accelerating glucose transport and triglyceride synthesis (lipogenesis), plus inhibiting lipolysis. Insulin also enhances the uptake of fatty acids derived from circulating lipoproteins by stimulating lipoprotein lipase activity in adipose tissue (Paradis *et al.,*1998).

1.1.9 Role of PPAR-γ in adipogenesis

Peroximase proliferator-activated receptor γ (PPAR-γ) is a nuclear hormone receptor which plays a central role in the control of adipocyte gene expression and differentiation. PPAR-γ is expressed predominantly in adipose tissue, and its expression is induced very early in the adipocyte differentiation process (Sharma *et al.*, 2007).When expressed ectopically, PPAR-γ converts fibroblasts into bona fide preadipose cells. Upon application of activators or PPAR-γ ligands, these cells differentiate into fat cells (Spiegelman *et al.*, 1997).

There are two additional PPAR isoforms, PPAR-α and PPAR-δ, whose expressions are less restricted but also occur at some level in certain adipose depots. Although PPAR-α activates the differentiation program, it appears to do so less efficiently than PPAR-γ. PPAR-δ did not stimulate adipogenesis even in response to the strongest PPAR-δ activators (Brun *et al.*, 1996). The major role of PPAR-γ in regulating adipogenesis is supported by the fact that thiazolidineadiones (TZD), which are high affinity, synthetic ligands for PPARγ, are potent inducers of adipocyte differentiation. PPARγ and its obligate heterodimeric partner, retinoid X receptor α (RXBα) in gene ablation studies reporting an absence of white adipose tissue have been placed as important transcription factors in regulating the gene expression leading to adipogenesis (Morrison *et al.*, 2000).

Hormones such as insulin, insulin-like growth factor (IGF)-1, growth hormone, glucocorticoids, and catecholamines play a prominent role in the promotion of adipogenesis.

They have effects on fat differentiation both in *vitro* and in *vivo*. The activity of all three isoforms of PPAR may be modified by one or more of these factors and make them more or less adipogenic (Brun *et al.*, 1996).

1. 2 Type 2 Diabetes: principles of pathogenesis

1.2.1 Diagnostic criteria for diabetes mellitus

Diabetes mellitus is a cluster of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is correlated with long-term damage, dysfunction, and failure of several organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Many diabetic individuals do not clearly match into a single class and therefore conveying a type of diabetes to an individual often depends on the circumstances present at the time of diagnosis.

Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency) is immune-mediated diabetes. This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin-dependent diabetes, type I diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas. Type 2 diabetes, which accounts for 90–95% of those with diabetes also referred to as non-insulin-dependent diabetes or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency (American Diabetes Association (ADA), 2005). The World Health Organization (WHO) criterion for diagnosing diabetes is a fasting plasma glucose at 126 mg/dl (7.0 mmol/l) or a 2-h glucose at 200 mg/dl (11.1 mmol/l) in an 75 g OGTT (WHO,1999). The criteria for the diagnosis of diabetes as specified in a position statement by ADA (2005) are presented in Table 1.1. Three ways to diagnose diabetes are possible, and each, in the absence of unequivocal hyperglycaemia, must be confirmed, on a subsequent day, by any one of the three

methods given in Table 1.1. The use of the haemoglobin A1c (A1C) for the diagnosis of diabetes is not recommended at this time.

Table 1.1 Criteria for the diagnosis of diabetes mellitus by ADA (2005)

1. Symptoms of diabetes plus casual plasma glucose concentration ≥ 11.1mmol/l (200 mg/dl) Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss, or
2. FPG ≥ 7.0 mmol/l (126 mg/dl). Fasting is defined as no caloric intake for at least 8 hours, or
3. 2-h post load glucose ≥ 11.1mmol/l (200 mg/dl) during an OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.
In the absence of unequivocal hyperglycaemia, the above criteria should be confirmed by repeat testing on a different day. The third measure (OGTT) is not recommended for routine clinical use.

The diagnostic criteria for diabetes adopted by the WHO and ADA are accepted internationally. Accordingly the International Diabetes Federation (IDF, 2005) has developed a global guideline which says detection programmes should use measurement of plasma glucose, preferably fasting, that following a positive screening test, diagnostic testing is required. For diagnosis, an OGTT should be performed in people with fasting plasma glucose (FPG) ≥ 5.6 mmol/l (≥ 100 mg/dl) and < 7.0 mmol/l (< 126mg/dl). Fasting or an OGTT should be performed where a random plasma glucose level) ≥ 5.6 mmol/l (≥ 100 mg/dl) and < 11.1 mmol/l (< 200 mg/dl) is detected on opportunistic screening (IDF, 2005).

1.2.2 Pathophysiology of type 2 Diabetes

Patients with type 2 diabetes have an impaired ability of insulin to stimulate muscle glucose disposal and inhibit hepatic gluconeogenesis and adipose tissue lipolysis (Reaven,1988, Reaven,1995a, Reaven, 1995b). Nonetheless as long as pancreatic β-cells can continue to secrete large amounts of insulin, gross decompensation of glucose homeostasis can be prevented. The development from normal to impaired glucose tolerance (IGT) is correlated with a deterioration of insulin resistance and an associated rise in both insulin and glucose. Further transition to diabetes incorporates overt β-cell failure with plasma insulin falling and glucose rising. This transition is seen as an inverted V-shape relationship of plasma insulin to plasma glucose (Reaven *et al.,*1968, Welborn *et al.,*1969, Savage *et al.,*1975, Hansen *et al.,*1986).

Pancreatic β-cells secrete insulin in a pulsatile fashion and release insulin in a biphasic manner, characterized by a "spike" lasting approximately 10 minutes (first-phase release) and followed by slowly increasing release (second-phase release). Both phases of insulin release are essential for sustaining normal glucose homeostasis (Gerich,1999). Several studies have shown that pulsatile insulin release is more effective than sustained release in promoting suppression of glucose production and stimulation of glucose utilization (Bratusch-Marrain *et al.,*1986, Paolisso *et al.,*1989). Probably at least 75% of insulin secretion is released in a very regular pulsatile fashion in healthy people. In contrast, type 2 diabetic subjects exhibit irregular oscillations of basal plasma insulin. Additionally, disturbed pulsatile insulin release is also a common feature in people prone to develop diabetes e.g. first-degree relatives of patients with type 2 diabetes. Assessment of high-frequency insulin pulsatility may be an important player in future tailoring of antidiabetic drugs and may come out to be significant as a predictor of type 2 diabetes in people at high risk for developing the disease (Schmitz *et al.,*2002).

It is not clear what factors would determine whether β-cells will fail in the face of prolonged insulin resistance. Glucose stimulation itself produces desensitization of the β-cell, or "glucose toxicity" (Lillioja *et al.,*1988a, Lillioja *et al.,*1987, Unger *et al.,*1985, Robertson,1989, Leahy *et al.,*1988). Thus, if insulin resistance results in an increase in glucose to maintain the signal

for increased insulin release, it is possible that eventually this over-stimulation leads to β-cell desensitization and initiates a vicious cycle, leading to worse and worse glycemia. Whether this desensitization is a general feature of the β-cell or is determined by one or two major genes requires further investigation (Lillioja *et al.,*1991).

1.2.3 Beta-cell apoptosis in the pathogenesis of human type 2 diabetes mellitus

Beta-cell mass is regulated by a balance of β-cell replication and apoptosis, as well as development of new islets from exocrine pancreatic ducts (neogenesis) (Finegood *et al.,*1995, Bonner-Weir,2000). Disruption of any of the pathways of β-cell formation, or increased rates of β-cell death, would result in decreased β-cell mass and thus reduced capacity to produce insulin. There is controversy as to whether β-cell mass is decreased in type 2 diabetes mellitus (Kloppel *et al.,*1985, Maclean *et al.,*1955, Stefan *et al.,*1982, Saito *et al.,*1979, Rahier *et al.,*1983, Clark *et al.,*1988, Guiot *et al.,*2001, Sakuraba *et al.,*2002).

These discrepancies are in part due to the lack of available data in humans. Furthermore, it is controversial whether β-cell apoptosis is truly increased in type 2 diabetes. A recent study by Butler *et al.,* (2003) gives new and convincing data indicating that increased apoptosis, rather than decreased neogenesis or replication, may be the main mechanism leading to reduced β-cell mass in type 2 diabetics. Butler *et al.,* (2003) reported that on autopsy obesity in non-diabetic humans is accompanied by a 50% increase in relative β-cell volume as compared with lean non-diabetic humans. However, the non-diabetic obese humans died younger than the non-diabetic lean humans and the difference found on autopsy may be due to age difference. Lean subjects with type 2 diabetes had a 41% deficit in relative β-cell volume compared with lean non-diabetic subjects. Once β-cell mass decreases below a critical level and insulin production no longer meets metabolic demands hyperglycaemia develops. Humans who have undergone 50% pancreatectomy have impaired glucose tolerance and insulin secretion in response to a hyperglycaemic clamp (Dresler *et al.,*1991, Robertson *et al.,*2002, Seaquist *et al.,*1996, Seaquist *et al.,*1994, Seaquist *et al.,*1992). On this basis it is possible to assume that a

60% reduction in β-cell mass in the face of insulin resistance may be sufficient to result in hyperglycaemia (Leonardi *et al.,*2003).

A series of studies (Maedler *et al.,*2002a, Maedler *et al.,*2002b) report on the roles of high glucose concentrations and different free fatty acids (FFAs) on β-cell proliferation, apoptosis and function in cultured human islets. While adipocytes store excess fatty acids in the form of triglyceride in lipid droplets, non-adipose tissues have a limited capacity for storage of lipids. In hyperlipidemic states, accumulation of excess lipid in non-adipose tissues gives rise to cell dysfunction and / or cell death. This lipotoxicity appears to be specific for saturated fatty acids in several tissues and is ameliorated by unsaturated fatty acids (Maedler *et al.,*2001a, De Vries *et al.,*1997, Cnop *et al.,*2001, Hardy *et al.,*2000, Listenberger *et al.,*2001). Although there are possible mechanisms to explain gluco- and lipotoxicity of β-cell apoptosis, it remains unclear what underlies the apoptotic loss of β-cells before hyperglycaemia and /or hyperlipidemia develops. A possibility for slowing the progression or even preventing type 2 diabetes may be to develop dietary and pharmacological strategies aimed at ameliorating increased β-cell apoptosis in people with a high risk for developing type 2 diabetes (Leonardi *et al.,*2003).

1.2.4 Genetic predisposition for development of type 2 diabetes

The cause of type 2 diabetes is most probably associated with a combination of genetic and environmental causes and, importantly, interactions between genes and environment. Despite the difficulties encountered, a number of genes and polymorphisms within them have now reproducibly been associated with risk of type 2 diabetes in a variety of studies. Disruptions of a variety of genes involved in insulin secretion or signalling, such as IRS-2, are known in animal models to result in alteration of fetal growth (Tamemoto *et al.*, 1994)

Recent genome-wide association studies (GWAs) have provided an important resource for furthering our understanding of type 2 diabetes disease mechanisms (Perry *et al.*, 2008). At the moment a small number of genes recognized through linkage scans or the candidate gene approach have been confirmed to be linked with type 2 diabetes (e.g., *PPARG, KCNJ11, CAPN10,* and *TCF7L2*) (Ng *et al.*, 2008).

GWAs have identified novel type 2 diabetes susceptibility loci inside or close to the *FTO* (fat mass and obesity associated)*, CDKAL1* (cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1), *CDKN2A/CDKN2B* (encoding the tumor suppressors p15[INK4b] and p16[INK4a], respectively), *IGF2BP2* (IGF2 binding protein 2), *HHEX/IDE* (homeobox, hematopoietically expressed/insulin degrading enzyme), and *SLC30A8* (zinc transporter) genes. With the exclusion of *FTO*, which modifies diabetes risk through increased adiposity (Frayling *et al.*, 2007), it is not proven how variation within these genes increases diabetes susceptibility, although initial work suggests that *CDKAL1* and *SLC30A8* might act via altered pancreatic β-cell function (Pascoe *et al.*, 2007, Sladek *et al.*, 2007, Steinthorsdottir *et al.*, 2007, Zeggini *et al.*, 2007). Usually, GWA studies begin with a fixed phenotype such as type 2 diabetes and subsequently search across a large range of potential genotypes for associations. In a reverse study it might initiate with a fixed genotype at a validated variant for example, the diabetes-associated single-nucleotide polymorphism (SNP) at *TCF7L2*, and explore across a range of possible phenotypes for new associations (McCarthy *et al.*, 2008).

There is a 2 to 4 times increased risk for developing type 2 diabetes with a positive family history. 15-25% of first-degree relatives of patients with type 2 diabetes develop impaired glucose tolerance or diabetes. In case of one parent had type 2 diabetes the lifetime risk (at age 80 years) to develop type 2 diabetes has been estimated to be 38% (Pierce *et al.*,1995).

The prevalence of type 2 diabetes in the offspring is reckoned to approach 60% by the age of 60 years when both parents are concerned (Tattersal *et al.*,1975). To differentiate genetic from non-genetic contributions concordance rates in monozygotic twins in additional of those in dizygotic twins have been used as only 50% of dizygotic twins genes share the environment (both intrauterine and extrauterine) (Stumvoll *et al.*,2005). Type 2 diabetes had been associated with a genetic polymorphism on chromosome 11p found close to the insulin gene (INS VNTR III), but only if the associated allele was transmitted from the father (Huxtable *et al.*, 2000).

As a substitute to both the thrifty genotype and thrifty phenotype hypotheses it was proposed that the association of low birth weight with type 2 diabetes in the Pima population might signal selective survival of low birth weight infants, with genetically established metabolic characteristics that were also related to the later development of diabetes (Hales *et al.*, 1992), Lindsay *et al.*, (2001). In the Pima Indian population, lower birth weight is associated with

parental diabetes, but in fact solely with diabetes in fathers (Lindsay *et al.*, 2000). Since obesity has a significant genetic component, insulin resistance occurring as a result of it could be considered genetic (Bouchard,1995). The ability of pancreatic β-cells to compensate for insulin resistance in obese individuals could explain why generally obese individuals who are insulin-resistant are not diabetic. So basically, one may attribute the pathogenesis of type 2 diabetes to a genetically determined inability to compensate for decreased tissue insulin sensitivity (Polonsky *et al.*,1996, Reaven,1995a).

1.2.5 Mitochondrial metabolism

The accumulation of ectopic triglyceride in liver, muscle and β-cells has suggested a defect in mitochondrial lipid oxidation in patients with type 2 diabetes, who have impaired oxidative capacity and small mitochondria in skeletal muscle (Kelley *et al.*,2002). PPARγ co-activator 1 (PGC1), a transcription factor for genes involved in mitochondrial fatty acid oxidation and ATP synthesis, was decreased in young, lean, insulin-resistant offspring of parents with type 2 diabetes, suggesting that an inherited defect in mitochondrial oxidative phosphorylation could lead to cellular lipid accumulation (Petersen *et al.*,2004). Decreased expression of PGC1 and related gene products could influence mitochondrial function in people with insulin-resistance and type 2 diabetes as shown gene expression profiling studies (Mootha *et al.*,2003, Patti *et al.*,2003).

1.2.6 Insulin resistance

1.2.6.1 Insulin resistance in obesity and type 2 diabetes

The term "insulin resistance" (IR) usually signifies resistance to the effects of insulin on glucose uptake, metabolism, or storage. Insulin resistance in obesity and type 2 diabetes is marked by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output (Reaven,1995a). These beneficial defects may result, in part, from impaired insulin signaling in all three- target tissues and, from down-regulation of the major insulin-responsive glucose transporter, GLUT4. Insulin attachment to its receptor, receptor phosphorylation and tyrosine kinase activity, and phosphorylation of insulin receptor substrates (IRSs) are decreased in muscle and adipocytes (Kahn *et al.*, 2000).

The tissue-specific modifications include the following: in adipocytes from obese humans with type 2 diabetes, IRS-1 expression is decrease, resulting in decreased IRS-1–associated phosphoinositide 3' kinase (PI3K) activity, and IRS-2 converts to the main docking protein for PI3K (Rondinone *et al.,*1997). On the contrary, in skeletal muscle of obese, type 2 diabetic subjects, IRS-1 and IRS-2 protein levels are normal but the PI3K activity correlated with both IRSs molecules is impaired (Kim *et al.,*1999).

1.2.6.2 Hyperinsulinaemia / insulin resistance in association with cardiovascular disease

The insulin resistance syndrome (IRS), also identified as the metabolic syndrome, is a cluster of cardiovascular risk factors often, but not always, associated with obesity. Reaven (1992) first drew attention to the association of insulin resistance (IR) and obesity, type 2 diabetes, high plasma triglycerides, and low plasma HDL cholesterol. Ever since its initial description,

there has been much experimental, clinical, and epidemiological data to support the association of this syndrome with cardiovascular disease (CVD) (McFarlane *et al.,* 2001).

One of the characteristic relationships between IR and cardiovascular risk factors is with "diabetic dyslipidaemia" (Sniderman *et al.,*2001). The exact mechanism by which IR causes CVD is not known. The increased obesity and unfavourable body fat distribution could to some extent explain more atherogenic features of cardiovascular risk factors in subjects with insulin-resistant type 2 diabetes mellitus compared to subjects with insulin-sensitive type 2 diabetes mellitus (Haffner *et al.*, 1999). Up to 75% of type 2 patients and 35% of type 1 patients die from cardiovascular events, such as myocardial infarction and cerebrovascular accident (Miettinen *et al.,*1998). In type 2 diabetic patients there is an increased insulin resistance that might be associated with hyperinsulinaemia, impaired glucose tolerance, hypertension and dyslipidaemia with especially raised serum triglycerides and decreased high density lipoprotein cholesterol (HDL) (Leung *et al.,*2001) (fig. 1.3).

Plasma LDL cholesterol levels in insulin-resistant subjects are similar to those in insulin-sensitive subjects. Qualitative variations in LDL cholesterol that results in "pattern B" distribution of LDL particles, which consists of smaller LDL particles are more sensitive to oxidation and therefore probably more atherogenic (Reaven *et al.,*1993b). Small dense LDL particles pass through the arterial wall faster and bind more vigorously to proteoglycans than larger LDL particles. The increased activity of hormone-sensitive lipase and as a result increased breakdown of stored triglycerides may be due to insulin resistance at the levels of adipose tissue (Fonseca *et al.,*2004). The existence of excess triglycerides in the circulation also influences other lipoproteins. The partial depletion of LDL and HDL cholesterol ester occurs as a result of exchanged VLDL triglycerides for cholesterol ester in LDL and HDL attributed to a protein in plasma called cholesterol-ester transfer protein (Egusa *et al.,*1985).
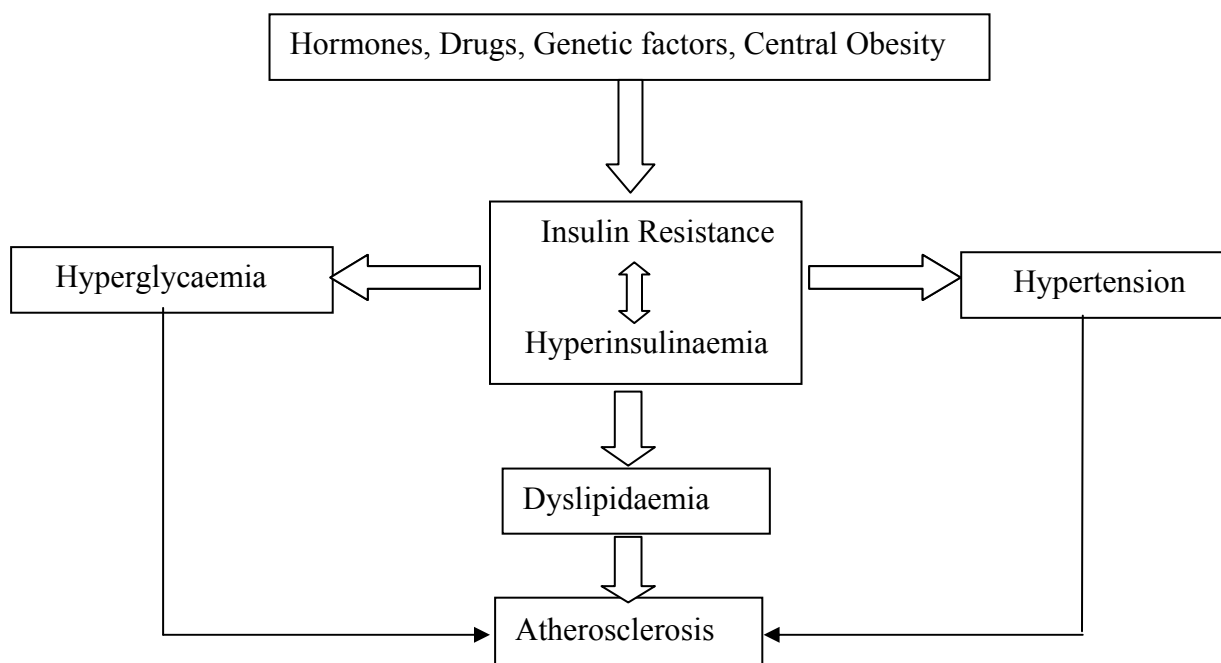
Figure 1.3 Association of insulin resistance with cardiovascular risk factors (adapted from Leung *et al.,*2001)

Two large observational studies, one in Canada and one in the UK, demonstrated a non-linear relationship between serum insulin levels and the incidence of ischaemic heart disease events. The risk was greatest at the highest insulin levels and there is some suggestion for a threshold effect. Insulin stimulates LDL cholesterol binding in both arterial smooth muscle cells and monocytes macrophages. It also increases the level of circulating plasminogen activator inhibitor 1 (PAI-1) in an environment of increased glucose and triglycerides, typical of type 2 diabetes mellitus (Ferri *et al.,*1999).

1.2.6.3 Abnormal insulin signaling, hyperinsulinaemia, and the vasculature

The precise role insulin plays in the pathogenesis of CVD is remains uncertain. Contrary, some of insulin vasodilator and anti-inflammatory properties should protect against atherosclerosis. Different mechanistic hypotheses have been proposed to describe this

controversy (McFarlane *et al.,*2001, Feener *et al.,*1997). One hypothesis is related to insulin being a growth factor that excites vascular cell growth and synthesis of matrix proteins. The other one refers to insulin signalling pathway that considers being responsible for abnormalities in glucose metabolism is also implicated in nitric oxide production. Therefore, the abnormal intracellular signalling that leads to hyperglycaemia may also be accountable for vascular disease due to loss of insulin's antiatherogenic properties, whereas hyperinsulinaemia continues to stimulate growth-promoting enzymes such as Mitogen-Activated Protein Kinase (MAPK). Additionally, abnormalities in expression and action of various peptides, growth factors, and cytokines, that include angiotensin II, endothelin-1, and IGF-I are correlated with imbalances in insulin homeostasis (Feener *et al.,*1997).

1.2.6.4 The role of Free Fatty Acid in obesity-related insulin resistance

An excess of circulating fatty acids may be a contributor to the development of insulin resistance. Plasma albumin-bound free fatty acids are derived largely from adipose tissue triglyceride stores via the action of the cyclic AMP-dependent enzyme hormone sensitive lipase. Fatty acids are also derived via the lipolysis of triglyceride-rich lipoproteins in tissues by the action of lipoprotein lipase (Eckel,1989). Insulin is essential to both antilipolysis and the stimulation of lipoprotein lipase. The most susceptible pathway of insulin action is the inhibition of lipolysis in adipose tissue (Jensen *et al.,*1989a). Therefore, when insulin resistance develops, the increased amount of lipolysis of stored triacylglycerol molecules in adipose tissue generates more fatty acids, which could in addition inhibit the antilipolytic effect of insulin, creating extra lipolysis.

Plasma FFA concentrations could be increased in obese subjects and in subjects with insulin resistance. Obesity-related insulin resistance precedes to reduced antilipolytic effect of insulin (Kishi *et al.,*1998). Highly increased activity of the sympathetic nervous system, which has been demonstrated in obese human subjects and type 2 diabetic patients might add to increased FFA production (Grassi *et al.,*1995, Scherrer *et al.,*1994, Hilsted *et al.,*1987).

FFA is taken up in liver and skeletal muscle cells. They negate the effects of insulin by increasing hepatic gluconeogenesis and by inhibiting glucose uptake and oxidation in skeletal

muscle (Randle *et al.,*1963, Gonzalez-Manchon *et al.,*1989, Felley *et al.,*1989). It has been suggested that this fatty acid-induced insulin resistance in liver and skeletal muscle may be a product of increased acetyl-CoA production and subsequent inhibition of glycolysis (Randle *et al.,*1963, Boden *et al.,*1994). The idea of a glucose-fatty acid cycle, which was originally illustrated by Randle *et al.,*(1963), has been called into question by Wolfe (1998). Wolfe provided evidence that glucose oxidation could directly regulate fatty acid oxidation by inhibition of fatty acid transport to the mitochondria. While Randle suggested that increased availability of FFA and fatty acid oxidation regulates glucose oxidation, Wolfe has developed a vice-versa concept in which the rate of glycolysis rather than the availability of fatty acids regulate fatty acid oxidation (Matthaei *et. al.*, 2000). Obesity may cause insulin resistance by a number of other mechanisms and these will be discussed in section 1.5.

1.2.6.5 Hypertension in relation to insulin resistance

Although it is well established that essential hypertension is frequently associated with IR the impact of this abnormality on blood pressure homeostasis is still a matter of debate. The association between essential hypertension and insulin resistance is a clearly established fact but the impact of insulin resistance on blood pressure homeostasis is still a topic of debate. The relationship between hypertension and IR is more significant in obese subjects. Obese subjects who lose reasonable amounts of weight had a significant decreases in blood pressure which correlated closely with the decline in fasting plasma insulin concentrations (McFarlane *et al.,*2001). A number of possible mechanisms have been suggested to explain how insulin resistance may cause hypertension (DeFonzo *et al.,*1991). Primary it is important to realise that insulin is a vasodilator when given intravenously to people of normal weight (Steinberg *et al.,*1994), with lesser effects on sodium reabsorption in the kidney (DeFonzo *et al.,*1975). Evidence indicates that sodium reabsorption is increased in Caucasian people but not Africans or Asians with the metabolic syndrome (Barbato *et al.,*2004). Sodium reabsorption is increased in Caucasian people while no increased observed in Africans or Asians with the metabolic syndrome (Barbato *et al.,*2004). Increased prevalence of hypertension in the metabolic syndrome could only moderately be attributed to insulin resistance when analysed

by concentrations of fasting insulin, or the HOMA-IR (Hanley *et al.,*2002). Hyperinsulinaemia stimulates hypertension via increased renal tubular reabsorption of sodium and water, increased sympathetic nervous system activity, proliferation of vascular smooth muscle cells, and modifications of transmembrane cation transport. Decreases in urinary sodium excretion by insulin at physiological concentrations mediated by binding to specific high-affinity receptors (Sechi *et al.,*1996). In black South African patients insulin resistance is an independent trait of essential hypertension and is to some extent rectified by treatment with a long-acting ACE inhibitor (Wing *et al.,* 1994). Nevertheless not all studies support the association of metabolic IR with essential hypertension. Obviously, hypertension is itself a complex disorder with many causes of the disease, and not all subjects with essential hypertension are insulin resistant (Fonseca *et al.,*2004).

1.3 Metabolic Syndrome

1.3.1 Defining the metabolic syndrome

Following the introduction of the concept of the 'Metabolic Syndrome" by George Reaven over a decade ago the amount of literature and research on this topic has been increasing exponentially. In the past 10 years this syndrome has received a number of different names including "Reaven's Syndrome", "Syndrome X", the "Metabolic Syndrome", "The Dysmetabolic Syndrome", and, more recently, the Insulin Resistance Syndrome (IRS) (Distiller, 2004). Reaven (1988) in his initial description of the syndrome identified five components. These were hyperinsulinaemia, glucose intolerance, hypertension, elevated triglycerides, and decreased HDL cholesterol. Since then numerous additional conditions and risk factors have been added, so that there is now an extensive list of metabolic abnormalities associated with the IRS (Table 1.2). The dominant underlying risk factors for the metabolic syndrome appear to be abdominal obesity and insulin resistance. Physical inactivity, aging, hormonal imbalance and genetic predisposition are also linked with metabolic syndrome.

Table 1.2 Components of the "Metabolic (Insulin Resistance) Syndrome" (adapted from Distiller, 2004)

Some degree of glucose intolerance	IFG or IGT, type 2 diabetes
Abnormal uric acid metabolism	
Dyslipidaemia	TG, HDL, LDL-particle size
Haemodynamic changes	Sympathetic nervous system activity Renal sodium retention Hypertension
Prothrombotic factors	PAI-1, Plasminogen
Markers of inflammation	CRP, WCC, IL-6, TNF-α
Endothelial dysfunction	Mononuclear cell adhesion Plasma concentration of cellular adhesion molecules Plasma concentration of asymmetric dimethylargine Endothelial-dependant vasodilatation

Abbreviations: IFG-Impaired Fasting Glucose; IGT-Impaired Glucose Tolerance; TG-Triglycerides; HDL-High Density Lipoprotein; LDL-Low Density Lipoprotein; PAI-1-Plasminogen Activator Inhibitor 1; CRP-C-Reactive Protein; WCC-White Cell Count; IL-6-Interleukin-6; TNF-α-Tumor Necrosis Factor-Alpha

The definition of the metabolic syndrome varies (Table 1.3). In the attempt to initiate the metabolic syndrome into clinical practice, several organizations have made an effort to devise simple criteria for its diagnosis. The first submission came in 1998 from a consultation group

on the definition of diabetes for the World Health Organization (Alberti *et al.*,1998). In 1999 it was suggested by the European Group for Study of Insulin Resistance (EGIR) that WHO definition of diabetes should be modified (Balkau *et al.*, 1999).

The term "insulin resistance syndrome" (IRS) was used by this group instead than metabolic syndrome. EGIR did not included patients with type 2 diabetes mellitus in their syndrome because insulin resistance was viewed primarily as a risk factor for diabetes This group concentrated more on abdominal obesity compared to WHO (Grundy *et al.*,2005). The European Group for the Study of Insulin Resistance and WHO both agree that either glucose intolerance or insulin resistance should be included for the diagnosis of IRS (Alberti *et al.*,1998, Balkau *et al.*,1999).

The National Cholesterol Education Program's Adult Treatment Panel III (NCEP: ATP III, 2001) specified that an increased fasting blood glucose (FBG) should be adequate to diagnose the metabolic syndrome. They agree that insulin resistance per se is not essential in order to diagnose the metabolic syndrome. In 2003, the American Association of Clinical Endocrinologists (AACE) adapted ATP III criteria to refocus on insulin resistance as the primary cause of metabolic risk factors (Einhorn *et al.*,2003). Like the EGIR (Balkau *et al.*,1999), AACE used the name "insulin resistance syndrome" and increased triglycerides, reduced HDL-C, elevated blood pressure, obesity and IGT were the main points for diagnosis. Basically a combination of the ATP III and WHO guidelines, the AACE guidelines do not identify the number of risk factors required to meet the definition of metabolic syndrome but rather consign this matter to individual clinical judgment. A family history of atherosclerotic cardiovascular disease (ASCVD) or type 2 diabetes, polycystic ovary syndrome, and hyperuricemia were another aspects used to inform clinical judgement. By the AACE's definition, once a person develops type 2 diabetes, the term insulin resistance syndrome no longer applies.

International Diabetes Foundation (IDF) published in 2005 new criteria that once more modified the ATP III definition. The abdominal obesity is greatly correlated with insulin resistance and IDF believed that other, more laborious measures of insulin resistance are unnecessary. The IDF clinical definition thus makes the existence of abdominal obesity necessary for diagnosis together with two other factors originally listed in the ATP III definition are sufficient for diagnosis. The present American Heart Association (AHA) and the

National Heart, Lung, and Blood Institute (NHLBI) statement, in contrast to IDF, maintains the ATP III criteria except for minor modifications such as the threshold for IFG was reduced from 110 to 100 mg/dL (6.2 to 5.6 mmol/l); this adjustment corresponds to the recently modified American Diabetes Association (ADA) criteria for IFG. The metabolic syndrome occurs in approximately 45% to 65% of individuals with impaired glucose tolerance in the USA and in 75% to 85% of patients with type 2 diabetes (Khan *et al.,*2003).

Lemieux and colleagues (2000) have suggested the importance of abdominal obesity and the so-called hypertriglyceridaemic waist phenotype as a central component. The association between obesity and the IRS has long been recognized. However, the level of obesity associated with increased risk for several diseases differs between populations. The WHO criteria that define overweight and obesity in terms of co morbidities are not necessarily appropriate for Asian populations. The International Obesity Task Force (IASO/IOTF) (2000c) together with the International Association for the Study of Obesity and supported by WHO (Western Pacific Region) redefined overweight as body-mass index (BMI) > 23 kg/m^2 and obesity as > 25 kg/m^2 in Asians. Central obesity was defined as waist circumference > 80 cm for women and > 90 cm in men (http://www.wpro.who.int). The IDF definition of IRS also includes ethnic specific waist circumference cut off points (see table 1.3).

The European Association for the Study of Diabetes (EASD) and the American Diabetes Association (ADA) issued a joint statement that has called into question the existence and reliability of the metabolic syndrome as a discrete clinical entity. The number of studies that been completed over the years regarding the prevalence of the metabolic syndrome and its associated cardiovascular risk, but the precise thresholds have not been established. It was suggested that metabolic syndrome requires much more study before its clarification as a "syndrome" is truly warranted and before its clinical utility is adequately defined (Kahn *et al.*, 2005, Cheng *et al.*, 2006). In the article published by Reaven, (2006) it was stated that the concept of the metabolic syndrome has little or no utility in clinical practice.

Table 1.3 Comparison of diagnostic criteria for the clinical diagnosis of metabolic syndrome suggested by the WHO, EGIR, NCEP/ATP III and IDF

CLINICAL MEASURE	WHO (1999)	EGIR (1999)	NCEP /ATP III, (2001)	IDF (2005)
Insulin resistance	Type 2 diabetes, IFG, IGT Plus any 2 of the following	Plasma insulin >75 percentile Plus any 2 of the following	None, But any 3 of the following	None
Abdominal Obesity Waist circumference Waist-to-hip ratio BMI	None Men > 0.90 Women > 0.85 > 30 kg/m^2	Men ≥ 94 cm Women ≥ 80cm	Men > 102 cm Women >88 cm	Europid ♦ Men ≥ 94 cm Women ≥ 80 cm Plus any 2 of the following
Blood Pressure	≥140/90 mm Hg	≥140/90 mm Hg	≥130/85mm Hg	≥130/85 mm Hg
Triglycerides	≥ 1.7 mmol/l (≥150 mg/dl)	≥ 1.7 mmol/l (≥150 mg/dl)	≥ 1.7 mmol/l (≥150 mg/dl)	≥ 1.7 mmol/l (≥150 mg/dl)
HDL-Cholesterol Men Women	< 0.9 mmol/l <1.0 mmol/l	<1.00 mmol/l In men or women	< 1.03 mmol/l < 1.29 mmol/l	<1.03 mmol/l ♣ <1.29 mmol/l ♣
Fasting plasma glucose (mmol/l)	≥ 7.0 mmol/l		≥ 6.16 mmol/l (≥ 110mg/dl) ♠	≥5.6 mmol/l (≥100mg/dl)
Other	Microalbuminuria	None	None	None

♠ The 2001 definition identified fasting plasma glucose of 110 mg/dl (6.1mmol/l) as elevated. This was modified in 2004 to be 100 mg/dl (5.6 mmol/l), in accordance with the ADA's updated definition of IFG

♣ These values have been updated from those originally presented to ensure consistency with ATP III cut-points

♦ South Asian and South-East-Asian men ≥ 80cm, women ≥ 80 cm; Japanese men ≥ 85cm, women ≥ 90 cm

1.3.2 Obesity, fat distribution and the components of metabolic syndrome

A French physician, Jean Vague, more than a half century ago first acknowledged the relationship between excess abdominal fat mass and insulin resistance (IR) and explained an association between a "masculine" or "android" obesity phenotype and diabetes (Vague,1956). Abdominal fat is made up of several different anatomic depots: subcutaneous fat and intra-abdominal fat. Subcutaneous fat can be separated into anterior and posterior or superficial and deep layers. Intra-abdominal fat can be separated into intraperitoneal and retroperitoneal sites. Intraperitoneal fat, also characterized as visceral fat, is composed of mesenteric and omental fat masses. Obese subjects have much larger absolute amount of each of these depots in upper-body compared to lean subjects. However, the comparative amount of abdominal fat with respect to total body fat mass is frequently similar in obese and lean groups. For instance, visceral fat constitutes about 10% of total body fat mass in lean and obese men (Abate *et al.,* 1995*).*

Whether there is a need to develop different BMI cut-off points for different ethnic groups is a become a point of discussion since growing evidence that the associations between BMI, percentage of body fat, and body fat distribution differ across populations and therefore, the health risks increase below the cut-off point of 25 kg/m^2 that defines overweight in the current WHO classification. An efforts to interpret the BMI cut-offs in Asian and Pacific populations were attempted twice previously (James *et al.*, 2002, Weisell, 2002), which contributed to the growing debates. Consequently, to shed the light on this debates, WHO organized the Expert Consultation on BMI in Asian populations (Singapore, 8-11 July, 2002) (http://www.who.int/bmi/index.jsp). Hong Kong Chinese have high BF% (body fat percentage) values at low BMIs and proposed BMI cut-off points for overweight and obesity as low as 23 and 26 kg/m^2, respectively (Ko *et al.*, 2001).

A WHO expert consultation reviewed scientific evidence that suggests that Asian populations have different associations between BMI, percentage of body fat, and health risks than do European populations. It reached a conclusion that the proportion of Asian people with a high risk of type 2 diabetes and cardiovascular disease is significant at BMIs lower than the existing WHO cut-off point for overweight (> or =25 kg/m^2). Nevertheless, available data do

not necessarily point to a clear BMI cut-off point for all Asians for overweight or obesity. The cut-off point for investigated risk varies from 22 kg/m^2 to 25 kg/m^2 in various Asian populations; for high risk it varies from 26 kg/m^2 to 31 kg/m^2. Therefore, no efforts were made to redefine cut-off points for each population separately. The consultation also decided that the WHO BMI cut-off points should be reserved as international classifications (WHO Expert Consultation, 2004).

Body fat distributions a better predictor of IR and cardiovascular risk than body mass (Abate *et al.,*1996). There is a better correlation between IR, type 2 diabetes, and hypertension with a central distribution of adiposity than with general increases in fat mass. Waist circumference serves as a clinical surrogate of intra-abdominal fat and correlates with insulin levels and IR (Fonseca *et al.,*2004). There are many large epidemiological and smaller physiological studies that have confirmed the association between abdominal obesity and insulin resistance, diabetes, and other components of the metabolic syndrome (Kissebah *et al.,* 1982, Folsom *et al.,*2000, Chan *et al.,*1994, Pouliot *et al.,*1992). Actually, excess abdominal fat is even associated with impaired insulin-mediated glucose uptake in lean adults (Ruderman *et al.,*1998).

It is not clear whether large waists are due to increases in subcutaneous adipose tissue or to visceral fat, therefore the use of computed tomography or magnetic resonance imaging could help with this distinction (Lee *et al.,*2004). Increases in intra-abdominal or visceral adipose tissue promotes a higher rate of flux of adipose tissue-derived free fatty acids to the liver by subcutaneous fat which in turn release lipolysis products into the systemic circulation and avoid more direct effects on hepatic metabolism (Aubert *et al.,*2003). There is a proof that after the meal the elevated free fatty acid release in upper body obese women comes from the non-splanchnic upper body fat, and not from the visceral depot (Guo *et al.,*1999). These results suggested that visceral fat might be an indicator for, but not a cause of, excess postprandial free fatty acids in obesity.

Results from the Framingham study show that each 10% weight gain is associated with a 6.5-mmHg increase in systolic blood pressure (Ashley *et al.,*1974). The relationship between hypertension and body fat is not only restricted to the morbidly obese individuals, but in fact there is a direct correlation between hypertension and body mass index throughout the entire range of body weight. The mechanisms by which obesity increases blood pressure are not well

understood (Lopez-Candales, 2001). However, increases in both plasma volume and cardiac output are thought to be crucial and these have been found to be sodium sensitive among obese adolescents. Fasting insulin is the best predictor of this sensitivity (Rocchini *et al.,*1989).

Hyperinsulinemia increases insulin-mediated glucose uptake in central hypothalamic neurons promoting sympathetic stimulation, through elimination of the normally inhibited effect of ventromedial cells from the hypothalamus on sympathetic centers (Reaven *et al.,*1996). Additional mechanisms that contribute to hypertension in obese individuals are activation of the renin angiotensin system by insulin resistance producing sodium reabsorption in the loop of Henle (Anderson, 1993), insulin-like growth factor I (Diez,1999), leptin (Mark *et al.,*1999) and physical compression of the kidneys due to accumulation of intrarenal fat and extracellular matrix (Lopez-Candales, 2001).

Free fatty acids (FFAs) released from adipocytes, particularly intra-abdominal adipocytes, can be transported to the liver where they stimulate synthesis of triglycerides and the assembly and secretion of VLDL (Fonseca *et al.,*2004). In general, with increases in free fatty acid flux to the liver, increased production of apoB-containing triglyceride-rich very low-density lipoproteins (VLDL) occurs (Lewis *et al.,*1995). The effect of insulin on this process is somewhat complex. In the setting of insulin resistance, increased flux of free fatty acids to the liver increases hepatic triglyceride synthesis; however, under physiological conditions, insulin inhibits rather than increases the secretion of VLDL into the systemic circulation (Lewis *et al.,*1996). This response, in part, is an effect of insulin on the degradation of apoB (Taghibiglou *et al.,*2002).

The increase in hepatic lipase located on the surface of hepatic endothelial cells accountable for an additional defect that affects lipoprotein metabolism in obesity(Nie *et al.,* 1998, Carr *et al.,* 2004). Hepatic lipase (HL) plays an essential role in LDL and HDL remodelling. High HL activity is related with small, dense LDL particles and with reduced HDL_2 cholesterol levels (Deeb *et al.,*2003). The primary target molecule for hepatic lipase is lipoprotein phospholipids (Grundy, 2004).

Central obesity-related triglyceride elevation could contribute to increases in small-dense LDL via cholesteryl-ester transport protein (CETP)-mediated transfer of triglyceride from VLDL and LDL, a process distinct from elevated hepatic lipase (Sibley *et al.*, 2003). Patients with

metabolic syndrome also fail to clear triglyceride-rich particles from their circulation following a meal which manifests as postprandial lipaemia and which is an independent cardiovascular risk factor (Reaven, 2002b).

1.3.3 Genetic susceptibility to metabolic syndrome

The pattern of metabolic syndrome alters significantly among individuals and populations, which indicates that genetic variability in regulation of the different risk components is responsible for these different patterns (Hanley *et al.,*2003). Additional data comes from differences in predisposition to metabolic risk factors and their patterns in different ethnic groups. Some populations (i.e. South Asians) demonstrate a high prevalence of metabolic syndrome even in the event of mild obesity (McKeigue,1996). The control of the pathways underlying the metabolic risk factors is highly complicated and vulnerable to genetic variations. Only when the nature of the genetic variation will be discovered we could fully comprehend the pathogenesis of the metabolic syndrome (Grundy, 2004).

1.3.4 Other manifestations of insulin resistance syndrome

Many other changes could accompany insulin resistance and they are not included in the diagnostic criteria for the metabolic syndrome. The list of these changes incorporates increases in apoB and C-III, uric acid, prothrombotic factors (fibrinogen, plasminogen activator inhibitor 1), serum viscosity, asymmetric dimethylarginine, homocysteine, white blood cell count, pro-inflammatory cytokines, the existence of microalbuminuria, non-alcoholic fatty liver disease and/or non-alcoholic steatohepatitis, obstructive sleep apnoea, and polycystic ovarian disease (Eckel *et al.,*2005). Cigarette smoking (Eliasson *et al.,*1994) and sedentary lifestyle (Lakka *et al.,*2003) could also result in many of the major criteria of the syndrome.

1.4 Non-traditional Cardiovascular Risk Factors associated with obesity and diabetes

Traditional cardiovascular risk factors do not sufficiently explain for the extent of cardiovascular disease in type 2 diabetes suggesting the presence of other risk factors which may include inflammation, abnormal fibrinolysis, and endothelial dysfunction (Fonseca, 2000). The number of different therapeutic strategies that are presently used in the management of dyslipidaemia and diabetes can improve these non-traditional risk factors. The example of that could be lipid management with statins that reduces markers of inflammation in plasma (Jialal *et al.,*2001b) and insulin sensitizers have a range of effects on many of these risk factors (Parulkar *et al.,*2001).

1.4.1 The role of endothelial cell dysfunction and oxidative stress in the pathogenesis of CVD

The control of vascular tone and maintenance of blood circulation, fluidity, coagulation, and inflammatory responses is influences by vascular endothelium which is an active and dynamic tissue. The endothelium controls vascular tone via the release of vasodilating and vasoconstricting substances. One of the most essential of vasodilating substance is nitric oxide (NO). In addition NO is vascular protective and inhibits inflammation, oxidation and vascular smooth muscle cell proliferation, and migration. Injure to the endothelium produces endothelial dysfunction with impaired release of NO and loss of its antiatherogenic protection (Hsueh *et al.,*2003).

The primary defect that connects insulin resistance and endothelial dysfunction is associated with deficiency of endothelial-derived nitric oxide. NO deficiency causes from decreased synthesis and/or release, in combination with too much consumption in tissues by high levels of reactive oxygen (ROS) and nitrogen (RNS) species, which are formed by cellular

disturbances in glucose and lipid metabolism. The alteration of the transcapillary passage of insulin to target tissues caused by endothelial dysfunction adds to impaired insulin action (Cersosimo *et al.,*2006).

Cardiovascular risk factors have an effect on many of the normal functions of the endothelium. Particularly, oxidized low-density lipoprotein cholesterol starts a series of events that begin with cell activation, endothelial dysfunction, local inflammation, and a procoagulant vascular surface. These events results in plaque formation and eventually plaque rupture and cardiovascular events (Gonzalez *et al.,*2003).

Clinical markers of oxidative stress include F2-isoprostanes, which are prostaglandin-like compounds formed in vivo from free radical-catalyzed peroxidation of arachidonic acid and which have emerged as novel and direct measures of oxidative stress. In patients with type 2 diabetes levels of F2-isoprostane found to be elevated in both urine and plasma (Gopaul *et al.,*1995, Ceriello *et al.,*2001, Davi *et al.,*1999). Nitrotyrosine, another marker of protein oxidation and indication of oxidative damage to DNA, is increased in patients with type 2 diabetes (Dandona *et al.,*1996, Gopaul *et al.,*1995, Ceriello *et al.,*2001).

The joining of mononuclear cells, such as monocytes and T lymphocytes, to the endothelium indicates the next step in atherogenesis; this joining is mediated by adhesion molecules present on the endothelial surface, such as vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM), and E-selectin. As soon as the monocyte migrates into the subendothelial space, it develops into a resident macrophage, takes up lipid largely via certain scavenger receptors such as SR-A and CD-36, and becomes a foam cell (Fig. 1.4). In the later stages of atherogenesis, smooth muscle cells travel to the surface and develop the fibrous cap of the lesion. Ultimately, lipid-laden macrophages discharge matrix metalloproteinase's resulting in plaque rupture and acute coronary syndromes such as myocardial infarction and unstable angina (Fonseca *et al.,*2004).
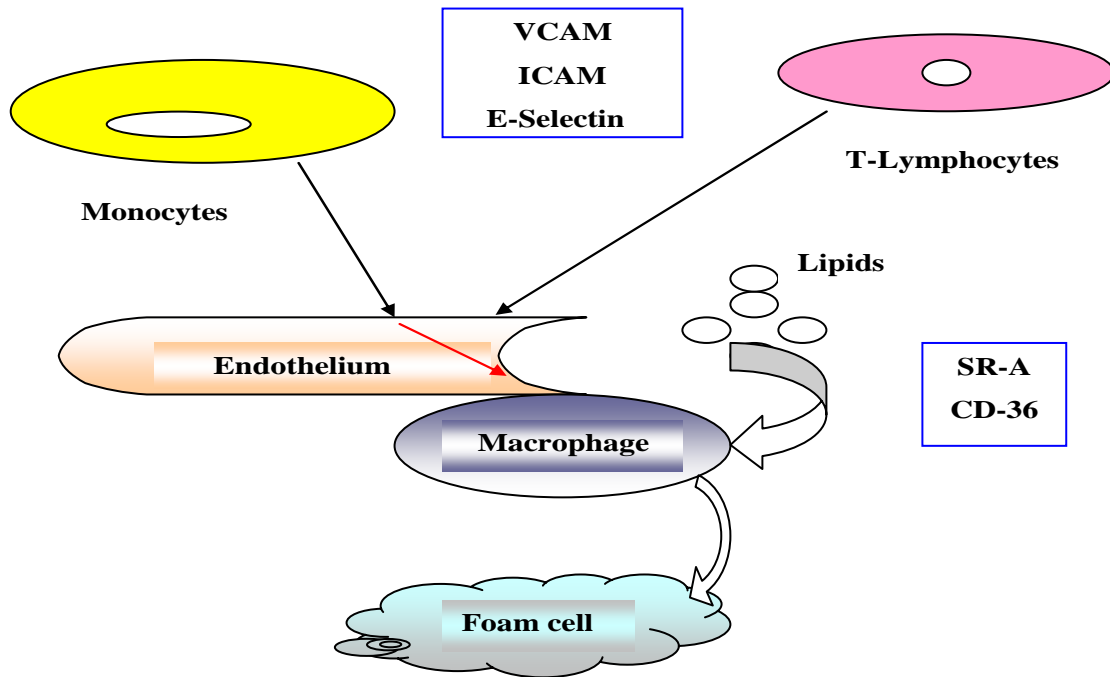
Figure 1.4 Binding effects of monocytes and T lymphocytes to the endothelium

Endothelial dysfunction has been reported in obese insulin-resistant subjects. In particular, an inverse relationship between obesity and endothelial function, as assessed by flow-mediated dilatation, or change in blood flow responses to methacoline, acetylcholine, or L-arginine, have been demonstrated (Arcaro *et al.,*1999, Steinberg *et al.,*1996, Perticone *et al.,*2001, Ziccardi *et al.,*2002). Since endothelial NO may mediate insulin-stimulated vasodilatation in skeletal muscle, it has been hypothesized that the physiological vasodilator action of insulin is blunted in obese subjects. In fact, indexes of insulin sensitivity that are linearly related to indexes of body fat distribution (BMI, waist-to-hip ratio, waist girth), may explain nearly half of the endothelial dysfunction associated with obesity (Esposito *et al.,*2002a).

1.4.2 Impaired Fibrinolysis and Prothrombotic State

The presence of a prothrombotic state could accelerate development of atherothrombotic cardiovascular disease in type 2 diabetes. This prothrombotic state consists of a diminished fibrinolytic capacity and an increased coagulability. Impaired fibrinolytic capacity emerge to be a feature of the metabolic syndrome of type 2 diabetes and can be a direct effect of visceral obesity (Banga, 2002).

Plasminogen activator inhibitor (PAI-1) is the most important physiological inhibitor of tissue-type plasminogen activator (t-PA) in plasma (Ridker *et al.*, 1993).The data published by Meigs *et al.*,(2000) demonstrated a positive association between fasting insulin levels and impaired fibrinolytic potential among people with normal glucose tolerance, independent of intermediary effects of obesity (such as BMI, waist circumference and waist-hip ratio) or lipid levels. However, markers of hypercoagulability, with the exception of von Willebrand factor (vWF) antigen in men did not show substantial associations with fasting hyperinsulinemia among subjects with glucose intolerance. Those subjects had higher PAI-1 and tPA antigen levels even after accounting for higher fasting insulin levels. According to the authors glucose intolerance may increase risk for acute thrombosis which mediated more by impaired fibrinolysis than by increased hypercoagulability, with fibrinolytic function in glucose intolerance further impaired by elevated fasting insulin levels.

Decreased fibrinolytic activity caused by elevated PAI-1 may hasten atherosclerosis by exposing vascular luminal wall surfaces to persistent and recurrent thrombi. Platelet function, PAI-1, and transcription factors associated with coagulation are inhibited by insulin. Dysregulation of these actions may add to the hypercoagulability in insulin-resistant states (Fonseca *et al.*,2004).

1.4.3 Inflammation and CVD in insulin resistant state

Inflammatory mediators play a principal role in the induction, development and rupture of atherosclerotic plaques. Thus markers may provide extra data about a patient's risk of

developing CVD and may become new targets for treatment. Some data suggested that inflammation is also involved in the development of Type 2 diabetes. In a number of prospective studies it was confirmed that increased levels of pro-inflammatory markers such as C-reactive protein (CRP) or reduced levels of anti-inflammatory markers such as adiponectin predict the development of Type 2 diabetes (Ziegler,2005).

Some inflammatory risk factors such as oxidized lipids, infectious agents, and cytokines produced from adipocytes or other inflammatory cells, accelerate production of IL-6, which serves as a "messenger" cytokine that stimulates the liver to produce inflammatory substances such as CRP (Jialal *et al.,*2001a). Hyperglycemia has been shown to stimulate proinflammatory cytokines and chemokine genes in monocyctic cells. Certain cytokines, such as tumor necrosis factor-α (TNF-α), impair insulin action in peripheral tissue and have a direct role in obesity-linked insulin resistance (Stentz *et al.,*2004). Figure 1.5 summarizes the theoretical link between cytokines secreted by adipose tissue (adipokines), inflammation, and CVD in obesity.
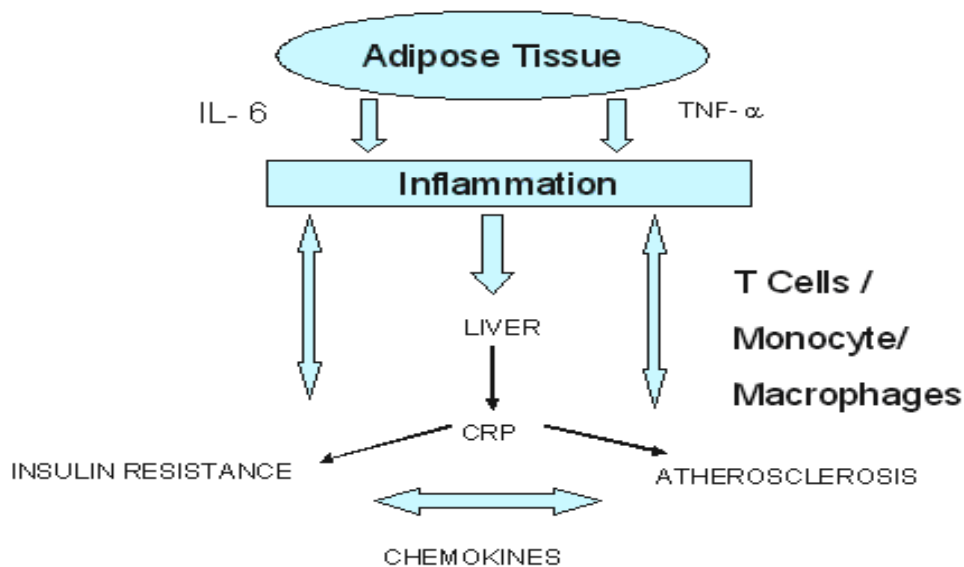
Figure 1.5 Interactions between inflammation, insulin resistance and atherosclerosis (adapted from Fonseca *et al.,*2004).

1.4.4 Hyperhomocysteinemia

Homocysteine (Hcy) have been recognized in a number of clinical and epidemiological studies as an important and independent risk factor for cardiovascular disease. Patients with severe hyperhomocysteinemia (HHcy) generally present with neurological abnormalities and premature arteriosclerosis. The reduced plasma Hcy improved endothelial dysfunction and reduced incidence of major adverse events after percutaneous coronary intervention.

The mechanisms by which Hcy causes atherosclerosis are not well-known. Several biological mechanisms have been suggested to elucidate cardiovascular pathological changes associated with HHcy. The number one of these mechanisms is endothelial cell damage and impaired endothelial function. The number two is dysregulation of cholesterol and triglyceride biosynthesis. The number three is stimulation of vascular smooth muscle cell proliferation. The number four is thrombosis activation and number five is activation of monocytes. To elucidate the vascular pathology of Hhcy four important biochemical mechanisms have been suggested, which include auto-oxidation through the production of reactive oxygen species, hypomethylation by forming *S*-adenosylhomocysteine (SAH), a potent inhibitor of biological transmethylations, nitrosylation by binding to nitric oxide or protein homocysteinylation by absorbing into protein (Yang *et al.*,2005).

In high-risk patients, such as with diabetes or with history of CHD, even reasonably increased total homocysteine (tHcy) concentrations are associated with coronary heart disease (CHD). In these patients an increased tHcy levels might trigger the event, resulting in a short-term association with the risk of CHD. Conversely, the tHcy concentration might be a marker of the degree of vascular disease (De Bree *et al.*, 2002).

Most cross-sectional and case-control studies have indicated a strong association between tHcy and vascular disease however the evidence provided by many prospective cohort studies has been evasive (Christen *et al.*, 2000). While elevated homocysteine levels increase risk for CHD in type 2 diabetes patients to a greater extent than among nondiabetic subjects (Hoogeveen *et al.*, 1998), fasting levels of homocysteine per se were not different when subjects with type 2 diabetes were compared to nondiabetic control subjects. Furthermore, the co-occurrence of specific features of the insulin resistance syndrome, especially hypertension

and central obesity, was associated with more marked elevations in homocysteine levels (Meigs *et al.*, 2001). Doncheva *et al.*, (2007) studied a group of male patients with CHD and found that 40.8% of them had increased tHcy levels, not associated with other lipid and non-lipid risk factors. The findings in the prospective, nested case-control study by Tanne *et al.*, (2003) were consistent with a strong predictive role of tHcy, independent of traditional risk factors or inflammatory markers, for incident ischemic stroke in patients at increased risk due to chronic CHD. A systemic review and meta-analysis published by Humphrey *et al.*, (2008) looked at homocysteine levels and CHD incidence. The risk of developing coronary heart disease increased nearly by 20% for each increase of 5 μmol/L of homocysteine in the overall analysis and the association between homocysteine and CHD was similar when analysed by sex, length of follow-up, outcome, study quality, and study design.

B vitamins are essential cofactors in the metabolism of homocysteine to methionine via the remethylation-pathway (vitamin B12, folic acid) and to cystathionine via the trans-sulphuration-pathway (vitamin B6). In the western world dietary deficiencies of folic acid, vitamin B12, and vitamin B6 seem to be identifiable among elderly people and it correspond to pathogenic factor related to the incidence of hyperhomocysteinaemia. A number of studies showed that dietary supplementation with folic acid and the vitamins B12 and B6 is an were effective in means to decrease plasma homocysteine (Sydow *et al.*,2001).

1.4.5 Vascular Wall Abnormalities - Carotid intima-media thickness (IMT)

Latest progress in technology has resulted in the development of techniques to identify early structural and functional changes that occur in the vessel wall. These changes consist of measurement of carotid IMT and aortic pulse wave velocity and arterial stiffness (Bokemark *et al.*,2002). IMT signifies a structural abnormality in the arterial wall and is a mostly good predictor of subsequent cardiovascular risk (Hedblad *et al.*,2000). The abnormal carotid IMT in patients with diabetes had been confirmed in a number of studies (Niskanen *et al.*,1996, Bokemark *et al.*,2002, Hedblad *et al.*,2000, Bonora *et al.*,1997, Redberg *et al.*,2002). Many of these studies have proposed an association between increased carotid IMT and IR (Howard *et al.*,1996). This observations are compatible with the potential effect of hyperinsulinaemia on

growth of vascular smooth muscle cells and extracellular matrix (Hsueh *et al.,*1999).In newly diagnosed patients with type 2 diabetes even without overt CVD there is an increase in carotid IMT (Wagenknecht *et al.,*1997). Increased arterial stiffness is another marker of early atherosclerosis that has been observed with increased frequency in patients with diabetes (Lehmann *et al.,*1997). It could be related to glycation of arterial collagen and elastin and accumulation of advanced glycation end products (Chappey *et al.,*1997). Some genetic effect or perhaps an early metabolic abnormality such as IR suggested to play role in nondiabetic young relatives of patients with diabetes that also had arterial stiffness (Riley *et al.,*1986, Hopkins *et al.,*1996, Fonseca *et al.,*2004).

1.4.6 Postprandial Hyperglycemia

Chronic hyperglycaemia has been shown to be responsible for multiple micro- and macrovascular complications as a result of hyperglycaemic damage through four major biochemical processes, including the polyol pathway, advanced glycation end products (AGEs), hyperglycaemia-induced activation of protein kinase C and increased hexosamine pathway flux and consequent over-modification of proteins by *N*-acetylglucosamine (Brownlee, 2005).

The mechanism through which increased postprandial glucose levels and lipid concentrations may damage endothelial cells on blood vessel walls appear to be complex (Ceriello,1997, Ceriello,1998, Ceriello *et al.,*1998a, Ceriello *et al.,*1998b, Ceriello *et al.,*1999, Ceriello *et al.,*2002). The activation of protein kinase C, increased expression of adhesion molecules, increased adhesion and uptake of leukocytes, increased production of proliferative substances such as endothelin, increased synthesis of collagen IV and fibronectin, decreased production of NO, and increased oxidative stress and inflammation are all included in these system. CRP is more strongly associated to postglucose load glucose than to fasting glucose in nondiabetic subjects (Festa *et al.,*2002a).

1.5 Adipocyte Endocrine System

In man and other mammals, a complex system has developed to maintain a stable supply of metabolic energy in an environment of uncertain and often limited food supplies. The adipose tissue is at the centre of this system. In times of nutritional surplus the energy is stored as triglycerides (TG) by adipose tissue. It also released energy in the form of free fatty acids (FFA) and glycerol during periods of caloric deprivation (Unger *et al.,*1999). Body weight within normal situations is sustained in narrow limits through regulation of both caloric intake and energy expenditure (Tataranni,1998, Ravussin *et al.,*1988). Neuronal systems perceive and react to input from hormones such as insulin and leptin that are secreted in proportion to body energy stores, and from the metabolism of circulating nutrients (such as glucose and FFAs). In feedback to this input, adaptive changes occur in energy intake, energy expenditure, and hepatic glucose production (fig.1.6).
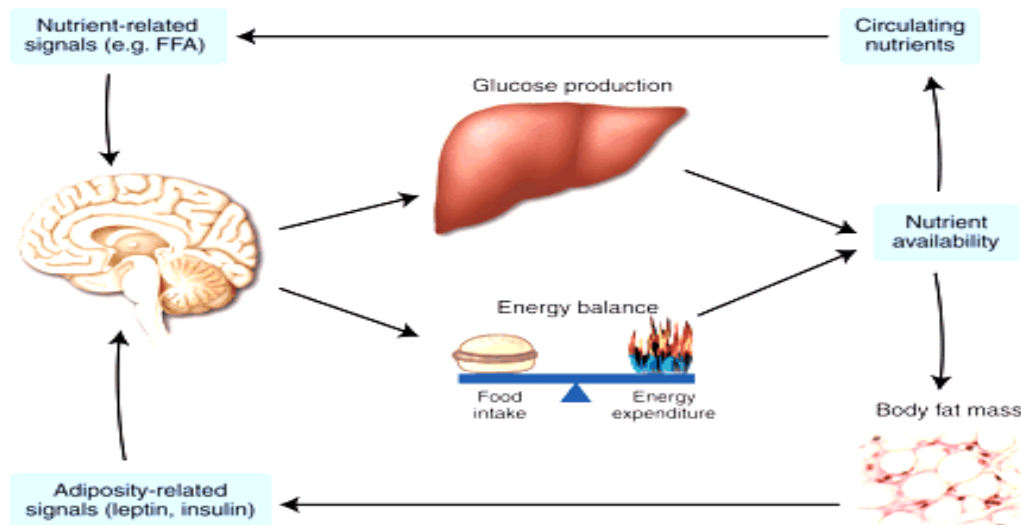
Figure 1.6 Model depicting the control of energy homeostasis and hepatic glucose metabolism by adiposity- and nutrient-related signals (Schwartz *et al.,* 2005).

However, obvious differences are observed in the predisposition of individuals to maintain energy balance, and data emerging over the past several years have established an additional role for the adipocyte - that of secretory cell (Fig. 1.7) (Kahn *et al.,* 2000). Adipose tissue can also function as multipotential endocrine and immune organ, secreting an array of cytokines, hormones, and other biochemically active molecules engaged in the regulation of insulin sensitivity and glucose homeostasis, hypothalamic activity, central sympathetic output, vascular tone, and reproduction, via endocrine/autocrine and paracrine effects (Diamond *et al.,*2002). A series of adipocyte-derived biologically active molecules that may influence the function and structural integrity of other tissues are referred to as 'adipocytokines'. Some examples of these substances are leptin, acylation-stimulating protein (ASP), tumor necrosis factor-α (TNF-α), plasminogen activator inhibitor-1 (PAI-1) and interleukin-6. ASP increases triglyceride synthesis by increasing adipocyte glucose uptake, activating diaglycerol acyltransferase, and inhibiting hormone-sensitive lipase (Cianflone *et al.,*1999, Murray *et al.,*1999). More recently, resistin has been identified as a novel adipose-specific cysteine-rich protein with a capacity to impair insulin sensitivity and glucose tolerance in rodents (Steppan *et al.,*2001).
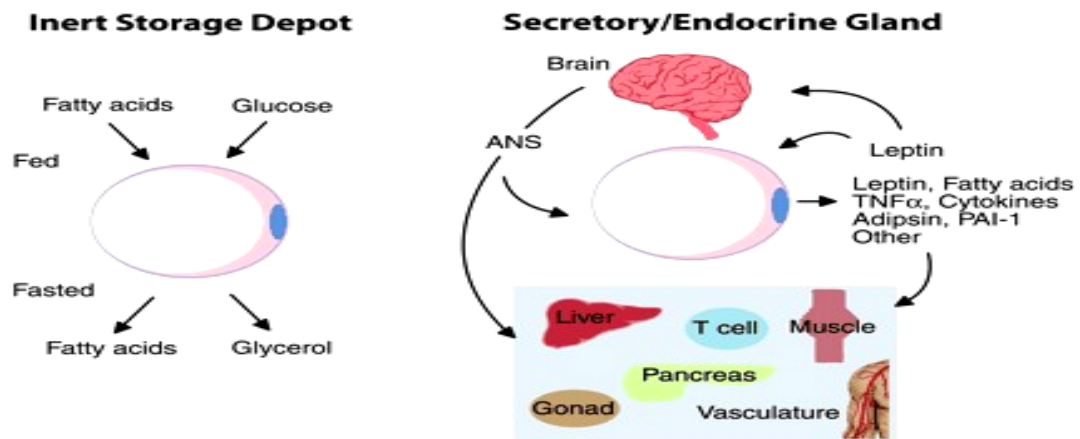
Figure 1.7 Evolving views of the biological functions of the adipocyte (Kahn *et. al.,* 2000).

1.5.1 Leptin and soluble leptin receptor

1.5.1.1 Regulation of leptin synthesis

Identification of the obese (ob) gene and its protein product, leptin, has increased our understanding of the pathophysiology of obesity. In ob/ob mice, a mutation in the ob gene prevents normal leptin production by adipose tissue, which causes both obesity and diabetes (Zhang *et al.,*1994). Treatment of ob/ob mice with leptin decreases body weight and normalizes blood glucose concentration (Pelleymounter *et al.,*1995). Leptin secretion and synthesis by adipocytes is shown to be in proportion to their triglyceride stores and correlate to body adiposity (Considine *et al.,*1996b) Adipose tissue is the prime source of leptin, but other tissues also express leptin, including placenta, ovaries, skeletal muscle and stomach (Hoggard *et al.,*1997a, Hoggard *et al.,*1997b, Spicer *et al.,*1997, Bado *et al.,*1998, Wang *et al.,*1998). Leptin is an adipocyte-derived hormone of 167 amino acid protein with an amino-terminal secretory signal sequence of 21 amino acids. The signal sequence is effective, and results in the translocation of leptin into microsomes with the subsequent removal of the signal peptide (Zhang *et al.,* 1994). Therefore, leptin circulates in the blood as a protein of 146 amino acid residues. The sympathetic nervous system, especially the β-adrenoceptor (β-AR) axis, has been implicated in the regulation of leptin gene expression. For example, the β-AR agonists, noradrenaline and isoprenaline decreased leptin gene expression in WAT in mice (Trayhurn *et al.,*1996) and decreased serum leptin (Stumvoll *et al.,*2000). The significance of β-AR in the control of leptin production in human subjects is uncertain, although there is evidence that the receptor may play an important role in the control of lipolysis in human omental and subcutaneous adipose tissue (Enocksson *et al.,*1995, Trayhurn *et al.,*1999).

1.5.1.2 Tissue sites of leptin synthesis

White adipose tissue (WAT) is the main location of leptin synthesis, but it is now obvious that leptin is also produced in other tissues. The brown adipose tissue (BAT) have been proved to be also a site of leptin production (Moinat *et al.,*1995, Tsuruo *et al.,*1996, Klingenspor *et al.,*1996, Deng *et al.,*1997, Dessolin *et al.,*1997, Cinti *et al.,*1997, Siegrist-Kaiser *et al.,*1997, Kutoh *et al.,*1998). Leptin expression in humans is much higher in subcutaneous than in omental adipose tissue (Hube *et al.,*1996, Montague *et al.,*1997, van Harmelen *et al.,*1998). An important new aspect to leptin biology has become apparent with the recognition that the placenta and ovary express the leptin gene and that they are sites of production of the hormone. The placenta also produces the leptin receptor gene, indicating that the organ is a target for the action of leptin as well as being a source of the hormone (Hoggard *et al.,*1997a). These statements propose that leptin may act in an autocrine manner (Fig.1.8). The expression of leptin by syncytiotrophoblasts (Hoggard *et al.,*1997b, Senaris *et al.,*1997, Bodner *et al.,*1999) has added support to the hypothesis of the significance of leptin in nutrient reposition.
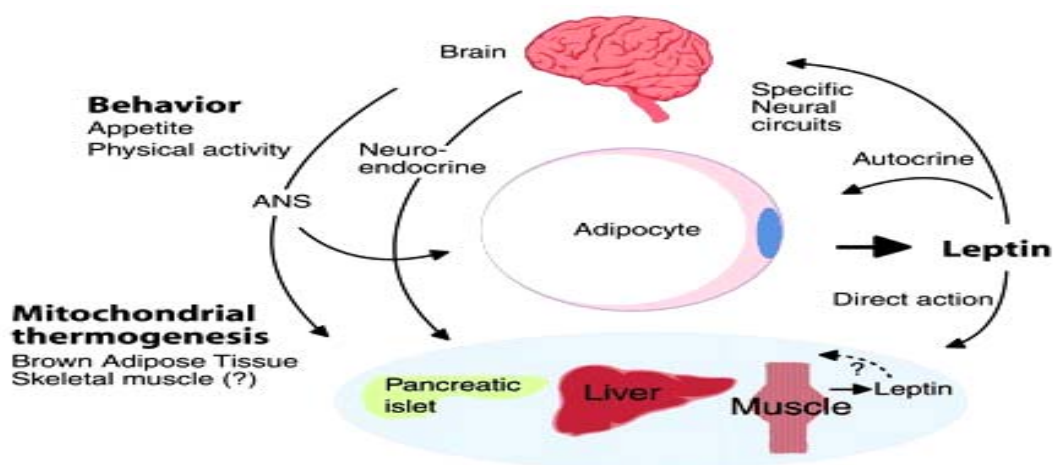
Figure 1.8 Multiple actions of leptin to regulate glucose homeostasis through autocrine, paracrine, endocrine, and neural circuits (Kahn *et. al.,* 2000).

1.5.1.3 Leptin, lipogenesis and FFA oxidation

In adipose tissue, insulin elevates lipoprotein lipase activity, releasing fatty acids from the triglycerides of VLDL and chylomicrons, and at the same time promotes glucose uptake, thus providing the substrate for glycerol-3-P synthesis and the storage of the fatty acids as triglycerides. Insulin also promotes the synthesis of fatty acids in adipose tissue by increasing the activity of acetyl-CoA carboxylase, the rate-limiting enzyme in fatty acid biosynthesis. In adipocytes, these lipogenic processes are generally suppressed by leptin, thus opposing insulin action (Iritani *et al.,*2000, Bai *et al.,*1996). Leptin also regulates LPL in a tissue-specific fashion. In adipose tissue, LPL activity is downregulated by leptin, whereas in muscle LPL activity is up-regulated (Arvaniti *et al.,*2001).

In nonadipocytes there is also convincing proof for the existence of adipose regulation of triglycerides (TGs). TGs content of normal nonadipocytes is typically maintained within a narrow range, irrespective of caloric intake (Unger *et al.,*1999). This is achieved, in part, by fatty acid-induced up-regulation of peroxisome proliferator-activated receptor (PPAR)α, and the enzymes acyl-CoA oxidase (ACO), and carnitine palmitoyl transferase I, which direct fatty acids into oxidative rather than lipogenic pathways (Zhou *et al.,*1998). As a result, unwanted fatty acids are oxidized, preventing accumulation as TGs (Shimabukuro *et al.,*1997). In leptin-insensitive or - resistant animals, the reverse action taking place when a marked increase in TGs build up in nonadipose tissue develops (Lee *et al.,*1997). These findings are evidence that a homeostatic system exists that is dependent on adipose tissue and dysfunctional in the absence of adipose derived leptin signaling (Diamond *et al.,*2002).

"The expression of two major enzymes of long chain FA oxidation, ACO, and carnitine palmitoyl transferase-1 (CPT-1) are remarkably increased in the adipocytes of hyperleptinemic rats in the course of the disappearance of their fat" (Zhou *et al.,*1997, Zhou *et al.,*1999). This discovery indicates that experimentally induced hyperleptinemia can transfer adipocytes from fat-storing cells into fat-burning cells (Wang *et al.,*1999b). The major increase in the expression of PPAR in WAT described in a studies by Kakuma *et al.,*(2000) and Zhou *et al.,*(1999) suggested that PPAR might be a proximal mediator of leptin action and, by up-regulating its target enzymes of FA oxidation, CPT-1 and ACO it play a role in the disappearance of adipocyte fat. In addition, it seemed possible that PPAR might be involved

in other changes in the expression profile of the overleptinized rats, such as the up-regulation of uncoupling proteins (UCP)-1 and 2, and of peroxisome proliferator-activated receptor γ coactivator (PGC)-1α and the down-regulation of lipogenic transcription factors and enzymes (Lee *et al.,*2002). The increase in fatty acid oxidation in non-adipose tissue is attributed to ability of leptin to stimulate the activity of CPT-1 and inhibit acetyl-CoA carboxylase (ACC), rate-limiting enzymes for fatty acid oxidation and synthesis, respectively (Bai *et al.,*1996).

1.5.1.4 Leptin and insulin

Insulin is an important regulator of energy homeostasis and stimulates glucose, free fatty acid and amino acid uptake by tissues and tissue anabolism. The link between leptin and insulin should be present in the regulation of energy homeostasis. In 1996, Kieffer and his colleagues reported that leptin receptors are expressed in the insulin producing β-cells within the pancreatic islets, suggesting that leptin might influence insulin secretion through a direct action on these cells. This hypothesis was investigated by several others, with apparently conflicting results (Dagogo-Jack *et al.,*1996, Fehmann *et al.,*1997, Poitout *et al.,*1998a, Poitout *et al.,*1998b, Russell *et al.,*1998).

Leptin transmits its effects by using both long (OB-Rb) and short (OB-Ra) receptors, which are present in pancreatic β-cells (Kulkarni *et al.,*1997), indicating the possibility of direct actions on insulin secretion. Some, but not all, in vivo studies have shown acute inhibitory effects of leptin on insulin secretion (Kulkarni *et al.,*1997, Cases *et al.,*2001). Notably, leptin-induced inhibition of insulin secretion has been observed in human islets (Kulkarni   *et al.,*1997, Lupi   *et al.,*1999), an effect that was associated with increased potassium channel permeability (Lupi *et al.,*1999).

The direct role of peripheral leptin in controlling glucose uptake and metabolism in skeletal muscle, the principal site of insulin-stimulated glucose disposal, remains controversial. In rats, some studies have shown acute, leptin-induced increases in muscle glucose uptake (Ceddia *et al.,*1999a); whereas others found that short-term in vivo leptin administration had the opposite effect on muscle glycogen synthesis and 2-deoxyglucose uptake (Harris,1998). Most studies

report that chronic leptin treatment, in either lean or obese rodents, enhances muscle glucose uptake and /or insulin responsiveness (Harris,1998, Wang *et al.,*1999a, Yaspelkis *et al.,*1999), suggesting that these effects might require long-term exposure. The importance of glucose in regulation of leptin expression and secretion have been suggested in a number of studies (Mizuno *et al.,*1996, Mueller *et al.,*1998). Conversely leptin modifies insulin sensitivity of muscle and liver to glucose uptake probably via CNS control and therefore it seems likely that glucose is regulated and is not a regulator (Margetic *et al.,*2002).

1.5.1.5 Leptin and obesity

Leptin is considered to be a major player in the regulation of body weight (Blum *et al.,*1997, Pelleymounter *et al.,*1995). The decrease in food intake and body weight following peripheral administration of leptin has been demonstrated in lean and ob/ob mice(Campfield *et al.,*1995, Halaas *et al.,*1995, Pelleymounter *et al.,*1995). While the role of leptin in ob mice is clear, its role in the pathogenesis of obesity in humans is still not well understood. In contrast to the ob mouse, most obese humans do not have any mutations in the coding region for leptin.

Leptin levels are elevated in obese subjects and a positive correlation between serum leptin and measures of adiposity was indicated in a number of studies. "Leptin resistance" could be associated with inability of elevated leptin levels to revise the obese state of obese subjects. Participation of a leptin receptor carrier protein in this kind of regulation has been suggested (Considine *et al.,*1995a, Considine *et al.,*1996a, Considine *et al.,*1996b).

1.5.1.6 Peripheral Leptin receptor (Ob-R)

Leptin receptors are divided into two groups: one group that has short (32-97 amino acid residues) intracellular domains and another that has a long 302-residue intracellular domain (Friedman,1998). The long form of the receptor (L-Rb) is predominantly expressed in the brain but, importantly, it is present at lower levels in multiple peripheral tissues, including

skeletal muscle, liver, and adipose tissue (Tartaglia *et al.*,1995, Lee *et al.*,1996, Ghilardi *et al.*,1996). Leptin receptors are part of the class I cytokine receptor family (Tartaglia,1997), which is labelled by a single membrane-spanning domain that affiliates with a class of protein tyrosine kinase termed the Janus kinases (JAK). JAKs link ligand binding of receptors to tyrosine phosphorylation of known signalling proteins such as phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK), as well as to a family of transcription factors known as the signal transducers and activators of transcription (STATS) (Hirano *et al.*,1994).

L-Rb is thought to function as the primary receptor that mediates leptin-induced signal transduction (Ghilardi *et al.*,1996); but, several studies have shown that short forms of the L-R are also able of transducing signals (Bjorbaek *et al.*,1997, Murakami *et al.*,1997, Fukuda *et al.*,1999). Some alternatively joined isoforms of leptin receptor have been identified (Ob-Ra, Ob-Rb, and Ob-Re). Ob-Rb is enhanced in the hypothalamus, the site of leptin's action on food intake and body weight. The activation of Ob-Rb by leptin in the hypothalamus produces the inhibition of neuropeptide Y/agouti-related protein neurons and activation of pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript neurons (Huang *et al.*, 2001). In addition, to the membrane-bound isoforms of the leptin receptor with varying cytoplasmic length, a soluble form of the soluble leptin receptor (sOb-R) could be verified. sOb-R characterizes the main leptin-binding compound in plasma resulting in a fraction of bound and a fraction of free leptin in plasma (Lammert *et al.*,2001). In contrast, to rodents, where an alternative splicing results in sOb-R (Yamaguchi *et al.*,1998) no m-RNA coding for sOb-R could be detected in humans. No m-RNA coding for sOb-R could be detected in humans which is quite the opposite to rodents, where an alternative splicing results in sOb-R (Yamaguchi *et al.*,1998). Latest work confirmed that sOb-R is generated by cleavage of the membrane-bound form of the Ob-R (Maamra *et al.*,2001).

During late stages of mouse pregnancy it was observed that levels of both OB-Re and leptin increased by up to 40-fold, which indicates that the soluble leptin receptor may modulate leptin's biological activity *in vivo* (Gavrilova *et al.*,1997). sOb-R levels are decreased in obesity compared to lean subjects and as a result of this the fraction of free leptin is increased (van Dielen *et al.*,2002). Circulating sOb-R levels are increased with the reduction of body weight through diet or surgical procedures. Thus in turn increases he fraction of bound leptin

(Laimer *et al.*, 2002). Therefore, sOb-R might act as a modulating factor of leptin action and plays a significant role in leptin resistance. The precise physiological mechanisms regulating sOb-R plasma concentration are not identified (Sandhofer *et al.*,2003).

1.5.2 The role of the novel adipocyte-derived hormone adiponectin in human disease

Adiponectin is the product of the apM1 gene with 244 amino acid protein, which is exclusively and significantly expressed in human adipose cells and also ascribed to as gelatin-binding protein-28 (Maeda *et al.*,1996, Nakano *et al.*,1996). This cytokine is a collagen-like protein that affiliates to the soluble defence collagen superfamily and has structural homology with collagen VIII and X and complement factor C1q (Kishore *et al.*,2000, Maeda *et al.*,1996, Scherer *et al.*,1995, Hu *et al.*,1996, Takahashi *et al.*,2000). The protein holds a signal sequence at the NH_2-terminal end followed by a short hypervariable region with no homology between different species, a collagen-like domain, and a C1q-like globular domain at the COOH-terminal end (Shapiro *et al.*,1998, Hu *et al.*,1996).

Adiponectin is ample in human plasma, with concentrations ranging from 5 to 30 μg/ml, thus accounting for approximately 0.01% of total plasma protein (Arita *et al.*, 1999). Adiponectin is an anti-inflammatory cytokine that improves insulin sensitivity (Nawrocki *et al.*,2004). In the liver, it inhibits both the manifestation of hepatic gluconeogenic enzymes and the rate of endogenous glucose production (Combs *et al.*,2001). In muscle, it increases glucose transport and increases fatty acid oxidation, effects that are partially attributed to the activation of AMP-kinase (Xu *et al.*,2003).

Yamauchi *et al.*, (2003) described the cloning of complementary DNAs encoding adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2) by expression cloning. AdipoR1 is profusely expressed in skeletal muscle, while AdipoR2 is mostly expressed in the liver. These two receptors are thought to be structurally and functionally distinct from G-protein-coupled receptors although they are predicted to contain seven transmembrane domains. Kadowaki *et*

al., (2005) have showed that AdipoR1 and AdipoR2 serve as receptors for globular and full-length adiponectin and mediate increased AMP-activated protein kinase, peroxisome proliferator-activated receptor-α ligand activities. Thus, the biological effects of adiponectin are modified by the relative circulating concentrations and properties of the different adiponectin isoforms as well as the tissue-specific expression of the adiponectin receptor subtypes. "Adiponectin-deficient mice develop premature diet-induced glucose intolerance and insulin resistance, increased serum nonesterified fatty acid (NEFA) levels, severe neointimal thickening and increased proliferation of vascular smooth muscle cells in mechanically injured arteries" (Kershaw *et al.*, 2004).

The pharmacological effect of adiponectin in reducing insulin resistance is related to a decrease in plasma fatty acid levels and in triglyceride content in muscle and liver in obese mice (Fruebis *et al.*, 2001, Yamauchi *et al.*, 2001). These observations may be due to enhanced expression of genes involved in β-oxidation and energy dissipation, such as acyl-CoA oxidase and uncoupling protein-2 (Yamauchi *et al.*, 2001). In addition to its activation of the 5'-AMP-activated protein kinase, adiponectin also stimulated phosphorylation of acetyl-CoA carboxylase, fatty acid oxidation, glucose uptake and lactate production in myocytes, and phosphorylation of acetyl-CoA carboxylase and reduction of molecules involved in gluconeogenesis in the liver (Yamauchi *et al.*, 2002). In humans a role for physiological concentrations of fasting plasma adiponectin in the regulation of skeletal muscle insulin receptor tyrosine phosphorylation has recently been demonstrated (Stefan *et al.*, 2002).

A physiological role for adiponectin has not been fully recognized. Adiponectin has been shown to reduce tumor necrosis factor (TNF)-α-induced monocyte attachment to cultured human aortic endothelial cells by inhibiting the expression of vascular cell adhesion molecule, intercellular adhesion molecule and E-selectin (Ouchi *et al.*, 1999). Adiponectin was shown to suppress phagocytic activity and lipopolysacharide-induced TNF-α production in cultured macrophages (Yokota *et al.*, 2000, Ouchi *et al.*, 2001), and was detected only in injured but not intact vessel walls (Okamoto *et al.*, 2000).

There seems to be a clear relationship between adiponectin and fat mass in humans. However, in contrast to leptin, adiponectin levels are significantly reduced among obese subjects in comparison with lean control subjects (Arita *et al.*, 1999). Adiponectin is the only adipose-specific protein identified to date that is negatively regulated in obesity (Matsubara *et*

*al.,*2002). Adiponectin similar to leptin levels appear to be gender-dependent, being higher among women than men(Yamamoto *et al.,*2002, Hotta *et al.,*2000, Nishizawa *et al.,*2002). In a large number of non-diabetic women with dyslipidaemia, Matsubara et al. (2002) have shown that plasma adiponectin is negatively correlated with serum triglyceride, atherogenic index, apoB or apoE, and positively correlated with serum HDL-cholesterol or apoA-I levels. These data suggest that low adiponectin concentrations are associated with some of the well-known risk factors for atherosclerosis (Fig.1.9). A relationship between hypoadiponectinemia and metabolic syndrome has been proposed (Matsuzawa *et al.,*1999).
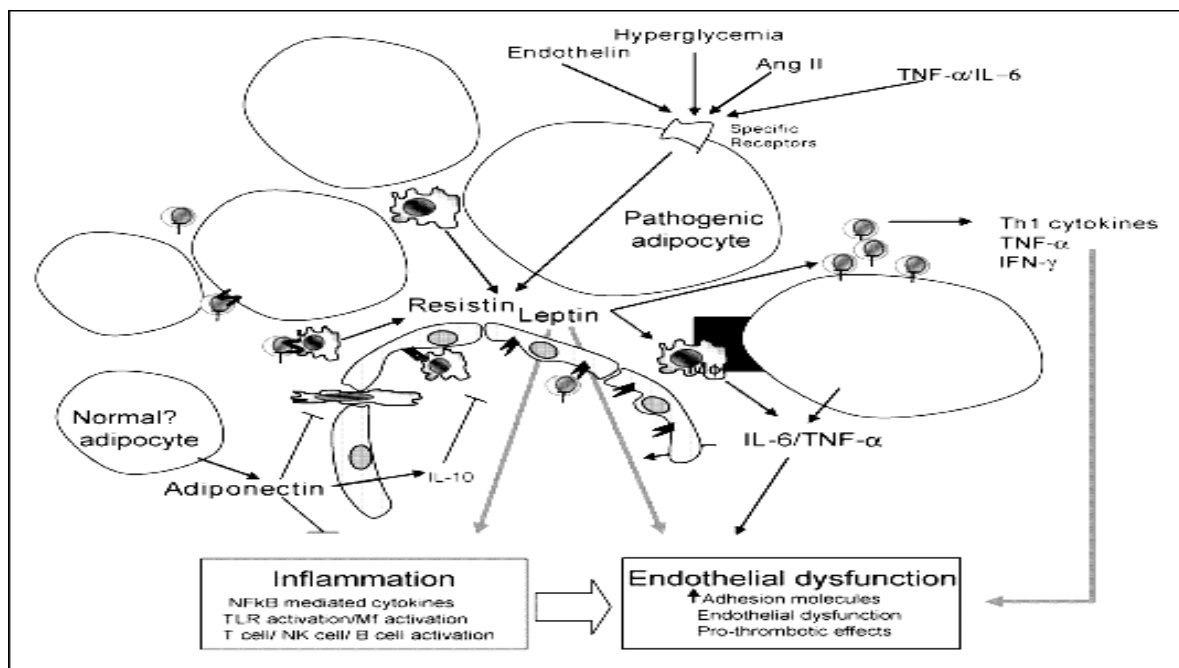
Figure 1.9 Role of adipocytokines in the metabolic syndrome

http://www.jpp.krakow.pl/journal/archive/1206/articles/01_article.html

Recently, Kadowaki *et al.,*(2005) have cloned adiponectin receptors in the skeletal muscle (AdipoR1) and liver (AdipoR2), which appear to comprise a novel cell-surface receptor family. Their research showed that AdipoR1 and AdipoR2 mediate AMP-activated protein kinase, peroxisome proliferator-activated receptor-α ligand activities, and glucose uptake and

52

fatty-acid oxidation by adiponectin and also function as receptors for globular and full-length adiponectin.

Expression Levels of AdipoR1/R2 decreased in obesity thus reducing adiponectin sensitivity, which may lead to insulin resistance. The pharmacological effect of adiponectin in reducing insulin resistance is related to a decrease in plasma fatty acid levels and in triglyceride content in muscle and liver in obese mice (Fruebis *et al.,*2001, Yamauchi *et al.,*2001). These observations may be due to enhanced expression of genes involved in β-oxidation and energy dissipation, such as acyl-CoA oxidase and uncoupling protein-2 (Yamauchi *et al.,*2001). In addition to its activation of the 5'-AMP-activated protein kinase, adiponectin also stimulated phosphorylation of acetyl-CoA carboxylase, fatty acid oxidation, glucose uptake and lactate production in myocytes (Yamauchi *et al.,*2002). In humans a role for physiological concentrations of fasting plasma adiponectin in the regulation of skeletal muscle insulin receptor tyrosine phosphorylation has been demonstrated (Stefan *et al.,*2002).

1.5.3 Resistin

A newly discovered hormone described in Nature by Steppan and colleagues (2001) is proposed as the essential link between obesity and type 2 diabetes. A distinctive signaling protein that was secreted by adipocytes was called "resistin" which means "for resistance to insulin". To build on their discovery, the researchers first examined normal mice. They discovered that resistin circulates normally in mouse blood. Resistin levels decreased in normal mice after a period of fasting, but increased again when the mice were fed. The researchers showed that in mice bred to have both diet-induced and hereditary diabetes the resistin levels are higher than in controls. The role of resistin in normal physiology is not known. Genetic or diet induced obese rodents as well as obese patients have significantly higher resistin gene expression and plasma levels (Steppan *et al.,*2001, Savage *et al.,*2001). Treatment with rosiglitazone, a drug that enhances insulin action by activating nuclear peroxisome proliferator-activated receptor γ (PPARγ), can noticeably down-regulate its mRNA expression in adipose tissue of obese mice (Steppan *et al.,*2001). "Administration of resistin was reported to impair glucose tolerance and insulin action, whereas administration of

an anti-resistin antibody significantly improved insulin action" (Steppan *et al.,*2001, Rajala *et al.,*2003a, Hotamisligil, 2003, Moon *et al.,*2003). It has been demonstrated that insulin-stimulated glucose uptake by adipocytes was enchanced by neutralization of resistin, and conversely reduced by addition of resistin (Steppan *et al.,*2001).

However, Savage and co-workers (2001) found that resistin mRNA levels were very low in freshly-isolated human adipocytes, and were undetectable in adipocytes from a severely insulin-resistant subject with a dominant-negative mutation in the PPARγ gene. Similarly, resistin is expressed at very low levels in several genetically obese rodents, its mRNA expression has been found to increase following treatment with thiazolidinediones (TZDs) PPARγ agonists and insulin itself in vivo, and yet suppressed by the same treatment in vitro (Way *et al.,*2001, Kim *et al.,*2001). The ambiguous function for resistin regarding the role it plays in obesity-induced insulin resistance in humans remains open to debate (Guo *et al.,*2004).

1.5.4 Acute-phase proteins and other systemic responses to inflammation

Cytokines are intercellular signalling polypeptides produced by activated cells and the majority of them have multiple sources, multiple targets, and multiple functions. The interleukin-6, interleukin-1β, tumor necrosis factor-α, interferon-γ, transforming growth factor β and interleukin-8 belong to the inflammation-associated cytokines (Kushner, 1993, Wigmore *et al.,* 1997). They are produced by a variety of cell types, but the most important sources are macrophages and monocytes at inflammatory sites (Gabay *et al,*.1999). Although they are produced by a variety of cell types, but the most essential sources are macrophages and monocytes at inflammatory sites (Gabay *et al,*.1999).

Interleukin-6 is the main stimulator of the production of most acute-phase proteins (Gauldie *et al.,*1987), while the other implicated cytokines affect subgroups of acute-phase proteins. Overproduction by the expanded adipose tissue mass indicated by elevated levels in proinflammatory cytokines including interleukin 6, resistin, and tumour necrosis factor α (TNFα) and C-reactive protein (Fernandez-Real *et al.,*2003, Trayhurn *et al.,*2004). Some data suggests that monocyte-derived macrophages exist in adipose tissue and might be in part the

source of the proinflammatory cytokines locally and in the systemic circulation (Weisberg *et al.,*2003, Xu *et al.,*2003). Glucocorticoids usually improve the stimulatory effects of cytokines on the production of acute-phase proteins (Baumann *et al.,*1987), while insulin decreases their effects on the production of some acute-phase proteins (Campos *et al.,*1994). The expression of genes for acute-phase proteins is regulated largely at the transcriptional level, but post-transcriptional mechanisms also take part (Jiang *et al.,*1995, Rogers *et al.,*1990).

1.5.4.1 Tissue necrosis factor alpha

Tumor necrosis factor α (TNF-α), also known as cachectin (Carswell *et al.,*1975, Beutler *et al.,*1985), is produced by neutrophils, activated T and B lymphocytes, NK cells, LAK cells, astrocytes, endothelial cells, smooth muscle cells and adipocytes. Mature human TNF-α is a polypeptide of 157 amino acid residues (mouse, rat, or rabbit TNF-α is one amino acid shorter). Human TNF-α shows no N-glycosylation (mouse TNF-α is N-glycosylated) (Vilcek *et al.,* 1991). The biologically active native form of TNF-α is a trimer (Jones *et al.,*1989, Eck *et al.,*1989). TNF-α does not possess a typical signal peptide sequence. TNF-α is, however, initially synthesized as a larger protein with the mature 17-kDa factor comprising the C-terminal portion of this precursor. Hydrophilic and hydrophobic domains are both present in the N-terminal sequence of the precursor. The presence of this precursor results in the occurrence of TNF-α as a membrane-bound form from which the mature factor is released by proteolytic cleavage (Kriegler *et al.,*1988, Luettig *et al.,*1989, Perez *et al.,*1990).

Two separate receptor types have been recognized that specifically bind TNF-α. The presence of one or both of these receptor types have been found almost in all studied cell types. Type TNF RII (or Type A, Type α, 75 kDa or utr antigen), is a transmembrane glycoprotein with molecular weight of 75 kDa (Dembic *et al.,*1990) and another one, TNF RI (or Type B, Type β, 55 kDa or htr antigen), is a transmembrane glycoprotein with molecular weight of 55 kDa (Schall *et al.,*1990, Loetscher *et al.,*1990). Every receptor type can bind TNF-α with high affinity and there is no proof that connection between the two receptor types is necessary for

signal transduction (Loetscher *et al.,*1990, Smith *et al.,*1990, Engelmann *et al.,* 1990a). Soluble forms of both types of receptors have been discovered in human serum and urine (Seckinger *et al.,*1989, Olsson *et al.,*1989, Engelmann *et al.,*1990b). The ability of these soluble receptors to neutralize the biological activities of TNF-α and function to modulate and localize the activities of TNF-α or function as a reservoir for the controlled release of TNFα had been recognized. TNF-α is an very pleiotropic molecule which is assignable to the ubiquity of its receptors, to its capability to trigger multiple signal transduction pathways, and to its capability to stimulate or restrain the expression of a vast number of genes, including those for growth factors and cytokines, transcription factors, receptors, inflammatory mediators and acute phase proteins (Vilcek *et al.,*1991, Kronke,1991). TNF-α plays an important role in normal host resistance to infections and to the growth of malignant tumors, function as an immunostimulant and a mediator of the inflammatory response. The extreme production of TNF-α has been implicated as playing a role in a number of pathological conditions, such as cachexia (progressive wasting) (Beutler *et al.,*1985, Oliff,1988), septic shock following infection with Gram-negative bacteria (Tracey *et al.,*1987), autoimmune disorders (Pujol-Borrell *et al.,*1987), and meningococcal septicemia (Waage *et al.,*1987).

TNF-α concentrations in human fat rise with obesity, correlate with abdominal adiposity, and decline during weight loss (Hotamisligil *et al.,*1995, Kern *et al.,*1995, Tsigos *et al.,*1999). In some experimental settings, TNF-α and its circulating p75 receptor fraction antagonize insulin action through effects on calcium-dependent GLUT-4 transport and phosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS-1) (Hotamisligil *et al.,*1994, Hotamisligil *et al.,*1996, Sayeed,1996).

Autocrine effects of TNF-α at the adipocyte promote leanness by inhibiting the activity of lipoprotein lipase, acetyl-CoA carboxylase, and glycerophosphate dehydrogenase, impeding lipogenesis, increasing lipolysis and accelerating fat cell apoptosis (Sethi *et al.,*1999, Torti *et al.,*1985, Zhang *et al.,*1996). TNF-α also reduces the deposition of triglycerides and promotes intracellular uncoupling (Berg *et al.,*1994, Greenberg *et al.,*1992). However, because TNF-α concentrations are not increased in venous output from subcutaneous fat tissue, the cytokine's primary actions appear to be autocrine or paracrine (Yudkin *et al.,*2000).

1.5.4.2 Interleukin-6

Human interleukin-6 [(h) IL-6] is a 184 amino acid protein with a molecular weight of 23-30kDa, which is N-linked glycosylated. Interleukin 6 is a major proinflammatory cytokine that produced in a variety of tissues, including activated leucocytes, adipocytes, and endothelial cells (Pradhan *et al.,*2001). The IL-6 receptor is a heterodimeric molecule consisting of an 80kDa IL-6 binding protein and a 130kDa accessory molecule, which is required for the high affinity form ($1x10^{-10}$M) that can transduce signals. The IL-6 receptor has been found to be on a wide range of cells including B and T cells, monocytes, myelomas, hepatocytes, hepatomas and astrocytomas up to 10000 per cell. A soluble circulating form of the 80kDa IL-6 binding protein is able to form a complex with IL-6 that can then associate with the membrane bound 130kDa molecule resulting in signal transduction (Mitsuyama *et al.*, 2006).

The in vivo infusion of human recombinant IL-6 in rodent models of glucose metabolism made known to induce gluconeogenesis, subsequent hyperglycemia, and compensatory hyperinsulinemia (Stith *et al.,*1994). In humans similar metabolic responses have been found after administration of subcutaneous recombinant IL-6 (Tsigos *et al.,*1997). Several studies have confirmed elevated levels of IL-6 and CRP among individuals both with features of the insulin resistance syndrome and clinically overt type 2 DM (Pickup *et al.,*1997, Grau *et al.,*1996, Ford,1999, Festa *et al.,*2000, Frohlich *et al.,*2000).

Up to one-third of circulating IL-6 originates from adipose tissue (Mohamed-Ali *et al.,*1997), with higher basal levels found in the preadipocytes compared to the mature fat cell. In vitro studies suggest a greater production of the IL-6 in visceral vs. subcutaneous fat (Fried *et al.,*1998). IL-6 release from adipose tissue is stimulated by TNFα and IL-1, and circulating IL-6 enters the CNS and binds to receptors present in the hypothalamus (Jones *et al.,*1993). Like TNFα, its anorexigenic effects result from stimulation of thermogenesis and satiety, with central effects on prostaglandin synthesis, corticotrophin releasing hormone, and activation of hypothalamic-pituitary-adrenal function. IL-6 is the key regulator of hepatic C-reactive protein and the hepatic acute phase response. IL-6 seems to correlate with insulin resistance and endothelial dysfunction (Yudkin *et al.,*1999, Galis *et al.,*1994). In hepatocytes in vitro, IL-6

reduces insulin-stimulated accumulation of glycogen (Kanemaki *et al.,*1998). Like TNFα, IL-6 inhibits lipoprotein lipase and induces lipolysis (Yudkin *et al.,*2000), and resultant increase of non-esterified fatty acids (NEFA) may impede nitric oxide mediated endothelial vasodilation (Robinson *et al.,*1995, Steinberg *et al.,*1997).

1.5.4.3 C - Reactive Protein

C-reactive protein (CRP) is a classical acute phase reactant and a member of the pentraxin family of innate immune response proteins (Du Clos, 2000). The human CRP molecule (MW 115,135) is composed of five identical nonglycosylated polypeptide subunits (MW 23,027), each containing 206 amino acid residues (Pepys *et al.*, 2003). CRP demonstrates calcium-dependent similarity for phosphate monoesters, such as phosphatidylcholine, but several other ligands of CRP have been labelled, including damaged cell membranes, small ribonucleoprotein particles, apoptotic cells and fibronectin (Du Clos *et al.*, 2004).

C-reactive protein is synthesized mainly within the endoplasmic reticulum of hepatocytes in response to proinflammatory cytokines particularly interleukin-6 (IL-6), as well as IL-1β and tumor necrosis factor α (TNF-α), which potentiate the IL-6 effect (Ross, 1999). Current data show that arterial tissue may generate CRP as well as complement proteins, and that CRP and complement, simultaneously with their mRNA, are substantially up-regulated in atherosclerotic plaque (Yasojima *et al.,*2001). Smooth muscle cells and macrophages seem to be the main producers, explaining that CRP may be generated by extra-hepatic mechanisms.

 Cytokines, which trigger the *de novo* hepatic production of acute phase reactants such as C-reactive protein (Pepys *et al.,*1983), have been shown to increase in acute coronary syndromes even in the absence of myocardial necrosis (Liuzzo *et al.,*1994). As a result, CRP has been examined as a surrogate marker of other inflammatory mediators such as IL-6 and TNF-α to better understand the inflammatory component of atherosclerosis (Rus *et al.,*1996, Sukovich *et al.,*1998). Some literature suggested that the CRP concentration might represent the vulnerability of the atheromatous lesion and the likelihood of a plaque to rupture (Ross,1999, Libby,1995, Maseri,1997).

High CRP levels are also observed in patients with dilated cardiomyopathy at increased risk of death and in patients with decompensated heart failure (Naidoo, 2003). Elevated CRP levels or association between CRP and disease progression have also been found in atrial fibrillation (Chung *et al.,*2001), obstructive sleep apnoea (Shamsuzzaman *et al.,*2002) and vascular dementia. Interestingly, CRP appears to be more weakly associated with measures of the burden of atherosclerosis, such as carotid intimal-media thickness or extent of coronary artery disease (Folsom *et al.,*2001).

A strong positive correlation between CRP and body mass index (as well as waist: hip ratio) has been revealed in many studies. This association with obesity is clear because hepatic CRP production is stimulated by IL-6, which originates mainly from adipose tissue (Naidoo, 2003). CRP also predicts the development of type 2 diabetes, suggesting a direct role for inflammation in the pathogenesis of insulin resistance and diabetes (Pradhan *et al.,*2001). In the Multiple Risk Factor Intervention Trial (MRFIT), C-reactive protein was predictive of future coronary mortality among high-risk smokers (Kuller *et al.,*1996). Whether silent mutations of CRP and IL-6 genes which have been shown to influence CRP levels influence disease progression, is not clear, but this may be another mechanism for the predisposition to early onset coronary disease in subjects with risk factors (Zee *et al.,*2002, Vickers *et al.,*2002). C-reactive protein concentrations as a general indicator of inflammation can vary by ethnic origin and within ethnic groups by fitness (LaMonte *et al.,*2002, Chambers *et al.,*2001). For instance, a study by Chambers *et al.,* (2001) found that concentrations of C-reactive protein were higher in healthy Indian Asians than in European White people and were related to greater central obesity and insulin resistance in Indian Asians. Currently it remains uncertain whether these differences when adjusted for other covariates will relate to different rates of development of diabetes and / or cardiovascular disease (Eckel *et al.,*2005).

1.6 Epidemiological studies of obesity and Type 2 Diabetes Mellitus

1.6.1 Prevalence of obesity world wide

On the basis of the standard WHO adult classification system (2000b), it is estimated that in the United States, overweight and obesity combined (BMI ≥25) affect 60% of the population; 32.2% of US adults are obese (BMI ≥30). These statistics reflects a marked increase since the late 1970s, when the combined prevalence of overweight and obesity was 47% and the prevalence of obesity was 15% (York *et al.,* 2004).

Nearly 30% of the US population is composed of diverse ethnic groups that are broadly categorized as Black or African American, Hispanic or Latino, Asians, Pacific Islanders, American Indians or Alaskan Natives, and Native Hawaiians. This proportion will increase to nearly 40% over the next 2 decades (Population Reference Bureau, 2000). In African-American and Hispanic-Americans racial / ethnic minority groups in the U.S. overweight and obesity emerged at higher rates compared with White Americans (Table 1.4). Asian-Americans have a comparatively low prevalence for obesity. Women and persons of low socio-economic status within minority populations appear to be particularly affected by overweight and obesity. Cultural factors that influence dietary and exercise behaviours are reported to play a major role in the development of excess weight in minority groups.

Table 1.4 The prevalence of overweight and obesity over the last decade across racial/ethnic groups in USA

Increase in Overweight and Obesity Prevalence Among U.S. Adults* by Racial / Ethnic Group				
	Overweight (BMI ≥25) Prevalence (%)		Obesity (BMI ≥ 30) Prevalence (%)	
Racial/Ethnic Group	1988 to 1994	1999 to 2000	1988 to 1994	1999 to 2000
Black (non-Hispanic)	62.5	69.6	30.2	39.9
Mexican American	67.4	73.4	28.4	34.4
White (non-Hispanic)	52.6	62.3	21.2	28.7

*Ages 20 and older for 1999 to 2000 and ages 20 to 74 for 1988 to 1994

Source: CDC, National Centre for Health Statistics, National Health, and Nutrition Examination Survey (Flegal *et al.*, 1998, Flegal *et al.*, 2002).

The report published in 1991 by US Centre for Disease Control and Prevention (CDC) indicated that the prevalence of obesity in 19 states varied between 15 and 19% with no single state above 20%. Eleven years later in 2002, 20 states had a prevalence between 20 and 24%, and in one state it exceeded 25% (Mokdad *et al.,* 1999, Mokdad *et al.,* 2001, Mokdad *et al.,* 2003). These alarming numbers are mirrored around the globe including many developing countries, where the adverse health consequences of overweight and obesity have begun to replace under nutrition and infection as the main causes of early death and disability (Caballero, 2001).

In the UK the proportion who were categorised as obese (BMI $\geq$30) increased from 13.2% of men in 1993 to 23.6% in 2004 and from 16.4% of women in 1993 to 23.8% in 2004 (http://www.ic.nhs.uk/pubs/hlthsvyeng2004upd). The description of what represents "European countries" has altered after new developments in central and Eastern Europe. It becomes more difficult to attain exact prevalence and incidence data in all age groups, in varying socio-economic settings, and in both sexes. The interest in Mediterranean countries was always considered to be important in regard to lifestyle factors, diet, and longevity (York *et al.,* 2004).

However, in Greece the prevalence of obesity in both men and women is one of the highest in Europe and is about twice that of men and women in Italy, France, and Spain. Denmark, Norway, and Sweden are in the lowest part of the distribution, with prevalence data of $\approx$10% for men and $\approx$12% for women. Interestingly, however, the prevalence data for neighbouring Finland, which is widely known as a country with some of the highest rates for cardiovascular disease, are $\approx$50% higher than rates in other Nordic countries (Heitmann, 2000, Lahti-Koski *et al.,* 2000, Lissner *et al.,* 2000). The causes for this are not identified but genetic factors probably play a role similar to that played in the prevalent hyperlipoproteinemic problems of the country. Table 1.5 shows an update from the International Obesity Task Force (IOTF) (http://www.iotf.org/media/iotfaug25.htm).

Table 1.5 BMI in Adults from European Countries (York *et al.,* 2004)*

Country	Overweight, % (BMI 25.0 –29.9)		Obesity, % (BMI ≥ 30)	
	Male	**Female**	**Male**	**Female**
Austria	48	29	12	17
Belgium	-	12	19	-
Bulgaria	37.7	27.7	10	9.2
Croatia	50.9	33.3	16.3	16.2
Czech Republic	48.5	31.4	24.8	26.2
Denmark	38.2	20.2	8.8	5.9
Germany	48.1	31.3	18.8	20.3
Finland	48	33	19.8	19.4
France	35	20.3	8.3	7.7
Hungary	42	28.3	21	21.2
Greece	51.1	36.6	27.5	38.1
Iceland	47.4	16.8	36.9	18.3
Israel	43.5	30.3	14.7	20.9
Italy	39.6	25.0	6.5	6.3
Lithuania	41.8	32.6	11.4	18.3
Malta	46	32	22	35
Netherlands	42	29.1	9.9	8.5
Norway	50.4	28.8	12.7	9.5
Portugal	42.3	31.9	13.2	16.2
Romania	38.8	31.5	21.1	27.4
Russian Federation	34.7	31	9.6	25.4
Continue…….				

	Overweight, % (BMI 25.0 –29.9)		Obesity, % (BMI ≥ 30)	
Country	**Male**	**Female**	**Male**	**Female**
Norway	50.4	28.8	12.7	9.5
Portugal	42.3	31.9	13.2	16.2
Romania	38.8	31.5	21.1	27.4
Russian Federation	34.7	31	9.6	25.4
Slovakia	37.8	23.6	13.1	13
Spain	59.9	48.1	11.8	15.7
Sweden	51.2	41.6	10	11.9
Switzerland	33.1	17.1	6.1	4.7
Tajikistan	17.8	11.4	3.2	2
Turkey	37.3	35	11.8	24.4
United Kingdom	43.9	32.8	18.7	21.1
Uzbekistan	10.2	8.9	8.4	6.1
Yugoslavia	28.1	28.4	25.5	40.9

Table 1.5 (continue) BMI in Adults from European Countries (York et al., 2004)*

*Updated data may be viewed at: http://www.iotf.org

Over the past 20 years the prevalence of obesity in Australia has been intensified. More recently the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab) (Dunstan *et al.*, 2001), a nationally representative survey, measured the prevalence of obesity and diabetes in adults aged 25 to 65. In 1980 the prevalence of obesity was 8% in women; in 2000, the prevalence had increased to 21.8%. In men the prevalence was 9.3% in 1980 and 19.1% in 2000. Currently the prevalence of obesity is 20.5% in Australian adults. Among men 67.4% have a BMI >25; among women, 52% have a BMI >25. The major increase in obesity has occurred since 1989. As reported by the Australian Institute of Health and Welfare (Armstrong

et al., 2000) there are a number of reasons for this and one of them is drop in activity. Despite decrease in dietary fat intake, the daily energy intake has increased (Cook *et al.,* 2001).

According to the data published by Zhou *et al.,* (2002), the prevalence of overweight (BMI 25.0 to 29.9) in Chinese adults aged 20 to 70 in the 1990s, was 22.4%, while prevalence of obesity (BMI ≥ 30) was 3.01%. The prevalence of overweight in Chinese men and women was 20.4% and 22.4%, respectively. The prevalence of obesity in Chinese men and women was 2.04% and 3.89%, respectively. The differences between urban and rural population were apparent in 1992 when the mean BMI of the urban population was 23.57 compared with 21.94 for rural communities (Ge,1995). Because China is a country in economic development, dietary fat intake increased during the 1980s and reached ≈30%, but has remained constant since then (Wang *et al.,* 2000, Chen *et al.,* 1994). The tendency of overweight and obesity increased rapidly in the past 17 years. The prevalence of overweight in the southern part of China (Guangzhou, Shanghai) increased more rapidly than in the northern part of the country. The prevalence of overweight reached 10% in 1998 even in the poor rural area of Shaanxi (Wu *et al.,* 2002).

Overweight and obesity are common among Hispanic women aged 15 to 49 (Martorell *et al.,* 2000b, Martorell, 2002,). The levels of overweight and obesity ranged from ≈30% to ≈50% in Bolivia, Brazil, Colombia, the Dominican Republic, Guatemala, Honduras, Mexico, Nicaragua, and Peru. In some countries prevalence rates come close to those of the United States. Overweight and obesity are common among Hispanic women aged 15 to 49 (Martorell *et al.,* 2000b, Martorell, 2002,). The levels of overweight and obesity were ~ 10% in country like Haiti and in sub-Saharan Africa. In the urban areas of Haiti obesity was more prevalent compared to the rural areas and in women with more education compared with those that have only primary schooling. By contrast, in 1999 obesity in Mexico was as common in urban areas as it was in rural areas and was more common among poorly educated women. Consequently with greater economic development the prevalence of obesity rises and begins to badly affect rich and poor alike; in more advanced countries obesity has become more common among poor and less educated people, as is becoming noticeable in Mexico. In 1988 the prevalence of overweight and obesity were 24% and 9%, respectively. By 1999 the corresponding values were 35% and 24% (Rivera *et al.,* 2002). High prevalence of overweight and obesity were found in North Africa and the Middle East. From 1995 to 1996 31.7% of Egyptian women

65

aged 15 to 49 were overweight; 23.5% were obese (York *et al.,* 2004). The national survey conducted in 1993 from primary healthcare clinics in Kuwait established that prevalence of obesity was 40.2% among women older than 18 years (al-Isa, 1997). The reported prevalence of obesity among women aged 20 to 60 in Morocco was 18.3% in 1998, while in 1997 in 1997 in Tunisia the prevalence was 22.7%. Rates in men remained low (i.e. 6.7% in Tunisia in 1997 and 5.7% in Morocco) in 1998 to 1999" (Mokhtar *et al.,* 2001).

1.6.2 Epidemiology of obesity in Africa

In sub-Saharan African there is a constant fight against poverty, worry about food, under-nutrition, and infectious diseases. The situation is aggravated by the HIV/AIDS epidemic (Food and Agriculture Organization of the United Nations, 2001). In the more industrialised parts of Africa obesity and overweight are rising however, leading to a coexistence of under nutrition and over nutrition in many African countries. The rising tendency of obesity in Africa also has been found in Mauritius, despite national programs to promote healthier diets and increased physical activity (Hodge *et al.,* 1996). The results from the prevalence studies in which 3677 subjects were followed up from 1987 to 1992 had indicated that the prevalence of overweight and obesity (BMI ≥ 25) increased from 26.1% to 35.7% in men and from 37.9% to 47.7% in women (York *et al.,* 2004).

In rural areas of Africa the prevalence of obesity is still relatively low. In Nigeria, for instance, only 1.2% of men and 3.2% of women had BMIs ≥ 25 (Okesina *et al.,* 1999). The data suggested that obesity in Africa is characterized by a gender difference, with women having 3 to 5 times the rate of obesity that was observed in men. In the overweight range (BMI ≥25), however, these differences are smaller (Hodge *et al.,* 1996). These data indicated that overweight and obesity emerges earlier in African women but that African men may eventually reach the same prevalence rates (Labadarios *et al.,* 2003).

Table 1.6 provides prevalence of obesity from some African countries, from migrant people of African descent and from other South-African studies. The lower rates of obesity were found

in countries like Ghana, Mali, and Tanzania when compared to South Africa, while in Mauritius the prevalence of obesity fall more or less between these extremes. African Americans had similar prevalence to those found in Africans from South Africa. Generally, these data suggest that the predominant pattern of malnutrition in adult South Africans, particularly in African women, is one of overweight and of remarkably high rates of abdominal obesity (Puoane *et al.,* 2002).

Table 1.6 Prevalence of obesity (body mass index ≥ 30 kg/m2) in South Africa and selected countries in Africa and the US (adapted from Puoane *et al.,* 2002)

Country	Year	Ages (Years)	Men	Women
			Prevalence of obesity (%)	
Ghana	1987/88	≥ 20	-	0.9
Mali	1991	≥ 20	-	0.8
Mauritius	1992	25 to 74	5	15
USA	1988-94	20 to 74	19.9	24.9
Tanzania	1986/89	35 to 64	0.6	3.6
South Africa				
Cape Peninsula, Africans	1990	25 to 64	13.9	48.6
Mangaung, Africans	1990	25 to 64	12.9	43.9
QwaQwa, Africans	1990	25 to 64	12.7	40.2
Cape Peninsula, Mixed	1982	25 to 64	7.2	31.4
South Western Cape, whites	1988	25 to 64	17.6	20.4
Durban, Indians	1990	25 to 69	3.5	17.6
Demographic& Health Survey, African	1998	15	7.7	30.5
Demographic& Health Survey, Mixed	1998	15	9.1	28.3
Demographic& Health Survey, Asian	1998	15	8.7	20.2
Demographic& Health Survey, White	1998	15	19.8	24.3

1.6.2.1 Epidemiology of obesity in South Africa

Obesity in Africa is associated with urbanization and changes in dietary patterns, socioeconomic circumstances, and physical activity levels. The prevalence of obesity in South Africa, as estimated using data collected in 1998 (Puoane *et al.,* 2002), was 10% for males and 30% for females, compared with data from the USA for the period 1999-2000 where the prevalence was 27.6% for men and 33.2% for women (Baskin *et al.,* 2005).

In 1940, Fox found that the African population consumed a typical traditional diet, where the fat intake was only 16% of the total calories. The 26% increase in fat intake was observed in an urban African community by 1990 (Mollentze *et al.,* 1993). The further analysis of this data revealed that those people who had lived in cities for most of their lives, already consumed a typical Westernized diet with 30% of calories derived from total fat, whereas those who had spent <20% of their lives in the city, derived only 22.5% of calories from total fat (Bourne, 1996). In South Africa an improvement in household income was positively associated with obesity, illustrating that at this stage of the demographic transition, obesity can be expected to have higher prevalence in the higher socio-economic strata. Increased in obesity in African women in both rural and urban areas, independent of the level of urbanization was associated with low physical activity (Kruger *et al.,* 2002).

The data about the nutritional status and dietary intake patterns in the African community, the large number of the South Africans with high poverty levels were gather in the past in a number of regional studies (Steyn *et al.,* 1998, Vorster *et al.,* 2000, Mollentze *et al.,* 1993, Bourne and Steyn, 2000). In the African communities that became more urbanized the rate of obesity increased and their diets became less sensible (Jooste *et al.,* 1988, Steyn *et al.,* 1990, Steyn *et al.,* 1998, Bourne and Steyn, 2000). Within South Africa abdominal obesity is the highest in white urban men and rural African women (Puoane *et al.,* 2002). The connection between abdominal obesity with diabetes and hypertension has been previously acknowledged in regional prevalence studies in the both African and mixed-ancestry communities of the Western Cape (Steyn *et al.,* 1996, Levitt *et al.,* 1993). Thus WHR and waist circumference are an important adult health markers for ongoing observation.

In South Africa, obesity in women appears to start at a young age. Puoane and co-workers (2002), show that 10% of women were obese at the ages of 15 to 24 years. Therefore it

seems that primary prevention of obesity should begin at a young age, particularly for girls. In African women, the elevated rate of obesity is primarily in the urban women. It was noted that women without education had lower BMIs than those with schooling, therefore the relationship between education and BMI become important.

These women tend to do more manual labour than their better-educated counterparts. There are two possible reasons to finding that women with tertiary education also had a lower BMI than those without. Firstly, this group of women may be aware of the connection between body weight and health, and secondly, one could anticipate that this female group would take more cognisance of the preferred body image of thinness that reaches them through the media. Obesity management is particularly compelled for older women in all the population groups (Puoane *et al.,* 2002).

A feature of the results reported by the various South African studies on different ethnic groups is the increase in the prevalence of both overweight and obesity with age in both males and females. After the age of 25 the increase in overweight was found to double in both males and females of all ethnic groups. With the exception of black subjects, the prevalence of overweight was found to be very similar in males and females after the age of 25. The prevalence rate of overweight in Indian women has been twice as high compared to White women of 25 years and older (Jooste *et al.,* 1988, Seedat *et al.,* 1990). According to the study by Benadé *et al.,* (1996) obesity was found to be rare in males of all ethnic groups and more women than men tended to become obese, as they grew older.

1.6.3 Epidemiology of diabetes world wide

Type 2 diabetes has reached epidemic proportions and affects more than 194 million individuals worldwide or 5.1% in the adult population. It is estimated that this will increase to 333 millions, or 6.3%, by 2025 (Table 1.7).

Table 1.7 Estimated prevalence of diabetes worldwide (adapted from Diabetes Atlas, second edition, © International Obesity Task Force, 2003)

All diabetes and IGT	2003	2025
Total world population (billions)	6.3	8.0
Adult population (billions) (20-79 years)	3.8	5.3
Number of people with diabetes (millions) (20-79 years)	194	333
World diabetes prevalence (%)(20-79 years)	5.1	6.3
Number of people with IGT (millions) (20-79 years)	314	472
IGT prevalence (%) (20-79 years)	8.2	9.0

Global estimates that the greatest increases in the prevalence of diabetes will occur in the developing regions of Africa, Asia, and South America (Zimmet *et al.,* 2001). In more developed countries, the prevalence of diabetes mellitus has reached about 6% (King *et al.,* 1998), and, even more alarmingly, among obese white adolescents, 4% had diabetes and 25% had abnormal glucose tolerance (Sinha *et al.,* 2002).

Type 2 diabetes is the most widespread diabetes in the world. The European Region and Western Pacific Region currently have the highest number of people with diabetes (48 million and 43 million, respectively). However the prevalence rate of 3.1% for the Western Pacific Region is significantly lower than 7.9% in the North American Region and 7.8% in the European Region as seen in figure 1.10 (http://www.eatlas.idf.org/media/).
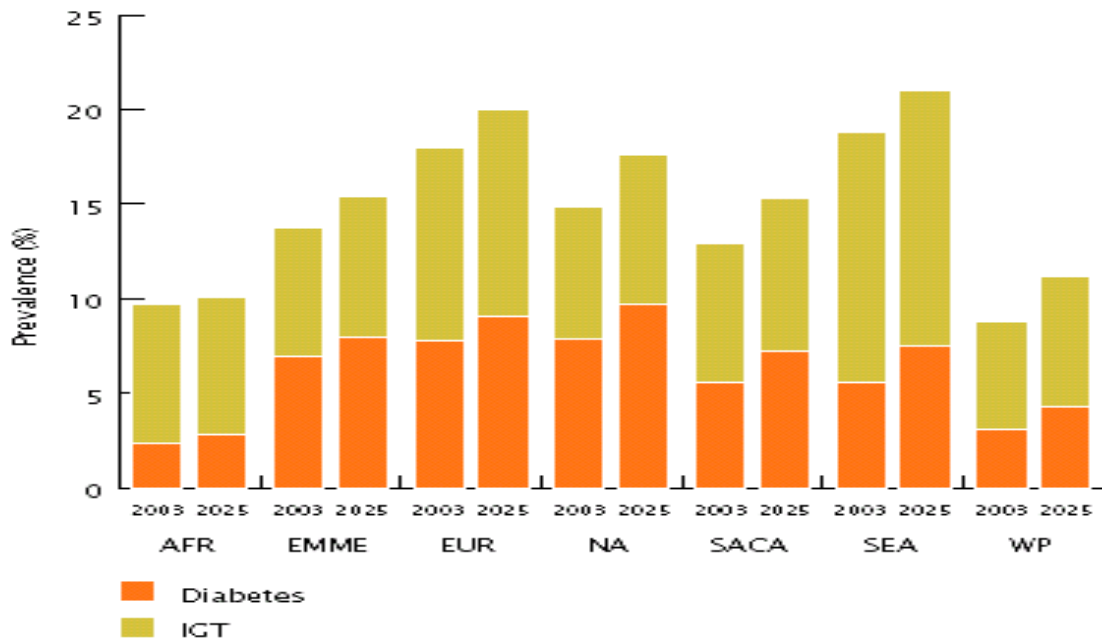
Figure 1.10 Estimate prevalence of diabetes and impaired glucose tolerance
(20-79 age groups) by region (Sourc*e*: Diabetes Atlas, second edition, © International
Obesity Task Force, 2003).

Abbreviations: AFR-Africa; EMME- Eastern Mediterranean and Middle East; EUR-Europe; NA-North
America; SACA- South and Central America; SEA- South-East Asia; WP-Western Pacific

Approximately 15 million Americans will develop type 2 diabetes mellitus (DM), which is
linked with an increased risk for cardiovascular disease, especially among women (Harris,
1995, Manson, 1996, Harris *et al.,* 1998). The secondary macrovascular and microvascular
injury is a typical of this disease; the economic and functional burdens are highest during mid-
to-late adulthood. "One third of individuals with type 2 diabetes are undiagnosed, and
approximately 20% have diabetic retinopathy or evidence of systemic vasculopathy at clinical
presentation" (Harris *et al.,* 1992).

According to the National Diabetes Statistics, (2002) the Pima Indians in Arizona have the

highest recorded prevalence of diabetes in the world. Overall, American Indian and Alaska Native adults are 2.6 times more prone to have diabetes than non-Hispanic whites of similar age (National Diabetes Statistics, 2002). The relationship between lower socioeconomic status or education level and higher prevalence of diabetes was described among white, black, Hispanic, and Japanese Americans in the United States (Auslander *et al.,*1992, Drury *et al.,*1987, Hazuda *et al.,*1992, Hendricks *et al.,*1991, Leonetti *et al.,*1992, Marshall *et al.,*1993). Known that large proportions of black and Hispanic populations live in poverty and have less than a high school education, socioeconomic status may strongly affect the prevalence of diabetes in these minority groups (Statistical Abstract of the United States,1994).

According to the data published by Yusuf *et al.,*(2001b) South Asians (in the UK and Canada) when compared with Europeans, do not display high rates of smoking, hypertension, or elevated cholesterol but still have higher rates of coronary heart disease (CHD). Conversely, smoking, hypertension, and diabetes are strongly linked with CHD among South Asians (Pais *et al.,*1996). High rates of diabetes has been reported among South Asian in the UK (10% to 19%), Trinidad (21%), Fiji (25%), South Africa (22%), Mauritius (20%), and Canada (10%) (Anand *et al.,*2000, McKeigue *et al.,*1989). The opposite was found in rural India where the prevalence of diabetes is 2% to 3% and roughly 8% in urban areas (Ramachandran *et al.,*1992). There is also increase in number of studies that provide evidence that elevations in blood glucose even in the nondiabetic range increases CHD risk among South Asian (Pais *et al.,*1996).

1.6.4 Epidemiology of cardiovascular diseases world wide

Cardiovascular disease (CVD) is the number one reason of death globally and is predictable to remain the leading cause of death. An approximate 17.5 million people died from cardiovascular disease in 2005, signifying 30 % of all global deaths. Of these deaths, 7.6 million were due to heart attacks and 5.7 million were due to stroke. Around 80% of these deaths occurred in low and middle-income countries (LMIC). An approximate 20 million people will die from CVD every year, mainly from heart attacks and strokes if right action is

not taken, by 2015 http://www.who.int/cardiovascular_diseases/en/. Women will remain to experience excessively high mortality from CVD. By 2040, women in study countries (Russia, Brazil, India, China, and South Africa) will represent a higher proportion of CVD deaths in comparison to men. It was projected that by 2040 women in China to be 49.5 percent of the population, so even if death rates no higher than now apply then, they will represent 54.6 percent of CVD deaths. The increase of CVD deaths in Brazil and China among working-aged women between 2000 and 2040 will be higher than for men. Projections suggest that for Coronary heart disease (CHD), mortality for all developing countries will increase by 120 percent for women and 137 percent for men. Estimations for the next two decades enclose tripling of CHD and stroke mortality in Latin America, the Middle East, and even sub-Saharan Africa, a proportion of increase that surpasses that for any other region, except for Asian and Pacific Island countries. Contrarily, the increase in more-developed nations, largely attributable to a growth of the population of older people at risk, will range between 30 percent and 60 percent (Yusuf *et al.*, 2001a). The data available from the World Health Organization (WHO) MONICA Project indicate that the coronary event rate (per 100,000) in men was highest in Finland (North Karelia, 835) and lowest in China (Beijing, 81). For women the maximum occurrence rate was in the UK (Glasgow, Scotland, 265) and the lowest in Spain (Catalonia, 35) and China (Beijing, 35). These data revealed results from 35 MONICA Project populations that were collected during the mid-1980s until the mid-1990s *(WHO, World Health Report, 2002b)*.

Data from the INTERHEART study (Yusuf *et al.*, 2004) showed that rates of CVD have risen greatly in low-income and middle-income countries with about 80 percent of the burden occurring in these countries. Nine potentially modifiable risk factors associated with myocardial infarction (MI) were identified. These varied by populations. The ability to prevent the premature cases of MI needs to apply a right attitude. The effect of the risk factors is remarkably noticeable in young men (population attributable risks [PAR] 93 percent) and women (PAR 96 percent), demonstrating that most premature MI is avoidable. Two-thirds of the PAR of an acute MI worldwide is related to smoking and abnormal lipids (Yusuf *et al.*, 2004).

 High blood cholesterol is predicted to cause about 4.4 million deaths (7.9 percent of total) which sum up to 18 percent of strokes and 56 percent of global CHD (*WHO World Health Report, 2002b)*. A blood cholesterol level of less than 5.0 millimoles per liter (mmol/L) is recommended for both primary and secondary prevention of CHD.

Approximately 66 percent of men and women in the UK have blood cholesterol levels of 5.0mmol/L and above (http://www.americanheart.org/downloadable/heart/). Approximately 600 million people with high blood pressure (HBP) are at risk of heart attack, stroke, and cardiac failure, according

to data published by World Health Organisation (http://www.who.int/cardiovascular_diseases/en/). In African-Americans hypertension develops at much earlier age compared to whites. The cause for this differences may be due to a complex interchange between environmental response to diet, stress, and a potential genetic/physiological difference in sodium/potassium excretion (Yusuf *et al.*,2001b). A study of hypertension in Canada, the United States and in six European countries, (Germany, Finland, Sweden, England, Spain and Italy), showed the average blood pressure (BP) was 136/83 mmHg in the European countries and 127/77 mmHg in Canada and the United States, among men and women ages 35–74. BP measurements for all age groups were highest in Germany and lowest in the United States (Wolf-Maier *et al.*, 2003). The measurement of blood pressure in England revealed that 34 percent of men and 30 percent of women have high blood pressure or are being treated for hypertension. The number of men and women not being treated for high blood pressure are 67 % and 78%, respectively. Of those being treated, just fewer than 60 percent carry on been hypertensive (http://www.bhf.org.uk/professionals/index.asp). In Asia, a steep increase in stroke mortality goes together with a rapid rise in the prevalence of hypertension. Projections suggest that in China, hypertension will increase from 18.6 percent to 25 percent between 1995 and 2025. In India, the equivalent figures are 16.3 percent to 19.4 percent (http://cpmcnet.columbia.edu/dept/ihn/faculty/pdf/race_against_time.pdf).

1.6.5 Pathophysiology of diabetes and CVD in Indian populations

Coronary heart disease (CHD) mortality is at least 40% higher in UK Indian Asians compared with European whites (Balarajan,1991, Balarajan,1995). Traditional coronary risk factors, including smoking, hypercholesterolemia, and hypertension, do not clarify their increased CHD risk compared with whites (McKeigue *et al.*,1993, McKeigue *et al.*,1991). While diabetes and insulin resistance are more common among Indian Asians (Mather *et al.*,1985, McKeigue *et al.*,1993), the exact mechanisms underlying the increased CHD mortality in Indian Asians are not known.

Myocardial infarction (MI) was seen to occur at a lower age in Indian population compared with the group population of countries to which they have migrated and mortality from MI was ten times higher (Misra,1999). Although insulin resistance may be involved in the early

event of risk factors and CHD in Indians, there is some data to suggest that other aspects of the lipid profile, such as the lipoprotein (a) (Lp[a]) level, affect risk in Indian patients (Gambhir *et al.*,2000). Lp(a) was an independent risk factor for CHD in type 2 diabetic patients in South India (Mohan *et al.*,1998).

Epidemiological studies have shown that South Asians also are more likely to have central obesity, increased waist/hip ratio (WHR), and glucose intolerance, compared with Caucasians (Hughes *et al.*,1997, Knight *et al.*,1992, McKeigue *et al.*,1991). Dhawan *et al.*, (1994) looked at the prevalence of diabetes, hyperinsulinaemia, and associated metabolic abnormalities in immigrant Asians, Asians in India, and native white British men. They concluded that central obesity in the subgroups of Asians studied showed a close association with hyperinsulinaemia and the risk of coronary artery disease. In this group of Asians a tendency to insulin resistance and its metabolic abnormalities appears to be genetically determined, environmental changes after migration having only a small additional effect. Studies have shown that the distribution of adipose tissue appears not to be markedly different in Asian Indian compared to African-American diabetic men or Swedish men when using comparable CT methods (Banerji *et al.*,1999). Visceral/total fat was 16.8% and 18.3% in Asian Indian and African American diabetic men, respectively (Banerji *et al.*,1997) and 19% and 18.4% in Asian Indian and Swedish men, respectively (Chowdhury *et al.*,1996). Contrary, anthropometry data such as BMI and WHR, indicated that migrant Asian Indians had lower overall obesity than Europeans, with a selective increase in central obesity (McKeigue *et al.*,1991).

One hypothesis that could account for the high frequency of both type 2 diabetes and premature CHD in Asian Indians had been proposed by Chandalia *et al.*, (1999). This hypothesis maintains that Asian Indians are more susceptible to the insulin resistance syndrome (McKeigue *et al.*,1989). The development of type 2 diabetes, which is an independent risk factor for CHD, associated with prolonged insulin resistance (Lillioja *et al.*,1993). The other coronary risk factors *e.g.* dyslipidemia and hypertension go together with insulin resistance (Haffner *et al.*,1988, Pyorala *et al.*,1985, Zavaroni *et al.*,1989). Ultimately, it is possible that insulin resistance affects CHD risk status via other mechanisms that are independent of the established risk factors. One of the most interesting observations of Chandalia *et al.*, (1999) was a strong tendency for insulin resistance in lean Asian Indians. The latter were much more insulin resistant than lean Caucasians. While the curve of insulin

sensitivity against percent body fat was relatively steep in Caucasians, this was not the case in Asian Indians. In the latter group increasing adiposity was accompanied by some decrease in insulin sensitivity, but decrements were relatively small. This finding strongly suggests that Asian Indian men living in the United States have relatively low insulin sensitivity even when their body fat content is in the normal range. The mechanisms accountable for the low insulin sensitivity in Asian Indians, whether due to physical inactivity, dietary differences, or hereditary factors, continue to be determined (Chandalia *et al.*,1999).

Asian Indian women had a higher rate of CHD than do other ethnic groups, despite similar conventional risk factors and lipid profiles. It may partly be explained by the differences in the prevalence of atherogenic HDL-C and low-density lipoprotein cholesterol (LDL-C) sizes and their subclass concentrations among Asian Indian women compared with Caucasian women (Bhalodkar *et al.*,2005). In a study conducted by Ranjith *et al.*, in 2005 they examined differences in major cardiovascular risk factors and clinical outcome in South African Asian Indians of different age groups and gender, who presented with acute coronary syndromes. Diabetes mellitus (21%) and hypertension (18%) were seen less frequently in young patients but this was confined to men only. Total cholesterol was elevated in 65 to 70% of all patients while high-density lipoprotein (HDL) levels were significantly lower in men compared with women for all age subsets. Hospital mortality was extremely low in young (1%) and middle-aged patients (2%), but was expectedly higher in older patients (8%; $p < 0.0001$). The most common familial vascular disease noticed in this study was a family history of CHD. Young patients had more aggressive disease, with 48% of those subjected to angiographic studies having triple vessel disease (TVD), and 14% undergoing coronary artery bypass grafting (CABG).

In the study by Chambers *et al.*,(2001) the researchers investigated CRP concentrations in a representative sample of Indian Asian and European white men living in West London, UK. They found that CRP levels were elevated in Indian Asians and were closely associated with increased central adiposity and markers of insulin resistance in Asians compared with Europeans. There is some conflict-ridden data in regards to increased WHR and prevalence of diabetes in Asian Indians are controversial (Raji *et al.*, 2001).

 Central adiposity and high are common features that shared by Indian Asians overseas (McKeigue *et al.*,1991), urban Indian Asians in India (Reddy *et al.*,1998), and Indian Asian

women (McKeigue *et al.*,1991), the findings of elevated CRP among Indian Asian men in West London are likely to be universal to other Indian Asian populations. The defined reasons underlying increased central obesity among Indian Asians compared with European whites are not known. The genetic factors in the first-degree relatives of Indian Asian CHD patients may play a major role in explanation of increased abdominal obesity in this racial group (Chambers *et al.*,2001). That is in agreement with the data that suggested that CRP concentrations may be influenced by genetic factors, although the molecular basis remains to be identified (Pankow *et al.*,2001). On the basis of the reported (Danesh *et al.*,2000) relationship between CRP and risk of CHD, Chambers *et al.*, (2001) estimated that increased CRP concentrations and/or the processes underlying elevated CRP are associated with an 14% increase in population CHD risk among Indian Asians compared with European whites. The extent of this effect on CHD risk is comparable to a rise in diastolic blood pressure of 5 mm Hg (van Den Hoogen *et al.*,2000) or an increase in total cholesterol of 0.5mmol/L (Verschuren *et al.*,1995). Therefore, their results suggested that inflammation or enhanced cytokine production and/or their acute phase consequences may contribute significantly to the increased CHD mortality in Indian Asians. Study by Chambers *et al.*, (2001) demonstrated that CRP concentrations were also closely associated with levels of HDL cholesterol, triglycerides, glucose, blood pressure, and a composite insulin resistance score in both racial groups. The similar data was reported in North American and European populations, which have additionally shown that CRP concentrations and other inflammatory markers, including white cell count and fibrinogen, are strongly correlated with plasma insulin and insulin-mediated glucose uptake (Festa *et al.*,2000, Frohlich *et al.*,2000, Hak *et al.*,1999).

In a study by Mohan *et al.*, (2005) it was established that CRP showed a strong association with coronary artery disease (CAD) and diabetes, even after adjusting for age and gender in an urban south Indian population. The association of body fat with diabetes seems to take place through hs-CRP. However, CRP didn't appear to mediate the relationship between body fat and CAD. The relationship between CRP and dietary nutrients was investigated in young Asian Indians residing in a major metropolitan city in north India. Raised CRP levels (>3 mg/L) were noted in 9% study subjects (8.6% males and 12.8% females).

Saturated fat appear to be the single most important nutrient contributing to increase in serum CRP levels after adjustment for other covariates. The probability of having a raised CRP level

in subjects eat more than 10% energy as saturated dietary fat were twice that compared to subjects having a normal saturated fat intake (Arya *et al.*,2006). Elevated CRP levels in adolescents and young adults in Asian Indians in north India were observed in 21.8% of the overweight subjects and 24.5% of the subjects with high (>85th percentile) percentage body fat (%BF). Levels of CRP correlated significantly with body fat (%), WHR, biceps skinfolds and triceps skinfolds for males only. The findings of significant prevalence of elevated CRP levels in adolescents and young adults having increased generalized and abdominal adiposity may be important for the development of metabolic syndrome and atherosclerosis in Asian Indian adults (Vikram *et al.*,2003).

1.6.6 Prevalence of diabetes and cardiovascular diseases in South Africa Indian population

The Indian population of South Africa is composed of two so-called races, Dravidian and Aryan, having distinct cultural and anthropological characteristics. The major Indian languages spoken in South Africa are Tamil, Telegu, Hindi, Gujarati, and Urdu. The three main religions are Hindu, Muslim, and Christian. The dietary habits, body build, colour of skin, marriage and social customs, cultural activities, home dress, and place of origin in India are considerably different in these groups (Seedat, 1982).

Walker (1973) felt that from the age of 5 years onwards the South African Indian population, which enjoys much better economic circumstances than the indigent rural population in India, did not have a life expectancy advantage over the latter population; the main causes of death had changed from infectious to degenerative diseases. The Indian population, a migrant Asian Indian group, in South Africa has a high rate of coronary artery disease. Important risk factors such as hypercholesterolemia, diabetes and smoking in men, and hypercholesterolemia and smoking in women were noted in a large survey of Indian subjects in South Africa (Seedat *et al.*,1990). In another large study by Sewdarsen *et al.*, (1991) fasting serum lipid and lipoprotein levels were measured in 620 consecutive male survivors of myocardial infarction and compared with those of 524 healthy male volunteer controls. Hypercholesterolemia and

hypertriglyceridaemia were present in the patient group and was associated with other non-lipid coronary risk factors. Hypercholesterolemia with or without associated hypertriglyceridaemia was the widely found abnormality: 125 (25%) patients showed hypercholesterolemia without associated hypertriglyceridaemia; 73 (12%) had both hypercholesterolemia and hypertriglyceridaemia and 89 (14%) had hypertriglyceridaemia without associated hypercholesterolemia. Obesity was significantly more common among hypertriglyceridaemic survivors, whereas diabetes and hypertension were seen more common in survivors with combined hypercholesterolemia and hypertriglyceridaemia. Among hyperlipidemic and normolipidaemic patients it was no significant difference in the degree of smoking and family history of coronary artery disease.

South African Indians have been found to have a high prevalence of type 2 diabetes (13%), with the prevalence of IGT being 6.9% (Omar *et al.,* 1985, Omar *et al.,* 1994). A 4-year prospective study showed that IGT in this population is associated with a strikingly high risk of progression to type 2 diabetes (50.4%; rate of progression 12.6%/year) (Motala *et al.,* 1993). From a cross-sectional analysis of the insulin response during an oral glucose tolerance test (OGTT), IGT in the South African Indian population was characterized by β-cell dysfunction (diminished early-phase insulin response and delayed hyperinsulinemia), which was also present in the transient IGT group (Motala *et al.,* 1997).

In a survey, conducted to find the prevalence of coronary heart disease risk factors in a sub-group of young Indian patients (< or = 45 years) who presented to the Coronary Care Unit at the R. K. Khan Hospital in Durban the most prevalent risk factors were: previous smoking (74%), and hypertriglyceridaemia (54%). Women constitute only 14% of the population that presented with an acute MI. Smoking was more common among men (81%) than in women (35%). Abnormal HDL cholesterol levels were detected in 43% and 9%, in men and women, respectively, showing a great gender difference. Hypertension was more common in young women with MI than in men, 38% and 19%, respectively. There was a strong familial link, 54% of the patients had a family background of coronary heart disease (CHD) while 42% and 41% had family members who suffered from diabetes mellitus and hypertension, respectively (Ranjith *et al.,*2002).

1.6.7 Prevalence of diabetes and cardiovascular diseases in South African Black population

According to South African Department of Statistics death due to diabetes mellitus (3%) and ischaemic heart disease (2.4%) were among the ten leading underlying natural causes of death in 2003-2004 (http://www.statssa.gov.za). The Medical Research Council of South Africa (Bradshaw *et al.,* 2003) has also published data showing that cardiovascular disease and diabetes mellitus are the second and tenth leading causes of death, respectively in South Africa. The possible explanations of these findings include availability and low cost of unhealthy food, and the use of labour-saving mechanical devices, which have greatly influenced excess calorie intake and decreased energy expenditure. The data from epidemiological studies have indicated that the prevalence of certain obesity-related disorders varies between black and white urban women in South Africa: mortality from ischaemic heart disease is very rare in black South Africans (8/100 000 vs. 55/100 000) (Isles and Milne, 1987), whilst hypertension (30% vs. 15%) (Seedat, 1983) and type 2 diabetes (7.0% vs. 3.6%) (Joffe and Seftel, 1994a) are more prevalent in black South Africans. It appears that at this stage of the epidemiological and nutrition development in Africa, hypertension, stroke, and type 2 diabetes mellitus are becoming major public health problems (Bourne *et al.,* 2002, Vorster, 2002), but that Africans are "protected" against CHD, possibly because of low total cholesterol levels and a high level of high-density lipoprotein cholesterol (Okesina *et al.,* 1999, Vorster, 2002).

The Heart of Soweto Study recorded data for 4162 patients with confirmed cases of cardiovascular disease (1593 newly diagnosed and 2569 previously diagnosed and under treatment) from Jan 1 to Dec 31, 2006, who attended the cardiology unit at the Chris Hani Baragwanath Hospital in Soweto, South Africa. Most patients were black Africans and the study population contained more women than men. Heart failure was the most universal primary diagnosis (704 cases, 44% of total). Moderate to severe systolic dysfunction was apparent in 415 (53%) of 844 identified cases of heart failure, 577 (68%) of which were attributable to dilated cardiomyopathy or hypertensive heart disease, or both. The investigators reported that on presentation, many patients had evidence of advanced disease and of the 844

patients diagnosed with heart failure, 31% of these had class III or IV heart failure according to the New York Heart Association (NYHA) functional classification system (Sliwa *et al.*, 2008).

At King Edward VIII Hospital in Durban from 1955 to 1980 CHD was diagnosed in 30% of Indian but only 2.7% of black patients admitted with cardiovascular disease. However, clinicians have noted that the incidence of CHD is progressively rising in the black population, and is likely to rise further as risk factor prevalences are altered by changes in lifestyle, westernisation, and migration to an urban or peri-urban environment (Seedat *et al.*, 1992).

Type 2 diabetes is most prevalent in the South African Indian population followed by the African and then the white population. This trend mirrors the levels of insulin resistance, with the Indian population being the most resistant and the white population the most insulin sensitive. Although Africans are more insulin resistant than the white population, their level of visceral fat is lower suggesting either that visceral fat plays no role in insulin resistant or, is more diabetogenic in African than white subjects (van der Merwe *et al.*, 2000, Ferris *et al.*, 2005).

Some urban and rural studies have shown that in South African black women obesity does not necessarily accompany hypertension, diabetes or hyperlipidaemia (Walker *et al.*, 1989, Walker *et al.*, 1991). In contrast, Omar and colleagues (1993) found that black women with diabetes did have a higher body mass index (BMI) than women without diabetes. Waist circumference could highly predict type 2 diabetes mellitus and to cluster with cardiovascular risk factors in subjects of mixed ancestry (Levitt *et al.*, 1999b, Charlton *et al.*, 2001a).

INTERHEART, an international, standardized, case-control study conducted in 52 countries, was designed to assess the association of CVD risk factors and acute myocardial infarction (AMI) (Rosengren *et al.*,2004, Yusuf *et al.*,2004). These relationships were investigated in the African population and in three ethnic subgroups (black, colored, and European/other Africans) and compared with those found in the overall INTERHEART study. The data from INTERHEART Africa study was the first to show that that only five risk factors account for 89.2% of the risk for an initial myocardial infarction. These factors include current/former tobacco smoking, self-reported hypertension and diabetes, abdominal obesity measured as the WHR, and lipoprotein ApoB/ApoA-1 ratio. This data confirmed that people from Africa who are exposed to known major CVD risk factors are at risk to develop AMI, as are other people

across the globe (Yusuf *et al.*,2004). The INTERHEART data showed heterogeneity in relation to the magnitude of the risk for AMI for the level of education and income among the three ethnic groups. These variations reflect that the three ethnic groups are at different points of the epidemiological transition and therefore the development of the CVD epidemic (Gillum,1996). During this study it was found that the low numbers of acute myocardial infarction cases were recruited among black Africans, particularly outside of South Africa.

In a recent publication by Kalk *et al.*, (2007) it was pointed out that the prevalence of CHD in the African diabetic population is much lower than in the White diabetic population of South Africa, 4.0 and 23.0 %, respectively. Although the lower frequency of CHD among the diabetic Africans was observed in this study, a substantial proportion, about 25%, were at high risk for CHD in the following decade, as estimated from the prevalence of traditional risk factors, which, however, may undervalue the risk in diabetic populations (McEwan *et al.*, 2004, Kalk *et al.*, 2007). This suggests that in the less-developed areas of sub-Saharan Africa, AMI in black Africans may still be relatively rare, and in these countries the black African population may be at an even earlier stage of the epidemiological development than those in South Africa. However, with growing urbanization and economic growth, the rates may increase and resemble those seen in South Africa (Steyn *et al.*,2005).

1.7 Nutrition and diseases of lifestyle

The adverse effects on human health are the results of quicken development of civilization (mainly industrialization, urbanization and nutrition). A gathering of problems named as 'civilization diseases' has become the focus of serious apprehension but review of available data indicates that this notion appears to add very little to our understanding of modern environmental effects on human health (Trnovec *et al.*, 2001). While the primary reason of the obesity epidemic is still uncertain, the enormous pressures on energy balance provided by the modern environment and lifestyle remain the most reasonable explanation (Zheng *et al.*, 2007).

The eating behaviour in modern society is characterized by the consumption of high-energy-density diets and often-unstructured feeding patterns, largely detached from seasonal cycles of

food availability. The privileged groups in economically emerging and developing nations have generally similar patterns of eating (Ulijaszek, 2002). Worldwide marketing and the methodical moulding of taste by giant corporations is a central characteristic of the globalization of the food industry (Barnett *et al.*,1994).

A recent survey in the USA found that only 38% of meals were home-made and that many people had never cooked a meal from basic ingredients (Gardner *et al.*,2000); an average restaurant meal supplies 1000–2000 kcal, i.e. up to 100% of the suggested daily intake for most adults, and the sizes of servings are mounting (Jacobsen *et al.*,2000). Additionally, there is a greater trend to snack between meals. In the United Kingdom, 75% of adults and 91% of children devour a snack food at least once a day. Such conduct is promoted from a young age: there are fast food restaurants in many schools in the USA, and soft drink vending machines are increasingly found in schools globally (Chopra *et al.*,2002).

1.7.1 Fatty acids, diabetes, and cardiovascular disease

The number of published data supports cardioprotective effects, beyond those that can be attributed to improvements in blood lipoprotein profiles, to the foods rich in ω-3 polyunsaturated fatty acids, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The main valuable effects include a reduction in sudden death (Albert *et al.*,1998, Siscovick *et al.*,1995), decreased risk of arrhythmia (Kang *et al.*,1994), lower plasma triglyceride levels (Harris,1997), and a reduced blood-clotting tendency (Agren *et al.*,1997, Mori *et al.*,1997). There is some evidence from epidemiological studies that α-linolenic acid (omega-3 fatty acid) reduces risk of myocardial infarction (Guallar *et al.*,1999) and fatal ischemic heart disease in women (Hu *et al.*,1999a). Several randomized controlled trials have recently demonstrated beneficial effects of both α-linolenic acid (de Lorgeril *et al.*,1999) and marine ω-3 fatty acids (GISSI- Prevenzione Investigators,1999, Singh *et al.*,1997, Von Schacky *et al.*,1999) on both coronary morbidity and mortality in patients with coronary disease. The food supply of ω-3 fatty acids can be found in fish, especially fatty fish such as salmon, as well as plant sources such as flaxseed and flaxseed oil, canola oil, soybean oil,

and nuts. The recommended two servings of fish per week could grant cardioprotective effects. Further studies are needed to agree on optimal doses of ω-3 fatty acids (including EPA, DHA, and α-linolenic acid) for both primary and secondary prevention of coronary disease as well as the treatment of hypertriglyceridemia (Krauss *et al.,*2000).

It has been recognized that dietary *trans*-unsaturated fatty acids can increase LDL cholesterol and reduce HDL cholesterol (Judd *et al.,*1994, Lichtenstein *et al.,*1999). *Trans* fatty acids found mainly in hydrogenated vegetable oils have a tendency to raise cholesterol levels relative to their non-hydrogenated counterparts (ASCN/AIN,1996, Report of the Expert Panel,1995). This increase seems to be less than take place with similar amounts of saturated animal fat or highly saturated vegetable oils, e.g., coconut and palm kernel oils. Some studies that used plasma or tissue levels of *trans* fatty acids as a measure of intake proposed that CHD risk is correlated with *trans* fatty acids derived from animal products, but not with those from hydrogenation of oils (Wahle *et al.,*1993). Besides, there is no clear dose-response effect for *trans* fatty acid intake and CHD risk. Based on this limited information, the American Heart Association (AHA) recommends limiting *trans* fatty acid intake, for example, by substituting soft margarine for hard. The AHA also supports the food industry to create more products with reduced *trans* fatty acid content (Krauss *et al.,* 1996).

The attention given to dietary medium-chain fatty acids (MCFAs) is due to their ability to be more readily absorbed from dietary medium-chain triacylglycerols (MCTs) than are long-chain fatty acids from, for example, vegetable oils. It has generally been declared that MCFAs do not increase plasma cholesterol, even though this claim is poorly documented. However, a study by Cater et al (1997) showed that, in comparison with oleic acid, MCTs have a cholesterol-increasing potential one-half of that of palmitic acid in mildly hypercholesterolemic middle-aged men, which corroborates results reported in the 1960s (Hashim *et al.,*1960, Roels *et al.,*1962). MCTs increase plasma triacylglycerol in contrast to long-chain triacylglycerols (LCTs) (Uzawa *et al.,*1964, Hill *et al.,*1990). A recent study by Tholstrup *et al.,*(2004) found that an intake of 70 g MCT oil/d for 21day increased total cholesterol, LDL-cholesterol, triacylglycerol, and glucose concentrations by, 11%, 12%, 22%, and 4%, respectively, relative to an equivalent intake of high-oleic sunflower oil. There is thus some unease concerning the cardiovascular effects of MCTs. It was suggested that combination of plant sterols with MCT oil could possible avert undesirable cardiovascular

effects (St-Onge *et al.*,2003c). It has been shown that when this combination is consumed, total and LDL-cholesterol concentrations are lower than those observed after LCTs consumption (St-Onge *et al.*,2003a, Bourque *et al.*,2003).

Groups of investigators have examined whether the composition of dietary FAs, as opposed to total fat consumption, can modulate insulin sensitivity and cardiovascular risk factors (Hu *et al.*, 2001, Rivellese *et al.*, 2002). Some epidemiological studies demonstrated that high intake of total and saturated fat is associated with insulin resistance, and this relationship may be dependent on increased body adiposity (Mayer-Davis *et al.*, 1997). Conversely, multiple cross-sectional studies have found that intake of both saturated and trans FAs is correlated with hyperinsulinemia and with risk of type 2 diabetes, independent of general obesity (Maron *et al.*, 1991, Marshall *et al.*, 1997, Parker *et al.*, 1993).

High intake of polyunsaturated FAs (PUFAs) does not look to have the same adverse effects and could even result in an increase in insulin sensitivity (Salmeron *et al.*, 2001). For instance, Summers *et al.* (2002) studied the effect of substituting dietary saturated fat with polyunsaturated fat on insulin sensitivity in healthy, obese, and type 2 diabetic subjects. Their results confirmed that an isocaloric diet enriched in polyunsaturated fat resulted in both an increase in insulin sensitivity measured by glucose clamp and a lowering of LDL cholesterol when compared with a diet rich in saturated FAs. Then again, it was not achievable in this study to conclude whether it was the increase in dietary PUFA or the decrease in saturated fat that created the relative benefits in the PUFA diet subgroup. Moreover, diets enriched in polyunsaturated fat have not reliably been shown to improve insulin sensitivity (Mayer *et al.*, 1993), and long-term intervention trials have not been carrying out. Inconsistency in the short-term studies are often imputable to the lapse to control for dietary FA [*e.g.* amount of monounsaturated FAs (MUFA)] and carbohydrate composition, total calories, physical activity, and population characteristics such as age, gender, and adiposity (Lara-Castro *et al.*, 2004). Favourable results of a high-MUFA diet on glycemic control in type 2 diabetes have been confirmed in a meta-analysis of randomized trials using isoenergetic high-MUFA diets (Garg,1998). "Short-term intervention studies in healthy volunteers have shown that the isocaloric substitution of MUFA for saturated fat (Perez-Jimenez *et al.*, 2001), or even substituting MUFA for carbohydrates, can have positive effects on insulin sensitivity" (Thomsen *et al.*,1999). Comparable findings were obtained in a 3-month trial that assessed

insulin sensitivity in healthy volunteers receiving diets varying in FA composition (ω-3 PUFA *vs.* MUFA *vs.* saturated fat). Significant increases in insulin sensitivity were achieved in the MUFA-enriched diet. This effect was larger when the total amount of fat was modest (<37% of calories) (Lara-Castro *et al.*,2004).

1.7.2 Fibre-rich foods

A number of longitudinal observational studies have described significant reverse associations between total fibre intake and both cardiovascular and all causes of mortality (Kromhout *et al.*,1982, Morris *et al.*,1977). Partly this occurrence may suggest the accompanying reverse association that is often observed between fibre and fat intake when calories are controlled (Anderson *et al.*,1992). In the Zutphen Study (Kromhout *et al.*,1982), men in the lowest quintile of dietary fibre intake exhibited a four times higher rate of CHD mortality compared with men in the highest quintile, even though total caloric intake was about the same. The rate of CHD mortality was reported to be inversely associated with fibre intake across 20 industrialized nations, but adjustment for fat intake removed the association (Liu *et al.*,1979). Likewise a 20-year cohort study of 1001 middle-aged men in Ireland and Boston described significant inverse association between fibre intake and risk of CHD, but the association reduced when other risk factors were controlled (Kushi *et al.*,1985). The 12 year study of 859 men and women aged 50 to 79 years, showed that a 6-g increment in daily fibre intake was correlated with a 25% reduction in ischemic heart disease mortality, independent of calories, fat, and other dietary variables (Khaw *et al.*,1987). In addition, various soluble fibres (e.g., oat products, psyllium, pectin, and guar gum) lower LDL cholesterol, mainly in hypercholesterolemic individuals. In one meta-analysis study it was concluded that for every gram increase in soluble fibre from these sources, LDL cholesterol would be expected to decrease by an average of 2.2 mg/dl (Brown *et al.*,1999). Higher intakes of fibre from oats and buckwheat could lower serum cholesterol and blood pressure levels as was reported in a study among 850 men in the Yi province of China (He *et al.*,1995). The mechanism by which fibre lowers blood cholesterol remains undefined. Evidence suggests that some soluble fibres bind

bile acids or cholesterol during the intraluminal formation of micelles (Anderson *et al.*,1986). As a result, decrease in the cholesterol content of liver cells leads to an up-regulation of the LDL receptors and thus increased clearance of LDL cholesterol, but increased bile acid excretion may not be adequate to account for the observed cholesterol reduction (Brown *et al.*, 1999). Other recommended mechanisms include inhibition of hepatic fatty acid synthesis by products of fermentation (production of short-chain fatty acids such as acetate, butyrate, propionate) (Nishina *et al.*,1990); changes in intestinal motility (Schneeman *et al.*,1985); fibres with high viscosity causing slowed absorption of macronutrients, leading to increased insulin sensitivity; and increased satiety, leading to lower overall energy intake (Brown *et al.*, 1999).

1.7.3 Homocysteine, folate, vitamins B6, B12, D, Coronary Artery Disease and Insulin Sensitivity

The effects folic acid and vitamins B6 and B12 have on the metabolism of homocysteine aroused interest in these vitamins. Elevated homocysteine in plasma has a strong epidemiological association with Coronary Artery Disease (CAD), peripheral vascular, and cerebrovascular disease (Boushey *et al.*,1995). Similar with other vitamin supplements, no clinical trials have been conducted to investigate if there is a clinical benefit to reduced homocysteine levels via increased intake of folic acid, B6, or B12 (Krauss *et al.*,1996). Homocysteine is a sulphydryl-containing amino acid produced from demethylation of dietary methionine. The link between increased concentrations of homocysteine with vascular disease is independent of other risk factors and is consistent across many studies. Reduction in plasma homocysteine levels could be achieved by dietary supplements of folic acid and B vitamins (Maxwell, 2000).

The B-Vitamin Treatment Trialists' Collaboration (2006) evaluated the design and statistical power of 12 randomized trials measuring the effects of lowering homocysteine with B-vitamin supplements on risk of cardiovascular disease. It was concluded that the individual trials may not have engaged a sufficient number of vascular events or have remained long enough to have a good chance on their own to identify reliably plausible effects of homocysteine lowering on

cardiovascular disease risk, but the combined analysis of these trials should have sufficient power to determine whether lowering homocysteine reduces the risk of cardiovascular events within a few years (Traber, 2007).

The normal metabolism of homocysteine demands a sufficient supply of folate, vitamin B6 vitamin B12, and riboflavin. Levels of these vitamins show an inverse relationship with those of circulating homocysteine. The association between these vitamins and vascular diseases has thus also come under enquiry (Pancharuniti *et al.*,1994). Increased coronary disease risk and fatal coronary artery disease have been correlated with lower folate levels. Lower levels of vitamin B6 also present an increased risk of atherosclerotic vascular disease in case-control and prospective studies. The risk of atherosclerosis associated with lower levels of vitamin B6 is independent of high homocysteine concentrations. Vitamin B12 deficiency is not associated with vascular disease (Krauss *et al.*, 2000).

A number of studies had describe a negative relationship between various anthropometric measures and intake of vitamin D or circulating concentrations of vitamin D has been reported by a number of studies (Kamycheva *et al.*, 2003, Parikh *et al.*, 2004). Data regarding association of hypovitaminosis D with insulin sensitivity is relatively constraint compared to available data for the effect of hypovitaminosis D on ß cell function. In study of a group of 34 men that included 7 subjects with diabetes a positive correlation was found between serum 25(OH) D concentration and insulin sensitivity (Lind *et al.*, 1995). The serum 25(OH) D concentration was negatively correlated with fasting insulin concentration, 1-h and 2-h insulin concentrations, and insulin area under the curve in 134 elderly nondiabetic men, independent of BMI, skinfold thickness, alcohol, smoking, and physical activity (Baynes *et al.*, 1997). These results indicated a positive association of 25 (OH) D concentrations with insulin sensitivity. In patients with type 2 diabetes addition of vitamin D to their diet reduced serum free fatty acids levels suggesting an improvement in insulin sensitivity (Inomata *et al.*, 1986). The data published by Chiu and co-workers*,* (2004) did show a positive correlation of 25(OH) D concentrations with insulin sensitivity and a negative effect of hypovitaminosis D on ß cell function. Subjects with low levels of vitamin D could be at higher risk of insulin resistance and the metabolic syndrome. Further studies will be required to explore the underlying mechanisms.

1.7.4 Antioxidants

The positive role that the major antioxidant nutrients vitamin E and vitamin C, and beta-carotene may have in prevention of several chronic disorders was confirmed in a number of epidemiological studies (Diplock *et al.*, 1998). Vitamin E is the combined name for a set of eight related tocopherols and tocotrienols and function as a chain-breaking antioxidant, preventing further lipid peroxidation. Of these, α-Tocopherol has been most studied as it has the highest bioavailability. α-Tocopherol has the highest bioavailability and has been researched most extensively. α-Tocopherol inhibits some of the cellular functions that may transform heart attack risk via mechanisms mediated by protein kinase C. α-Tocopherol supplementation also decreases monocyte superoxide production through inhibition of protein kinase C. α-Tocopherol mediates nitric oxide production in endothelial cells. Vitamin E is depleted in humans during oxidative stress. Plasma vitamin E concentrations depleted much slower in non-smokers than in cigarette smokers as a result of their increased oxidative stress (Traber, 2007). The disparity in vitamin E levels were acquired by the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study with a low-dose vitamin E supplementation (50 mg/d) and the Cambridge Heart Antioxidant Study (400-800 mg/d). The data from GISSI-Prevenzione (300 mg/d) and HOPE (400 mg/d) trials indicated the absence of relevant clinical effects of vitamin E on the risk of cardiovascular events (Marchioli *et al.*, 2001).

The recommended intake of vitamin E of 1000 mg/d is without risk, because vitamin E has very low toxicity. Large intake of beta-carotene must be viewed with caution because it has been shown to be detrimental to a population at high risk of lung cancer when administered after many years of high risk (smoking) behaviour (Diplock *et al.*, 1998). Clinical trials with beta-carotene supplements have been inadequate, and therefore their use as a preventive intervention for cancer and coronary heart disease should be discouraged (Marchioli *et al.*, 2001).

A number of dietary factors, such as trace elements, including selenium, copper, zinc, and manganese are proposed to act as antioxidants and have been recommended to protect against coronary heart disease. Some of them function as cofactors for enzymes with antioxidant activity (e.g., glutathione peroxidase and superoxide dismutase) (Tribble, 1999). Antioxidant

dietary intake had shown in some studies to have a positive effects on cardiovascular disease which may be attributed to other chemicals present in foods, such as flavonoids (Cherubini *et al.*, 2005).

1.7.5 Carbohydrates

The long-term usefulness and safety of the diets restricted in carbohydrate but high in protein and fat are not known, despite their popularity over some period of time(Golay *et al.*,1996b). The relative achievement of diets severely restricted in carbohydrate calories over the first few days may be due to water losses (Fisler *et al.*,1987). The reduction in weight over weeks to months associates to reductions in total energy intake, which are possible in part to be a consequence of the ketosis that accompanies carbohydrate restriction (Cahill Jr.,1976).

There are a number of safety issues during the active phases of weight reduction include mineral, electrolyte (Fisler *et al.*,1987), and vitamin deficiencies, whereas the continued consumption of a diet high in fat and protein and low in carbohydrate during the maintenance period may result in an atherogenic lipoprotein profile and reductions in renal function and skeletal mass (Larosa *et al.*,1980). Also, the relative absence of other major constituents of a healthy diet such as fruits, vegetables, milk products, legumes, and whole-grain products raises concerns about adequacy of micronutrient intake (Krauss *et al.*,2000). The type of carbohydrate consumed is now also being seen as relevant to the development of obesity. Diets rich in complex carbohydrates or "fibre" (i.e., nonstarch polysaccharide) are protective (Howarth *et al.*,2001), whereas energy-dense diets, as well as drinks that are rich in refined sugars, promote weight gain (Yao *et al.*,2001).

Samaha and co-workers, (2003) found that severely obese subjects with a high prevalence of diabetes and the metabolic syndrome lost more weight in a six-month period on a carbohydrate-restricted diet than on a fat- and calorie-restricted diet. The higher decline in overall caloric intake could contribute to greater weight loss in the low-carbohydrate group preferably than a direct effect of macronutrient composition.

In subjects who reduced more than 5 percent of their initial weight on the carbohydrate-restricted diet the improvements in insulin sensitivity were independent of weight loss and a

greater reduction in triglyceride levels. These findings must be interpreted with care, however, since the extent of the overall weight loss relative to the subjects severe obesity was small, and it is unclear whether these benefits of a carbohydrate-restricted diet extend beyond six months. No harmful effects on other serum lipid levels were found. The black subjects had a lesser overall weight loss compared to the white subjects. The researchers suggested that future studies should explore whether greater weight loss in this population can be achieved by more effective incorporation of culturally sensitive dietary counselling (Samaha *et al.*,2003).

1.7.6 Proteins

Most Americans consume protein in surplus of their needs (McDowell *et al.*,1994, National Research Council.Food and Nutrition Board, 1989). Since extra protein is not properly utilized by the body it provides a burden for its degradation. Moreover, meat protein is the most expensive source of calories in the food budget. Protein foods from animal sources (with the exemption of low-fat and non-fat dairy products) are in general higher in fat, saturated fat, and cholesterol. In general nutrient adequacy, long-term palatability, and maintenance of the diet become major worries with the diets high in protein and limited carbohydrates. An average of 15% total energy or ≈50 to 100 g/d should be adequate to meet most needs while there are many conditions in which extra protein may be needed such as growth, pregnancy, lactation, and some disease states (Goff *et al.*, 1995). In the epidemiological Nurses' Health Study a positive health advantage from a high protein intake was observed. Women in the highest protein intake group had a 26% lower rate of cardiovascular disease compared to women in the lowest protein intake group (Hu *et. al.*,1999b).

Clinical intervention studies have presented reliable evidence that an ad libitum high-protein diet from mixed sources in free-living overweight people increases the amount of weight lost in a 6-month weight-loss program (by 3.8 kg) compared with a high-carbohydrate diet by enhancing satiety (Skov *et al.*,1999). Additionally, weight-loss studies in overweight women have shown that diets with a high ratio of protein to carbohydrate have positive effects on markers of disease risk, including body composition, blood lipids, and glucose homeostasis,

and that these benefits may be arbitrated to a degree by the effect of protein on satiety and by a lower glycemic load because of a lower carbohydrate intake (Layman *et al.*,2003a, Layman *et al.*,2003b). Insulin sensitivity may be enhanced by inevitable loss of lean body mass during weight loss on a higher protein intake (Baba *et al.*,1999, Piatti *et al.*,1994), while this has not been observed at very low energy intakes (Vazquez *et al.*,1995). Subjects with insulin resistance or type 2 diabetes, that been overweight, showed fat loss by 1-2 kg over 12 weeks when using a high-protein weight-loss diet (28–30% of energy from protein) from mixed sources. It was particularly noticeable in women with an isocaloric high-carbohydrate weight-loss diet (Farnsworth *et al.*,2003, Parker *et al.*,2002).

It is recognized that different protein sources have different effects on the release of insulin (Gannon *et al.*,1988, Westphal *et al.*,1990), and this may be important to the mechanism of action of both the enhanced satiety and the differential fat loss (Latner *et al.*,1999). Some fears have been raised out regarding the effect of high-protein diets on serum lipids and subsequent cardiovascular disease risk since foods and dietary patterns high in protein may vary in saturated fat and nutritional composition. There is some proof that high-protein diets increase calcium excretion and increase bone loss, which particularly needs clarification (Kerstetter *et al.*,1999). The study by Noakes *et al.*,(2005) showed similar reductions in creatinine clearance with both dietary patterns as a consequence of body mass change. These results call off the main concerns that diets high in meat protein may have deleterious effects on renal function and bone turnover. A excess of protein in the system also increases urinary calcium loss, which may facilitate osteoporosis (Barzel *et al.*,1998).

1.7.7 Effect of dietary calcium and calcium supplements on body weight

Calcium homeostasis is essential to a large number of physiological processes in the body. Previous epidemiological and experimental data have suggested that increasing dietary calcium accelerated weight and fat loss secondary to energy restriction, with a substantially greater effect exerted by dietary (dairy) sources of calcium when compared with a supplemental (calcium carbonate) source (Andersen *et al.*,1986, Fleming *et al.*,1994, Lind *et al.*,1993, McCarron *et al.*,1984).

A study by Cummings *et al.*,(2004) showed that calcium acutely stimulated postprandial fat oxidation and suppressed carbohydrate oxidation. The effects over the 6 h postprandial period were very similar in regards to dairy or non-dairy calcium meals. The subjective sensations of hunger and satiety after food intake have not been affected by high dairy calcium and vitamin D diet, but spontaneous food intake over the subsequent 24h period was significantly suppressed, as stated by Ping-Delfos *et al.*,(2004).

High calcium diets suppress calcitriol levels, which result in reduced lipogenesis and increases in lipolysis, uncoupling protein 2 expression, and adipocyte apoptosis (Shi *et al.*,2002, Sun *et al.*,2004a, Zemel,2004), thereby reducing lipid filling and adiposity (Shi *et al.*,2001a, Sun *et al.*,2004b). However, these information do not present an explanation for the substantially greater effects observed with dairy vs. supplemental sources of calcium (Zemel *et al.*,2005a).

It was suggested by Zemel *et al.*, (2005b), that suboptimal dairy and calcium intakes may contribute to increased total and central adiposity and obesity in already-obese African-American adults. Suboptimal dairy and calcium intake may also intervene with successful weight and fat loss during energy restriction. These data are consistent with their other's earlier studies (Zemel *et al.*,2004).

Recently it was shown that 1,25-dihydroxyvitamin D stimulates 11ß-HSD-1 expression and cortisol production in human adipocytes (Morris *et al.*,2005, Zemel *et al.*,2003). Given that high calcium diets suppress 1,25-dihydroxyvitamin D levels, the authors proposed that loss of central adiposity on high dairy diets my be attributable, in part, to suppression of 1,25-dihydroxyvitamin D levels and a consequent reduction in cortisol production by visceral adipocytes (Zemel *et al.*,2005a).

Animal studies and epidemiological studies have suggested that calcium supplementation (with Ca^{++} supplements or dairy products) may be associated with weight loss in human adults. However, Trowman and colleagues, (2006) in their systematic review and subsequent meta-analysis included a total of thirteen randomized controlled trials and found no association between the increased consumption of either Ca^{++} supplements or dairy products and weight loss after adjusting for differences in baseline weights between the control and intervention groups (Trowman *et al.*,2006).

The review by Barr (2003) of randomized trials of increased dairy product or calcium intake obtained little evidence to support the hypothesis that calcium or other components of dairy

products have a measurable impact on body weight in generally healthy humans. Significantly greater weight loss in the calcium-supplemented group was found in merely one of 17 randomized trials of calcium supplementation (Recker *et al.*,1996), and it could be contended that this is close to what would be expected on the basis of chance alone (Barr, 2003).

The relation between dairy intake and incidence of insulin resistance syndrome (IRS) was examined in the Coronary Artery Risk Development in Young adults (CARDIA) study. As a result of this study the researchers found that dairy consumption was inversely associated with the incidence of all IRS components among overweight individuals but not among leaner individuals. Each daily occasion of dairy consumption was associated with 21% lower odds of IRS and was similar for blacks and whites and for men and women. The association between dairy intake and IRS could not be explained by other dietary factors, including macronutrients and micronutrients (Pereira *et al.*,2002).

1.7.8 Dietary salt and its affects on blood pressure

The majority of available data indicates that a high intake of salt negatively affects blood pressure. As summarized in a recent AHA advisory (Kotchen *et al.*,1998), such data include results from observational studies of diet and blood pressure and clinical trials of reduced salt intake. Meta-analyses of randomized trials have shown that on average, reducing sodium intake by ≈80 mmol (1.8 g)/d is correlated with systolic and diastolic blood pressure reductions of ≈4 and 2 mm Hg in hypertensive and lesser reductions in normotensive subjects (Cutler *et al.*,1997, Graudal *et al.*,1998). The blood pressure response to variations in salt intake differs among individuals, in part because of genetic factors (Hunt *et al.*,1998) and other host factors such as age (Law *et al.*,1991).

In the Dietary Approaches to Stop Hypertension (DASH) study, a healthy diet termed the DASH combination diet substantially reduced blood pressure in both nonhypertensive and hypertensive individuals (Appel *et al.*,1997, Svetkey *et al.*,1999). This dietary guide highlights fruits and vegetables (5 to 9 servings per day) and low-fat dairy products (2 to 4 servings per day). The diet was rich in potassium, magnesium, and calcium. The reduction in systolic and diastolic blood pressure by 3.5 and 2.1 mm Hg, respectively, was observed among

nonhypertensive individuals. Resultant blood pressure reductions in hypertensive were striking: 11.4 and 5.5 in persons with stage 1 hypertension. Black Americans had greater blood pressure reductions than did non-black Americans (Svetkey *et al.*,1999).

The reduced risk of stroke have been linked to the diets rich in potassium (Ascherio *et al.*,1999). In order to asses whether reduction in sodium intake levels and an increase in potassium intake levels are critical components of blood pressure (BP) control Maseko *et al.,* (2006) studied the relationship between hypertension awareness and treatment, and 24- hour urinary sodium and potassium excretion rates in urban, developing communities in South Africa. Their study showed that hypertension awareness and treatment were not associated with electrolyte excretion rates either when considered alone or after adjusting for age, gender, body mass index, alcohol and tobacco intake, the presence of diabetes mellitus and the type of antihypertensive therapy (multivariate regression analysis).

In addition, the proportion of patients who were vigilant of their hypertension, were receiving treatment for it, and who had 24-hour sodium intake values above the RDA for sodium intake (80%) was similar to the proportion noted in those who were unaware of their hypertension (73%), and to normotensives (84%). They proposed that the lack of relationship between either hypertension awareness and treatment, and sodium and potassium intake levels suggests that current recommendations for a reduced sodium and increased potassium intake in hypertensives do not translate into clinical practice in urban, developing communities of South Africa (Maseko *et al.*, 2006).

In another study Charlton *et al.*,(2005a) investigated whether habitual intakes of sodium (Na^{++}), potassium, magnesium, and calcium vary across South African ethnic groups and to assess whether the blood pressure-cation association differs according to ethnic status. Ethnic differences in calcium intake were apparent, with black subjects having particularly low intakes. The differences with respect to sources of dietary Na^{++} were obvious, with more than 70% of total non-discretionary Na^{++} being provided by bread and cereals in rural black South Africans compared with 49% to 54% in urban dwellers.

All ethnic groups had Na^{++} intakes in excess of 6 g/d of salt, whereas potassium intakes in all groups were below the recommended level of 90 mM/d. White normotensive subjects had significantly higher median urinary sodium concentrations compared to normotensive black and mixed-ancestry subjects, however no differences were found between white and black

hypertensive subjects. Mixed-ancestry normotensive subjects had a lower urinary potassium excretion than white normotensive subjects, but no other ethnic differences were found. Urinary calcium levels in both normotensive and hypertensive individuals were significantly different between all three groups. Black subjects had nearly less than half of those of white subjects. No differences were found in urinary magnesium excretion across ethnic groups. The authors concluded that dietary differences may contribute to ethnically related differences in blood pressure (Charlton *et al.*,2005b). The considerably high dietary intake of potassium, magnesium, and calcium comes from food sources and diets rich in these minerals provide a variety of other nutrients it recommended to increase mineral intake through foods rather than supplements. Women who need to meet current guidelines for osteoporosis prevention or treatment calcium supplement should be implemented (Krauss *et al.*,2000).

1.7.9 Genetics influences in nutrition

The application of genomic knowledge to adjust population-based dietary recommendations is not without threat since gene-diet interactions are complex and poorly understood. Identification of genetic variation that happened as an effect of diet as a selective pressure helps to identify gene alleles that have an effect on nutrient utilization. Any genetic variation that presents an unusual nutritional requirement will almost certainly be conflicting with life and early development and consequently not viable. Fascinatingly, polymorphisms for the aldolase B enzyme, which metabolizes fructose, have been discovered (Esposito *et al.*, 2002b). The gene for this enzyme is extremely polymorphic, but until recently, many of these polymorphisms were regarded as silent. Polymorphisms presented as disease alleles only when fructose was added in high quantities to the food supply as a sweetener. This is a situation where a transformation in environment has threatened a normally silent allele to the degree that it begins to present as a disease allele (Stover, 2006).

Some recent findings indicate a vital role for genetic susceptibility to the insulin resistance syndrome and dyslipidaemia. Development and progression of these diseases largely associated with nutrition. Genetic background may reciprocate with regular dietary fat

composition, affecting predisposition to the insulin resistance syndrome and individual responsiveness to changes in dietary fat intake (Lopez-Miranda *et al.*, 2007). Both omega-6 and omega-3 fatty acids control gene expression. Omega-6 fatty acids do not have these same anti-inflammatory effects as does omega-3 fatty acids. Since inflammation is at the base of many chronic diseases, dietary intake of omega-3 fatty acids it is very important particularly in persons with genetic variation, as for example in individuals with genetic variants at the 5-lipoxygenase (Simopoulos, 2006). Genetic effects have been recognized for plasma lipoprotein reactions to dietary fatty acids, cholesterol, and fibre; blood pressure responses to sodium; and homocysteine responses to folic acid. Furthermore, there is increasing evidence, mainly from animal models, for the roles of specific genes in influencing susceptibility to diet-induced obesity (Krauss *et al.*,2000).

1.7.10 Prevention strategies and nutrition recommendations

In a number of studies of primary and secondary prevention of coronary artery disease it was clearly recognised the gains of therapies intended to reduce levels of total and LDL cholesterol. It was recommended that individuals with LDL cholesterol levels that are higher than current National Cholesterol Education Program (NCEP) marks for primary or secondary prevention decrease their intake of dietary saturated fat and cholesterol to levels below those recommended for the general population (NCEP,1994). The upper limit for such individuals is <7% of total energy for saturated fat and <200 mg of cholesterol per day and the lower intake levels in both cases could be of further benefit in reducing LDL cholesterol levels.

The American Heart Association (AHA, 1988) does not specifically advocate proportionate reduction in other types of fat, however diets that are very low in saturated fat may also be very low in total fat (<15% of energy). In recent years much attention was given to monosaturated fatty acids as an appropriate substitute for saturated fatty acids. Whilst their final outcome on serum lipids and lipoproteins is not much different from that of polyunsaturated fatty acids they may have some advantages (Dreon *et al.*,1990, Ginsberg *et al.*,1990, Grundy *et al.*,1990, Mensink *et al.*,1989, Mensink *et al.*,1992).

Unlike polyunsaturated, monounsaturated fats are not as susceptible to oxidation, which may play a role in atherogenesis. As a result the AHA recommends a monounsaturated fatty acid intake in the range of 10% to 15% of total calories (NCEP,1994). Diets with very low total fat intake have been tested with favourable results in studies of persons at high risk, but such diets have not been demonstrated to be of value for the general population and may have adverse consequences, including potential nutrient deficiencies in certain subgroups such as children, pregnant women, and the elderly (Gould *et al.*,1995, Ornish *et al.*,1990, Schaefer *et al.*,1995). For this reason, the AHA supports the recommendation of the World Health Organization for a lower limit of 15% of calories as total fat (Food and Agriculture Organization.,1994.). Additionally, the AHA recommends that for the general population, the level of fat intake in the diet should be guided by emphasis on adequate consumption of fruits, vegetables, and grains; a healthy weight goal; and dietary intake of saturated fatty acids and cholesterol appropriate to individual risk for CHD. The AHA highlights restraint of saturated fatty acid intake because this is the strongest dietary determinant of plasma LDL cholesterol levels (Hegsted *et al.*,1965). The AHA recommendation is to increase fibre intake in the diet (Vanhorn ,1997). This goal can be achieved by an emphasis on consumption of vegetables, cereals, grains, and fruits. Although there are studies showing that specific fibre supplements are associated with lowered LDL or glucose, there are no long-term trials showing relations between these supplements and cardiovascular disease. Therefore, at this time, fibre supplements are not recommended for heart disease risk reduction. Diets high in unrefined carbohydrates also incline to be high in both soluble and insoluble fibre. The decrease in plasma total and LDL cholesterol levels as part of a fat-modified diet can be achieved by consumption of the foods rich in soluble fibre, including oats, barley, beans, soy products, guar gum, and pectin found in apples, cranberries, currants, and gooseberries (Bell *et al.*,1990, Ripsin *et al.*,1992, Whyte *et al.*,1992). Total dietary fibre intake of 25 to 30 g/d from foods, not supplements, will make certain an eating pattern high in complex carbohydrates and low in fat.

There is general understanding in the scientific community that salt restriction can improve blood pressure in hypertensive individuals, but there are no clear data that allow definition of a desirable upper limit for salt intake. The average of sodium chloride intake in the United States could range from 7.5 to 10.0 g/d. The AHA has chosen to support the recommendation

of the US Dietary Guideline Committee to limit sodium chloride intake to 6 g/d. There was no increase in cardiovascular risk or raise blood pressure in normotensive persons without other cardiovascular risk factors with slightly higher intakes (6.0 to 7.5 g/d). The recommended guideline is an admittedly subjective recommendation for avoiding excessive salt intake instead than an attempt to impose low salt intake (Krauss *et al.*,1996). Sodium chloride restriction in hypertensive persons was conducted in therapeutic trials and provided some evidence of modest but significant reductions in blood pressure (Australian National Health and Medical Research Council, 1989, Cutler *et al.*,1991, Midgley *et al.*,1996). However, there is significant variation among blood pressure responses to sodium chloride restriction, and there is no simple, reliable test to accurately predict salt sensitivity.

The number of issues should be addressed to reverse dietary trends in obesogenic environment by public education and clear food labelling. The reduction in serum cholesterol levels and number of deaths from coronary heart disease was significantly reduced in Finland and Norway when they changed the high-fat, energy-dense diets consumed by their populations (Norum,1997, Puska *et al.*,1995). Introduction of a national intervention programme in Singapore has decreased the levels of some cardiovascular risk factors and of childhood obesity (Cutter *et al.*,2001). Mauritius organized a comprehensive programme that has reduced mean serum cholesterol levels, hypertension, smoking, and heavy alcohol use. The program involves the use of the mass media, pricing policy, widespread educational activity in the community, workplaces and schools, and other legislative and fiscal measures (Dowse *et al.*,1995). But, the resources of countries to formulate comprehensive interventions of this kind are increasingly limited by the norms and trade laws associated with globalization. A medical model approach has been the major concept in prevention strategies. The identification of various risks and risky behaviour and, as a result, identification of individuals at greatest risk should be included in this model. The International Heart Foundation has concentrated on the need for individuals to eat less fat and salt. However, this tactic disregards the repeated and expensive failures to change diets solely through the improvement of knowledge (Beaglehole, 2001).

1.7.11 Weight management programs

To reach and maintain a healthy body weight it need to rely on strategies that self-sufficient of the expected or healthy body weight to be attained. Weight gain comes with aging, specifically between the ages of 25 and 44 years (Williamson *et al.*,1990), and independently related to development of coronary heart disease (Willett *et al.*,1995) and stroke (Rexrode *et al.*,1997). The exact definitions of weight gain persists to be uncertain with some authors suggesting limits of >2.27 kg (St Jeor *et al.*,1997) and <5 kg (Rexrode *et al.*,1997). The balance between energy intake and energy expenditure need to be maintain to reach a successful weight management in children and adults. The improvement in blood lipids levels, blood pressure and reduction in a risk for heart disease, stroke, and certain cancers may accompany loss of excess weight and long-term maintenance of a healthy weight (Stunkard,1996).

In many individuals with increased abdominal or visceral fat, even moderate weight reduction may result in improvement in many metabolic CHD risk factors, mostly those associated with insulin resistance, including low HDL level, elevated triglyceride level, and small dense LDL (Blackburn,1995; Goldstein,1992). Physical activity in combination with a diet low in calories, especially those coming from fat, and to a certain extent rich in complex carbohydrates and fibre should be encouraged to maintain a long term healthy body weight (Blair,1993, Grilo,1994).

1.7.12 Physical Activity

National Academy of Sciences, (2002) issued the dietary reference intake (DRI) report that targets a daily caloric intake based on the amount of physical activity performed by an individual. The DRI report recommends that total energy expended be at least 1.6–1.7 times an individual's resting energy expenditure in order to maintain body weight in the ideal range (body mass index of 18.5–25 kg/m^2), as well as decrease the risk of cardiovascular disease.

Physical activity is an essential management strategy for weight reduction (Stefanick *et al.*,1998), preservation of the reduced state (Doucet *et al.*,1999, McGuire *et al.*,1999), and prevention of weight gain (US Department of Health and Human Services,1996). To maintain physical and cardiovascular fitness it is essential to have a regular physical activity. In the beginning, for sedentary individuals, engaging in a moderate level of physical activity, such as intermittent walking for 30 to 45 minutes, is recommended (Pate *et al.*,1995). It may also be helpful to concentrate on reduction in sedentary time such as time spent watching television. Approximately 33% of African-American men and 43% of women report no leisure time physical activity compared with 25% and 28% among white American men and women, respectively (Yusuf *et al.*,2001b).

The NCEP encourages moderate physical activity contributing to $\sim$ 837 kJ/day (200 kcal/day) of total energy expenditure. Regular exercise as a way to improve insulin sensitivity, to acutely lower blood glucose levels, and to improve cardiovascular status is highly recommended by ADA (Franz, 2003). Then again, exercise by itself has only a modest effect on weight (Bouchard *et al.*,1993) but is essential for the long-term maintenance of weight loss (Pavlou *et al.*,1989, Maggio *et al.*,1997). Therefore, exercise is a useful addition to other weight loss strategies and is most helpful in weight maintenance.

1.7.13 Nutritional status of South African population

South Africa is in a state of nutrition transition and as with other societies this is reflected in the emergence of obesity, sometimes in conjunction with protein malnutrition. The event like these may be articulated differently in a multi-ethnic society with its major ethnic groups (Blacks, Whites, Coloureds and Indians) and diverse socio-economic status (Benadé *et al.*,1996). Not much is known about the effect of urbanization on nutritional status of South Africans. In KwaZulu-Natal, Margo et al. (1978) found a higher incidence of nutritional anaemia in urban children from a "city slum" area than in rural control children. Byarugaba (1991) examined the effect of urbanization on the health of black preschool children in the

Umtata district in the Eastern Cape. Urbanization was associated with several risk factors for under-nutrition, such as increased poverty, overcrowding, decreased availability of clean water, diarrhoea, less breastfeeding and the replacement of "mother-care" with "maid-care". In this study, urbanization was associated with less underweight and stunting, but with more wasting (acute malnutrition).

A comparison of fat and carbohydrate proportions of energy intake for adults aged 15-64 years, with that of black 'adults' (age range not provided) from the city of Johannesburg in 1940 shows a relative 10.9 % reduction of carbohydrate (from 69.3 % to 61.7 % of energy in 1990) and an increase of 59.7 % in fat intake (from 16.4 % to 26.2% of energy in 1990) over 50-year period (Bourne *et al.*,2002). The complexity of the effects of urbanization on nutritional status is further demonstrated by Bourne et al. (1993) who showed in the BRISK study (n=983 adult Africans, aged 15-64 years from the Cape Peninsula) that although the subjects followed a prudent diet regarding fat and carbohydrate intake, their diet was low in fibre, vitamins and minerals. Urbanization, and especially migration of men from rural areas to earn money in urban areas, has led to broken homes and destitution with consequent malnutrition in the rural areas (Westcott *et al.*,1977).

A cross-sectional analytical study by Charlton et al. (1997) was undertaken to investigate the macronutrient intake and cardiovascular risk factor profile of community-dwelling older coloured (mixed descent) South Africans. A randomly drawn sample of 200 subjects aged 65 years and above, residing in Cape Town was studied. The mean daily energy intake was 7984 kJ and 6979 kJ for men and women, respectively. The recommended energy intakes were less than two-thirds in 29% of the subjects. Dietary fat intake comprised 32.4% of total energy intake, which is in line with the prudent dietary guidelines. The insufficient fibre intake (mean = 17(8) g/day) was accredited to the low consumption of fruit and vegetables. There are only a few regional, cross-sectional studies that have attempted to quantify physical activity patterns in representative samples of South Africans who are undergoing transition. In two large cross-sectional studies of risk factors in urban black communities in the Western Cape Province, 30-40% of men and women reported being inactive or minimally active in their work or leisure time (Levitt *et al.*,1993, Steyn *et al.*,1991). Furthermore, in a sample (n=980) from a peri-urban community of the Western Cape, lack of physical activity was a significant risk factor for type 2 diabetes (relative risk =1.67) (Levitt *et al.*,1999b). In the past when indigenous

populations followed a traditional lifestyle, diabetes was virtually absent, as observed in Tanzania (McLarty *et al.*,1990).

1.8 Aims and objectives of the study

It is known that type 2 diabetes and ischaemic heart disease (IHD) are more common amongst Indian communities that have emigrated from the Indian subcontinent to Great Britain (McKeigue *et al.*,1991) and South Africa (Omar *et al.*,1994, Walker *et al.*,1993) than amongst the non-migrant population. Thus, environmental factors such as diet and socio-economic status may be involved in the pathophysiology of these diseases in the Indian population residing in South Africa.

Studies carried out in our unit have shown that visceral fat area (Punyadeera *et al.*,2001a) and total triglyceride concentration (AUC) during a 7-hour OGTT are both higher in White than Black South African obese women. This may explain the well known rarity of IHD within the Black South African population (Walker *et al.*,1993). Higher fasting cortisol levels have also been observed in White obese women in comparison to Black obese women (Punyadeera *et al.*,2001b) and this may contribute to the higher visceral fat levels of the former group.

We initiated this study to elucidate the possible role of metabolic factors such as cortisol, lipid and free fatty acid levels (FFA), and body composition, diet and socio-economic factors in the pathogenesis of obesity-related disorders in two ethnic groups of the South African population, namely Indian and African. This will allow us to identify modifiable risk factors associated with the development of metabolic syndrome and type 2 diabetes. No studies have been able to establish a clear relationship between abdominal obesity, insulin resistance and disease prevalence in Indian and African populations.

In the present study African and Indian subjects were investigated because of the much higher prevalence of CVD and type 2 diabetes in the latter group. Female subjects were studied because obesity is far more common in South African females than males (Puoane *et al.*, 2002). The hypothesis to be tested in this thesis is that ethnic differences in metabolism in

conjunction with environmental factors such as diet and socio-economic status explain the higher prevalence rates of obesity-related disorders in the South African Indian compared to the African population.

The objectives of the present study were as follows:

1. To assess the body fat distribution in both ethnic groups & to determine how it influences glucose & lipid metabolism.
2. To assess the role of anthropometric, metabolic and hormonal factors in determining insulin resistance in each ethnic group.
3. To compare the aetiology of type 2 diabetes between the two ethnic groups, particularly with reference to beta cell function and insulin resistance.
4. To examine the relationship of anthropometric and metabolic indices with blood pressure, in each ethnic group.
5. To ascertain the role adipokines play in the aetiology of insulin resistance in each ethnic group.
6. To determine the impact of socio-economic and dietary status on intermediary metabolism in each ethnic group.

CHAPTER 2 - STUDY DESIGN AND METHODS

2.1 Subjects selection

The study population consisted of two groups of women one Indian and one African. Subjects were subdivided into lean, obese and overweight groups, according to WHO criteria (2000b). Each ethnic group included:

- 20 lean (BMI<25) women
- 20 obese (BMI>30) women
- 20 obese type 2 diabetic patients
- 10 overweight (BMI 25-29.9) women

They all volunteered for the extended 5 hours oral glucose tolerance test (OGTT). All participants were recruited from an urban population of women residing in the Greater Johannesburg area, via word-of-mouth, newspaper advertisements & flyers distributed throughout Johannesburg hospital and major community centres.

Selection criteria included: age (35-60), ethnicity (Indian or African) and diabetes status.

An exclusion criterion was the presence of any metabolic or endocrine disorder and these patients were not included in our study. Those excluded from the study were:

- 1 women with Crohn's disease,
- 4 women with hyperthyroidism
- 4 women with polycystic ovarian syndrome

Diabetic patients were disqualified from the study if they were receiving insulin treatment. The OGTT identified diabetes (WHO,1999) in five women from the obese Indian group and two from the obese African group. We advised them of their condition and they were referred to a diabetic clinic or to their family practitioner. The newly diagnosed diabetic patients from the original obese groups were moved to the diabetic obese group, and new patients were recruited into the obese groups. Patients having abnormal clinical or laboratory findings were

referred for future medical attention to their respective clinician. Each participant completed questionnaires relating their health history, physical activity, food intake, and socioeconomic status.

This study protocol was approved by the Human Ethics Committee of the University of Witwatersrand, Faculty of Health Sciences, clearance number: M970618 (Appendix 1). The participants were required to sign an informed consent, explaining purpose, procedures, risks, and benefits of the study.

2.2 Oral glucose tolerance test

Diabetic patients were instructed to stop the intake of oral hypoglycaemic tablets 24 hours before the onset of the study. Twenty four-hours prior to the study the subjects were asked to refrain from strenuous physical exercise, smoking, alcoholic beverages, oral metabolic agents, (i.e. oral contraceptives, β-blockers, glucocorticoids, nicotinic acid and thiazides), and other drugs that might have influenced lipid or carbohydrate metabolism.

Supine blood pressure was measured at the onset of the test using a sphygmomanometer (GURR, Surgical Instruments (Pty.) LTD. Johannesburg, South Africa). Blood samples were obtained in the morning after a 10-11 hour overnight fast. An intravenous needle was inserted into the antecubital vein of the forearm and normal saline (0.9% NaCl) was infused slowly into the line to maintain flow. The forearm was covered with a heating blanket. Only water was permitted during the investigation. Each subject was given a standard OGTT consisting of 75g glucose (according to the WHO criteria, 1985) in 200ml of water over a 2 min period.

A study of South African women of African and White origin was conducted previously in our department. Ethnic differences in glucose, lactate, insulin, C-peptide, des 31, 32 proinsulin and FFA levels were observed between 2 + 5 hours during an extended 7 hour OGTT and for this reason we decided to use an extended 5 hour OGTT in the present study. Throughout this period subjects were under the supervision of qualified nurses.

During 5 hour OGTT blood samples were collected from each subject into 10 ml. Vacutest red top tubes (for serum collection), in 5 ml. grey top tubes with sodium fluoride and potassium oxalate anticoagulant (for glucose and lactate measurements) and in 5 ml. purple top

EDTA tubes (for HbA$_{1c}$ measurement). Samples were centrifuged for 10minutes at 2500 rpm and the serum/plasma transferred in aliquots to 5 ml. plastic tubes.

Samples were assayed immediately for glucose, lactate, HbA$_{1c}$, cortisol and lipids; otherwise they were stored at -20ºC until necessary /required. Fasting blood samples were drawn for the measurement of HbA$_{1c}$, full lipid profile, leptin and soluble leptin receptor, TNFα, IL-6 and CRP concentrations. Blood samples for glucose, lactate, glycerol, insulin, proinsulin, C-peptide and FFA were collected at baseline, 30, 60, 90, 120, 180, 240 and 300 minutes during a 5-hour OGTT. Samples for cortisol assay were collected at baseline, 30, 60, 90 and 120 minutes. Most of the laboratory investigations (insulin, total proinsulin, intact proinsulin, C-peptide, FFA, leptin, leptin receptor, IL-6 and TNF-α) were performed by the candidate. The other tests (HbA$_{1c}$, glucose, lactate, hs-CRP, lipid profiles and glycerol) were performed in the routine laboratory in the Department of Chemical Pathology. The biochemical investigations are described in the Appendix 2. All assays were performed according to principles of good laboratory practice using intra-laboratory and external quality control samples.

2.3 Anthropometric measurements

2.3.1 Weight and height

In the present study the volunteers were weighed using an electronic calibrated weighing scale (Modern scale Co. (Pty) Ltd., Johannesburg, South Africa). Shoes were removed and the subject's height was measured to the nearest 0.5 cm with a wall-mounted stadiometer.

2.3.2 Waist /Hip ratio (WHR)

Measurements of waist and hip circumferences were based on skeletal reference points as advocated by World Health Organization guidelines (1988b). To determine WHR, the waist circumference was measured as the midpoint between the lower rib margin and the iliac crest and the hip circumference was measured as the widest circumference over the great trochanters (Van Der Kooy *et al.*, 1993). WHR has been the most widely used index of abdominal obesity in epidemiological studies, because it is easy to measure (Taskinen, 1993). With increasing obesity the accuracy in WHR measurement to assess visceral fat declines. Severe pitfalls of this measurement have been recognized (Despres *et al.*, 1991, Busetto *et al.*,1992). The WHR is greatly influenced by obesity and it cannot be properly used to estimate visceral adiposity, particularly in obese women. Some data suggest that waist circumference might be preferential to WHR for assessment of visceral obesity (Taskinen, 1993).

2.3.3 Body Mass index (BMI)

The prevalence of overweight and obesity is usually determined by using BMI, defined as the weight in kilograms divided by the square of the height in metres (kg/m^2). A BMI of 20 kg/m^2 to 25 kg/m^2 is defined as normal. A BMI of 25 kg/m^2 to 29.9 is defined as overweight and a BMI of over 29.9 kg /m^2 as obese (WHO, 2000b). This cut-off points were derived primarily in European populations to correspond to risk thresholds for a wide range of chronic diseases and mortality (WHO, 1998). Nevertheless, there is ongoing discussion as to whether these criteria for obesity and overweight are suitable for non-European populations. Data from Razak *et al.*, (2007) study supported the belief that "normal ranges" for obesity using BMI cut points derived in European populations may be misleading when applied to populations such as South Asians, Chinese, and Aboriginals. In a number of studies the use of BMI as a marker of body fat and metabolic abnormalities in different ethnic groups indicated that percentage body fat was higher for a given BMI in South Asians (Lear *et al.*, 2007). To our knowledge there is no recommended cut-off points for the classification of obesity using BMI, WHR or

WC in Black or Indian population from South Africa and further investigation are needed to establish these anthropometric indices to apply to the latter groups.

BMI is limited in its prediction of correct body fat for a given individual for a number of reasons: Overweight may or may not be due to increases in body fat. It may also be due to an increase in lean muscle. A large build person heavy in muscle relative to height can give a BMI in the obese range and yet not be obese. Professional athletes may be qualifying as 'overweight' due to their large muscle mass, but under no circumstances could they be regarded as fat regardless of BMI. Likewise, BMI will categorize individuals with a small build mass relative to their height, as being underweight. Individuals with short legs in relation to their upper body height, have a higher BMI. BMI is itself not a direct measure of fatness. It is only a generalized measure of proportional weight (Brozek, 1963).

2.3.4 Body composition analysis: tetrapolar bioelectrical impedance

BODYSTAT® 1500 is the registered trademark of BODYSTAT (Isle of Man) LIMITED and was used to measure body composition.

2.3.4.1 Principle of the BODYSTAT 1500 unit

BODYSTAT 1500 unit operates on a principle that is based on Bioelectrical Impedance Analysis (BIA). The principle of this technique is that the body's lean compartment, comprising approximately 73% of electrolytic water, conducts electricity far better than the body's fat compartment, which is very low in body water content (between 5-10%). These two compartments have very different impedance to a high frequency electrical current. Water is a good conductor of electricity and the less fluid in the body the higher therefore the resistance factor in the body. A tiny torch-battery operated electrical current is sent via the supine body via electrodes attached to the right foot and hand. During the procedure the impedance value, which is associated to that subject's body fat and lean mass, is registered on the BODYSTAT 1500 LCD display screen. This number, collectively with the other details of age, height,

weight, and gender are used by the regression equations to analyse the data and within seconds produces a personal body composition analysis.

2.3.4.2 Preparing subject for measurement

Height and weight was accurately determined. The subject removed the right shoe and sock or stocking, and lay flat with the arms and legs spread slightly as shown in the diagram (Fig. 2.1). NO parts of the body were touching one another.
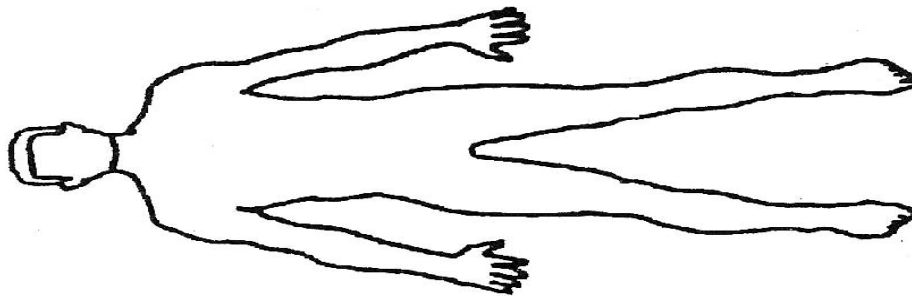
Figure 2.1 Subject position (Brozek *et. al.*,1963)

2.3.4.3 Placement of electrodes and leads

The BODYSTAT 1500 has two sets of main leads that are then divided into a red and black lead. It was important to ensure that the red (injecting) leads were connected to the electrodes just behind the finger and toe as shown in the illustration (Fig 2.2). The black (measuring) leads were then connected to the electrodes on the right wrist and right ankle.

RIGHT HAND

Black   Red
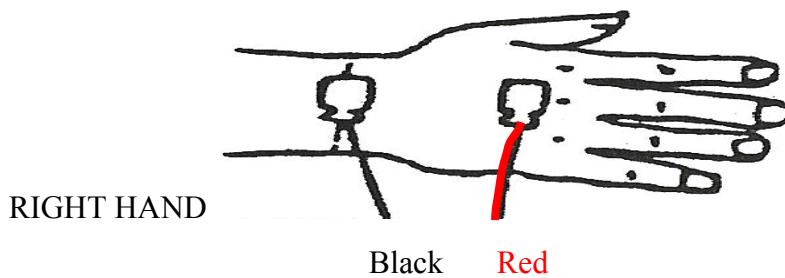
BLACK LEAD: On the wrist next to the ulnar head

RED LEAD: Behind the knuckle of the middle finger

RIGHT FOOT

Black   Red
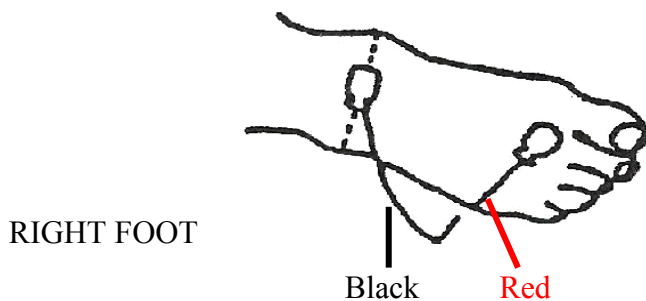
Figure 2.2 Placement of the electrodes

BLACK LEAD: On the ankle at the level of and between the medial and lateral malleoli (the large protruding bones on the sides of the ankle)

RED LEAD: Behind the second toe next to the big toe

2.3.4.4 Calculation of the basal metabolic rate (BMR)

Energy expenditure will be affected by the nature and level of physical activity, body composition, age and gender. The BMR predictive equation used by the BODYSTAT 1500 unit is the Brozek and Grande formula (1963):

Percentage of body fat = 457/D - 414.2, where D = body density in units of gm/cm^3.

This is based on the measured lean weight of a subject and not on total body weight. Lean weight is identified as everything that is not fat and, as such, includes the skeleton, muscles, viscera (brain, heart, liver, intestines) and total body water content. Fat-free mass represents a key determinant of the energy expenditure (Nelson *et al.*, 1992).

2.3.4.5 Estimated average (Energy) Requirement (E.A.R.)

To estimate the total daily energy expenditure level of a person, an assessment of the average activity level during a 24-hour period should be made when entering the data in the BODYSTAT 1500 unit. This can only be a subjective assessment as detailed in table 2.1. Activity level was graded from 1 to 5 (from very low to very high) for statistical analysis.

Table 2.1 Activity level (Brozek *et. al.,*1963)

Level of Activity	General
Very Low - Movement Restricted	Generally inactive
Low/Medium - Office/Light Work	Recreational activities for short duration and at low intensity
Medium - Weekend Recreation	Sporadic involvement in recreation activities for short duration and at moderate intensity
Medium/High - Moderate Exercise	Moderate job activity and moderate exercise 3 times per week
Very High - Vigorous Exercise at Competitive Level	Consistent job activity and vigorous exercise 4 times per week

2.3.5 Computerised axial tomography (CT-Scan)

2.3.5.1 Scan parameters and position of a patient

Subcutaneous and visceral fat were measured using CT Toshiba X Vision scanner (Tokyo, Japan). This scanner is a whole-body computed tomography system, which is designed to acquire transverse image data from any region of the body for diagnostic purposes.
The scan parameters were as follow:

- 10 mm slice thickness; 120 kV; 250 mAs and 1.5s
- 34-480 mm field of view (FOV), depending on the size of the patient.
- The area of subcutaneous fat and visceral fat were clearly defined with CT density in the range of -130 to -30 Hounsfield units.

The patient remained in a supine position when CT images were taken. Subcutaneous adiposity was measured using CT-Scan at 4 levels i.e. the umbilicus, L4/5 lumbar disc, widest diameter of the pelvis, and mid-thigh (calculated as the total distance from the iliac crest to the knee joint, divided by 2). These measurements were derived from two scanograms. The first scanogram included the umbilicus (marked with a metallic coin), iliac crest and symphysis pubis. The second scanogram extended from the symphysis pubis to the knees (Punyadeera *et al.*, 2001a). The total subcutaneous fat area was calculated as a mean ± SEM from the four slices (Fig.2.3). Upper body subcutaneous fat was calculated as a mean ± SEM of the CT-scan measurements at the top two levels, i.e. umbilicus and iliac crest. Lower body subcutaneous fat was calculated as a mean ± SEM of the CT-scan measurements at the bottom two levels, i.e. symphysis pubis and mid-thigh. Abdominal visceral fat was the mean of measurements at level 1 (umbilicus) and level 2 (iliac crest). The areas of subcutaneous and visceral fat (Fig.2.4) were calculated separately using the anatomical boundaries as described by Kvist *et al.*, (1986).

2.3.5.2 Region of Interest (ROI) Calculation

Areas, averages, standard deviations, and other data that can be extracted from a ROI is automatically calculated and then displayed. The following lists some of the major features of the system related to ROI calculation:

- up to a maximum of 10 ROIs can be displayed on the monitor
- the shape of the previous rectangular or circular ROI can be reused
- the CT data of the point indicated by the cursor can be displayed
- the display of statistical calculation results can be vertically scrolled
- a free ROI can be rotated and moved
- a polygonal ROI can be rotated, moved, changed in size/shape, and made into a curvilinear one.
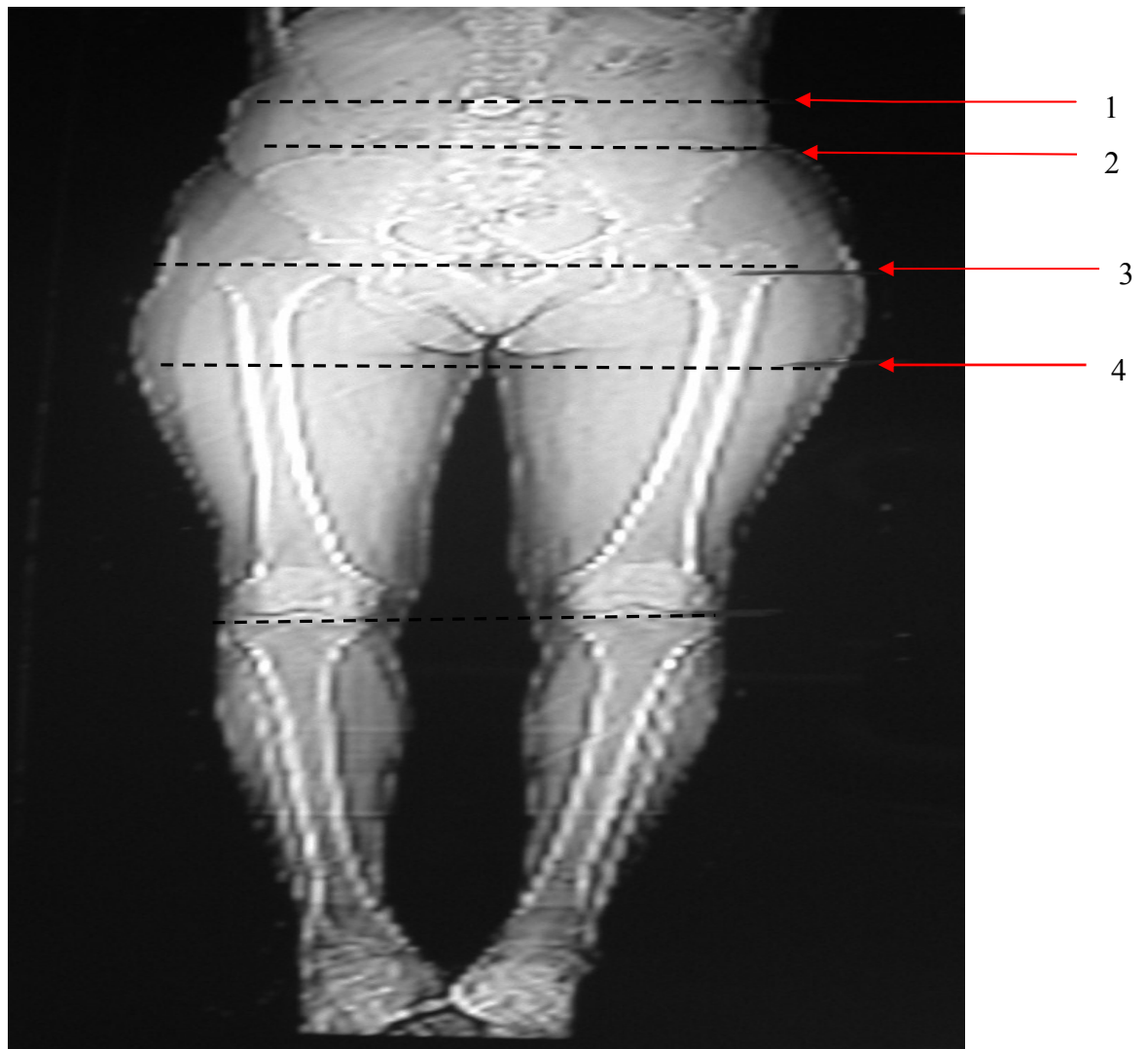
Figure 2.3 CT Scan image of measument areas levels

1-Umbilicus; 2- Iliac crest; 3- Symphysis pubis; 4- Mid-thigh

Subcutaneous Fat   **Visceral Fat**
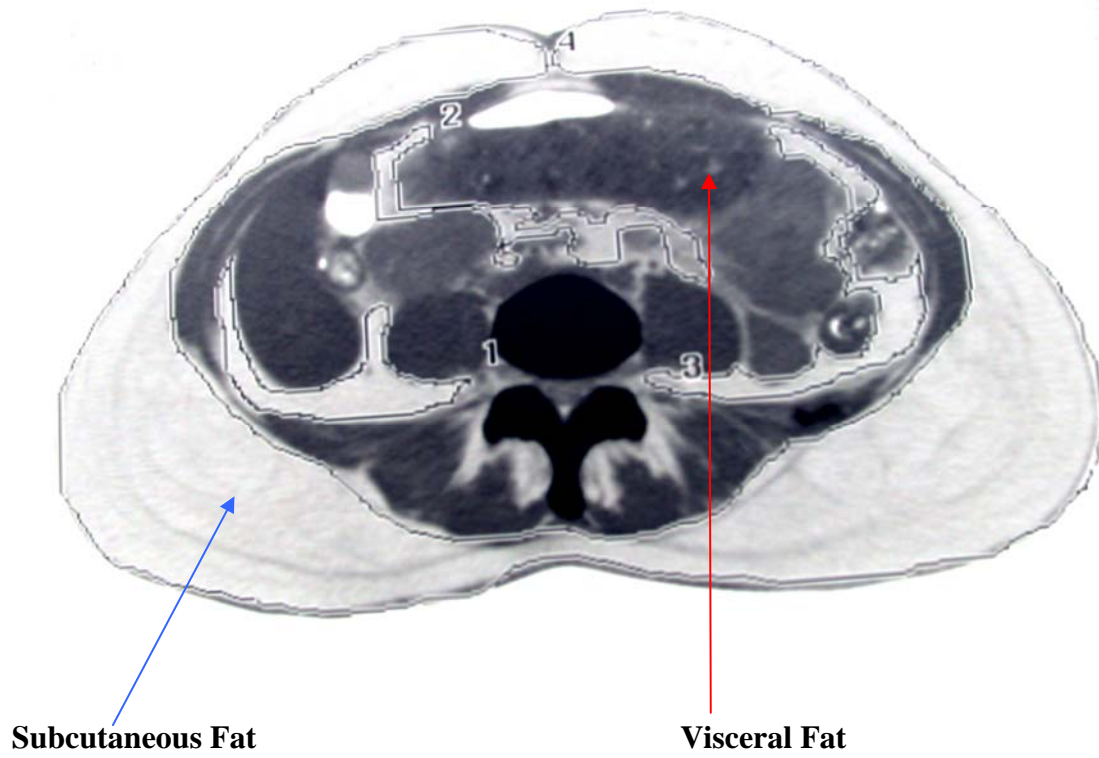
Figure 2.4 CT-Scan image of subcutaneous and visceral fat in area between umbilicus and iliac crest

2.4 Evaluation of β-cell function and hepatic insulin extraction

2.4.1 Evaluation of β-cell secretory ability

The formula known as insulinogenic index (Kadowaki *et al.*, 1984) was used to calculate pancreatic β-cell secretory ability

$$\Delta I_{30} / \Delta G_{30} = (I_{30} - I_0) / (G_{30} - G_0)$$

Where:

I_0 = Insulin levels at fasting

I_{30} = Insulin levels at 30 minutes post 75 g oral glucose load

G_0 = Glucose levels at fasting

G_{30} = Glucose levels at 30 minutes post 75 g oral glucose load

Several studies have documented that insulinogenic index is a more accurate method for assessing β-cell activity than the HOMA-derived method and is the best method for assessing first-phase insulin secretion (Wareham *et al.,* 1995 Phillips *et al.,* 1994b).

2.4.2 Calculation of area-under- the- curve (AUC)

To calculate total concentrations of glucose, lactate, insulin, total proinsulin, intact proinsulin, des-31, 32 proinsulin, C-peptide and free fatty acids during the 5-hour OGTT the trapezoidal rule was used to approximate the area under a curve (AUC). This method, which has sensitive application, consists of connecting points on the concentration-time curve with straight-line segments and then using the area under this polygon to approximate the actual area under the curve. It is named the trapezoid method since each segment of the polygon is a trapezoid. The total sum of these individual areas represents the total area under the curve to assess the overall secretion over a specific time period.

The area under the curve was calculated by using the formula illustrated bellow:

AUC= ((V1+V2)/2) x (t2-t1)) + ((V2+V3)/2) x (t3-t2)) +…. + ((V8+V7)/2) x (t8-t7))

Where: V1=baseline concentration; t1= baseline time; V2 = unit concentration at specified time (t2); t2 = time at the specified unit concentration (V2).

2.4.3 Calculation of insulin resistance via homeostatic model analysis (HOMA)

Insulin resistance was measured using the Homeostasis Model of Insulin Resistance, known as the HOMA-IR index (Matthews *et al.,* 1985). Using the fasting insulin and fasting glucose levels in the following formula, HOMA-IR was calculated:

[Fasting insulin (µU/ml) x Fasting glucose (mmol/l)] / 22.5
Or
[Fasting insulin (pmol/l) x Fasting glucose (mmol/l)] / 155.25

There is a good correlation between the HOMA-IR and the euglycaemic clamp which is the "gold standard" for the measurement of insulin resistance (Matthews *et al.,* 1985). The HOMA-IR is used extensively in population studies assessing insulin resistance.

2.4.4 Calculation of fasting and postprandial hepatic insulin extraction (HIE)

The molar ratio of C-peptide to insulin was used in this study. This ratio has been widely used as a non-invasive method to assess hepatic extraction of insulin in both humans and animal models (Osei *et al.,* 1994; Joffe *et al.,* 1994b). Although, Polonsky *et al.,* (1984) have extensively described the pitfalls and limitations of the use of simple molar ratios at each time point as a reflection of HIE during a non-steady state, the use of molar ratios of C-peptide / Insulin was suggested as a good indication of HIE (Radziuk *et al.*, 1985).

2.5 Food intake questionnaire assessment

Nutrient intake was assessed using a validated quantified food frequency questionnaire. The interviews were conducted in English with the assistance of the day ward nurse who is familiar with most of the official languages of South Africa. Food intake questionnaire used in our study was validated for the South African black population. In the time when we started our study it was not any validated food intake questionnaire available that was designed for the South African Indian population.

The list of food items in the questionnaire (Appendix 3) comprised the most frequently consumed items by South Africans based on many years of experience of dietary intake studies carried out and published in PhD thesis by Mackeown J M, (1999) of the MRC/WITS Dental Research Institute, Johannesburg and the Birth-to Twenty (Bt20) Research Programme. Underreporting, often biases food intake, which obscures interpretation of the results; however adjustment for energy intake provides some correction (Goldberg *et al.*, 1991). Underreporting of energy intake was assessed as reported by energy intake divided by basal metabolic rate (EI/BMR). Low energy reporters (LERs) were defined as those having an EI/BMR <1.14, according to data published by Lau *et al.*, (2006). Nutrient analyses were carried out using the following software: Food finder III Version 1.1.2 2002 Nutritional Intervention Research Unit and Research Information Systems Division, Medical Research Council, South Africa. The data was calculated as a daily intake. Further statistical analyses were performed on the SAS System for Windows Release 8.02 SAS Institute Inc., Cary, NC, USA 1999-2001.

2.6 Socio-economic status evaluation

Socio-Economic status (SES) was assessed from the education or number of household amenities. The level of education achieved and the numbers of household amenities (television, radio, microwave, telephone, fridge, motor vehicle, washing machine, electricity, video machine) were recorded in a Questionnaire (Appendix 4).

Education status was graded as follows (adapted from Richter, 2002):

Grading	Educational Level	Age (years)
1	< Standard 5	<12
2	Standards 5-7	12 - 14
3	Standards 8-12	14 - 18
4	Post - matric (including tertiary education)	>18

Household amenities were graded as follows (adapted from Richter, 2002):

Grading	Number of Household amenities
1	Less than 1
2	From 1-3
3	From 4-5
4	> 5

2.7 Statistical analysis

Statistical analyses were performed using package 8.2 SAS/STAT Enterprise Guide V2 software (SA Institute, Inc., Cary, NC, U.S.A.) in consultation with a statistician. One of the main aims of this study was to investigate the relationship between anthropometry and metabolism. Therefore, the lean, overweight and obese groups were combined and analysed using correlation analyses. The lean, overweight and obese subjects in each ethnic group were combined to increase the chance of detecting a clinically relevant effect between the ethnic groups. Diabetic subjects were excluded from the analysis because they display distinct disease-specific metabolism abnormalities from non-diabetic subjects that may unduly influence the results of the correlation analyses. The diabetic subjects were therefore studied by comparing their metabolite, anthropometric and demographic data with those of the obese, no-diabetic subjects with which they had similar mean BMI levels.

All data are shown as mean ± standard error of the mean (SEM), adjusted for age. Standard error is defined as the ratio of the sample standard deviation to the square root of the sample size. The Kolmogorov-Smirnov and Shapiro-Wilcoxon tests were used to test for normality. The statistical significance of differences between the means across groups was evaluated using ANOVA two sample Student's t-test with age included as an independent variable. Spearman rank correlation coefficients in parallel with canonical correlation were used to analyse relationships between continuous variables. The effect of age was removed by calculating a partial correlation coefficient. The frequency procedure, controlling for race, was used to establish prevalence of metabolic syndrome. Multiple linear regression models with step-down backward selection were used to select dominant independent variables. The coefficient of determination (R^2) was simultaneously monitored. A major drop in R^2 after excluding particular independent variables enabled selection of those independent variables that are the most important determinants of the dependent variable under analysis. All P values were two-tailed, and values of less than 0.05 were considered to indicate statistical significance. Problems of collinearity or multicollinearity were resolved by examining both the regression weights and zero order correlations, and where collinearity was observed, such variables were not included together in the same regression model.

CHAPTER 3 – RESULTS

This chapter includes tables and figures containing results for the clinical and anthropometric data, metabolic measurements, food questionnaire evaluation and socio-economic status assessment in groups sub-divided by ethnicity, BMI and disease status i.e. diabetic and non-diabetic. This was done to determine whether ethnicity, body habitus or diabetes had any influence on these parameters, and was further investigated using multiple regression analysis. Metabolic and hormonal levels were measured in the fasting and postprandial state to ensure that a complete picture of metabolic status and control was captured, whilst lipids and adipokines levels were measured in the fasting state only.

We were able to divide the type 2 diabetic Indian patients into the following groups based on the anti-diabetes drugs they were taking:

- ➢ 1 patient on Metformin (Glucophage).
- ➢ 7 patients on Gliclazide (Diamicron).
- ➢ 5 patients on combined therapy of Gliclazide and Metformin.
- ➢ 1 patient on herbal medicine of unknown origin.
- ➢ 6 newly diagnosed patients, not on treatment.

The diabetic African group was similarly divided based on their anti-diabetes drugs usage as follows:

- ➢ 3 patients on Metformin.
- ➢ 9 patients received Gliclazide.
- ➢ 3 patients on combined therapy of Gliclazide n and Metformin.
- ➢ 4 patients (1 newly diagnosed) not on treatment
- ➢ 1 patient on a diet control regimen

Originally we had not intended to include overweight women (BMI 25-29.9) in our study, but as quite a number of overweight volunteers came forward we allowed them into the study to increase the number of the subjects. Including overweight subjects into the study would normalise the distribution of metabolic and anthropometric variables allowing us to use

parametric statistical analysis. The overweight group consisted only of 10 subjects for logistical reasons and it was not statistically viable to make comparisons of this group across the ethnic populations; however we combined overweight with lean and obese in each ethnic group when we examined interethnic differences in non-diabetic subjects. The main objective of our study was to examine the relationship of anthropometric and metabolic indices in each ethnic group; therefore lean, overweight and obese subjects in each ethnic group were combined to increase chances of detecting a clinically relevant effect between ethnic groups and detecting significant relationships within each ethnic group.

We did not include diabetic patients in the combined groups because diabetic patients are known to exhibit a cluster of metabolic syndrome risk factors; thus it was not our aim to assess relationship between metabolic variables by combining diabetic patients with non-diabetics.

Body composition and CT-scan analysis are described in this chapter in order to discover whether any differences in such variables between Indian and African groups, would have any influence on glucose and lipid metabolism, insulin sensitivity, adipokine levels and blood pressure. Insulin sensitivity was assessed using HOMA-IR and the prime determinants of HOMA-IR were analysed in each ethnic group using multiple regression analysis. Socio-economic and dietary status may have an impact on the development of obesity-related disorders and therefore such data was collected using questionnaires and compared across subject groups. The influence of socio-economic status on metabolic and anthropometric variables was studied using multiple regression analysis.

3.1 Clinical characteristics, body composition and CT-scan analysis

Clinical and anthropometric data for Indian and African groups are presented in table 3.1. The diabetic Indian group had statistically significantly higher age ($p<0.05$), lower diastolic pressure ($p<0.05$) and shorter duration of diabetes ($p<0.05$) than the diabetic African group. Blood pressure and the majority of the anthropometric variables in both ethnic groups were higher in obese than lean subjects. Age, waist circumference and waist/height ratio were

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significantly higher (p<0.05) in diabetic than obese subjects in both ethnic groups but systolic pressure and waist/hip ratio (WHR) were higher in diabetic than obese subjects only in the African group.

Table 3.2 shows data for body composition and CT-scan analysis. Measurements of total subcutaneous fat were significantly higher in lean Indian than African women but lower in obese Indian than African subjects (p<0.05). The upper body subcutaneous fat (UBSF) was significantly higher in lean Indian than African women but lower in obese Indian than African subjects (p<0.05). The obese subjects had significantly higher UBSF compared to lean subjects in both ethnic groups (p<0.0001). Differences in lower body subcutaneous fat (LBSF) were found between obese Indian subjects and obese African subjects (higher in African, p<0.05). In the African group LBSF was significantly higher in obese compared to lean and diabetic subjects (p<0.05 and 0.0001, respectively). In the Indian group the obese subjects had significantly higher LBSF compared to lean subjects (p<0.0001).

Visceral fat was significantly higher in diabetic and lean Indian subjects than African subjects. Visceral /subcutaneous fat ratio was significantly higher in diabetic and obese Indian than in diabetic African and obese African. Activity level was higher in obese African than obese Indian and diabetic African subjects (p<0.05).

Table 3.1 Clinical and anthropometric data

Groups Variables	LI (N=20)	OBI (N=20)	DI (N=20)	LA (N=20)	OBA (N=20)	DA (N=20)
Age (years)	44.0±1.7	47.0±1.9	52.0±1.2 [a, c]	42.0±1.4	43.0±1.2	47.6±1.5 [c]
Weight (kg)	56.1±1.4	81.2±2.3 [b]	85.0±2.8	56.0±1.6	85.4±2.1 [b]	88.7± 2.8
BMI (kg/m^2)	22.9±0.4	32.9±0.6 [b]	34.8±1.0	22.4±0.4	34.4±0.7 [b]	36.2 ±1.0
HC (cm)	94.4±0.9	112.3±1.9 [b]	111.3±2.2	95.1±1.6	114.8±1.4 [b]	114.3 ±2.1
WC (cm)	72.7±1.5	92.5±1.8 [b]	99.3±2.0 [c]	72.0±1.0	91.2±1.7 [b]	98.1±1.7 [c]
Height (cm)	156.3±1.1	156.7±1.1	156.2±1.5	157.9±1.6	157.4±1.3	156.4±1.0
Waist/Height Ratio	0.47±0.01	0.59±0.011 [b]	0.64±0.01 [c]	0.46±0.01	0.58±0.01 [b]	0.63±0.01 [c]
Waist/Hip Ratio	0.77±0.01	0.83±0.02 [b]	0.90±0.02	0.76±0.01	0.80±0.02	0.86±0.02 [c]
Systolic BP (mm/Hg)	113.5±2.7	125.4±3.9 [b]	132.4±3.6	114.5±1.9	122.9±2.1 [b]	133.5±3.8 [c]
Diastolic BP (mm/Hg)	72.7±2.2	81.8±2.4 [b]	82.5±2.0 [a]	75.8±1.6	82.2±1.7 [b]	86.8± 2.7
Duration of diabetes (years)	-------	-------	2.96±0.7 [a]	-------	-------	4.23±1.02

Data shown as Mean values ± SEM; adjusted for age

[a] $p<0.05$ Indian vs. African; [b] $p<0.05$ Obese vs. Lean; [c] $p<0.05$ Diabetic vs. Obese

Abbreviations: LI- lean Indian; OBI-obese Indian; DI-diabetic Indian; LA-lean African; OBA-obese African;

DA-diabetic African; HC-hip circumference; WC-waist circumference

Table 3.2 Body composition and CT-scan analysis

Groups Variables	LI (N=20)	OBI (N=20)	DI (N=20)	LA (N=20)	OBA (N=20)	DA (N=20)
BMR (Kjoule)	5762 ±93.3	6226 ±106 [b]	6046 ±98.3	5941±129	6392±94.8 [b]	6216±74.9
Fat (%)	25.6±0.8	42.5±0.7 [b]	45.3 ±1.1 [c]	23.9 ±1.0	43.4 ±0.9 [b]	46.2±1.1
Activity Level	2.35 ±0.23	2.45 ±0.18 [a]	2.15 ±0.17	2.65 ±0.2	3.05 ±0.14	2.5 ±0.11 [c]
Subcutaneous Fat (cm^2)	185.9 ±7 [a]	340.9±14 [a, b]	357.3 ±21	155.9 ±12	389.5±16 [b]	367.1±16
UBSF (cm^2)	210.0±10 [a]	415.7±24 [a, b]	467.6 ±30	152.1 ±16	486.5±23 [b]	488.1 ±24
LBSF (cm^2)	169.9 ± 8	290.9±11 [a, b]	284.4 ±21	158.5± 10	332.4±14 [b]	279.6±15 [c]
Visceral Fat (cm^2)	51.3 ±6.2 [a]	96.7 ±7.8 [b]	117.5 ±9.9 [a]	30.3 ±2.9	81.4 ±6.4 [b]	93.9 ±6.2
Visceral/Subcut Fat Ratio	0.27 ±0.03	0.29 ±0.03 [a]	0.34 ±0.03 [a]	0.21±0.02	0.21±0.01	0.26 ±0.02 [c]

Data shown as Mean values ± SEM; adjusted for age;

[a] $p<0.05$ Indian vs. African; [b] $p<0.05$ Obese vs. Lean; [c] $p<0.05$ Diabetic vs. Obese

Abbreviations: UBSF –upper body subcutaneous fat; LBSF – lower body subcutaneous fat

Results in combined groups (excluding diabetics) are presented in table 3.3. Age, waist/hip ratio, visceral fat and visceral/subcutaneous fat ratio were higher (p<0.05) in the Indian than African group.

Table 3.3 Clinical and Anthropometric data in the combined* Indian and African groups

Groups / Variables	INDIAN (N=50)	AFRICAN (N=50)	Groups / Variables	INDIAN (N=50)	AFRICAN (N=50)
Age (years)	45.4 ± 1.1 [a]	42.2 ± 0.9	Systolic BP (mm/Hg)	121 ± 2.2	120 ± 1.6
Weight (kg)	68.6 ± 1.9	70.4 ± 2.2	Diastolic BP (mm/Hg)	78 ± 1.5	80 ± 1.1
Height (cm)	156.2 ± 0.7	157.5 ± 0.9	BMR (Kjoule)	5941 ± 64.9	6135 ± 76.8
HC (cm)	103.4 ± 1.4	105.5 ± 1.6	Body Fat (%)	34.7 ± 1.2	33.9 ± 1.4
WC (cm)	82.9 ± 1.6	81.2 ± 1.5	Subcutaneous Fat (cm^2)	264.7 ± 15.0	270.3 ± 21.4
BMI (kg/m^2)	28.0 ± 0.68	28.3 ± 0.83	UBSF (cm^2)	313.6 ± 19.9	309.9 ± 28.3
Waist/Height Ratio	0.53 ± 0.01	0.52 ± 0.01	LBSF (cm^2)	230.8 ± 11.1	242.6 ± 15
Waist/Hip Ratio	0.80 ± 0.01 [a]	0.77 ± 0.01	Visceral Fat (cm^2)	77.9 ± 6.5 [a]	56.1 ± 5.5
Activity Level	2.59 ± 0.14	2.83 ± 0.1	Visceral/Subcut Fat Ratio	0.30 ± 0.02 [b]	0.21 ± 0.01

Data shown as Mean values ± SEM; adjusted for age;

[a] p<0.05 Indian vs. African; [b] p<0.0001; * Obese, overweight, and lean combined

3.2 Fasting beta-cell parameters, intermediate metabolic indices and lipids levels

The assessments of beta-cell function are shown in table 3.4. The obese Indians had significantly higher ($p < 0.05$) fasting glucose, des 31, 32 proinsulin, C-peptide, HOMA-IR levels and lower proinsulin/insulin ratio than obese African subjects. The levels of intact proinsulin and proinsulin /insulin ratio were significantly lower in lean Indian than lean African women. In the diabetic groups there were statistically significant differences (higher in Indian, $p < 0.05$) for intact proinsulin, des 31, 32 proinsulin and C-peptide levels. Obese African and Indian subjects had higher levels of Insulinogenic index than diabetics African and Indian ($p < 0.001$).

Non-diabetic subjects in both ethnic groups were divided into groups with/without metabolic syndrome, according to the ATP III/NCEP, (2001) criteria. HOMA-IR levels were significantly different between subjects with and without metabolic syndrome in the Indian and African groups ($p = 0.0127$ and 0.001, respectively). Adjustment for waist circumference did not have any influence on the results.

To establish the possible interference of Gliclazide and Metformin with measurements of fasting glucose and insulin we compared diabetic patients in the Indian and African groups, with and without use of Gliclazide and Metformin. The fasting glucose and insulin levels were not significantly different with and without use of Gliclazide and Metformin in diabetic Indian group ($p = 0.341$, 0.768, 0.916 and 0.414 respectively).The fasting glucose and insulin levels were not significantly different with and without use of Gliclazide and Metformin in diabetic African group ($p = 0.499$, 0.947, 0.763 and 0.211 respectively). Thus we may state that in our group of diabetic patients the use of different anti-diabetic drugs did not influence results in the present study.

Fasting lactate levels were significantly higher in the diabetic Indian than diabetic African group. Obese Indian subjects had significantly higher fasting lactate levels than lean Indian subjects. Both ethnic groups displayed significantly different fasting lactate levels between

diabetic and obese subjects (higher in diabetics). To establish the effect of Metformin on lactate levels diabetic patients in both ethnic groups were divided into group that consisted of the patients using Metformin and the group that was not on Metformin. There were no significant differences in lactate levels between the groups.

Glycerol concentrations were significantly lower in lean Indian than obese Indian and lean African subjects (p=0.005 and 0.004, respectively). Obese Indian women had elevated triglyceride and cortisol levels compared to obese African women. Obese African women had lower cortisol levels than both diabetic and lean African subjects. Cholesterol and LDL cholesterol were higher in lean Indian than African group (table 3.5).

In groups combined by ethnicity (excluding diabetics) the fasting glucose, insulin, C-peptide, des 31, 32 proinsulin, HOMA-IR levels were significantly higher and proinsulin / insulin ratio lower in Indian than African females (table 3.6). Cholesterol, triglycerides and LDL cholesterol levels were statistically significantly (p<0.05) higher and glycerol levels lower in Indian than African subjects (table 3.7).

Table 3.4 Fasting beta-cell parameters

Groups \ Variables	LI (N=20)	OBI (N=20)	DI (N=20)	LA (N=20)	OBA (N=20)	DA (N=20)
Glucose (mmol/l)	4.6 ±0.07	5.0 ±0.13 [a,b]	9.0 ±0.79 [c]	4.4 ±0.12	4.4 ±0.11	8.8 ±0.94 [c]
HbA1c (%)	5.6 ±0.07	6.0 ±0.31	8.2 ±0.45 [c]	5.6 ±0.08	5.6 ±0.08	9.1 ±0.57 [c]
Insulin (pmol/l)	74.5 ±8.8	124.4±14.2 [b]	138.5 ±14.5	62.1±8.3	94.1±14.9	111.9 ±13.5
Intact proinsulin (pmol/l)	2.1 ±0.34 [a]	4.8 ±0.85 [b]	12.2 ±1.73 [a,c]	3.4 ±0.39	5.6 ±0.74 [b]	7.6 ±1.01
des 31,32-split proinsulin (pmol/l)	4.9 ±0.62	13.6 ±1.97 [a,b]	26.1 ±4.4 [a,c]	4.6 ±0.75	7.0 ±2.08	10.7 ±2.55
C-peptide (pmol/l)	758 ±97	1475 ±168 [a,b]	1031±102 [a,c]	756±99	672±98	678±84
HOMA -IR	2.23 ±0.27	4.28 ±0.52 [a,b]	7.54 ±0.74 [c]	1.87 ±0.28	2.83 ±0.51	6.56±1.26 [c]
HIE	11.0 ±1.0	12.4 ±1.68	8.4 ±0.93 [c]	14.9 ±3.0	9.51 ±1.58	6.9±0.82
Proinsulin/ insulin ratio	0.03 ±0.01 [a]	0.04 ±0.01 [a]	0.11 ±0.02 [c]	0.08 ±0.02	0.09 ±0.01	0.09±0.01
Insulinogenic index	214 ±29.2	285±43.8	27.7 ±6.3 [c]	212 ±61.1	347±66.2	45.2±12.7 [c]

Data shown as Mean values ± SEM; adjusted for age

[a] p<0.05 Indian vs. African; [b] p<0.05 Obese vs. Lean; [c] p<0.05 Diabetic vs. Obese

Abbreviation: HIE-Hepatic Insulin Extraction

Table 3.5 Fasting intermediate metabolic indices and lipids

Groups Variables	LI (N=20)	OBI (N=20)	DI (N=20)	LA (N=20)	OBA (N=20)	DA (N=20)
Lactate (mmol/l)	1.11 ± 0.1 [b]	1.51± 0.11[c]	2.08 ±0.12 [a]	1.31± 0.13	1.21± 0.12	1.72±0.10 [c]
Glycerol (mmol/l)	0.15± 0.01 [a]	0.19 ± 0.02 [b]	0.20 ± 0.02	0.18 ± 0.01	0.22 ± 0.02	0.22 ± 0.02
FFA (mmol/l)	0.50± 0.04	0.58 ± 0.07	0.63 ± 0.06	0.56 ± 0.07	0.55 ± 0.05	0.56 ± 0.08
Cortisol (nmol/l)	315 ± 27.8	313 ± 20.5 [a]	330 ± 26.6	301 ± 22.6	238 ± 19.4 [b]	377± 30.0 [c]
Cholesterol (mmol/l)	5.25 ± 0.2 [a]	5.21 ± 0.18	5.24 ± 0.23	4.37 ± 0.21	4.93 ± 0.19	4.78± 0.22
HDL (mmol/l)	1.54 ±0.09	1.25 ± 0.06 [b]	1.76 ± 0.40	1.45 ± 0.1	1.31 ± 0.1	1.17 ± 0.05
LDL (mmol/l)	3.24±0.17 [a]	3.30 ± 0.18	3.10 ± 0.22	2.58 ± 0.17	3.12± 0.18 [b]	2.98 ± 0.2
Triglycerides (mmol/l)	1.05± 0.11 [b]	1.44 ± 0.11[a]	2.11 ± 0.36	0.79 ± 0.1 [b]	1.09 ± 0.12	1.41 ± 0.11

Data shown as Mean values ± SEM; adjusted for age

[a] p<0.05 Indian vs. African; [b] p<0.05 Obese vs. Lean; [c] p<0.05 Diabetic vs. Obese

Table 3.6 Fasting beta cell parameters in the combined* Indian and African groups

Variables / Groups	INDIAN (N=50)	AFRICAN (N=50)
Glucose (mmol/l)	4.83 ± 0.07 [b]	4.43 ±0.07
HbA1c (%)	5.77 ± 0.14	5.62 ±0.05
Insulin (pmol/l)	98.6 ±7.28 [a]	75.1 ±7.11
Intact Proinsulin (pmol/l)	3.47 ±0.41	4.52 ±0.42
des 31,32 split Proinsulin (pmol/l)	9.46 ±1.06 [a]	5.39 ±0.93
C-peptide (pmol/l)	1029 ± 94.3 [a]	679 ± 56.9
HOMA-IR	3.16 ± 0.28 [a]	2.23 ± 0.24
HIE	11.0 ± 0.83	11.8 ±1.41
Proinsulin / insulin ratio	0.04 ± 0.004 [b]	0.08 ± 0.01
Insulinogenic index	225.8± 23.9	276.2 ± 39.5

Data shown as Mean values ± SEM; adjusted for age;

* Obese, overweight, and lean combined; [a] $p<0.05$; [b] $p<0.0001$ Indian vs. African

Table 3.7 Fasting intermediate metabolic indices and lipids in the combined*
Indian and African groups

Groups / Variables	INDIAN (N=50)	AFRICAN (N=50)
Lactate (mmol/l)	1.34 ± 0.08	1.24 ± 0.07
Glycerol (mmol/l)	0.16 ± 0.01 [a]	0.20 ± 0.01
FFA (mmol/l)	0.55 ± 0.03	0.55 ± 0.04
Cortisol (nmol/l)	308 ±14.0	271 ±14.6
Cholesterol (mmol/l)	5.25 ± 0.11 [a]	4.66 ± 0.12
HDL (mmol/l)	1.38 ± 0.05	1.38 ± 0.06
LDL (mmol/l)	3.32 ± 0.11 [a]	2.84 ± 0.11
Triglycerides (mmol/l)	1.21 ± 0.07 [a]	0.97 ± 0.07

Data shown as Mean values ± SEM; adjusted for age;

[a] $p < 0.05$ Indian vs. African; * Obese, overweight, and lean combined

3.3 Serum adipokines measurements

Serum leptin levels were significantly ($p < 0.05$) higher in lean Indian than lean African subjects. TNF-α concentrations were lower in diabetic and lean Indian subjects in comparison with diabetic and lean African subjects, respectively. Leptin was lower and soluble leptin receptor higher in diabetic than obese and in lean than obese subjects in both ethnic groups. Serum CRP levels were significantly higher in obese than lean subjects in both ethnic groups (table 3.8). When obese, overweight and lean subjects were combined serum TNF-α concentrations were significantly ($p < 0.05$) lower and serum leptin levels were significantly higher in Indian than African subjects (table 3.9).

Table 3.8 Adipokines markers

Groups / Variables	LI (N=20)	OBI (N =20)	DI (N =20)	LA (N =20)	OBA (N =20)	DA (N =20)
CRP (mg/l)	1.74 ± 0.4	6.66 ± 1.2 [b]	8.97 ± 1.6	2.06 ± 0.5	9.95 ± 3.5 [b]	11.4 ± 3.1
TNF-α (pg/ml)	2.97 ± 0.9 [a]	2.50 ± 0.8	2.26 ± 0.4 [a]	6.51 ± 1.4	4.03 ± 1.3	7.06 ± 1.4
IL-6 (pg/ml)	2.17 ± 0.5	1.62 ± 0.2	3.87 ± 1.1 [c]	2.01 ± 0.5	2.17 ± 0.3	3.15 ± 0.6
Leptin (ng/ml)	29.5 ± 2.7 [a]	50.8 ± 2.1 [b]	43.6 ± 2.1 [c]	13.8 ± 2.5	51.3 ± 2.1 [b]	40.6 ± 2.5 [c]
Leptin Receptor (U/ml)	27.2 ± 2.7	14.8 ± 1.1 [b]	20.5 ± 1.7 [c]	31.5 ± 2.9	16.2 ± 0.9 [b]	21.0 ± 1.7 [c]

Data shown as Mean values $\pm$ SEM; adjusted for age;

[a] $p<0.05$ Indian vs. African; [b] $p<0.05$ Obese vs. Lean; [c] $p<0.05$ Diabetic vs. Obese

Table 3.9 Adipokines markers in the combined* Indian and African groups

Groups / Variables	INDIAN (N=50)	AFRICAN (N=50)
CRP (mg/l)	4.08 ± 0.6	5.14 ± 1.5
TNF-alpha (pg/ml)	2.54 ± 0.5 [a]	5.22 ± 0.9
IL-6 (pg/ml)	1.88 ± 0.2	2.04 ± 0.3
Leptin (ng/ml)	41.3 ± 2.0 [a]	34.2 ± 2.9
Leptin Receptor (U/ml)	20.3 ± 1.4	22.7 ± 1.6

Data shown as Mean values $\pm$ SEM; adjusted for age; [a] $p<0.05$ Indian vs. African

* Obese, overweight, and lean combined

3.4 Results for 5-hour OGTT

Results for the 5-hour OGTT were calculated as area under the curve (AUC). Plasma glucose concentrations during the 5-hour OGTT are illustrated in figure 3.1. Differences in plasma glucose were found in diabetic vs. obese and obese vs. lean in both ethnic groups ($p<0.05$). Obese and lean Indian subjects were much more hyperinsulinemic in comparison with African subjects (figure 3.2), and in both ethnic groups insulin levels were higher in obese than lean and diabetic subjects. Significantly higher C-peptide and des 31, 32 split proinsulin values were observed for diabetic, obese and lean Indian women compared to the respective African group (figure 3.3 and 3.4, respectively). Serum intact proinsulin concentrations were higher in diabetic Indian than African group (figure 3.5). Hepatic insulin extraction (HIE) had significant difference between obese and lean Indian subjects and proinsulin/insulin ratio was significantly lower in obese and lean Indian than the respective African groups (figure 3.6). No statistically significant differences ($p<0.05$) were found between diabetic, obese or lean Indian and African subjects for serum free fatty acid, cortisol (2 hour OGTT) and plasma lactate (figure 3.7, 3.8, and 3.9, respectively). Plasma glycerol concentrations were lower in lean Indian women in comparison with African women (figure 3.10).

In combined groups of lean, overweight and obese subjects plasma glucose, serum insulin, des 31, 32 split proinsulin and C-peptide were significantly higher and plasma glycerol levels, HIE and proinsulin/insulin ratio were significantly lower in the Indian group. No statistically significant interethnic differences were found for plasma lactate, serum intact proinsulin, free fatty acids and cortisol (2 hour OGTT) concentrations (figures 3.11, 3.12, 3.13, 3.14, 3.15 and 3.16).
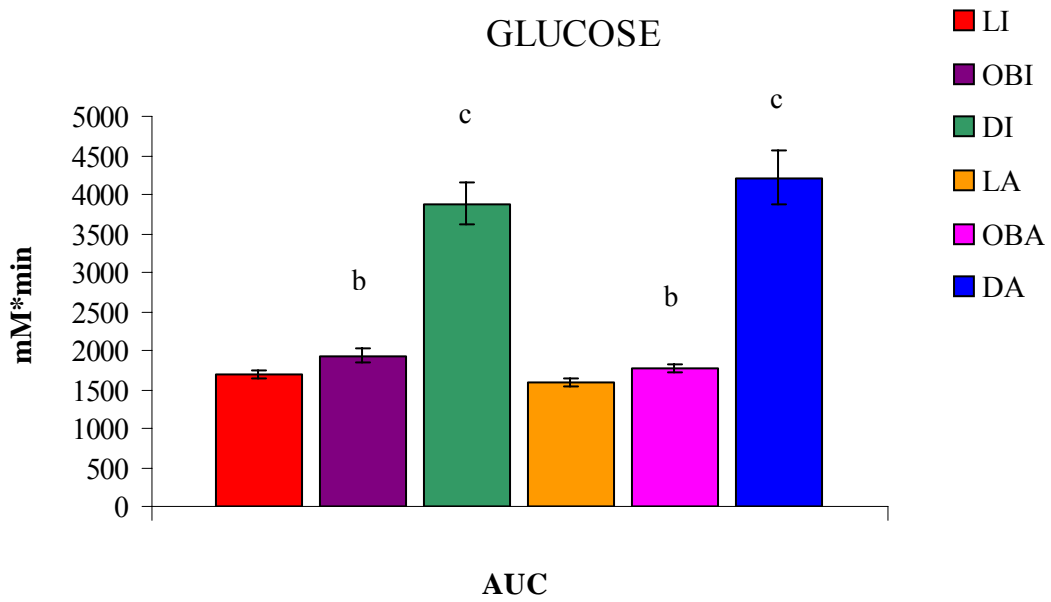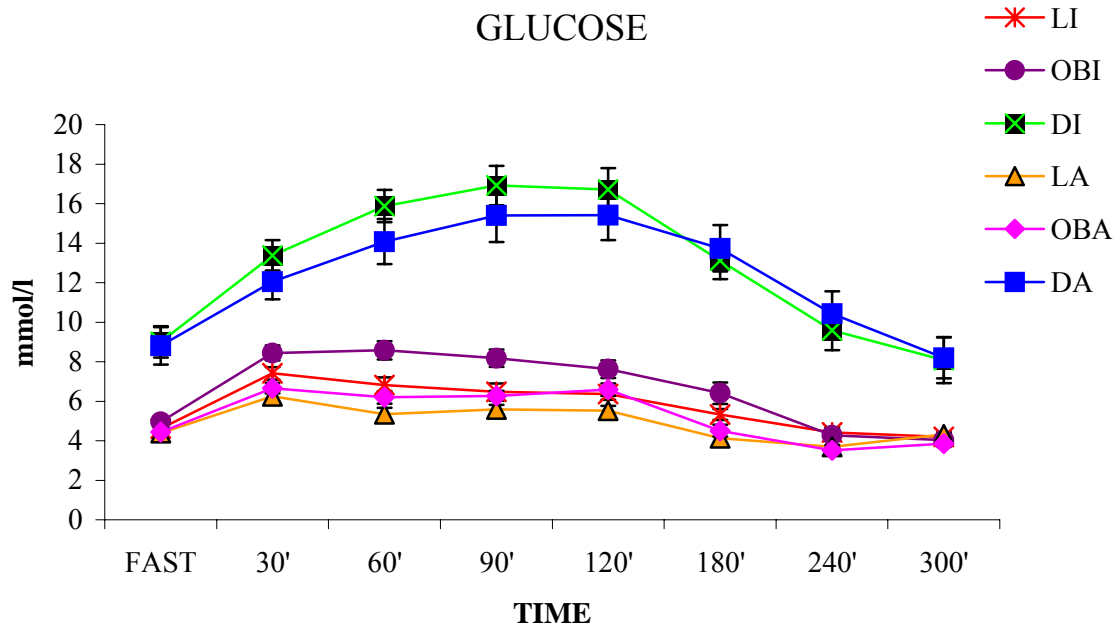
Figure 3.1 Plasma glucose concentrations during 5-hour OGTT

Data shown as Mean values ± SEM; adjusted for age

[a] p<0.05 Indian vs. African; [b] p<0.05 Obese vs. Lean; [c] p<0.05 Diabetic vs. Obese
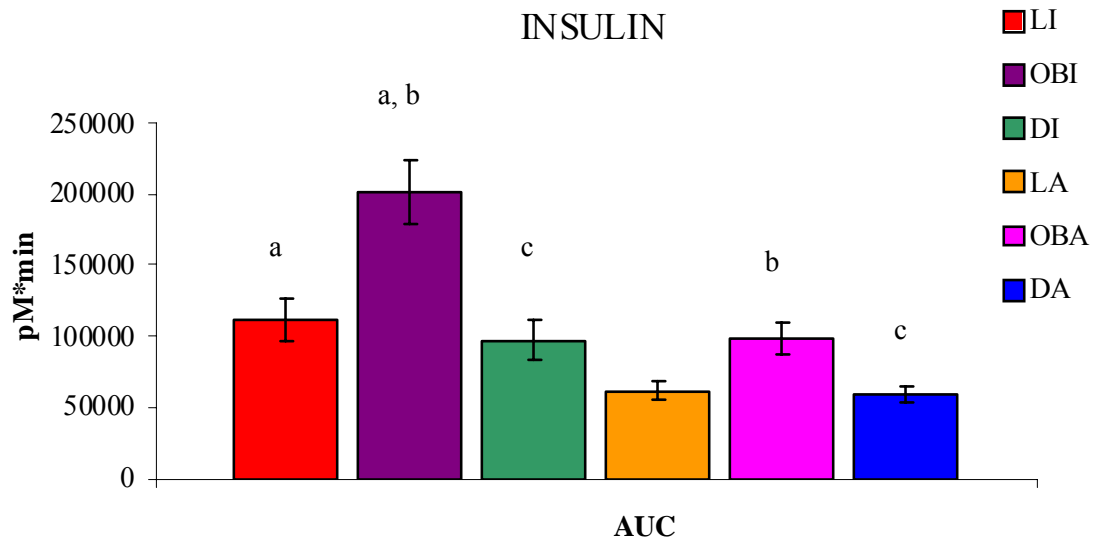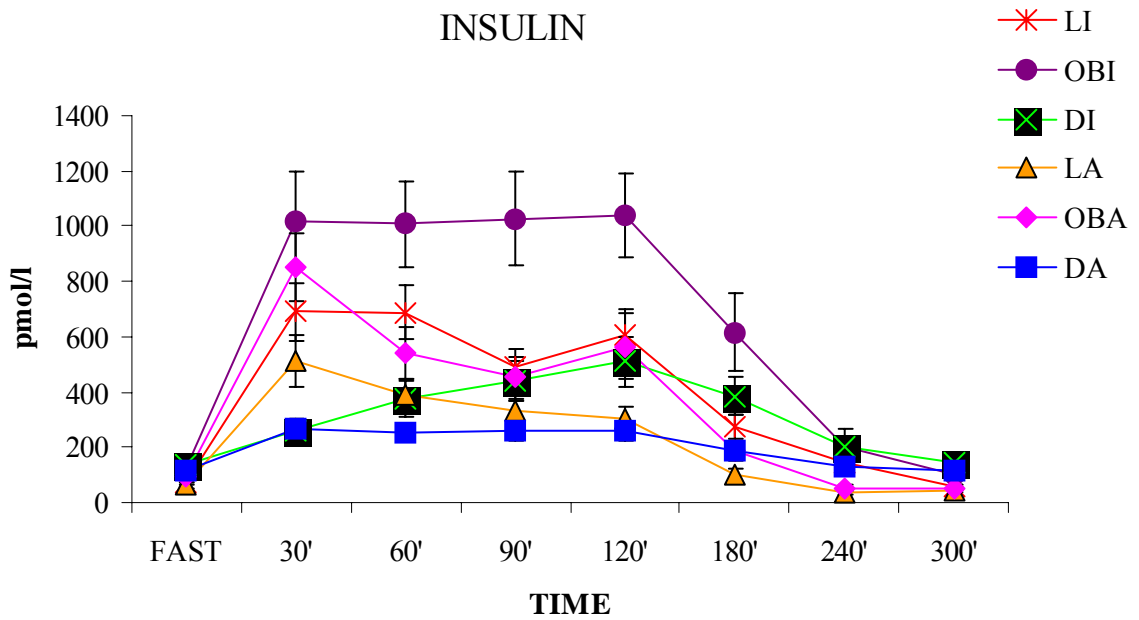
Figure 3.2 Serum insulin concentrations during 5-hour OGTT

Data shown as Mean values ± SEM; adjusted for age

[a] p<0.05 Indian vs. African; [b] p<0.05 Obese vs. Lean; [c] p<0.05 Diabetic vs. Obese
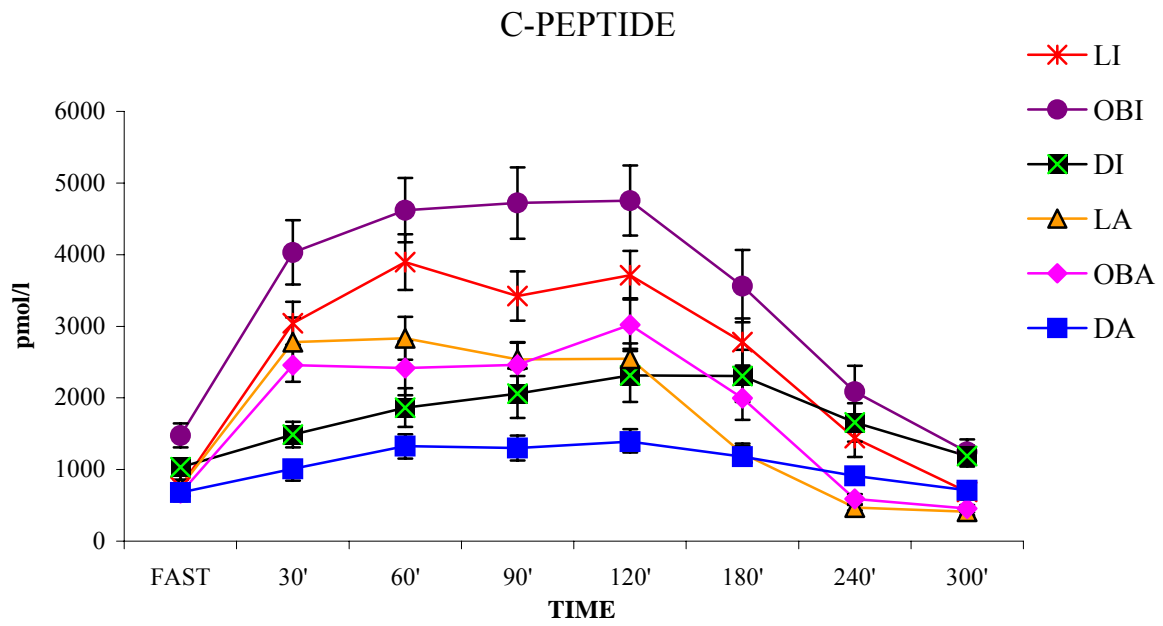
C-PEPTIDE

Figure 3.3 Serum C-peptide concentrations during 5-hour OGTT

Data shown as Mean values ± SEM; adjusted for age

[a] p<0.05 Indian vs. African; [b] p<0.05 Obese vs. Lean; [c] p<0.05 Diabetic vs. Obese

des 31,32 split PROINSULIN

des 31,32 split PROINSULIN
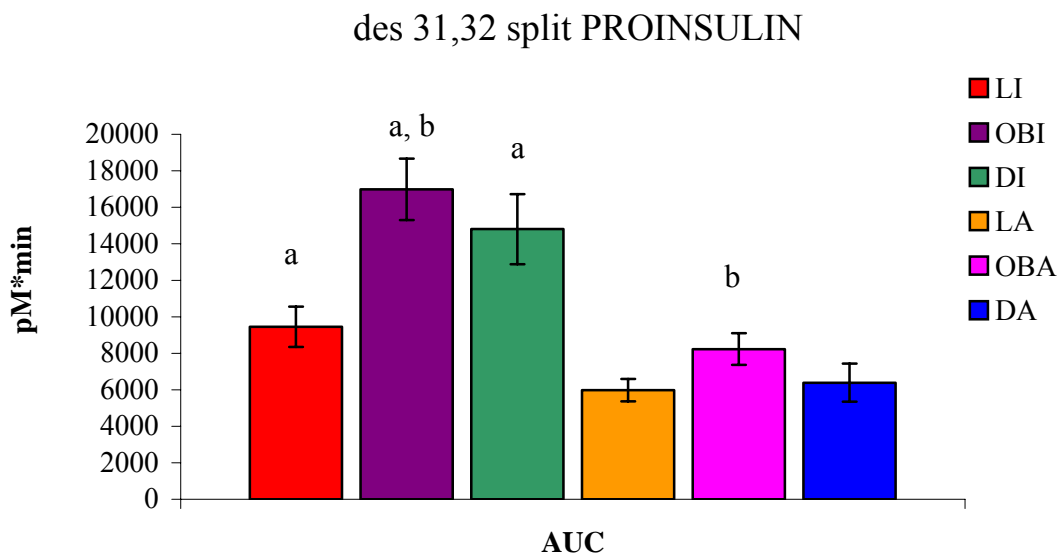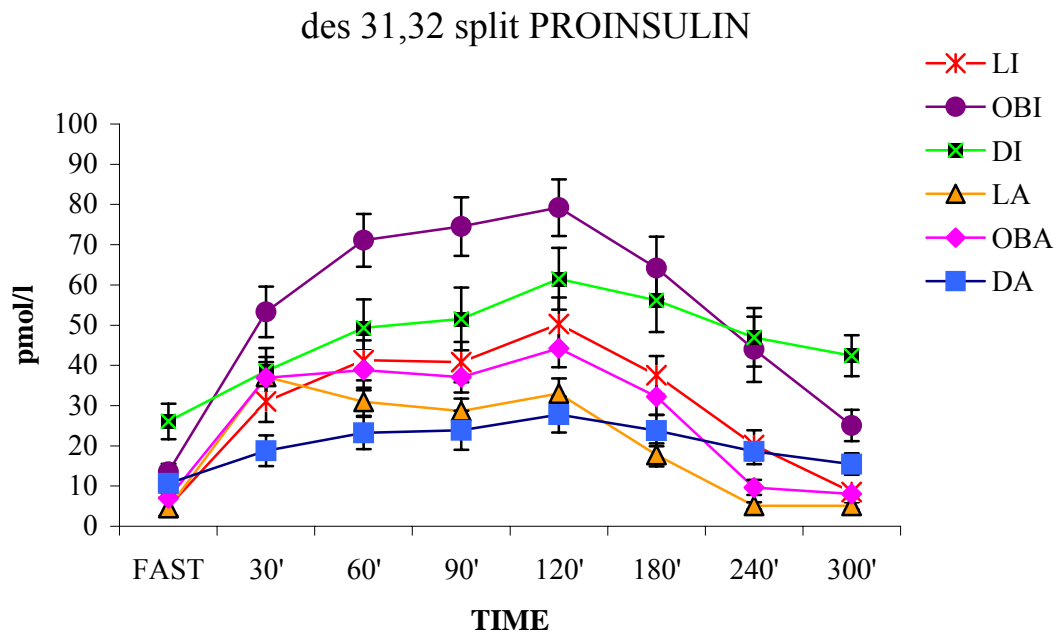
Figure 3.4 Serum des 31, 32 split proinsulin concentrations during 5-hour OGTT

Data shown as Mean values ± SEM; adjusted for age

[a] p<0.05 Indian vs. African; [b] p<0.05 Obese vs. Lean; [c] p<0.05 Diabetic vs. Obese

INTACT PROINSULIN
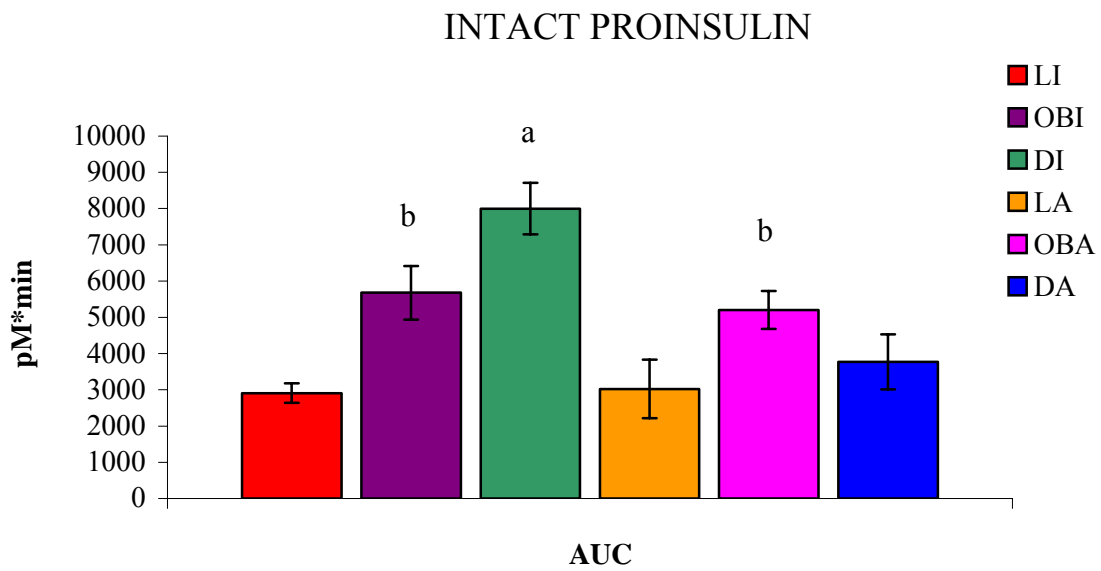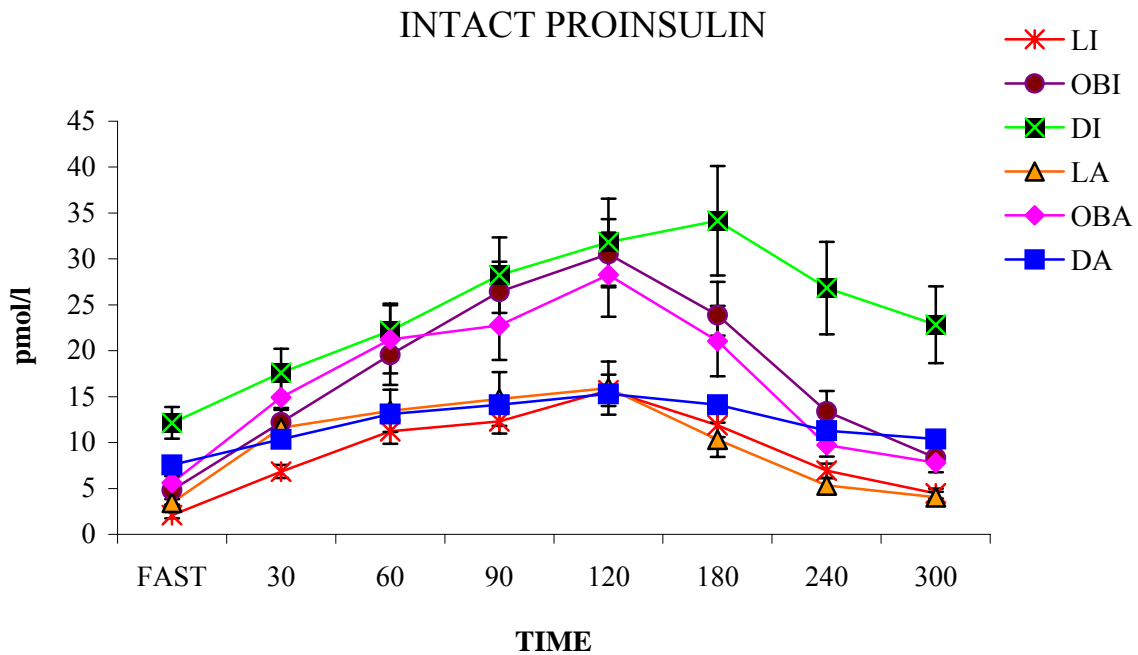
INTACT PROINSULIN

Figure 3.5 Serum intact proinsulin concentrations during 5-hour OGTT

Data shown as Mean values±SEM; adjusted for age

[a] p<0.05 Indian vs. African; [b] p<0.05 Obese vs. Lean; [c] p<0.05 Diabetic vs. Obese
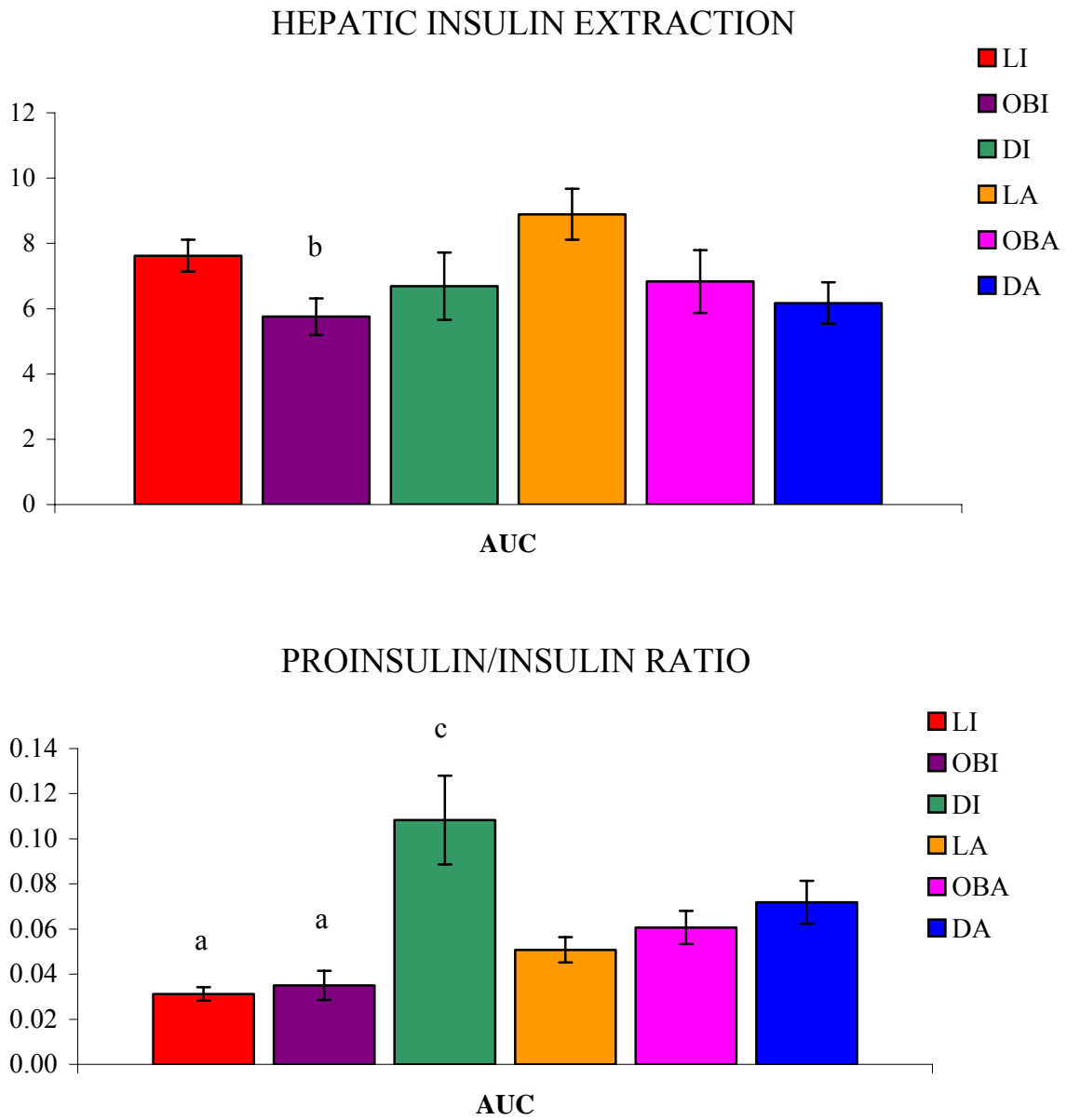
Figure 3.6 Hepatic insulin extraction (HIE) and proinsulin/insulin ratio during
5-hour OGTT

Data shown as Mean values ± SEM; adjusted for age

[a] p<0.05 Indian vs. African; [b] p<0.05 Obese vs. Lean; [c] p<0.05 Diabetic vs. Obese
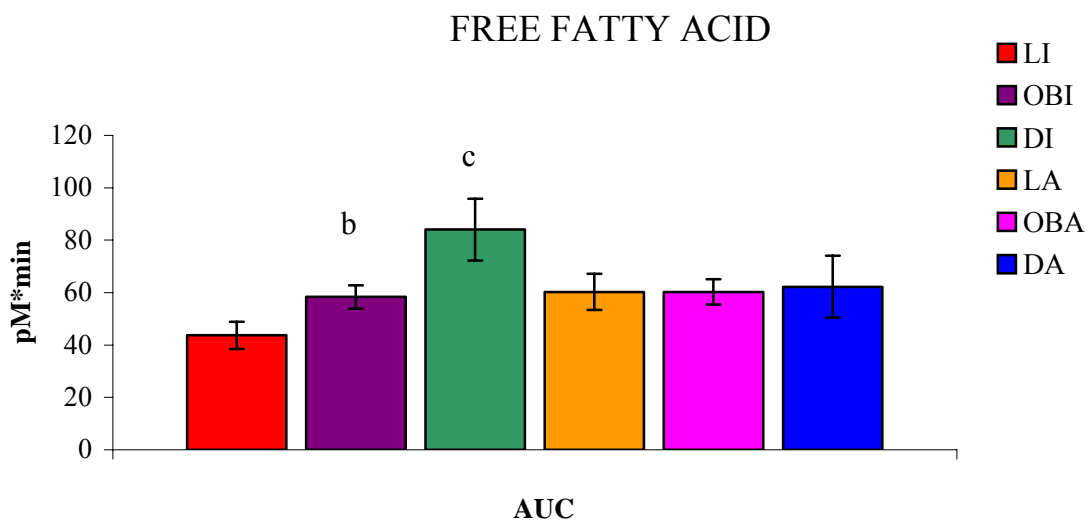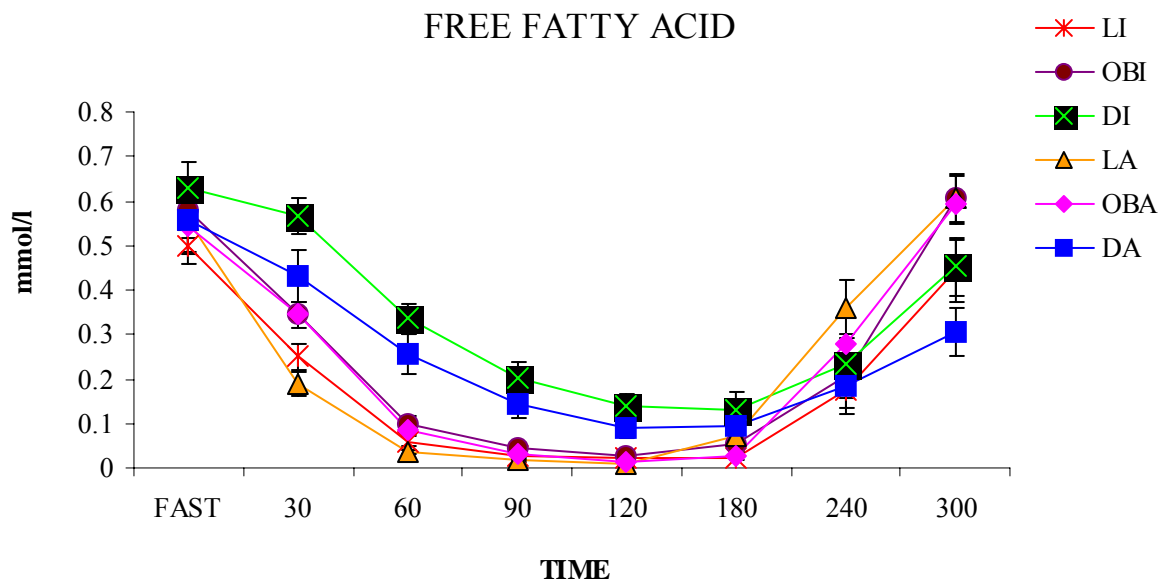
Figure 3.7 Serum free fatty acid concentrations during 5-hour OGTT

Data shown as Mean values ± SEM; adjusted for age

[a] $p < 0.05$ Indian vs. African; [b] $p < 0.05$ Obese vs. Lean; [c] $p < 0.05$ Diabetic vs. Obese
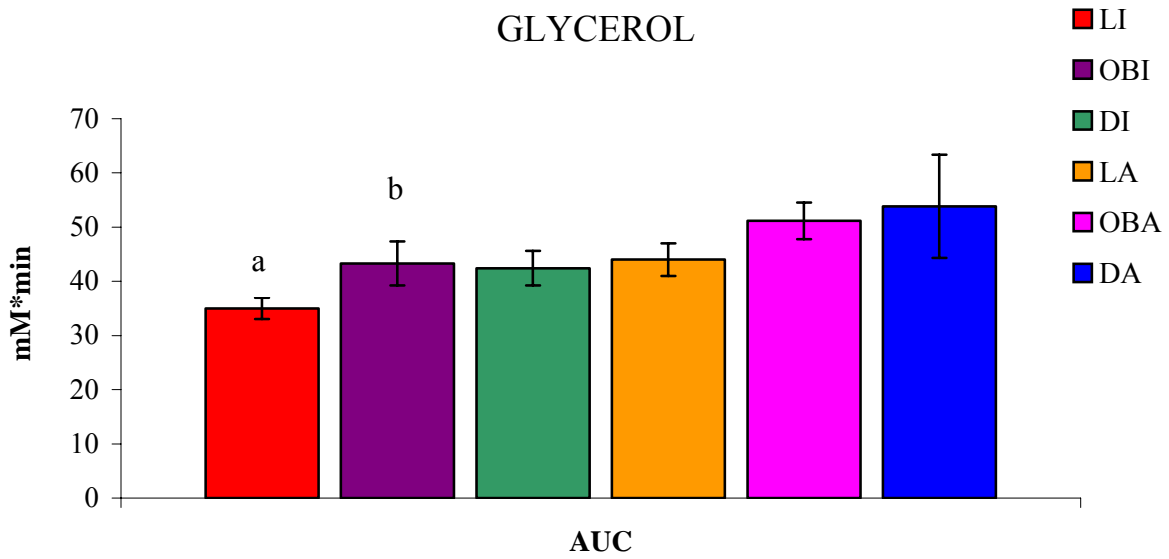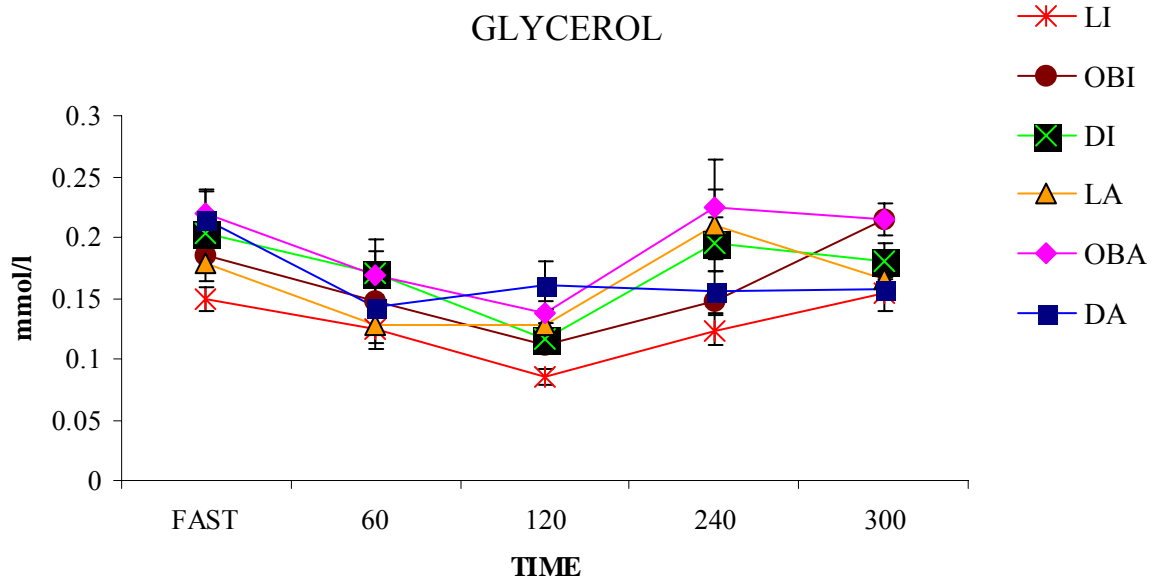
Figure 3.8 Plasma glycerol concentrations during 5-hour OGTT

Data shown as Mean values ± SEM; adjusted for age

[a] $p<0.05$ Indian vs. African; [b] $p<0.05$ Obese vs. Lean; [c] $p<0.05$ Diabetic vs. Obese
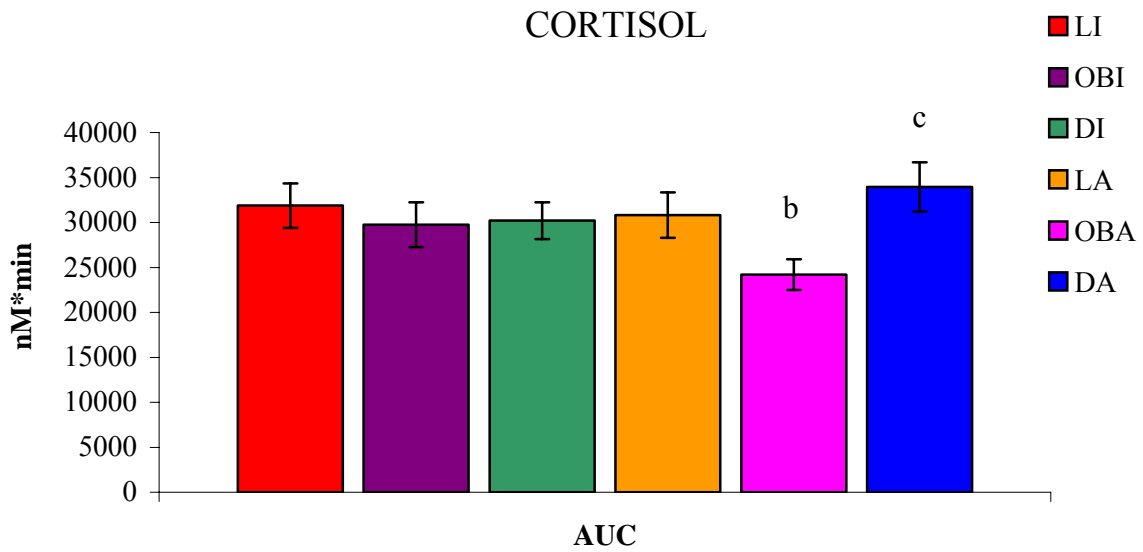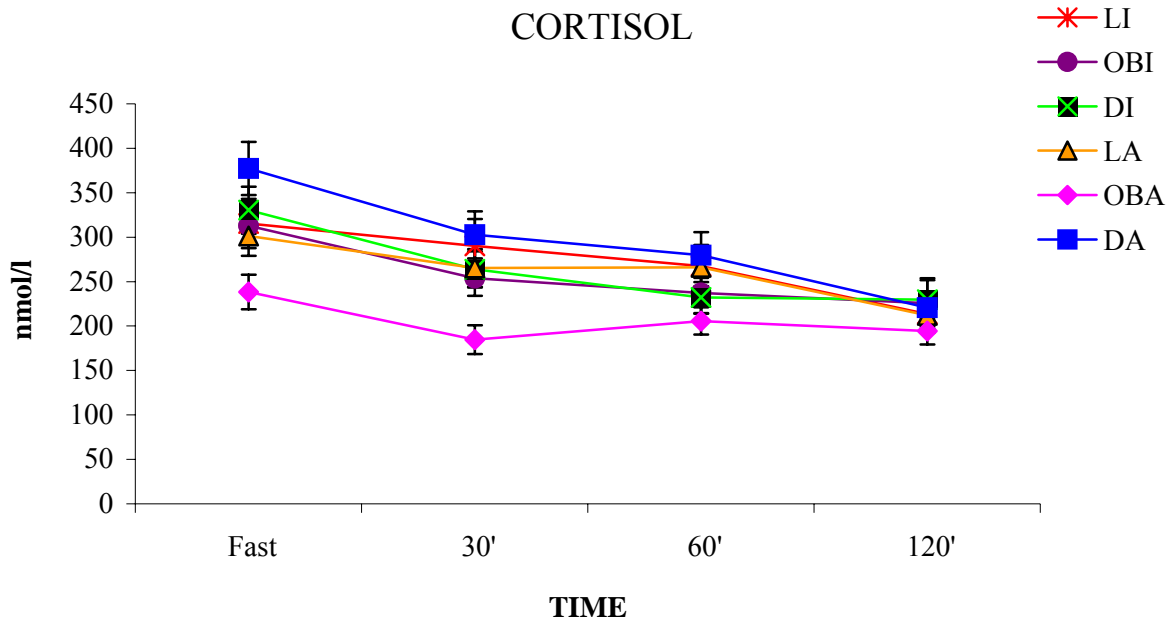
Figure 3.9 Serum cortisol concentrations during 2-hour OGTT

 Data shown as Mean values ± SEM; adjusted for age

[a] p<0.05 Indian vs. African; [b] p<0.05 Obese vs. Lean; [c] p<0.05 Diabetic vs. Obese
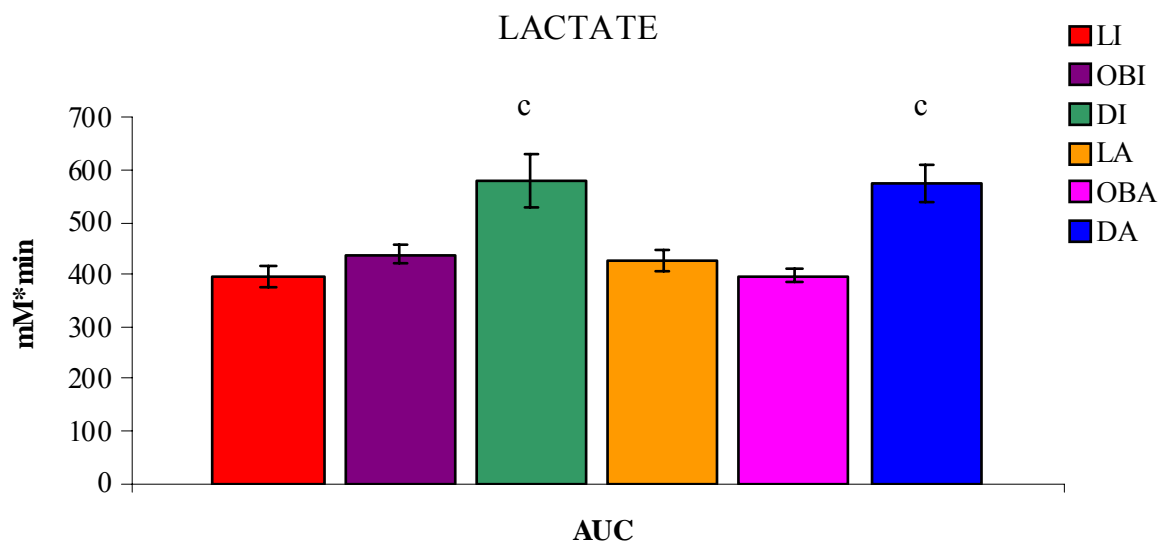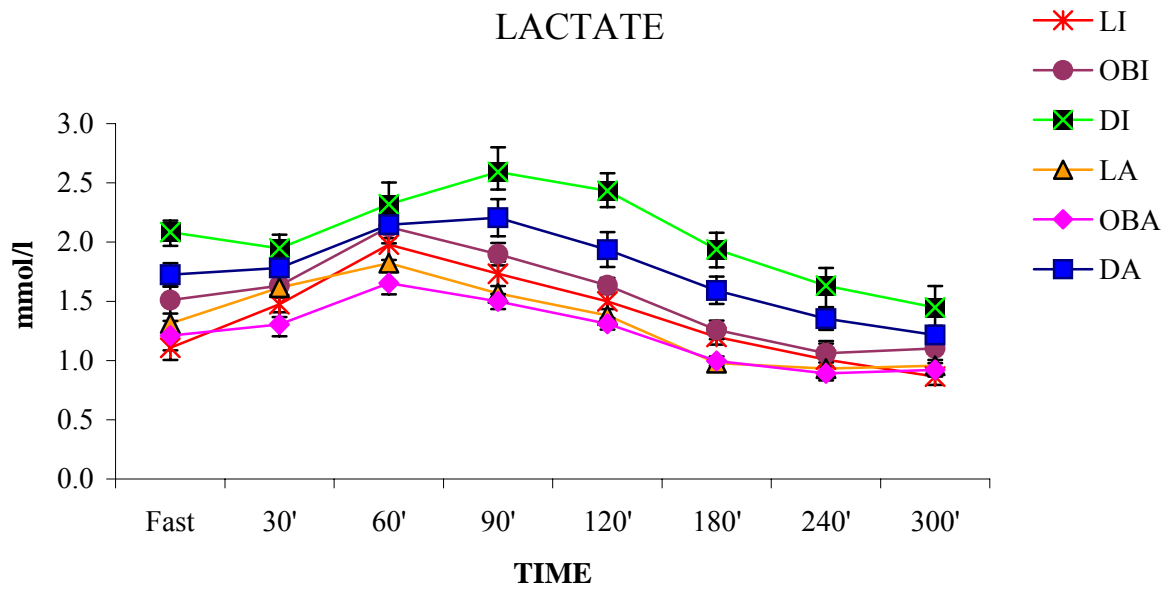
Figure 3.10 Plasma lactate concentrations during 5-hour OGTT

Data shown as Mean values ± SEM; adjusted for age

[a] p<0.05 Indian vs. African; [b] p<0.05 Obese vs. Lean; [c] p<0.05 Diabetic vs. Obese
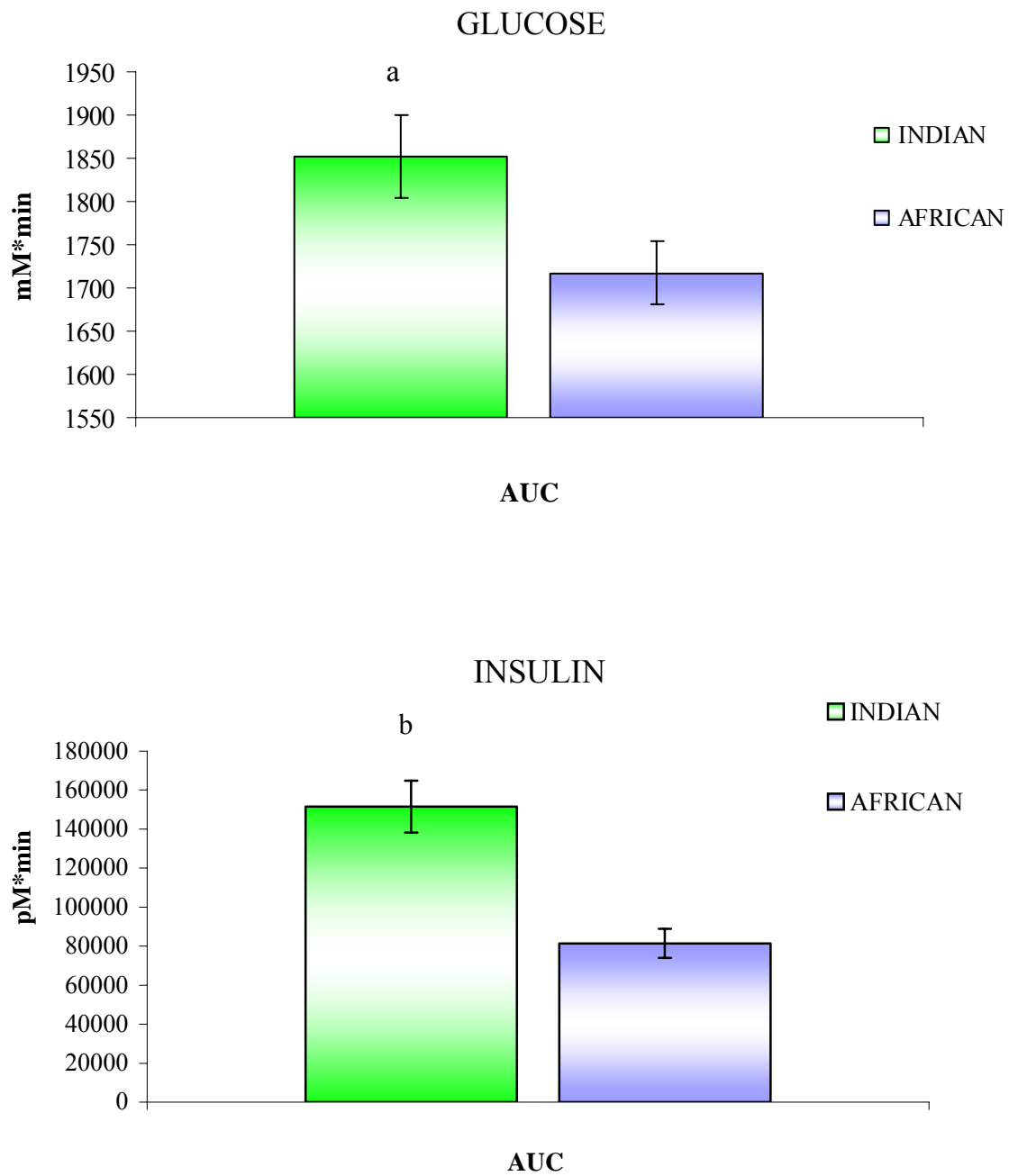
GLUCOSE

INSULIN

Figure 3.11 Plasma glucose and serum insulin concentrations during 5-hour OGTT in the combined* Indian and African groups

Data shown as Mean values ± SEM; adjusted for age;

* Obese, overweight, and lean combined; [a] p<0.05; [b] p <0.0001 Indian vs. African

C-PEPTIDE

HEPATIC INSULIN EXTRACTION
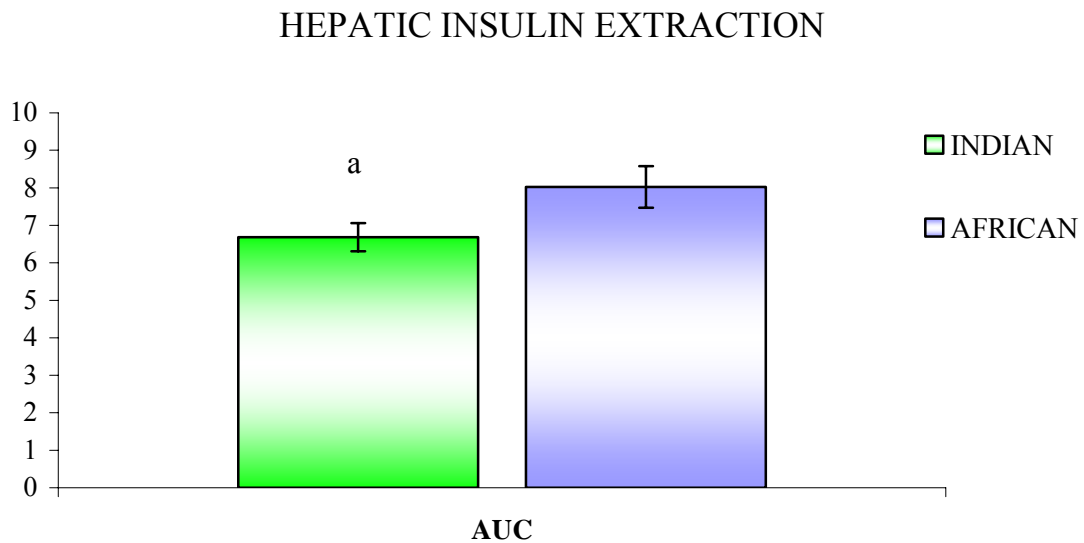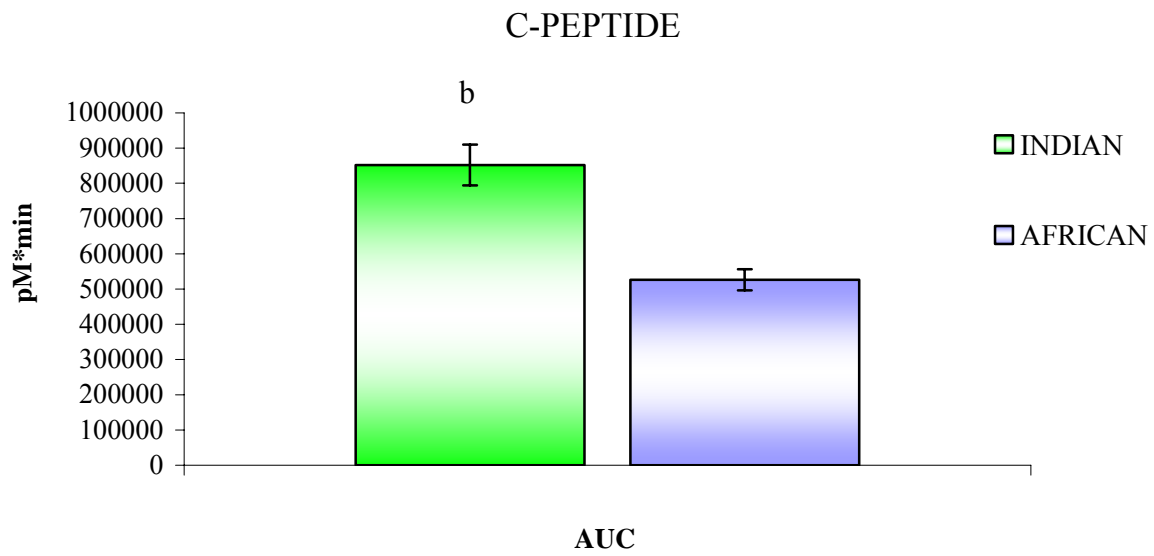
Figure 3.12 Serum C-peptide concentrations and hepatic insulin extraction during 5-hour OGTT in the combined* Indian and African groups

Data shown as Mean values ± SEM; adjusted for age;

* Obese, overweight, and lean combined; [a] $p<0.05$; [b] $p <0.0001$ Indian vs. African;

INTACT PROINSULIN

des 31,32 split PROINSULIN
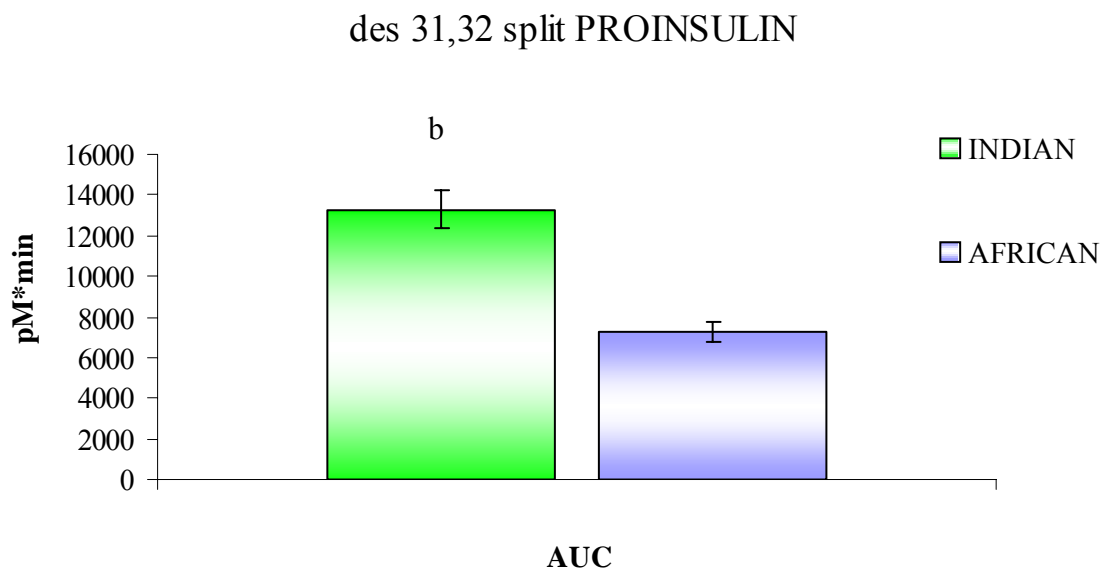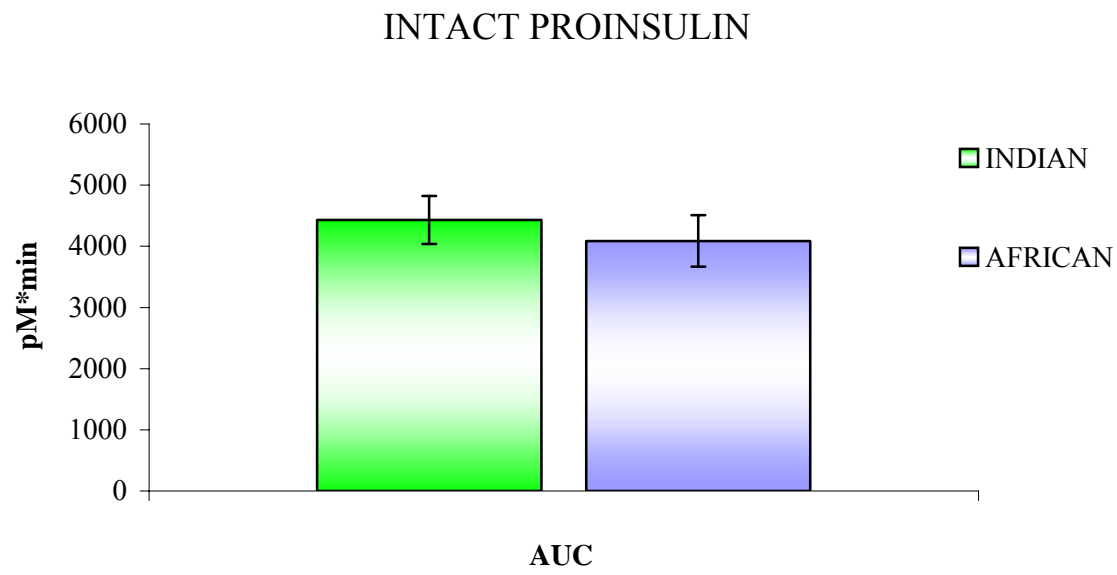
Figure 3.13 Serum intact proinsulin and des 31,32 split proinsulin concentrations during 5-hour OGTT in the combined* Indian and African groups

Data shown as Mean values ± SEM; adjusted for age;

* Obese, overweight, and lean combined; [b] p <0.0001 Indian vs. African;

PROINSULIN/INSULIN RATIO
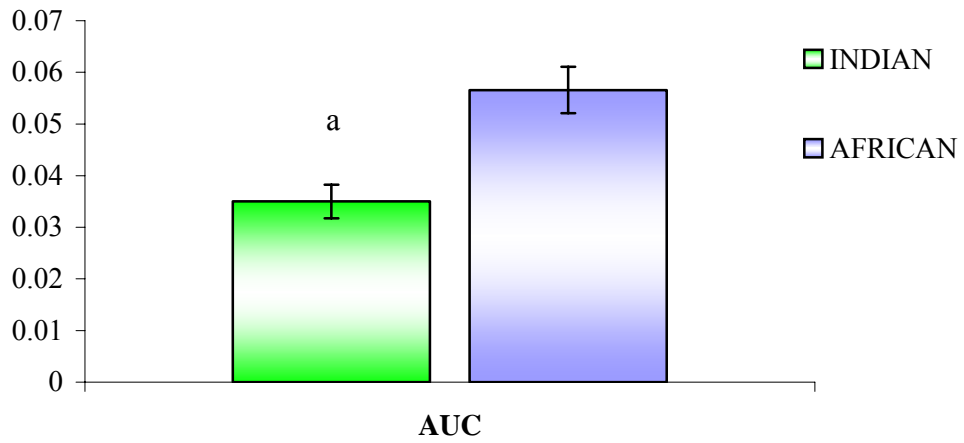
Figure 3.14 Proinsulin/Insulin ratio during 5-hour OGTT in the combined*
Indian and African group

Data shown as Mean values ± SEM; adjusted for age;

* Obese, overweight, and lean combined; [a] p<0.05 Indian vs. African
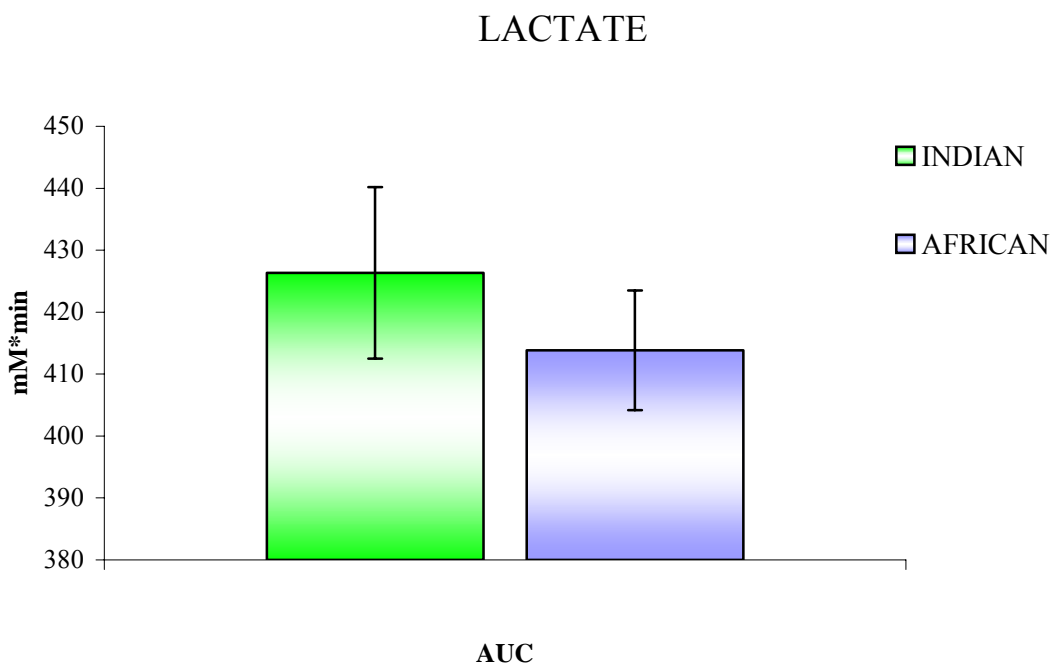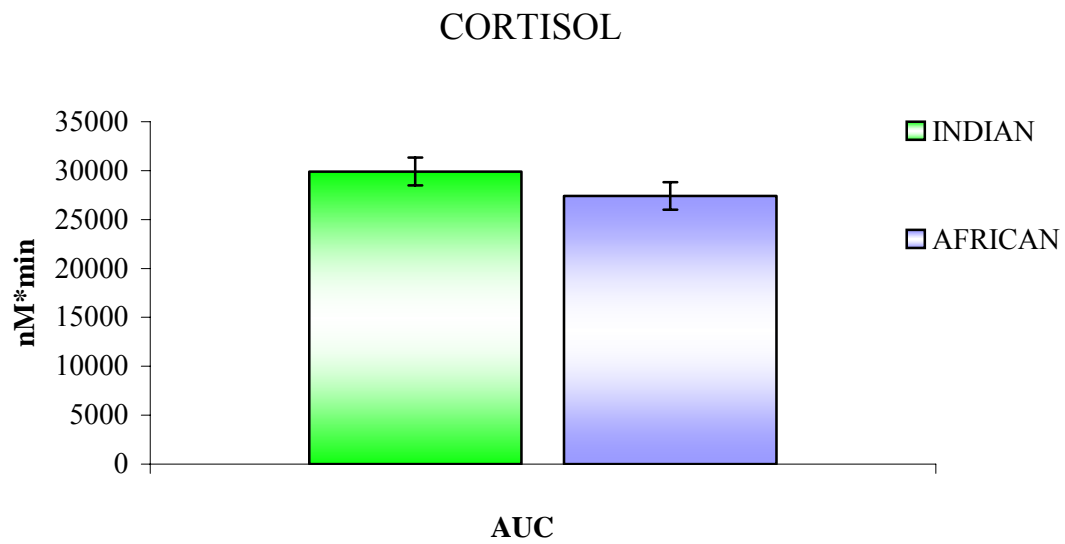
CORTISOL

LACTATE

Figure 3.15 Serum cortisol and plasma lactate concentrations during 5-hour OGTT in the combined* Indian and African groups

Data shown as Mean values ± SEM; adjusted for age;

*Obese, overweight, and lean combined; [a] $p < 0.05$ Indian vs. African; [b] $p < 0.0001$

FREE FATTY ACID
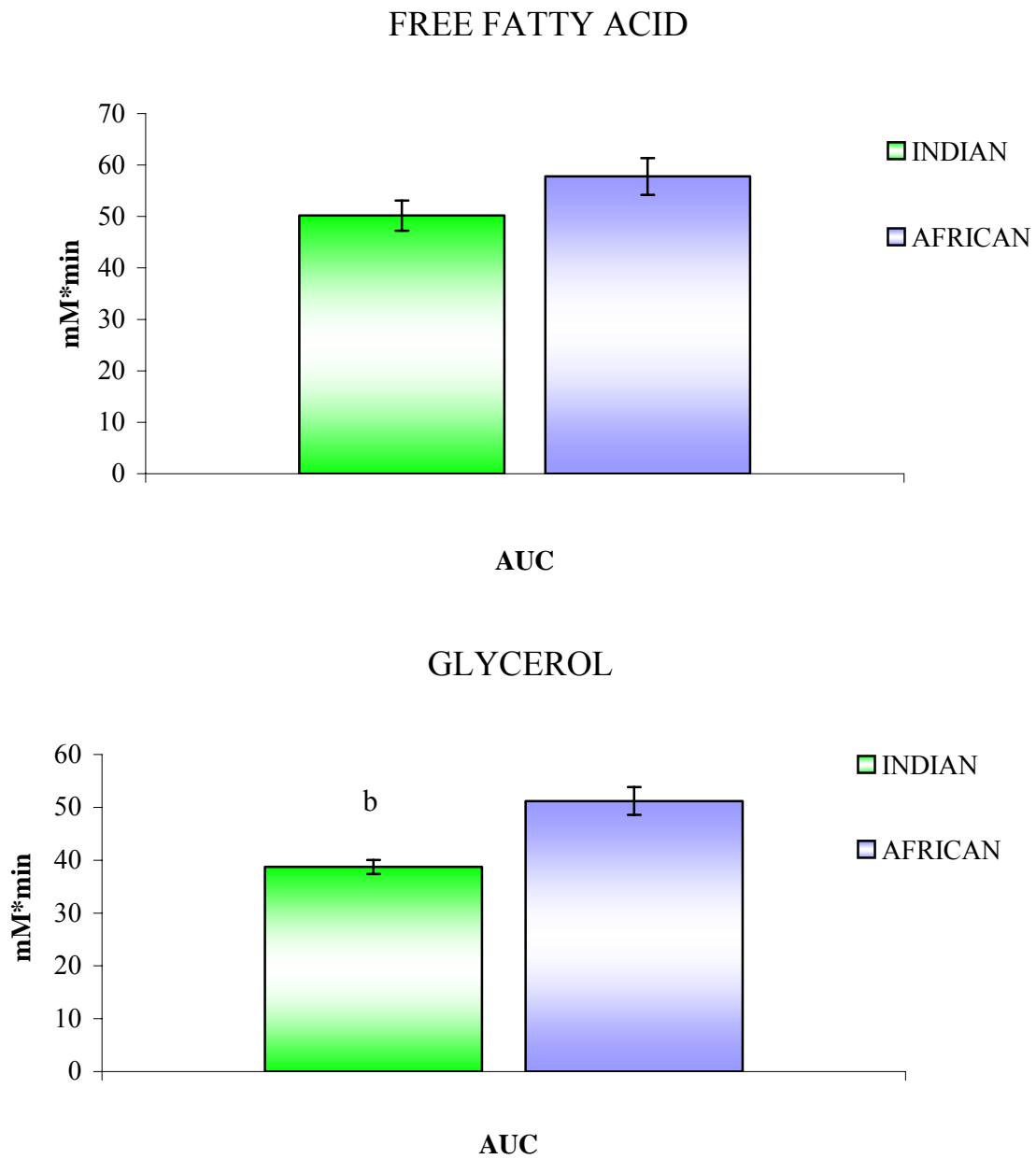
GLYCEROL

Figure 3.16 Serum free fatty acids and plasma glycerol concentrations during

5-hour OGTT in the combined* Indian and African groups

Data shown as Mean values ± SEM; adjusted for age;

* Obese, overweight, and lean combined; [a] $p<0.05$; [b] $p <0.0001$ Indian vs. African;

3.5 Prevalence of metabolic syndrome

The definition of metabolic syndrome is fully described in section 1.3 and was diagnosed using the NCEP: ATP III, (2001) criteria. The highest (85%) prevalence of metabolic syndrome was found in the diabetic Indian group. Obese Indian and African females exhibit similar prevalence of metabolic syndrome (35 and 40%, respectively). Overweight African group had 10% prevalence of metabolic syndrome compared to 0% in the Indian overweight group. Lean Indian had 5% prevalence of metabolic syndrome, while lean African had 0% prevalence of metabolic syndrome (Table 3.10).

Table 3.11 represent prevalence of metabolic syndrome components according to the NCEP: ATP III, (2001) criteria in the combined non-diabetic and diabetic Indian and African groups, respectively. The data indicated that in the combined Indian group the prevalence of HDL <1.29 mmol/l was 42% compared to 46% in the combined African group (p=NS). The prevalence of triglycerides >1.7 mmol/l was higher in the combined Indian group than in the combined African group (20% and 6%, respectively, p<0.05). Systolic BP > 130 mmHg and diastolic BP > 85 mmHg were similar across the groups. No significant differences were found for the prevalence of increased WC or increased fasting glucose between the combined Indian and African groups.

Table 3.10 Prevalence of metabolic syndrome (NCEP: ATP III, 2001 definition)

Indian					
	Diabetic N=20	Lean N=20	Obese N=20	Overweight N=10	Total N=70
With MS	17 (85)	1 (5)	7 (35)	0 (0)	25 (36)
Without MS	3 (15)	19 (95)	13 (65)	10 (100)	45 (64)

African					
	Diabetic N=20	Lean N=20	Obese N=20	Overweight N=10	Total N=70
With MS	15 (75)	0 (0)	8 (40)	1 (10)	24 (34)
Without MS	5 (25)	20 (100)	12 (60)	9 (90)	46 (66)

Data expressed as N number (%)

Abbreviation: MS-Metabolic syndrome

Table 3.11 Prevalence of metabolic syndrome components (NCEP: ATP III, 2001 definition) in the combined* Indian and African groups

Groups / MS Components	Indian		African	
	Non-diabetic* N=50	Diabetic N=20	Non-diabetic* N=50	Diabetic N=20
Systolic BP (mm/Hg)	14 (28)	13 (65) [a]	14 (28)	12 (60) [a]
Diastolic BP (mm/Hg)	10 (20)	7 (35)	13 (26)	10 (50)
WC (cm)	14 (28)	18 (90) [a]	14 (28)	19 (95) [a]
Fasting Glucose (mmol/l)	1 (2)	18 (90) [a]	0 (0)	13 (65) [a]
Triglycerides (mmol/l)	10 (20) [b]	8 (40)	3 (6)	7 (35) [a]
HDL (mmol/l)	21 (42)	7 (35)	23 (46)	12 (60)

Data expressed as N number (%)

* Lean, overweight and obese combined; [a] p<0.05 diabetic vs. non-diabetic;

[b] p<0.05 Indian non-diabetic vs. African non-diabetic

3.6 Socio-economic status

Details describing socio-economic status evaluation are given in section 2.7. Household amenities were found to be more prevalent ($p<0.05$) in the diabetic, obese and lean groups of Indian subjects compared to the respective groups of African subjects. Lean Indian women had significantly higher education status in comparison with lean African women. There were significant differences in education in diabetic vs. obese African and obese vs. lean Indian women (table 3.12). Household amenities and education displayed statistically significant ($p<0.05$) differences in the combined (obese, lean and overweight) group, the values being higher in the Indian subjects (figure 3.17).

Table 3.12 Socio-economic data

Groups Variables	LI (N=20)	OBI (N=20)	DI (N=20)	LA (N=20)	OBA (N=20)	DA (N=20)
Education	3.35 ±0.15 [a]	2.70 ±0.16 [b]	2.40 ± 0.13	2.35 ± 0.17	2.70 ± 0.15	2.10 ± 0.21 [c]
Household amenities	3.85 ± 0.08 [a]	3.80 ± 0.09 [a]	3.70 ± 0.11 [a]	3.00 ± 0.16	3.35 ± 0.15	3.15 ± 0.18

Data shown as Mean values ± SEM; adjusted for age;

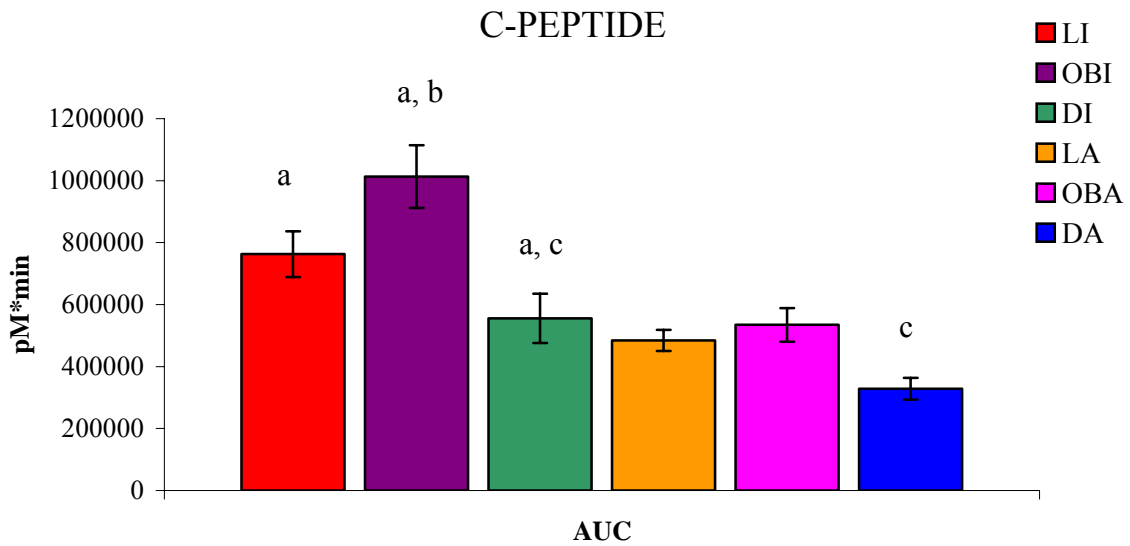[a] p<0.05 Indian vs. African; [b] p<0.05 Obese vs. Lean; [c] p<0.05 Diabetic vs. Obese


Figure 3.17 Socio-economic status in the combined* Indian and African groups

* Obese, overweight, and lean combined; [a] p<0.05 Indian vs. African

3.7 Food intake questionnaire assessment

Food intake data was gathered using a validated food frequency questionnaire (Appendix 3) as described in section 2.6. Original statistical analysis included 145 nutrients and the selection was made on basis of the most meaningful nutrient variables that may modify the anthropometric, metabolic and hormonal profiles in our study groups. The food intake questionnaire assessment is presented for all subjects and also with the exclusion of low energy reporters (LERs). Table 3.13 indicates percentage of the study population left from the original sample after taking into account this ratio of 1.14 as a cut-off point for discrimination between LERs (≤ 1.14) and "accurate" reporters.

Table 3.13 Data related to the number of subjects categorized as LERs

Subjects / Groups	Total before LERs exclusion (N)	After LERs exclusion (N)	After LERs exclusion (%)
INDIAN *	50	19	38
AFRICAN *	50	39	78
LI	20	7	35
OBI	20	6	30
DI	20	7	35
OWI	10	6	60
LA	20	18	90
OBA	20	11	55
DA	20	9	45
OWA	10	10	100

* Obese, overweight, and lean combined, excl. diabetics

 Abbreviations: LI- lean Indian; OBI-obese Indian; DI-diabetic Indian; OWI-overweight Indian;

 LA-lean African; OBA-obese African; DA-diabetic African; OWA-overweight African

Food intake questionnaire data is presented in tables 3.14 and 3.15. There were statistically significant ($p<0.05$) differences between lean Indian and lean African women for most of the variables (higher in African), with the exception of chloride, total carotenoids, vitamin C, total trans-FA, lycopene, chromium and starch. The diabetic group displayed interethnic differences (higher in Africans) in total energy, total protein, plant protein, animal protein, total carbohydrate, starch, total dietary fibre, magnesium, phosphorus and zinc.

Significant differences (p<0.05) were found between obese Indian and African women in total protein, plant protein, total carbohydrate, total dietary fibre, magnesium, phosphorus and zinc (higher in African) with lower intake in total sugar and starch.

Table 3.16 and 3.17 represents food intake questionnaire data after excluding low energy reporters. Lean Indian females had significantly lower total energy intake, total protein, plant protein, and total carbohydrate, total dietary fibre, magnesium, zinc, vitamin D and vitamin E in comparison with lean African subjects. Similarly, there were also significant differences between obese Indian and obese African for consumption of plant protein, total dietary fibre, magnesium, phosphorus and zinc. Diabetic Indian subjects had statistically significantly lower intake in total protein, plant protein, animal protein, total dietary fibre, magnesium, phosphorus zinc, total iron but higher intake of starch, fibre and chloride in comparison with diabetic African patients. Added sugar was significantly lower and plant protein was higher in diabetic Indian patients than in obese Indian group. Diabetic African group had lower vitamin C intake when compared to obese African group. Obese African subjects had significantly lower vitamin D values compared to lean African subjects. Food intake data, after exclusion of LER's, showed no interethnic differences in diabetics, obese and lean groups for the consumption of total fat, and saturated, monounsaturated, polyunsaturated and total *tran*s fatty acids.

The combined groups have statistically significantly higher food intake (p<0.05 and 0.0001) for African than Indian subjects for most of the variables with the exception of polyunsaturated and total trans-FA, chloride, total carotenoids, vitamin C and chromium where there were no differences and lycopene and starch where intake were significantly higher in Indians (table 3.18). Table 3.19 represents food intake in combined African and Indian group after excluding low energy reporters. There were statistically significantly higher intake (p<0.05 and 0.0001) for African than Indian women for most of the variables, with the exception of animal protein, total fat, monounsaturated, polyunsaturated and total trans-FA, sodium, chloride, vitamin C, folate, vitamin B12, total carotenoids and chromium, where intake were similar and lycopene and starch where intake were significantly higher in Indians.

Table 3.14 Food intake questionnaire assessment-Part 1

Groups / Variables	LI (N=20)	OBI (N=20)	DI (N=20)	LA (N=20)	OBA (N=20)	DA (N=20)
Total Energy (kJ)	5730 ±381 [a]	6047 ±348	5841 ±323 [a]	9759 ±560	7364 ±570 [b]	7163 ±516
Total Protein (g)	52.6 ±5.23 [a]	59.9 ±4.0 [a]	60.2 ±3.6 [a]	90.9 ±5.9	76.5 ±6.1	82.2 ±6.2
Plant Protein (g)	15.3±1.02 [a]	15.3±0.79 [a]	19.0±1.04 [a,c]	29.4 ±2.04	20.9±1.78 [b]	24.7 ±1.54
Animal Protein (g)	35.4±4.7 [a]	43.6±3.7	40.4±3.0 [a]	60.1 ±4.7	54.9 ±4.9	57.0 ±5.3
Total Fat (g)	50.1 ±3.8 [a]	53.3 ±4.4	51.9 ±4.2	75.6 ±5.7	58.7 ±5.9 [b]	52.9 ±5.1
Saturated FA (g)	16.6 ±1.7 [a]	17.6 ±1.5	15.7 ±1.4	25.9 ±2.2	21.3 ±2.3	17.7 ±1.9
Monounsaturated FA (g)	15.5 ±1.2 [a]	17.7 ±1.8	16.9±1.4	23.5 ±1.9	18.9 ±1.8	17.6 ±1.7
Polyunsaturated FA (g)	13.7 ±1.0 [a]	13.4 ±1.1	14.9 ±1.4	19.3 ±1.5	13.0 ±1.5 [b]	12.7 ±1.4
Total trans FA (g)	0.78±0.17	1.33 ±0.53	0.76 ±0.27	0.97±0.18	0.66 ±0.08	0.76± 0.14
Total Carbohydrate (g)	161 ±12.2 [a]	167 ±9.2 [a]	156 ±8.7 [a]	295 ±17.2	211±15.2 [b]	204 ±16.0
Added sugar (g)	33.3 ±5.3 [a]	34.5 ±5.3	17.2 ±3.6 [c]	59.1±6.0	37.3 ±3.6 [b]	17.6 ±4.6 [c]
Total sugar (g)	50.0 ±6.2 [a]	48.3 ±4.4 [a]	36.9 ±3.7	82.1 ±7.0	68.9 ±6.4	42.4 ±7.3 [c]
Starch (g)	22.8 ±4.5	29.5 ±5.2 [a]	32.4 ±7.4 [a]	20.8 ±3.9	15.7 ±4.1	12.2 ±2.3
Total Dietary fibre (g)	14.5 ±1.1 [a]	12.4 ±0.7 [a]	14.7 ±1.2 [a]	23.8 ±1.8	17.3 ±1.4 [b]	19.7 ±1.4

Data shown as Mean values ± SEM; adjusted for age

[a] p<0.05 Indian vs. African; [b] p<0.05 Obese vs. Lean; [c] p<0.05 Diabetic vs. Obese

Table 3.15 Food intake questionnaire assessment-Part 2

Groups / Variables	LI (N=20)	OBI (N=20)	DI (N=20)	LA (N=20)	OBA (N=20)	DA (N=20)
Magnesium (mg)	200±15.6 [a]	192±8.7 [a]	206±12.6 [a]	354±22.5	274±23.0 [b]	298±18.3
Phosphorus (mg)	889±82 [a]	903±51 [a]	940±59 [a]	1511±101	1198±105 [b]	1229±91
Zinc (mg)	6.2 ±0.54 [a]	6.5 ±0.39 [a]	6.7 ±0.39 [a]	10.6±0.77	8.9±0.72	9.5±0.83
Calcium (mg)	594±84.0 [a]	584±52.6	570±56.4	881±90.2	782±95.9	637 ±73.4
Total iron (mg)	7.1±0.56 [a]	7.1 ±0.54	6.9 ±0.48	11.2 ±1.03	8.1±0.58 [b]	8.1 ±0.68
Sodium (mg)	1104±91 [a]	1178 ±78	1221 ±80	1734 ±135	1326 ±130 [b]	1207 ±95
Chloride (mg)	895±115	1008 ±121	1079±154	1193±110	1064±134	782±89
Total carotenoids (mcg)	2020±293	1826±242	1935±297	1973±310	1507±278	2124±329
Folate (mcg)	158 ±15.0 [a]	159±12.5	183 ±12.6	227±18.5	181.3±15.1	165.1±13.2
Vitamin B12 (mcg)	3.1±0.40 [a]	3.5±0.33	3.4 ±0.25	5.1±0.51	4.5±0.47	4.2±0.58
Vitamin C (mg)	82.3±13.0	89.5±11.8	53.0±9.4 [c]	114.7±13.6	117.0±14.7	61.1±12.8 [c]
Vitamin D (mcg)	2.7 ±0.36 [a]	3.1±0.36	3.4±0.44	6.4±0.88	3.2±0.51 [b]	3.9±0.73
Vitamin E (mg)	7.7 ±0.75 [a]	7.0±0.63	8.4±1.06	11.2±0.99	6.9±0.92 [b]	7.5±0.99
Lycopene (mcg)	71 ±21.6	132±54.4	135±45.6	60.3±16.3	35.2±15.2	40.1 ±18.8
Chromium (mcg)	37.2±4.9	45.3±5.7	42.8±7.3	51.5±5.5	38.4±5.6	38.8±5.0

Data presented as Mean values ± SEM; adjusted for age

[a] $p < 0.05$ Indian vs. African; [b] $p < 0.05$ Obese vs. Lean; [c] $p < 0.05$ Diabetic vs. Obese

Table 3.16 Food intake questionnaire assessment (excluding LERs) -Part 1

Groups / Variables	LI (N=7)	OBI (N=6)	DI (N=7)	LA (N=18)	OBA (N=11)	DA (N=9)
Total Energy (kJ)	7496±468 [a]	7839±420	7423±386	10151±545	9125 ±557	9050 ±702
Total Protein (g)	69.9±9.3 [a]	79.1±6.0	77.6±2.5 [a]	95.0±5.8	92.7 ±6.3	103±7.1
Plant Protein (g)	17.4±1.5 [a]	17.8±1.6 [a]	22.9±1.3 [a,c]	30.0±2.2	26.9 ±1.4	30.5±1.7
Animal Protein (g)	48.1±9.6	60.3±5.5	53.5±2.4 [a]	63.5±4.5	64.8 ±5.4	71.7±6.7
Total Fat (g)	68.0±4.6	71.7±8.3	69.9±6.3	79.4±5.7	74.2 ±6.6	69.4±6.8
Saturated FA (g)	25.0±2.0	23.6±2.6	21.1±2.6	27.4±2.2	27.1 ±2.5	24.2±2.4
Monounsaturated FA (g)	20.6±1.8	25.4±3.5	22.7±2.0	24.6±1.9	23.5 ±2.2	22.6±2.3
Polyunsaturated FA (g)	16.1±1.6	16.8±2.5	20.3±2.5	20.0±1.6	16.6 ±1.9	15.9±2.0
Total trans FA (g)	0.85±0.10	3.37±1.52	1.4±0.72	1.06±0.19	0.81 ±0.10	1.08±0.26
Total Carbohydrate (g)	207±19.0 [a]	213±12.0	191±14.9	304±17.4	261 ±14.6	253±25.6
Added sugar (g)	47.5±10.9	53.5±9.2	23.1±8.9 [c]	62.2±6.2	43.4 ±5.0	23.8±8.5
Total sugar (g)	77.1±7.5	66.4±7.8	46.1±6.4	85.6±7.4	83.8 ±7.9	59.1±13.8
Starch (g)	27.9±7.8	41.7±10.0	50.6±14.9 [a]	19.2±3.4	20.7 ±6.5	13.4±2.0
Total Dietary fibre (g)	16.3±2.0 [a]	12.7±1.4 [a]	16.1±2.4 [a]	24.7±1.9	21.7 ±1.3	25.2±1.1

Data shown as Mean values ± SEM; adjusted for age

[a] $p<0.05$ Indian vs. African; [b] $p<0.05$ Obese vs. Lean; [c] $p<0.05$ Diabetic vs. Obese
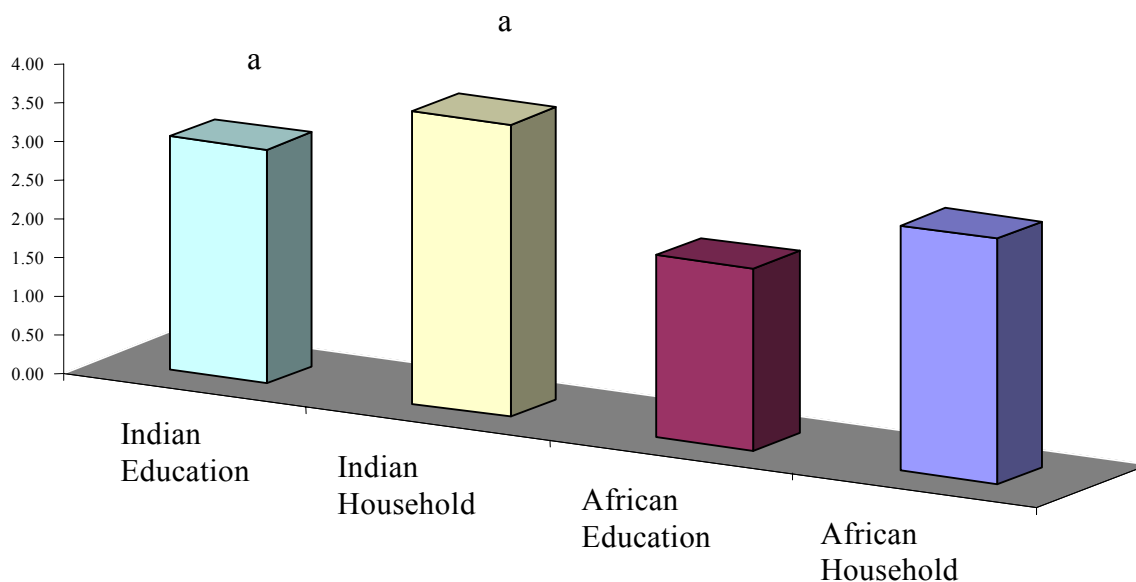
Table 3.17 Food intake questionnaire assessment (excluding LERs) -Part 2

Groups / Variables	LI (N=7)	OBI (N=6)	DI (N=7)	LA (N=18)	OBA (N=11)	DA (N=9)
Magnesium (mg)	257±20.9 [a]	216±16.8 [a]	243±21.2 [a]	367±22.9	345 ±23.6	371±18.7
Phosphorus (mg)	1250±107	1098±95.4 [a]	1200±69 [a]	1580±99	1508±106	1569±96
Zinc (mg)	8.2±0.92 [a]	7.7±0.78 [a]	8.5±0.38 [a]	11.1±0.75	10.8 ±0.75	12.5±1.10
Calcium (mg)	958±121	684±95	802±75	925±95	1027 ±116	870±88
Total iron (mg)	8.7±1.07	8.5±0.96	7.4±0.60 [a]	11.8±1.04	9.7 ±0.58	10.7±0.74
Sodium (mg)	1344±181	1520±99	1561±96	1812±138	1730 ±138	1561±106
Chloride (mg)	1359±119	1288±236	1611±265 [a]	1210±116	1413 ±158	1011±125
Total carotenoids (mcg)	1771±357	2338±628	1990±671	2039±341	1982 ±409	2587±456
Folate (mcg)	201.4±27.0	201.9±27.8	210.9±20.8	234.4±19.5	226.9±15.7	211.2±13.9
Vitamin B12 (mcg)	4.69±0.67	4.52±0.68	4.11±0.40	5.36±0.53	5.42 ±0.49	5.53±0.68
Vitamin C (mg)	86.8±26.2	105.3±21.2	71.0±22.3	123.2±13.6	126.8 ±20.0	66.9±18.2 [c]
Vitamin D (mcg)	3.3±0.66 [a]	4.3±0.58	3.8±0.68	6.9±0.9	3.9 ±0.8 [b]	5.8±1.1
Vitamin E (mg)	8.5±0.6 [a]	8.8±1.2	11.3±2.4	11.7±1.0	8.8 ±1.4	10.3±1.3
Lycopene (mcg)	131.7±52	238.9±119	137.8±64.3	58.9±18.1	30.9±11.9	13.5±10.8
Chromium (mcg)	46.3±9.4	59.5±12.7	64.4±12.8	52.7±5.5	44.9±8.2	49.1±6.0

Data presented as Mean values ± SEM; adjusted for age

[a] $p < 0.05$ Indian vs. African; [b] $p < 0.05$ Obese vs. Lean; [c] $p < 0.05$ Diabetic vs. Obese

Table 3.18 Food intake questionnaire assessment in the combined* Indian and African groups

Groups / Variables	INDIAN (N=50)	AFRICAN (N=50)	Groups / Variables	INDIAN (N=50)	AFRICAN (N=50)
Total Energy (kJ)	6131± 230 [b]	8691± 373	Magnesium (mg)	206.7± 8.6 [b]	317.3± 14.6
Total Protein (g)	60.7± 3.2 [b]	86.3± 3.82	Phosphorus (mg)	938.1±43.1 [b]	1387± 65.9
Plant Protein (g)	16.4± 0.7 [b]	25.4± 1.3	Zinc (mg)	6.79± 0.33 [b]	9.88± 0.46
Animal Protein (g)	42.9±2.8 [b]	60.1± 3.0	Calcium (mg)	586± 40.2 [a]	860± 61.1
Total Fat (g)	54.5±2.5 [a]	68.3± 3.6	Total iron (mg)	7.32± 0.34 [a]	9.77± 0.56
Saturated FA (g)	17.7±0.9 [a]	24.0±1.4	Sodium (mg)	1204± 54.5 [a]	1548± 83.0
Monounsaturated FA (g)	17.8 ±0.9 [a]	21.6± 1.1	Chloride (mg)	996.6± 75.9	1173±79.8
Polyunsaturated FA (g)	14.4±0.7	16.3±0.9	Vitamin C (mg)	89.7±7.7	105.8± 9.3
Total trans FA (g)	1.02±0.23	0.86 ±0.08	Folate (mcg)	165.8±8.5 [a]	209.8±11.4
Cholesterol (mg)	200.0±13.8 [b]	342.5± 26.2	Vitamin B12 (mcg)	3.48±0.23 [b]	5.13±0.32
Total Carbohydrate (g)	167.2 ± 6.5 [b]	255.7 ± 11.1	Total carotenoids (mcg)	1914±173	1632±184
Added sugar (g)	31.8± 3.2 [a]	49.5 ± 3.3	Vitamin D (mcg)	3.1±0.3 [a]	5.0± 0.5
Total sugar (g)	47.9± 3.0 [b]	75.5 ± 3.9	Vitamin E (mg)	7.40± 0.41	9.38 ± 0.67
Starch (g)	28.5 ± 3.5 [a]	18.9 ± 2.6	Lycopene (mcg)	88.6± 22.7 [a]	41.6± 9.2
Total Dietary fibre (g)	14.3 ± 0.6 [b]	20.5 ± 1.1	Chromium (mcg)	42.6± 3.6	45.9 ± 3.85

Data shown as Mean values ± SEM; adjusted for age;

* Obese, overweight, and lean combined; [a] p<0.05; [b] p<0.0001 Indian vs. African

Table 3.19 Food intake questionnaire assessment in the combined* Indian and African groups (excluding LERs)

Groups / Variables	INDIAN (N=19)	AFRICAN (N=39)	Groups / Variables	INDIAN (N=19)	AFRICAN (N=39)
Total Energy (kJ)	7733±263 [b]	9699±348	Magnesium (mg)	252.4±12.6 [b]	353.6±14.2
Total Protein (g)	79.0±4.82 [a]	95.5±3.68	Phosphorus (mg)	1203±58.4 [a]	1563±62.8
Plant Protein (g)	19.6±1.1 [b]	28.1±1.3	Zinc (mg)	8.6±0.52 [a]	11.0±0.46
Animal Protein (g)	56.6±4.5	66.2±2.9	Calcium (mg)	780±63.1 [a]	989±67.4
Total Fat (g)	69.7±3.4	77.3±3.5	Total iron (mg)	8.6±0.53 [a]	11.0±0.61
Saturated FA (g)	23.6±1.2 [a]	27.4±1.4	Sodium (mg)	1497±82.1	1766±83.4
Monounsaturated FA (g)	22.8±1.4	24.2±1.1	Chloride (mg)	1317±110	1342±85
Polyunsaturated FA (g)	17.2±1.1	18.5±1.0	Vitamin C (mg)	100.4±12.7	109.6±10.3
Total trans FA (g)	1.70±0.53	1.00±0.10	Folate (mcg)	202.6±14.4	235.2±12.4
Cholesterol (mg)	247±17.8 [a]	394±29.7	Vitamin B12 (mcg)	4.7±0.38	5.8±0.34
Total Carbohydrate (g)	208.0±8.8 [b]	283.9±10.6	Total carotenoids (mcg)	1949±270	1809±218
Added sugar (g)	41.1±6.3 [a]	56.0±3.8	Vitamin D (mcg)	3.4±0.37 [a]	5.9±0.58
Total sugar (g)	64.8±4.6 [a]	83.9±4.3	Vitamin E (mg)	8.3±0.50 [a]	10.9±0.74
Starch (g)	36.5±6.5 [a]	20.8±2.9	Lycopene (mcg)	150.2±44.9 [a]	41.5±9.57
Total Dietary fibre (g)	16.3±1.2 [a]	22.8±1.1	Chromium (mcg)	51.8±6.4	50.7±4.42

Data shown as Mean values ± SEM; adjusted for age;

* Obese, overweight, and lean combined; [a] $p<0.05$; [b] $p<0.0001$ Indian vs. African

Table 3.20 represents a comparison of total protein, total fat and total carbohydrate expressed as percentage of total energy intake in combined Indian and African groups. Statistically significant differences were found for fat (higher in Indian) and carbohydrate (higher in African). Similar pattern was observed after excluding low energy reporters in these groups.

Table 3.20 Percentage of total energy from macronutrients in the combined* Indian and African groups

Groups Variables	INDIAN* (N=50)	AFRICAN* (N=50)	INDIAN** (N=19)	AFRICAN** (N=39)
	% Of Total Energy Intake			
Total Protein	16.5	16.7	17.2	16.6
Total Fat	33.1 [a]	29.3	34.0 [a]	29.9
Total Carbohydrate	46.2 [a]	49.7	45.2 [a]	49.3

Data presented as percentage of total energy; [a] $p<0.05$; [b] $p<0.0001$ Indian vs. African

* Obese, overweight, and lean combined; ** excluding LER's

In combined African group ratio of protein to carbohydrate (=0.34) didn't differ from the results calculated in this group after exclusion of LER's. Ratios of protein to carbohydrate in Indian group were as follows: 0.36 in combined group and 0.39 when LER's were excluded. Ratios of protein to carbohydrate between combined African and Indian groups were statistically significant only when LER's were excluded (p=0.032).

3.8 Correlation and regression data analysis

Spearman rank correlation coefficients were used to analyse relationships between continuous variables. Beta cell variables that were investigated for correlation with anthropometric variables were: insulin (fasting and AUC), proinsulin/insulin ratio (fasting and AUC), and HOMA and insulinogenic index. As the main aim of our study was to investigate the factors associated with obesity- related changes in metabolic and hormonal parameters in two ethnic groups we decided to combine lean, obese and overweight subjects. It would not be statistically viable to use small groups in correlation and regression analysis. It is statistically valid to combine the different BMI groups because many of the metabolic differences between obese and lean subjects are due to the BMI differences. The best way to show this is to combine the groups and run multiple regression analysis including BMI, or other anthropometric variables as an independent variable. The diabetic patients were not included in the combined groups for correlation and regression analysis due to the fact that diabetic patients are known to exhibit a cluster of metabolic disturbances that may not be due to anthropometric differences. Therefore, including them in regression analysis may obscure relationships in the non-diabetic subjects that are driven by BMI. The diabetic subjects were therefore analysed by comparing them with BMI-matched non-diabetic subjects.

Multiple linear regression models for step-down backward selection were used to select dominant independent variables (see section 2.8) and results presented as R-Square and p values. All variables that stay in the model were significant at the 0.05 level. Independent variables included in the initial regression models were the ones that had statistically significant correlation with dependent variables in the Spearman test. Age was included in all regression models as an independent variable. Collinearity between variables was considered and independent variables like weight, height and waist/height ratio were excluded from the model where BMI also correlated significantly with dependent variables. No correlation analyses were performed for the food questionnaire assessment due to the small sample number after exclusion of low energy reporters.

3.8.1 Anthropometric parameters versus beta cell function in the combined African group (excl. diabetics)

Table 3.21 shows the results of correlation analyses between beta-cell function and anthropometric variables in the combined African group. Waist circumference had significant correlation with visceral fat in the combined African group (r= 0.816, p<0.0001).

No correlations were found between anthropometric variables and HOMA or proinsulin/insulin ratio. Age, WC, BMI, body fat (%), visceral and subcutaneous fat were included in regression model as independent variables and insulin (AUC) as the dependent variable. Linear regression analysis demonstrated that visceral fat could explain 26% of the variance in total insulin (p=0.014). Age, HC, BMI, waist/height ratio, body fat (%), visceral and subcutaneous fat had significant correlations with insulinogenic index. However the relationship didn't persist in a regression model.

Table 3.21 Anthropometric parameters versus beta cell function in the combined[*] African group

Variables	Insulin AUC	Insulinogenic index
BMI	0.369 [a]	0.391 [a]
HC	0.239	0.372 [a]
WC	0.381 [a]	0.379 [a]
Waist/Height	0.368 [a]	0.381 [a]
Body Fat (%)	0.375 [a]	0.350 [a]
Subcutaneous fat	0.347 [a]	0.386 [a]
Visceral fat	0.563 [a]	0.414 [a]

Data presented as Spearman Correlation Coefficient (r); adjusted for age

[*] Obese, overweight, and lean combined; [a] $p<0.05$; [b] $p<0.0001$

3.8.2 Anthropometric parameters versus beta cell function in the combined Indian group (excl. diabetics)

Table 3.22 shows the results of correlation analyses between anthropometric parameters and beta cell function in the combined Indian group. Waist circumference had significant correlation with visceral fat in the combined Indian group (r=0.778, p<0.0001).

No correlations were found between anthropometric parameters vs. proinsulin/insulin ratio (AUC and fasting) and insulinogenic index. Age, waist/height ratio and visceral fat were included in regression model as independent variables and insulin (AUC) as dependent variable. Linear regression analysis demonstrated that waist/height ratio could explain 29% of the variance in insulin (AUC) (p=0.0012). Age, BMI, HC and visceral fat were each

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significant predictors (58%) of fasting insulin in a regression model (p=0.021, 0.006, 0.003 and 0.005, respectively). Age, BMI, HC, WC, body fat (%), subcutaneous and visceral fat were included in regression model as independent variables and HOMA as dependent variable. BMI, HC and visceral fat were the only independent variables that could explain 55% of the variance in HOMA (p=0.007, 0.009 and 0.023, respectively).

Table 3.22 Anthropometric parameters versus beta cell function in the combined[*] Indian group

Variables	Insulin Fasting	Insulin-AUC	HOMA
BMI	0.521 [a]	0.295	0.502 [a]
HC	0.405 [a]	0.187	0.387 [a]
WC	0.619 [b]	0.435 [a]	0.609 [a]
WHR	0.552 [a]	0.464 [a]	0.548 [a]
Waist/Height	0.664 [b]	0.483 [a]	0.657 [b]
Body Fat (%)	0.548 [a]	0.327	0.532 [a]
Subcutaneous fat	0.498 [a]	0.290	0.480 [a]
Visceral fat	0.546 [a]	0.391 [a]	0.554 [a]

Data presented as Spearman Correlation Coefficient (r); adjusted for age

[*] Obese, overweight, and lean combined; [a] p<0.05; [b] p<0.0001

3.8.3 Anthropometric parameters versus intermediate metabolic variables, lipids and adipokines in the combined African group (excl. diabetics)

Table 3.23 represents correlation data between anthropometric parameters versus intermediate metabolic variables, lipids and adipokines in the combined African group (excl. diabetics). Not included in the table were the following correlations: fasting glucose correlated positively with WHR (r=0.352 [a]). HC had positive correlations with fasting and total (AUC) glycerol (r=0.395 [a] and 0.393 [a], respectively).

Visceral fat positively correlated with cholesterol (r=0.451 [a]). WC, waist/height ratio and visceral fat had positive correlation with triglycerides (r=0.354 [a], 0.428 [a] and 0.403 [a], respectively). Age, waist/height ratio and visceral fat were included in a regression model as independent variables and triglycerides as dependent variable. Waist/ height ratio had 21% association with triglycerides (p=0.021). We replaced waist/height ratio with WC in another regression model and likewise WC had 21% association with triglycerides (p=0.027).

BMI and body fat (%) negatively correlated with HDL cholesterol (r=-0.355 [a] and -0.360 [a], respectively). However the relationship didn't persist in a regression model. Age was the only independent variable that could explain 15% of the variance in HDL cholesterol (p=0.04). No correlations were found between IL-6 versus CRP and TNF-α in the combined African group. Leptin and soluble leptin receptor didn't have any correlations with triglycerides, FFA (AUC and fasting) or CRP when corrected for age and BMI. No correlations were found between TNF-α and glycerol (fasting and total) when corrected for age and HC.

Age, BMI, waist/height ratio, body fat (%), visceral and subcutaneous fat were included in regression model as independent variables and total glucose as dependent variable. Age and subcutaneous fat were the only independent variables that could explain 32% of the variance in total glucose (p=0.015 and 0.013, respectively). We replaced in another regression model waist/height ratio variable with WC and results were similar, indicating a strong association between subcutaneous fat and glucose (AUC) in the combined African group.

Age, WC, HC, BMI, body fat (%), visceral and subcutaneous fat were included in regression model as independent variables and LDL cholesterol, leptin, soluble leptin receptor and CRP as dependent variables. Visceral fat was the only independent variable that could explain 35 %

of the variance in LDL cholesterol and 40 % of the variance in CRP (p=0.001 and 0.0002, respectively). Body fat (%) and subcutaneous fat were significant determinants (85%) of the variance in leptin (p=0.0035 and 0.016, respectively). Subcutaneous fat had 49% association with soluble leptin receptor (p=<0.0001). Leptin level in the combined African and Indian lean groups correlated significantly with body fat (%), total subcutaneous and visceral fat, upper and lower body subcutaneous fat when corrected for age and ethnicity (r=0.390, 0.403, 0.330, 0.366 and 0.407, respectively, with p<0.05 for all). We included age, ethnicity, body fat (%), subcutaneous and visceral fat, upper and lower body subcutaneous fat in a regression model as independent variables and leptin as dependent variable. Ethnicity and total subcutaneous fat were the only independent variable that could explain 44% of the variance in leptin (p=0.007 and 0.024, respectively).

Age, WC, BMI, visceral and subcutaneous fat were included in regression model as independent variables and cortisol (fasting and total) as dependent variables. Linear regression analysis demonstrated that WC was the only independent variable explaining 23% of the variance in fasting and 19% in total (AUC) cortisol (p=0.009 and 0.012, respectively).

Table 3.23 Anthropometric parameters versus intermediate metabolic variables, lipids and adipokines in the combined[*] African group

Variables	Glucose AUC	Cortisol fasting	Cortisol-AUC	LDL	Leptin	Leptin receptor	CRP
BMI	0.344[a]	-0.370[a]	-0.352[a]	0.409[a]	0.842[b]	-0.670[b]	0.563[a]
HC	0.296	-0.329	-0.346	0.373[a]	0.763[b]	-0.592[a]	0.382[a]
WC	0.356[a]	-0.475[a]	-0.453[a]	0.383[a]	0.787[b]	-0.620[a]	0.532[a]
Waist/Height	0.396[a]	-0.440[a]	-0.386[a]	0.348[a]	0.826[b]	-0.518[a]	0.582[a]
Body Fat (%)	0.387[a]	-0.323	-0.323	0.439[a]	0.837[b]	-0.654[b]	0.558[a]
Subcutaneous fat	0.395[a]	-0.383[a]	-0.395[a]	0.403[a]	0.864[b]	-0.661[b]	0.485[a]
Visceral fat	0.355[a]	-0.435[a]	-0.357[a]	0.567[a]	0.814[b]	-0.664[b]	0.635[b]

Data presented as Spearman Correlation Coefficient (r); adjusted for age;

[*] Obese, overweight, and lean combined; [a] $p<0.05$; [b] $p<0.0001$

3.8.4 Anthropometric parameters versus intermediate metabolic variables, lipids and adipokines in the combined Indian group (excl. diabetics)

Table 3.24 represents correlation data between anthropometric parameters versus intermediate metabolic variables, lipids and adipokines in the combined Indian group. Not included in the table were following correlations: Visceral fat had positive correlation with fasting lactate (r=0.416[a]). Height had negative correlation with TNF-α (r=-0.355[a]).

No correlations were found between IL-6 versus CRP and TNF-α in the combined Indian group. No correlations were found between glucose (fasting and AUC) and anthropometric variables in the combined Indian group. Leptin and soluble leptin receptor didn't have any correlations with triglycerides, FFA (AUC and fasting) or CRP when corrected for age and BMI. Positive correlation was found between leptin and TNF-α (r=0.289 [a]). No correlations were found between TNF-α and glycerol (fasting and total) when corrected for age and HC.

Age, WC, HC, BMI, body fat (%), visceral and subcutaneous fat were included in regression model as independent variables and FFA (AUC), glycerol (AUC), triglycerides, leptin, soluble leptin receptor and hs-CRP as dependent variables. Linear regression analysis demonstrated that HC as independent variable could explain 37% of the variance in FFA (AUC) (p=0.002). BMI had 32% association with glycerol (AUC), 40% association with soluble leptin receptor and 35% association with hs-CRP (p=0.009, 0.0002 and 0.007, respectively). Visceral fat was the only independent variable that could explain 36% of the variance in triglycerides (p=0.0017). Body fat (%) was the only independent variable that could explain 57% of the variance in leptin (p=<0.0001). Age, BMI and WHR were included in regression model as independent variables and HDL cholesterol as dependent variable. Age and WHR had 39% association with HDL cholesterol (p=0.016 and <0.0001, respectively).

Table 3.24 Anthropometric parameters versus intermediate metabolic variables, lipids, and adipokines in the combined[*] Indian group (excl. diabetics)

Variables	FFA AUC	Glycerol AUC	HDL	Triglycerides	Leptin	Leptin receptor	CRP
Weight	0.584[a]	0.477[a]	-0.337	0.490[a]	0.594[a]	-0.652[b]	0.511[a]
BMI	0.488[a]	0.422[a]	-0.350[a]	0.424[a]	0.609[a]	-0.629[a]	0.482[a]
HC	0.550[a]	0.490[a]	-0.184	0.361[a]	0.588[a]	-0.561[a]	0.551[a]
WC	0.564[a]	0.496[a]	-0.462[a]	0.455[a]	0.535[a]	-0.616[a]	0.447[a]
Waist/Height	0.467[a]	0.426[a]	0.483[a]	0.435[a]	0.554[a]	0.625[a]	0.438[a]
Body Fat (%)	0.441[a]	0.397[a]	-0.318	0.411[a]	0.637[b]	-0.631[a]	0.507[a]
Subcutaneous fat	0.508[a]	0.459[a]	-0.152	0.353[a]	0.613[a]	-0.584[a]	0.576[a]
Visceral fat	0.528[a]	0.448[a]	-0.255	0.671[b]	0.451[a]	-0.534[a]	0.417[a]

Data presented as Spearman Correlation Coefficient (r); adjusted for age

[*] Obese, overweight, and lean combined; [a] $p < 0.05$; [b] $p < 0.0001$

3.8.5 Anthropometric parameters, beta cell, intermediate metabolic and adipokines levels versus blood pressure measurements in the combined African group (excl. diabetics)

Systolic BP correlated positively with BMI, HC, WC, and waist/height ratio, body fat, subcutaneous and visceral fat when corrected for age (r=0.437 [a], 0.373 [a], 0.491 [a] and 0.411 [a], 0.406 [a], 0.436 [a], 0.402 [a], respectively). We included in a regression model as independent variables age and anthropometric parameters that had correlation with systolic BP, excluding waist/height ratio due to collinearity. WC was the only independent variable that could explain 25% of the variance in systolic BP (p=0.004). Systolic BP had negative correlation with lactate (AUC) when corrected for age and BMI (r=-0.378 [a]).

Diastolic BP had positive correlations with BMI, WC, WHR, waist/height ratio, and body fat, subcutaneous and visceral fat (r=0.358 [a], 0.491 [a], 0.490 [a], 0.420 [a], 0.326 [a], 0.371 [a] and 0.525 [a], respectively). We included in a regression model as independent variables age and anthropometric parameters that had correlation with diastolic BP, excluding WHR and waist/height ratio due to collinearity. Age and WC were the only independent variables that could explain 40% of the variance in diastolic BP (p=0.038 and 0.002, respectively). Diastolic BP correlated positively with glucose (AUC), fasting C-peptide and HOMA (r=0.378 [a], 0.310 [a] and 0.327 [a], respectively) when corrected for age and BMI. We therefore performed a regression analyses and included in a model as independent variables age, WC, glucose (AUC), fasting C-peptide and HOMA. WC and glucose (AUC) were the only independent variables that could explain 43% of the variance in diastolic BP (p=0.001 and 0.0009, respectively) in combined African group.

Systolic BP displayed a positive correlation with leptin (r=0.401, p=0.004) and a negative correlation with soluble leptin receptor (r=-0.363, p=0.01) in the African group. However, when leptin and leptin receptor were included as independent variables in a regression model with the anthropometric parameters, such as BMI, HC, WC, body fat (%), subcutaneous and visceral fat, only WC remained significant, explaining 26% of the variance in systolic BP (p=0.004). Diastolic BP correlated positively with leptin (r=0.457, p=0.001) and CRP

(r=0.373, p=0.008) and correlated negatively with leptin receptor (r=-0.428, p= 0.002) in the African group. We included in a regression model BMI, WC, subcutaneous and visceral fat, leptin, leptin receptor and CRP as independent variables and diastolic blood pressure as dependent variable. Age and WC were the only variables that displayed a significant correlation, explaining 44% of the variance in diastolic BP (p=0.038 and 0.002, respectively) in the African group.

3.8.6 Anthropometric parameters, beta cell, intermediate metabolic and adipokines levels versus blood pressure measurements in the combined Indian group (excl. diabetics)

Hip circumference and subcutaneous fat were the only anthropometric variables that had positive correlations with systolic BP in the combined Indian group (r=0.375 [a] and 0.338 [a], respectively). Systolic BP correlated positively with fasting intact proinsulin and fasting proinsulin/insulin ratio when corrected for age and BMI (r=0.345 [a] and 0.334 [a], respectively). Age, HC, subcutaneous fat and fasting intact proinsulin were included in regression model as independent variables. Age was the only independent variables that could explain 31% of the variance in systolic BP as dependent variable (p=0.005).

Diastolic BP had positive correlations with BMI, HC, WC, body fat (%), subcutaneous fat and visceral fat (r=0.386 [a], 0.405 [a], 0.347 [a], 0.413 [a], 0.399 [a] and 0.380 [a], respectively). When these variables and age were included in a multiple regression analysis BMI and body fat (%) were the only independent variables that could explain 46% of the variance in diastolic BP (p=0.004 and 0.001, respectively). Diastolic BP correlated positively with glucose (AUC), fasting intact proinsulin, fasting proinsulin/insulin ratio and lactate (AUC) when corrected for age and BMI (r=0.367 [a], 0.317 [a], 0.316 [a] and 0.290 [a], respectively). Age, BMI, body fat (%), glucose (AUC), fasting proinsulin/insulin ratio and lactate (AUC) were included in regression model as independent variables. BMI, body fat (%) and glucose (AUC) were the only independent variables that could explain 49% of the variance in diastolic BP (p=0.006, 0.001 and 0.043, respectively).

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In the Indian group a positive correlation between leptin and systolic BP was observed (r=0.312, p=0.029). The relationship did persist in a regression model with leptin, and HC as independent variables and systolic BP as dependent variable: age and leptin explained 33% of the variance in systolic BP (p=0.002 and 0.02, respectively). No correlations were found between adipokines and diastolic BP in the Indian group. BMI and body fat (%) were the only independent variables in a regression model that could explain 45% of the variance in diastolic BP in the Indian group (p=0.004 and 0.001, respectively).

3.8.7 Anthropometric parameters, insulin, intermediate metabolic variables, lipids levels, and blood pressure versus socio-economic status in the combined African group (excl. diabetics)

Education and household amenities had positive correlations with insulin (AUC) and negative correlations with HIE (AUC) when corrected for age and BMI (r=0.440[a], 0.319[a], and -0.621[b], -0.312[a], respectively). Education correlated negatively with proinsulin/insulin ratio (AUC) and had positive correlation with insulinogenic index (r=-0.445[a], and 0.344[a], respectively). Household amenities had positive correlation with HDL cholesterol (r=0.332[a]). No other correlations were found between anthropometric variables, lipids, adipokines and blood pressure versus socio-economic status in the combined African group when corrected for age and BMI.

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3.8.8 Anthropometric parameters, insulin, lipids and blood pressure versus socio-economic status in the combined Indian group (excl. diabetics)

Education had negative correlations with BMI, WC, waist/height and body fat (%), subcutaneous and visceral fat (r=-0.422 [a], -0.430 [a], -0.436 [a], -0.456 [a], -0.431 [a] and -0.449 [a], respectively). In the combined Indian group education had negative correlations with insulin (AUC), diastolic BP and positive correlation with proinsulin/insulin ratio (AUC) when corrected for age and BMI (r=-0.364 [a], -0.299 [a] and 0.353 [a], respectively). Age, visceral fat and education were included in a regression models as independent variables with insulin (AUC) and diastolic BP as dependent variables. Education could explain 32% of the variance in insulin (AUC) (p=0.0010) whereas visceral fat was the only independent variable that had 36% interaction with diastolic BP (p= 0.003).

Household amenities had negative correlations with WHR and triglycerides (r=-528 [a] and - 0.459 [a], respectively). Age, WHR and household amenities were included in a regression model as independent variables with triglycerides as dependent. Household amenities were the only independent variables that could explain 18% of the variance in triglycerides (p=0.004). No other correlations were found between socio-economic status versus anthropometric parameters, insulin, lipids, adipokines and blood pressure in the combined Indian group.

3.8.9 HOMA-IR versus lipids, intermediate metabolic parameters, adipokines and socio-economic status in the combined African group (excl. diabetics)

No correlations were found between HOMA-IR versus lipids, intermediate metabolic parameters, adipokines and socio-economic status when corrected for age and BMI in the combined African group (excl. diabetics). Diastolic BP had a positive correlation with HOMA-IR (r=0.374, p=0.009). HOMA-IR correlated negatively with fasting proinsulin/insulin ratio (r=-0.644 [b]), but no correlations were found with total (AUC) proinsulin/insulin ratio.

3.8.10 HOMA-IR versus lipids, intermediate metabolic parameters, adipokines and socio-economic status in the combined Indian group (excl. diabetics)

HOMA-IR had positive correlations with lactate (AUC and fasting) (r=0.360 and 0.411, p=0.017 and 0.003, respectively) and negative correlation with HDL cholesterol (r=-0.328, p=0.022) when corrected for age and BMI. HOMA-IR correlated negatively with total (AUC) proinsulin/insulin ratio (r=-0.425, p=0.002), but no correlations were found with fasting proinsulin/insulin ratio. No correlations were found between HOMA-IR versus adipokines and socio-economic status when corrected for age and BMI in the combined Indian group (excl. diabetics).

CHAPTER 4 - DISCUSSION

The present study was carried out in an attempt to explain the possible role of body fat distribution, intermediate metabolic indices, aspects of beta-cell function, certain adipokines, socio-economic status and dietary intake on the development of obesity-related disorders in two South African ethnic groups, namely Indian and African women. To our knowledge this is the first time that these variables have been compared in these ethnic groups in a single study.

4.1 Body fat distribution & other anthropometric variables

There are many indications that in some ethnic groups (particularly of Asian origin), the risk of diabetes starts to increase rapidly at BMI or waist circumference levels that fall within the acceptable range for Europeans (Seidell,2000). This raises an important issue regarding the use of BMI in the definition of obesity in different races and predicting disease risk. Raji *et al.*, (2001) suggested that, even at a lower BMI, Asian Indians are profoundly insulin resistant and have increased total abdominal fat compared to European population, which may explain their increased prevalence of diabetes and coronary heart disease. Lowering the BMI cut-off points in Asian populations may be beneficial in early identification of individuals at high risk for developing obesity-related disorders. In the present study the criteria used for defining high waist circumference were those specified by NCEP: ATP III, 2001. To our knowledge there are no established cut-off points for South African Indian and African population, therefore in our estimation of metabolic syndrome we applied equal cut-off points in both ethnic groups. Bioelectrical impedance analysis was used to measure percentage body fat levels. This method has been admonish in terms of its accuracy however, the BODYSTAT technology has been scientifically validated for both its accuracy of measurement of impedance *in vivo* and in the application of its unique regression equation (unpublished) to determine body composition with particular reference to the obese population group (Smye *et al.*, 1993). The accuracy of

bioelectrical impedance in estimating the body fat-fat mass is comparable to densitometry with lower predictive error or standard error of 2.7% (Lukaski *et al.*, 1986). Our results revealed a strong correlation between BMI and body fat (%) assessment by BODYSTAT in Indian and African groups (r= 0.987* and 0.987*, respectively, *p< 0.0001). Therefore, it is unlikely that the large ethnic difference in TNF-α, lipids, leptin or HOMA-IR were due to ethnic differences in body fat % levels that were undetected using the BODYSTAT technology.

Statistically significant differences in anthropometry between the obese and overweight subjects were not found and this may be a result of the small sample size in the overweight group. In our study we found that visceral fat accumulation was greater in diabetic and lean Indian subjects than in diabetic and lean African groups. This fat depot is more lipolytically active than subcutaneous fat (Kissebah *et al.*, 1989, Bjorntorp , 1991) due to higher β-adrenoreceptor mediated catecholamine-induced lipolysis and greater resistance to the antilipolytic activity of insulin (Hoffstedt *et al.*, 1997). Measures of subcutaneous fat were higher in lean Indian than African women but lower in obese Indian than African subjects (Present study). Our data therefore confirms the suggestion of Raji *et al.*, (2001) that at any given BMI, Indian subjects have more visceral fat than other ethnic groups and may explain their higher risk for obesity-related disorders at lower BMI than non-Indian populations.

The upper body subcutaneous fat level was significantly higher in lean Indian than lean African women but lower in obese Indian than obese African subjects. The upper body subcutaneous fat was much more pronounced in obese subjects compared to the lean subjects in both ethnic groups. This may be an important predictor of metabolic abnormalities in the obese group of women. The literature suggests that individuals with peripheral obesity ('pears') possess fat distributed subcutaneously in gluteofemoral areas and the lower part of the abdomen, and are at little risk of metabolic complications. Individuals with upper-body obesity ('apples') accrue fat in subcutaneous and visceral deposits and predisposed to the development of metabolic and cardiovascular complications (Lafontan *et al.*, 2003). On the other hand, accumulation of fat in lower body subcutaneous adipose tissue depots linked to a lower probability of insulin resistance and type 2 diabetes than when it deposits in centrally located sites (Livingston, 2006). Lower body peripheral fat may safeguard the effect of excess ingested energy, and central body fat may be involved in the pathogenesis of insulin resistance and type 2 diabetes (Livingston, 2006).

The study by Virtanen *et al.*, (2002) demonstrated that insulin-stimulated glucose uptake rate per gram of tissue was higher in visceral, compared to subcutaneous adipose tissue, regardless of the magnitude of obesity. One reason for higher glucose uptake in visceral adipose tissue in comparison with subcutaneous adipose tissue may be different sizes of the adipocytes in these depots; visceral adipocytes are smaller than subcutaneous and visceral fat contains more adipocytes per gram than subcutaneous fat, which may facilitate glucose uptake (Rebuffe-Scrive *et al.*, 1989). Therefore, despite the fact that the intra-abdominal visceral adipose tissue is metabolically more active than sc adipose tissue per tissue weight, the large size of the subcutaneous adipose tissue depot makes it an important regulator of whole-body glucose metabolism (Virtanen *et al.*, 2002). In the present study subcutaneous fat did explain some of the variance in total glucose levels in the combined African group while no correlations were found between glucose (fasting and AUC) and anthropometric variables in the combined Indian group. The reason for this difference needs further investigation.

Waist/height ratio has been proposed as an alternative to waist circumference for the evaluation of abdominal adiposity which takes into account differences in height (Hsieh *et al.*,1995a, Hsieh *et al.*,1995b), but the usefulness of this parameter has been questioned (Sattar *et al.*,1998). Some previous studies reported that waist/height ratio had a stronger association with cardiovascular risk factors than waist circumference alone (Hsieh *et al.*,1995a, Hsieh *et al.*,1995b). In our study correlations of waist/height ratio and waist circumference with cardiovascular risks factors demonstrated that waist/height was no better than WC at predicting CVD risk factor levels. However, waist/height ratio was the best predictor of insulin (AUC) in the Indian group.

4.2 Beta-cell evaluation and insulin resistance

Metformin belongs to biguanide class of oral anti-diabetic drug. The half life of metformin is 6.2 hours. Metformin absorption is comparatively slow and may prolong over about 6 hours. The initial elimination of metformin is quick with a half-life varying between 1.7 and 3 hours. 4-5% of the absorbed dose that accounts in the terminal elimination phase is slow and could prolong with a half-life between 9 and 17 hours (http://www.drugs.com/metformin.html).

Subjects with type 2 diabetes were asked to stop oral anti-diabetes drugs 24 hours prior to the OGTT; therefore drug-induced interference with measures of beta cell function from metformin was probably avoided. However for Gliclazide at the time of the OGTT drug orally administered 24 hours beforehand would still be present in the circulation.

To establish the effect of Gliclazide and Metformin on measurements of fasting glucose and insulin we compared diabetic patients in the Indian and African groups, with and without use of Gliclazide and Metformin. The fasting glucose and insulin levels were not significantly different between those groups. Thus we may state that in our group of diabetic patients the use of different anti-diabetic drugs did not influence results in the present study.

Our results clearly indicated that obese Indians had significantly higher fasting glucose, des 31, 32 proinsulin, C-peptide and HOMA-IR levels and lower proinsulin/insulin ratio than obese African subjects whilst the fasting levels of intact proinsulin and proinsulin /insulin ratio were significantly lower in lean Indian than lean African women. This suggests that lean and obese Indians have better β-cell proinsulin-processing efficiency than Africans, probably due to higher secretory load imposed on beta cells by a higher level of insulin resistance in the Indian subjects. Obese and lean Indian subjects were far more hyperinsulinemic in comparison with African subjects during the 5-hour OGTT, again suggesting greater insulin resistance in Indians.

In the present study HOMA-IR was significantly different between groups with metabolic syndrome and without metabolic syndrome in non-diabetic subjects in both ethnic groups. Adjustment for waist circumference did not have any influence on the results suggesting that metabolic syndrome in both ethnic groups is associated with insulin resistance. Diabetic Indian women in our study had significantly higher fasting intact proinsulin, des 31, 32 split proinsulin and C-peptide levels than diabetic African women. The similarity in insulin levels of the two diabetic groups in the presence of a significant difference in C-peptide concentrations may reflect differences in hepatic insulin clearance.

A hyperbolic relationship between insulin secretion and action is referred to as the disposition index and suggest that insulin sensitivity and insulin secretion are linked through a negative feedback loop. This model might explain enchanced β cell function when individuals becomes more insulin resistant (Bergman *et al.*, 1981, Kahn *et al.*, 1993). The greater insulin resistance and higher levels of proinsulin and split proinsulin are markers of risk for type 2 diabetes

(Pradhan *et al.*, 2003) and it is known that type 2 diabetes is more common in the Indian population of South Africa (Omar *et al.,* 1985, Omar *et al.,* 1994, Motala *et al.*, 2003a). The current study shows that serum insulin, des 31, 32 split proinsulin and C-peptide are significantly higher and hepatic insulin extraction (HIE) and proinsulin/insulin ratio significantly lower in the Indian group compared to the African group. This suggests higher insulin resistance, which is compensated for by reduced HIE and improved proinsulin-processing in the non-diabetic Indians compared to the non-diabetic African group. The higher proinsulin-processing efficiency observed in the Indian compared to the African subjects in conjunction with the higher prevalence of diabetes in the former population group suggests that in Indian subjects the secretory and proinsulin-processing burden placed at the β-cells by the high level of insulin resistance eventually leads to β-cells "burn out" and the development of glucose intolerance and eventual type 2 diabetes.

In the combined African group linear regression analysis demonstrated that visceral fat could explain some of the variance in insulin (AUC). In the combined Indian group linear regression analysis demonstrated that waist/height ratio had an association with total insulin and age, whilst BMI, HC and visceral fat were significant predictors of fasting insulin. BMI, HC and visceral fat were the only independent variables that could explain variance in HOMA-IR in the combined Indian group. Thus, visceral fat is a strong determinant of insulin levels in both ethnic groups and may partially explain higher insulin levels in Indian females. Furthermore, elevated insulin, proinsulin and split proinsulin levels have been shown to be CVD risk factors (Reaven, 1992, Haffner *et al.,*1993, McFarlane *et al.*, 2001) and the higher levels in the Indian group mirror the higher prevalence of CVD in this population group.

4.3 The relationship of anthropometric and metabolic variables with blood pressure

In data collected annually in England between 1991-1996 that compared blood pressure (BP) levels and hypertension rates in people of white, black (combining black-Caribbean, black-African and black-other), and South Asian origin (combining Indians, Pakistanis and Bangladeshis) the age-adjusted mean BP levels and hypertension rates of older adults were highest among blacks, while South Asian men showed BP levels and hypertension rates similar to black men and South Asian women had mean BP levels and hypertension rates similar to white women (Primatesta *et al.*, 2000).The study by Morar *et al.*, (1998), that compared the blood pressure profile in Indian and black students in South Africa, showed that young black people had higher blood pressure readings than young Indian participants in the absence of metabolic abnormalities.

We found in our study that in both ethnic groups BP was higher in obese than lean subjects. Systolic blood pressure was higher in diabetics than obese subjects in the African and Indian groups but only reached statistical significance in the Africans. In our study diabetic Indian women had significantly lower diastolic BP than diabetic Africans. No ethnic differences in BP were observed in the non-diabetic subjects.

Some authors suggest that BMI, a general indicator of obesity, is a better correlate of blood pressure (BP) than the WHR among African Americans (Adams-Campbell *et al.*,1994), whereas other investigators found that waist circumference was positively correlated with BP in Nigerians, Jamaicans and African Americans (Okosun *et al.*,1998). Gupta *et al.*, (2007) discussed in their study a significant positive correlation of BMI, waist-size and WHR with systolic BP and diastolic BP in an urban Indian population.

The relationship between indices of adiposity (WC, waist-to-hip ratio, BMI or skin-fold thickness) and ambulatory or conventional BP was determined in 300 randomly selected individuals of African descent living in an urban community in South Africa (Majane *et al.*, 2007). Their results showed that WC is the only clinical index of adiposity that is associated with 24-h and conventional BP measurements independent of other adiposity indices in a community with a high prevalence of obesity.

This data is in agreement with our study where in the combined African group WC was the only independent predictor in a regression model that could explain variance in systolic BP. Hip circumference (HC) was the only anthropometric variable that had positive correlations with systolic BP in the combined Indian group. Age and WC were the only independent variables that had an association with diastolic BP in the combined African group. BMI and body fat (%) were strong predictors of the variance in diastolic BP in the combined Indian group. Ethnic differences in the determinants of blood pressure in relationship to anthropometric variables showed the importance of abdominal obesity in the African group whilst in Indian group the total body fat was more important.

Leptin-mediated sympatho-activation emerge as on of the major mechanisms leading to the development of obesity-induced hypertension. Leptin also negatively shifts the renal pressure-natriuresis curve, leading to relative sodium retention (Haynes, 2005). The study by Wada *et al.*, (2006) showed that leptin was positively related with diastolic BP independent of insulin resistance among the subjects in the normal blood pressure range, but this relationship was not found among the subjects having higher blood pressure. Although the association between leptin and diastolic BP was independent of BMI, BMI adjustment had attenuated the association remarkably, suggesting that leptin is a physiological mediator/or a marker/ of diastolic BP elevation in obesity.

The finding that increasing plasma leptin, to levels similar to those found in obesity, raises arterial pressure in non-obese rats is consistent with the hypothesis that leptin is an important link between obesity, sympathetic activity and hypertension. The complex relationships between leptin and long-term blood pressure regulation is further illustrated by the finding that lower body obesity causes greater increases in leptin than visceral obesity, even though visceral obesity is more closely associated with hypertension (Hall *et al.*, 2000). A number of clinical studies have shown increased plasma leptin levels in subjects with essential hypertension and a significant positive correlation between leptin and blood pressure independent of body adiposity both in normotensive and in hypertensive individuals (Beltowski, 2006).

In our study systolic and diastolic BP had positive correlations with leptin and negative correlations with leptin receptor in the African group, but this relationship didn't persist in a regression model when adjusted for BMI, HC, WC, body fat (%), subcutaneous and visceral

fat. Waist circumference was the only variable that explained significant variance in systolic and diastolic BP in the African group. In the Indian group systolic BP correlated positively with leptin and the relationship did persist in a regression model after adjustments for anthropometric covariates. The reason for the presence of a correlation between leptin levels and BP in the Indian but not African subjects is not known, but few studies indicated similar results.

El-Gharbawy *et al.*, (2002) evaluated associations of leptin with blood pressure (BP) in normotensive and hypertensive, matched for BMI, African American individuals. The plasma leptin was higher in hypertensive women than in normotensive women. No significant correlations were found between leptin and BP after adjustment for obesity and insulin resistance; although leptin independently predicted 28% of the variability of heart rate in hypertensive men and 18% of the variability of lithium clearance in hypertensive women. Authors proposed that although there is no direct or independent association of leptin with BP leptin may contribute to hypertension in these women by increasing renal tubular sodium reabsorption.

The study by Schutte *et al.*, (2005a) showed that while leptin levels were the same in overweight/obese hypertensive and normotensive African women, it was directly and positively associated with systolic blood pressure and pulse pressure only in overweight/obese hypertensive African women after adjustment for obesity, insulin-resistance, hyperinsulinaemia and age. In related study leptin levels were similar in obese African and Caucasian women, but association with diastolic blood pressure and total peripheral resistance was found only in obese Caucasians, but not in obese Africans (Schutte *et al.*, 2005b).

Insulin resistance has been found to be a common feature of essential hypertension (Baron *et al.*, 1993). Ferrannini *et al.,* (1997) have analyzed the relationship between insulin sensitivity and blood pressure and have shown that insulin sensitivity contributes to blood pressure variability. The increase in systolic blood pressure of 1.7 mm Hg and an increase of diastolic blood pressure of 2.3 mm Hg were found for each 10-unit increase in insulin resistance (in a molar value it is a decrease of 10 µM/min per kilogram).

Possible mechanisms by which insulin resistance and hyperinsulinaemia contribute to the pathogenesis of hypertension include: activation of the sympathetic nervous system, increased activity of the Na/H exchange pump, increased retention of renal sodium, and increased salt

sensitivity. These mechanisms are not well understood, and it is not clear how important insulin resistance is to their development (Pi-Sunyer, 2004).

The association of essential hypertension with insulin resistance in black South Africans was clearly demonstrated in a case-controlled study. The fasting insulin concentration was significantly greater in the hypertensive than the normotensive subjects. The direct assessment of insulin-mediated, whole-body glucose disposal and the derived insulin sensitivity index was significantly reduced in hypertensive group by some 30% and 40%, respectively. The authors concluded that the pathogenic role of this association is unlikely to be dominant in this group and the overall role of insulin resistance in influencing cardiovascular risk factors in black South Africans remains ill-defined (Wing *et al.*, 1994).

Diastolic BP in our study correlated positively with glucose (AUC), fasting C-peptide and HOMA-IR in the combined African group. In a regression model WC and glucose (AUC) were the only independent variables that could explain variance in diastolic BP. Diastolic BP correlated positively with glucose (AUC), fasting intact proinsulin, fasting proinsulin/insulin ratio and lactate (AUC) in the combined Indian group.

In regression model BMI, body fat (%) and glucose (AUC) were the only independent variables that had an association with diastolic BP in the combined Indian group. Thus, in both ethnic groups, postprandial glucose levels were significant determinants of diastolic blood pressure, independently of anthropometric variables. Other studies have also demonstrated correlations between blood pressure and plasma glucose levels (Jarrett *et al.*, 1978, Khoo *et al.*, 2000, Mancia *et al.*, 2005) and that arterial stiffness and left ventricular diastolic dysfunction are both related to glucose levels (Miyazato *et al.*, 2002, Gordin *et al.*, 2008), and that responsiveness to ACE inhibitors is better in diabetic patients with good glycaemic control (Jenkins *et al.*, 1990, Chan *et al.*, 1995).

4.4 Lipids and markers of lipolysis

In the study by Seedat *et al.,*(1992) on risk factors and coronary heart disease in black subjects resident in Durban, South Africa it has been showed that only 7.5% of the subjects (11.2%-males and 3.5%-females) had hypertriglyceridaemia. This contrasts with their study of coronary artery disease in the Indian population of Durban in which they found that 20.5% had hypertriglyceridaemia. Coronary heart disease is not very common among African diabetic population which may be explained by contributions of low total cholesterol levels and probably lesser insulin resistance and its consequences; renal disease may be an important additional risk factor (Kalk *et al.*, 2007).

A number of risk factors for CHD were studied by Seftel *et al.*, (1993) in groups that included urban and rural blacks, Indians of higher and lower socio-economic status, Coloureds of higher and lower socio-economic status, and middle-class White South African male scholars. The prevalence and severity of CHD risk factors, such as total cholesterol, LDL, HDL, the HDL/LDL ratio, apolipoprotein B, apolipoprotein A-I, insulin and fibrinogen was much higher in Indian groups, the whites and both coloured groups than the two African groups.

The higher socio-economic group as well as urban, but not rural blacks had poorer risk profile for development CHD. A cross-sectional (BRISK) study to determine the lipid and lipoprotein profile of the urban black South African population of the Cape Peninsula revealed that the cholesterol level was low compared with other South African groups studied (Oelofse *et al.*, 1996).

The impact of urbanisation of black South Africans on risk factors for cardiovascular disease (CVD) was assessed by Vorster, (2002) when they compared rural and urban Africans who participated in the Transition and Health during Urbanisation of South Africans (THUSA) study. The available data suggested that black South Africans might be protected against ischaemic heart disease (IHD) because of favourable serum lipid profiles and low homocysteine values. Ranjith *et al.*, (2005) examined differences in major cardiovascular risk factors and clinical outcome in South African Asian Indians of different age groups and

gender, who presented with acute coronary syndromes (ACS). Total cholesterol was elevated in 65 to 70% of all patients while high-density lipoprotein (HDL) levels were significantly lower in men compared with women for all age subsets.

In the present study cholesterol, triglycerides and LDL cholesterol levels were significantly higher in the Indian than African group thus supporting the data from the studies discussed above. In African and Indian subjects, anthropometric markers of abdominal obesity were major determinants of fasting lipid levels. Therefore the higher visceral fat and WHR in the Indian population may explain their more atherogenic lipid profiles. Many studies have shown associations between measures of abdominal fat and lipid levels (Zoratti, 1998, Banerji *et al.*, 1999, Despres *et al.*, 2000, Punyadeera *et al.*, 2001a, Crowther *et al.*, 2006).

Glycerol and FFA levels normally rise with increasing fat mass because adipose tissue, via lipolytic breakdown of triglycerides, is the major source of circulating glycerol and FFA (Jensen, 1997, Duncan *et al.*, 2007). Glycerol is thought to be a better indicator of adipocyte lipolytic activity (Robinson *et al.*, 1998). In the present study correlations were observed between both these variables and anthropometric measurements, particularly BMI (Indian group only) and hip circumference (both ethnic groups). The correlation with hip circumference is intriguing since studies in South Africa have shown that lipolytic rate is higher in femoral than abdominal adipose tissue in obese and diabetic African females (van der Merwe *et al.*, 2001) whereas studies in European populations demonstrated the opposite (Arner, 2005).

Fasting and total glycerol levels were higher in African than Indian females when all BMI groups were combined. This cannot be due to differences in anthropometry since BMI and hip circumference, which were the main determinants of glycerol levels, were the same in both ethnic groups. An alternative hypothesis may be that the higher TNF-α levels in African subjects may lead to greater lipolysis, as studies have shown that TNF-α can increase lipolytic rate (Arner, 2005, Langin *et al.*, 2006). However, in our study no correlations were found between TNF-α and glycerol (fasting and total) in either ethnic group.

Studies from South Africa have shown that lipolytic rate *in situ* is higher in subcutaneous adipose tissue sites in African than European males and females (van der Merwe *et al.*, 1999) and that isolated adipocytes from African females are more resistant to the anti-lipolytic activity of insulin than adipocytes taken from white females (Buthelezi *et al.*, 2000). These

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data plus the data from the current study suggest that adipocyte lipolytic rate is higher in African than both Indian and European females, but the metabolic consequences of this are uncertain.

No significant differences were observed in FFA levels between lean, obese and diabetic subjects in the African group, while in the Indian group there were significant differences between obese vs. lean and diabetic vs. obese. The exact reasons for these results are not clear. The high FFA levels in diabetic Indian subjects could be the result of high insulin-resistance and subsequent reduction of the antilipolytic effect of insulin.

It has been suggested that the higher FFA levels observed in African than white females may explain the higher level of insulin resistance in the former population (van der Merwe *et al.*, 2000, Buthelezi *et al.*, 2000) whereas in the present study the Indian females were more insulin resistant than the African females and FFA levels were not significantly different between the 2 groups.

4.5 Cortisol and lactate

It has been suggested that cortisol-metabolising enzymes may play a key role in determining body fat distribution. Increased regeneration of cortisol from cortisone within adipose tissue by 11 beta-hydroxysteroid dehydrogenase (HSD) type 1 (11HSD1) has been proposed to cause visceral fat accumulation (Bujalska *et al.*, 1997). However, in a study by Westerbacka *et al.*, (2003) visceral adipose fat mass was not associated with indices of cortisol metabolism; indeed, after adjusting for the effects of whole-body and liver fat, increased visceral fat was associated with lower cortisol metabolite excretion. They concluded that alterations in 11HSD1 and hepatic 5alpha-reductase activity are associated with generalized, rather than central, obesity in humans. In the present study waist circumference, but not visceral fat had a negative association with fasting and total cortisol (AUC) in the combined African group. The reasons for this association in the African and not the Indian group are not known and warrant further investigation.

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The presence of enhanced cortisol secretion in patients with type 2 diabetes is debated. According to the study by Reynolds *et al.*, (2003) the decline in plasma cortisol after glucose administration was poorly predictive of features of the metabolic syndrome. In type 2 diabetic subjects, hypothalamic-pituitary-adrenal activity is enhanced in patients with diabetic complications and the degree of cortisol secretion is related to the presence and number of diabetic complications as revealed in a study by Chiodini *et al.*, (2007). The current study demonstrated that diabetic African females had higher cortisol levels than non-diabetic obese African subjects. This difference was not observed in the Indian females. Whether this ethnic difference is related to a higher prevalence of diabetic complications in African than Indian females is not known. However, African diabetics did have a longer duration of diabetes and slightly higher HbA1c levels than the Indian diabetic group.

Differences in cortisol concentration in our study were observed between obese African and Indian women (higher in Indian). In another South African study obese white women were found to have higher fasting cortisol levels than obese black women (van der Merwe *et al.*, 2001). The present study also demonstrated that obese African women had lower cortisol (fasting and AUC) levels than lean African subjects. This supports other studies (Reynisdottir *et al.*, 1994, Wabitsch *et al.*, 1995) and may be explained by the higher cortisol clearance rates observed in obese as opposed to lean subjects (Pasquali *et al.*, 1993). This difference in cortisol levels was not seen in obese and lean Indian subjects, again suggesting that cortisol clearance by adipose tissue maybe higher in African than Indian subjects.

Adipose tissue production of lactate is influenced in vitro by changes in glucose, insulin, and epinephrine concentrations, while in vivo lactate production is regulated by the animal's nutritional state and chronically by the degree of obesity. During fasting the adipose tissue may provide lactate for hepatic gluconeogenesis and for hepatic glycogen synthesis after food ingestion (DiGirolamo *et al.*, 1992).

A number of our diabetic patients were treated with Metformin as an oral anti-diabetic drug. The most serious potential side effect of Metformin is lactic acidosis; this complication is very rare, and seems limited to those with impaired liver or kidney function. To establish the effect of Metformin on lactate levels, diabetic patients in both ethnic groups were divided into groups that consisted of the patients using Metformin and the group that was not on Metformin. There were no significant differences in lactate levels between the groups.

In a study to determine the primary mechanism by which Metformin improves glycemic control in patients with type 2 diabetes and its effects on lactate metabolism it has been shown that Metformin treatment did not significantly alter the mean fasting plasma lactate concentration or the rate of plasma lactate turnover. However, the rate of plasma lactate oxidation increased by 25 %, whereas the rate of conversion of plasma lactate to plasma glucose decreased by 37 % (Stumvoll *et al.*, 1995).

Fasting lactate levels in our study were significantly higher in the diabetic Indian than diabetic African group. Obese Indian subjects had significantly higher fasting lactate levels than lean Indian subjects. Both ethnic groups displayed significantly different fasting lactate levels between diabetic and obese subjects (higher in diabetics). There was a positive correlation between HOMA-IR and fasting lactate in the combined Indian group. No correlations were found in the combined African group between HOMA-IR and lactate levels. The data from the Indian subjects support other data showing that fasting lactate levels rise with increasing insulin resistance (Digirolamo *et al.*, 1992, Lovejoy *et al.*, 1992).

The absence of a relationship between fasting lactate levels and HOMA-IR in African subjects maybe related to the narrower range of HOMA-IR values, however fasting lactate was higher in diabetic than non-diabetic Africans. Studies have also shown that postprandial lactate levels correlate negatively with insulin resistance (Lovejoy *et al.*, 1990, Lovejoy *et al.*, 1992), however in our study there was a positive correlation between these variables in the Indian females and in both ethnic groups total lactate was higher in diabetic subjects.

4.6 Adipokines

The significant role of TNF-α in insulin resistance was confirmed in a number of studies. TNF-α inhibit the transduction of insulin signaling and down-regulate glucose transporter GLUT-4 and insulin receptor substrate-1(Hotamisligil,1999, Stephens *et al.*,1997, Hoffstedt *et al.*,2000, Bertin *et al.*,2000). Both TNF-α and IL-6 plasma levels have been found to be elevated in obese people, as compared with levels of age- and disease-matched normal-weight people (Ziccardi *et al.*,2002). Increased TNF-α levels amongst patients in a stable phase after

MI were associated with an increased risk of recurrent coronary events in a study from the secondary prevention Cholesterol and Recurrent Events (CARE) trial (Ridker *et al.,*2000b). Plasma levels of TNF-α have also been shown to correlate with measures of atherosclerosis as assessed by carotid intima-media thickness in healthy middle-aged men (Skoog *et al.,*2002). The results from our study didn't support these findings. Serum TNF-α didn't correlate with HOMA or other cardiovascular risk parameters in either of the ethnic groups. We also found that TNF-α concentrations were lower in diabetic and lean Indians compared to diabetic and lean African subjects and when obese, overweight and lean subjects were combined serum TNF-α concentrations were significantly lower in Indian than African subjects despite the higher HOMA levels in the Indian group. Not all studies report positive correlations between serum TNF-α levels and BMI or insulin resistance (Carey *et al.*, 2004) and one study has shown that TNF-α release from adipocytes has little influence on systemic TNF-α levels and therefore TNF-α effects are paracrine rather than endocrine (Mohamed-Ali *et al.*, 1997).

Since TNF-α stimulates IL-6 production in many tissues (Heinrich *et al.,*1990), it is not surprising that exogenous TNF-α can likewise promote IL-6 synthesis in adipocyte cell cultures (Berg *et al.,*1994). It is thus reasonable to postulate that increased production of IL-6 by hypertrophied adipocytes is simply a direct consequence of their increased production of TNF (McCarty,1999). CRP was the strongest correlate of IL-6 in the data by Ridker *et al.,*(2000c), which is consistent with IL-6 being the main stimulant for the hepatic production of CRP.

In the present study we didn't find interethnic differences for IL-6 levels. Diabetic Indian women had significantly higher IL-6 values compared to obese Indian women and this was also observed in the African group although the difference was not statistically significant. Other studies have also found higher IL-6 levels in diabetic subjects (Deepa *et al.*, 2006, Cardellini *et al.*, 2007, Pitsavos *et al.*, 2007) but not all studies support this (Carey *et al.*, 2004). No correlations were found between IL-6 and anthropometric variables in the combined African and Indian groups. Therefore, IL-6 cannot explain higher prevalence of diabetes or CVD in the Indian population. No correlations were found between IL-6 versus CRP and TNF-α in combined African and Indian groups in our study.

Pradhan *et al.,* (2001) found a stronger relationship between baseline elevations of CRP and incident diabetes than that seen for IL-6. This may partially indicate the significantly longer plasma half-life of CRP that thereby may provide a more stable indication of sub-clinical inflammation. In a study by Blake *et al.,*(2002) of all the inflammatory and lipid parameters, CRP was the strongest predictor of cardiovascular risk in univariate analyses. Plasma levels of CRP are good independent predictor of future myocardial events, stroke, peripheral vascular disease and vascular death among individuals without known cardiovascular disease (Ridker,2001). Moreover, circulating levels of CRP are elevated in human obesity and correlate with body weight, visceral fat and insulin resistance (Tchernof *et al.,*2002). Diabetic patients are reported to have increased CRP values (Ford,1999); in this regard, links between CRP and the insulin resistance syndrome have also been reported (Festa *et al.,*2000).

Inflammatory markers such as CRP, fibrinogen and TNF-alpha were associated with type 2 diabetes in North-Indian subjects, but only CRP was associated with development of accelerated atherosclerosis and subsequent CHD (Ahmad *et al.,* (2007). As a general index of inflammation, CRP concentrations vary by ethnic origin and within ethnic groups by fitness (Chambers *et al.,*2001, LaMonte *et al.,*2002). For instance, concentrations of CRP were higher in healthy Indian Asians than in European white population and were related to greater central obesity and insulin resistance in Indian Asians (Chambers *et al.,* 2001).

These data were supported in the present study by the observation that serum CRP levels were significantly higher in diabetic and obese subjects compared to lean subjects in both ethnic groups. Visceral fat and BMI were the only independent predictors of CRP levels observed in the combined African group and in the combined Indian group, respectively. However, despite the fact that the Indian population of South Africa have been reported (Seedat *et al.,* 1990, Omar *et al.,* 1985, Omar *et al.,* 1994, Motala *et al.,* 1993) to have a higher prevalence of ischaemic heart disease, cardiovascular risk factors and type 2 diabetes, in the present study CRP levels in combined African and Indian groups were similar. Thus, within these ethnic groups, CRP may possibly act as a marker for increased risk of CVD and diabetes but it does not explain ethnic differences in obesity- related disease prevalence rates.

The interaction between insulin sensitivity and leptin concentration may be important in the regulation of body weight. It has been hypothesized that insulin resistance itself protects against weight gain. Segal *et al.,* (1996) suggested that insulin resistance might help prevent

obesity by increasing plasma leptin concentrations. They also demonstrated that insulin resistance, independent of adiposity, was associated with elevated plasma leptin concentrations. Although the researchers found that insulin did not regulate leptin production acutely, they proposed that chronically elevated plasma insulin concentrations possibly stimulate obese (Ob) gene expression. However, the increase in plasma leptin concentration observed with increasing adiposity suggest that obese humans may be resistant to the putative effects of leptin in modulating food intake and energy expenditure (Considine *et al.*,1996b).

Black women had higher leptin levels compared to white women, despite comparable BMI's (Der Merwe *et al.*, (1999). The higher leptin levels in the black population may represent a form of leptin resistance due to defective leptin signalling, resulting in hyperglycaemia and a possible over-expression of hypothalamic NPY (Schwartz *et al.*, 1996). The study by Buyukbese *et al.*, (2004) in the eastern Mediterranean area of Turkey, showed that the leptin levels were lower in diabetic obese women than obese non-diabetic women. Tatti *et al.*, (2001) in their study compared leptin concentration in moderately obese type 2 diabetics, age and weight matched, with non-diabetic controls. The leptin levels were lower in the diabetic population only when both sexes were combined and were higher in the females of both groups. When plasma leptin was included in a multiple linear regression model with plasma leptin as a dependent variable, BMI, waist/hip ratio and fasting serum insulin levels were significantly related to leptin in the non-diabetic population, while no relationship reached the level of statistical significance among the diabetics. The in vitro data by Ceddia *et al.*, (1999b) provided some evidence that leptin might modulate insulin secretion from pancreatic islets. The above studies support data from our study. Thus, in both ethnic groups, leptin levels were lower in diabetic than non-diabetic obese subjects.

The Indian group are not iso-obese with the African subjects, even when matched for BMI as they differ significantly with regard to visceral and subcutaneous fat area. Furthermore, lean Indian subjects have more visceral and subcutaneous fat than BMI-matched lean Africans and also have higher leptin levels. Regression analysis demonstrated that the higher leptin levels are partially explained by the higher subcutaneous fat mass but the major determinant of leptin levels in the lean subjects is ethnicity. This suggests that some unknown factor, specific to ethnicity is influencing leptin levels in this group of subjects. Also, in our study there was a significant positive correlation between serum leptin and total (AUC) insulin levels in both

ethnic groups. In the African group leptin had a positive correlation with total (AUC) glucose levels while in Indian group leptin had positive correlation with fasting insulin levels.

Results from Hube *et al.*, (1996) indicated that leptin expression was lower in omental than subcutaneous adipose tissue, possibly due to differences in fat cell size and/or sympathetic innervations. In another study, multiple regression analyses pointed out that percentage body fat was the most important determinant of leptin for all obese subjects (males and females), while for women subcutaneous fat was the most important parameter, and for men alone total abdominal fat. These results suggested that subcutaneous fat seems to be an important factor related to leptin levels (Wauters *et al.*, 1998), and this supports data from the present study where, in the combined African group body fat (%) and subcutaneous fat were significant determinants of the variance in leptin. Body fat (%) was the only independent variable that could explain variance in leptin in the combined Indian group. Subcutaneous fat and BMI had an association with soluble leptin receptor in the combined African group and in the combined Indian group, respectively. No correlations were found between leptin and FFA or glycerol in both ethnic groups.

Ogier *et al.*, (2002) in their study found that soluble leptin receptor (sOB-R) levels were lower in obese and overweight than lean subjects and were inversely correlated to leptin and percentage of body fat. A gender difference was observed in sOB-R levels, which were higher in obese and overweight men than in obese and overweight women. The relationship of sOB-R with the degree of adiposity suggested that high soluble leptin receptor levels might enhance leptin action in lean subjects more than in obese subjects.

Ogawa *et al.*, (2004) in their study measured the serum soluble leptin receptor level in healthy Japanese subjects and in type 2 diabetic patients. Serum leptin and soluble leptin receptor levels were not significantly different between healthy subjects and diabetic patients. The researchers concluded that the serum soluble leptin receptor level was negatively correlated with HOMA-IR and serum leptin level and positively correlated with HDL-cholesterol level and serum adiponectin level, independent of age, sex, and BMI, in the Japanese population.

Leptin and soluble leptin receptor in our study didn't have any correlations with lipids, hormones or adipokines when corrected for age and BMI in the combined African group. However, in the Indian population a positive correlation was found between leptin and TNF-α. Leptin could modulate TNF-alpha production and macrophage activation (Loffreda *et al.*,

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1998). In adipose tissue of several rodent models of obesity the overproduction of TNF-alpha has an important role in the pathogenesis of insulin resistance in these species (Bastard *et al.*, 2006).

Levels of the sOb-R are decreased in obese subjects compared to lean controls, which results in an increased fraction of free leptin. After the surgical procedures or in body weight loss through diet the levels of circulating sOb-R are significantly increased. sOb-R may play a role as a modulating factor of leptin action and leptin resistance, but the exact physiological mechanisms regulating sOb-R plasma concentration are not known (Sandhofer *et al.*, 2003).

Serum sOB-R concentrations did not differ significantly between normal-weight Japanese men and women, but were significantly higher in underweight subjects than in normal-weight subjects. In contrast, overweight and obese subjects had significantly lower sOB-R concentrations than did normal-weight subjects. Therefore, the researchers hypothesized that a reduction in serum sOB-R concentrations in overweight and obese persons may reflect down regulation of hypothalamic leptin receptor production as a result of an increase in circulating leptin and might be an important factor in leptin resistance (Shimizu *et al.*, 2002).

The results from the study by Lewandowski *et al.*, (1999) indicated that there was no significant difference in free or bound leptin levels between the normal and insulin-dependent diabetic subjects either during pregnancy or postpartum, but female insulin-dependent diabetic subjects had significantly higher soluble leptin receptor levels and so they speculated that high soluble leptin receptor levels might be implicated in the development of the leptin resistance in this group.

Serum leptin levels in our study were significantly lower and soluble leptin receptor higher, in diabetic than obese subjects in both ethnic groups. As expected, obese subjects had higher leptin levels and lower soluble leptin receptor than lean subjects in both ethnic groups. Interethnic differences for leptin levels were observed in the lean group of women with higher levels in the Indian subjects and when obese, overweight and lean subjects were combined serum leptin levels were significantly higher in Indian than African subjects. This is an intriguing result, since obesity is more common in the African than Indian populations of South Africa (Puoane *et al.,* 2002). We may therefore hypothesize that the lower leptin level in lean African females may lead to higher dietary intake and thus lead to an increased prevalence of obesity in this group. Caloric intake was higher in lean African than Indian

females in this study. However, this hypothesis must be evaluated in a longitudinal study of leptin levels and weight gain.

Insulin resistance are linked with low serum adiponectin concentrations. However, ethnic differences in adiponectin levels probably do not explain the higher levels of insulin resistance observed in the Indian subjects in the present study as already demonstrated in a previous study, which was specifically designed to investigate whether interethnic variations in insulin resistance might be due to differences in plasma adiponectin levels. The group of subjects included black, white and Asian-Indian males and females. Serum adiponectin levels were found to be similar in black and Asian-Indian subjects, but significantly lower compared to BMI-matched white subjects. The correlation between HOMA and adiponectin was analyzed in each ethnic group, but significant negative correlation was only observed in white subjects (Ferris *et al.*, 2005). Another study aimed to determine differences in fasting adiponectin concentrations between Caucasian and African women. The results showed no differences for overweight and obese women, but normal weight African women had marginally lower adiponectin levels than their Caucasian counterparts. Multiple regression analyses showed that only HOMA-IR significantly contributed to the variance in adiponectin levels of African women, whereas leptin, triacylglycerol levels and HOMA-IR contributed significantly to adiponectin variance in Caucasian women. Ethnicity played a significant role in adiponectin levels (Schutte *et al.*, 2007).

4.7 Nutritional intake

The rapid increase in prevalence of diabetes mellitus may emerge as a main public health problem in South Africa. Several epidemiological studies have shown that the prevalence of type 2 diabetes is soaring, at 13% in the Indian community of Durban and at 7% in black urban women (Omar *et al.*, 1994, Joffe *et.al.*,1994a). The effect of urbanization and unhealthy lifestyles are important contributors to this rising prevalence (Knowler *et al.*,2002). One of the aims of lifestyle change is for the patient to lose weight. It is noteworthy that modest weight loss pays large dividends in correcting insulin resistance, dyslipidaemia, reducing blood

pressure, improving dysglycaemia and ultimately reducing cardiovascular events (Zimmet et al., 2003, Higgins *et al.,*1988). The importance of regular physical activity combined with education about food selection is advised. The benefits of smoking cessation are substantial in the short- and long-term, and go beyond a reduction in cardiovascular risk (Buse *et al.*, 2007). In the current study the total energy intake was much higher in the African group despite similar BMI and body fat (%) when compared to the Indian subjects. This suggests that the African subjects may have higher basal metabolic rate (BMR) and /or physical activity levels than the Indian group. Indeed our data does show higher levels of both these variables in the African group but only reaching statistically significant differences for physical activity in the obese African subjects. Future studies using more accurate measure of BMR and physical activity must be performed to confirm these findings.

Trans fatty acids originate primarily in hydrogenated vegetable oils tend to elevate cholesterol levels in contrast to their non-hydrogenated counterparts (ASCN/AIN,1996, Report of the Expert Panel,1995). Much higher increases found in similar amounts of saturated animal fat or highly saturated vegetable oils, e.g., coconut and palm kernel oils have much higher increases.

The intake of *trans* fatty acids was analysed using plasma or tissue levels of *trans* fatty acids and it was proposed that CHD risk is associated with *trans* fatty acids derived from animal products but not with those from hydrogenation of oils (Wahle *et al.*,1993). Hu *et al.*, (2001) and Rivellese *et al.*, (2002) had examined whether the composition of dietary FAs, as opposed to total fat consumption, can modulate insulin sensitivity and cardiovascular risk factors. These findings indicated that a higher intake of polyunsaturated fat could be beneficial, while a higher intake of saturated fat and trans-fat could negatively affect glucose metabolism and insulin resistance. Epidemiological studies have show that high intake of saturated fat is associated with insulin resistance, and this relationship may be dependent on increased body adiposity (Mayer-Davis *et al.*, 1997). High intake of both saturated and *trans* FAs is related with hyperinsulinaemia and with a risk of developing type 2 diabetes, independent of general obesity as shown in multiple cross-sectional studies (Maron *et al.*, 1991, Marshall *et al.*, 1997, Parker *et al.*, 1993). High intake of polyunsaturated FAs (PUFAs) does not emerge to have the same adverse effects and may even result in an increase in insulin sensitivity (Salmeron *et al.*, 2001).

Food intake data in our study, after exclusion of LER's, showed no interethnic differences in diabetics, obese and lean groups for the consumption of total fat, and saturated, monounsaturated, polyunsaturated and total *trans* fatty acids. In combined groups (excluding diabetics and LER's), saturated fatty acid values were significantly higher in African group, but no differences were observed in total fat, monounsaturated, and polyunsaturated, and total *trans* fatty acid intake. However, it is interesting to note that dietary intake of all these different types of fat tended to be higher in Africans than Indian with the exception of total *trans* fatty acid intake which was higher in Indians, particularly obese Indians. However this difference did not reach statistical significance.

The normal metabolism of homocysteine requires sufficient source of folate, vitamin B6 vitamin B12, and riboflavin" (Pancharuniti *et al.*,1994). Increased risk of developing coronary disease can be associated with lower folate levels (Robinson *et al.*,1998). In the present study, folate intake was surprisingly lower in Indian subjects, but this statistical difference was lost when LER's were removed.

A study by Charlton *et al.*,(2005a) demonstrated ethnic differences in calcium intake across South African ethnic groups, with black subjects having particularly low intakes. The groups included black, mixed ancestry and white men and women from Cape Town. Food intake questionnaire data in our study, after excluding low energy reporters, show that calcium and magnesium intake was significantly lower in the combined Indian group than in the combined African group. The differences in calcium intake in this population group could be explained by higher consumption of dairy products, notably powdered milk.

Food intake data from our study, after exclusion of LER's, showed that diabetic Indian had statistically significant lower intake in total dietary fibre, magnesium, phosphorus zinc, but higher intake of starch, fibre and chloride in comparison with diabetic African patients. Added sugar was significantly lower in diabetic Indian patients than in obese Indian group.

The table below (Table 4.1) compares nutritional intake in diabetic patients from the present study and published data from South Africa. Diabetic Africans had comparable total energy and total fibre intake with published data. The data for other nutrients showed significant differences between results from our study and the data published by Nthangeni *et al.*, (2002). Such differences may be related to the different methods used for assessing dietary intake, and this is a well-known problem when comparing nutritional intake across different studies.

Furthermore, differences in socio-economic status between the study groups and differences in geographical location may also explain the differences in dietary intake between the studies.

Table 4.1 The average energy estimate and nutritional intake per day in diabetic women

Groups / Variables	African diabetic women (Present study)	Indian diabetic women (Present study)	Urban African diabetic women (Nthangeni et al., 2002)	Rural African diabetic women
Total Energy (kJ)/d	7163	5841	7381	6987
Total Protein (g)	82.2	60.2	65	59
Plant Protein (g)	24.7	19.0	39	39
Animal Protein (g)	57.0	40.4	26	20
Total Fat (g)	52.9	51.9	28	27
Saturated FA (g)	17.7	15.7	7.2	6.1
Monounsaturated FA (g)	17.6	16.9	9.0	8.9
Polyunsaturated FA (g)	12.7	14.9	7.4	8.2
Total Carbohydrate (g)	204	156	288	274
Added sugar (g)	17.6	17.2	4	2.1
Total sugar (g)	42.4	36.9	16	14
Total Dietary fibre (g)	19.7	14.7	21	19

Lean Indian subjects in our study had significantly lower energy intake and total carbohydrate, total dietary fibre, magnesium and zinc intake in comparison with lean African subjects. Similarly, there were also significant differences between obese Indian and obese Africans for consumption of total dietary fibre, magnesium, phosphorus and zinc, with all intakes higher in the African group.

Food intake data in combined African and Indian groups (excluding diabetics and LER's) showed that there were statistically significantly higher intake for African than Indian women for most of the food variables, with the exception of sodium, chloride, vitamin C, folate, vitamin B12, total carotenoids and chromium, where intake were similar and lycopene and starch where intake were significantly higher in Indians. Lower intake of tomato-based products explains the lower lycopene levels in Africans. Sugar in plants is stored as starch and the greater starch intake in Indian subjects might be due to higher use of vegetables and fruits in their diet.

The table below (Table 4.2) compares the nutritional intake in African and Indian subjects from the present study with published South African data. The total energy intake, total protein, animal protein, cholesterol, vitamins C and D, and calcium intake in the African group from our study was much higher than the results from the published data, and may reflect study differences in measurement of dietary intakes and differences in socio-economic status of study populations.

Dietary macronutrient intake in urban blacks and Indians according to the data published by Vorster *et al.*, (2001) were as follows: total carbohydrate 161.6 and 102.5, respectively; added sugar 45.8 and 48.4, respectively and total dietary fibre 14.6 and 13.1, respectively. These published data are similar to our results with the exception of total carbohydrate intake where the intakes in our study were considerably higher. However, both studies show that total carbohydrate intake was higher in Africans than Indians.

Table 4.2 The average energy estimate and nutritional intake per day in African and Indian population

Groups Variables	African group	Indian group	Urban African population	South African adult women	Middle class urban African population
	(Present study)	(Present study)	(Bourne *et al*., 1993)	(Steyn *et al*., 2006)	(MacIntyre *et al*., 2002)
Total Energy (kJ)/d	8691	6131	5799	6808	8010
Total Protein (g)	86.3	60.7	49	59.3	59.5
Plant Protein (g)	25.4	16.4	22	28.1	30.2
Animal Protein (g)	60.1	42.9	28	31.0	29.1
Total Fat (g)	68.3	54.5	42	41.9	58.8
Saturated FA (g)	24.0	17.7	15	12.3	18.3
Monounsaturated FA (g)	21.6	17.8	15	14.3	20.3
Polyunsaturated FA (g)	16.3	14.4	8	9.3	14.8
Total Carbohydrate (g)	255.7	167.2	198	232.0	283.6
Added sugar (g)	49.5	31.8	38	38.9	-
Total Dietary fibre (g)	20.5	14.3	13	18.0	17.1
Calcium (mg)	860	586	358	388.4	405
Total iron (mg)	9.77	7.32	6	8.9	8.8
Zinc (mg)	9.88	6.79	6.8	8.4	8.2
Sodium (mg)	1548	1204	979	1193.4	-
Vitamin B12 (mcg)	5.13	3.48	3.5	3.5	1.11
Vitamin D (mcg)	5.0	3.1	-	1.8	-
Vitamin C (mg)	105.8	89.7	32	47.0	43.2
Folate (mcg)	209.8	165.8	147	230.8	209

Mean nutrient intakes in our study were compared to the Recommended Dietary Allowances (RDAs) or to the Adequate Intakes (AIs) according to the data published by National Academy of Sciences (http://www.nap.edu.). The results from our study showed that total protein intake in African and Indian groups was 47% and 24%, respectively higher than that of RDAs. This same pattern was observed for the total carbohydrate intake with 49% higher in Africans and 22% higher in Indians than that of RDAs. However, the total dietary fiber intake fell below the RDAs in both ethnic groups, 22% lower Africans and 75% lower in Indians. Our study also showed that intakes of calcium, folate and total iron appeared to be the most deficient in the diet in both ethnic groups when compared to that recommended by the National Academy of Sciences (RDAs/AIs: 1000mg, 400mcg and 18mg, respectively).

Vitamin D deficiency has been identified as a potential novel cardiovascular disease risk factor but many studies suggested that the link between hypovitaminosis D and metabolic disorders, including obesity, metabolic syndrome, type 2 diabetes and CVD requires further investigation, particularly for those most at risk of these combined conditions (Martins *et al.*, 2007, Michos *et al.*, 2008, McGill *et al.*, 2008). The lack of relationship of vitamin D and metabolic syndrome has been reported previously (Reis *et al.*, 2007). However, in the large USA NHANES dataset, it was found that abdominal obesity as measured by waist alone, in addition to metabolic syndrome, was related to low vitamin D_3, notably affecting mixed-ethnicity participants equally (Ford *et al.*, 2005).

The main source of body iron is derived from the diet. Dietary iron is present as either heme (derived from meat and meat products) or nonheme (cold breakfast cereal) iron. In recent prospective studies, intake of total or nonheme iron was not linked with the risk of type 2 diabetes, but heme iron was associated with elevated risk (Jiang *et al.*, 2004, Lee *et al.*, 2004, Rajpathak *et al.*, 2006).

Development of cardiovascular disease (CVD) have been linked with involvement of Vitamin E in the prevention of oxidation of unsaturated lipids in the low-density lipoprotein (LDL) particle that activates a complex sequence of events and directs to the development of atherosclerotic plaque (Pryor, 2000). The results from clinical trials with beta-carotene supplements have been distressing so far and the consumption of vitamin E as a preventive intervention for cancer and coronary heart disease should be discouraged. The relevant clinical affects of vitamin E on the risk of cardiovascular events were absent in the results of the

GISSI-Prevenzione (300 mg/d) and HOPE (400 mg/d) trials (Marchioli *et al.*, 2001).

The present study results also reflect those from published data suggesting a protective association of vitamins E, D and iron intake with development of CVD in African population. Lean Indian females in our study had significantly lower vitamin D and vitamin E intakes in comparison with lean African subjects. In combined ethnic groups (excluding diabetics and LER's) African women had significantly higher vitamin E and D than Indian women. Our data, after exclusion of LER's, showed that diabetic Indian subjects had statistically significantly lower total iron intake in comparison with diabetic African patients. In combined ethnic groups (excluding diabetics and LER's) African women had significantly higher iron intake in comparison to Indian women (10.9 vs. 8.3, 5.9 vs. 3.4 and 11.0 vs. 8.6, respectively). This could be attributed to the larger consumption of eggs, beef, and chicken, fish, peanut butter and sunflower oil in the African population.

A comparison of fat and carbohydrate dietary intake expressed as % of total energy intake for adults aged 15-64 years in 1990, with that of black 'adults' (age range not provided) from the city of Johannesburg in 1940 shows a relative 10.9% reduction of carbohydrate (from 69.3 to 61.7% of energy in 1990) and an increase of 59.7% in fat intake (from 16.4 to 26.2 of energy in 1990) over a 50-year period (Bourne *et al.*,2002). As a continuation of this trend we found a 19.4% decrease of % carbohydrate intake (49.7% of total energy intake) and 11.8% increase of % total fat intake (29.3% of total energy intake) in the combined African group, from the 1990 data.

There are not inadequate data to determine a defined level of fat intake; therefore neither an Adequate Intake (AI) nor Recommended Dietary Allowance (RDA) was set by National Academy of Sciences. The total fat as 20- 35 percent of energy have been estimated as an Acceptable Macronutrient Distribution Range (AMDR). The ranges for carbohydrate and protein intake as a percent of total energy intake were estimated in a region of 45-65 and 10-35, respectively in adults (http://www.nap.edu.).

Our results for total protein, total fat and total carbohydrate when expressed as % of total energy intake were in agreement with that recommended by National Academy of Sciences in both ethnic groups before and after excluding LERs. In combined African and Indian groups in our study a comparison of total protein, total fat and total carbohydrate proportions of energy intake, expressed as a %, indicated significant differences for fat (higher in Indian) and

carbohydrate (higher in African). A similar pattern was found after excluding low energy reporters in these groups. Greater proportional intake of energy from fat in the Indian diet may predispose this population to cardiovascular disease (Hu *et al.*, 1997, Jakobsen *et al.*, 2004). Greater total energy intake in African than Indian females may explain the higher prevalence of obesity in the African population. Furthermore the higher proportional intake of carbohydrates in the African group may promote fat storage through maintaining higher blood levels of insulin (Sheard *et al.*, 2004).

4.8 Influence of socio-economic status

Socio-economic status in the present study was based on household items as well as education level of participants in the study. Although cultural differences might exist between two ethnic groups, the general assessment of socio-economic status should be applied to both groups equally. The study by Xie *et al.*, (2003) described the overall diet and potential effects of gender, ethnicity, family income, and parents' education on dietary patterns in adolescents aged 11 to 20 years who participated in a cohort study in 12 Southern California communities between 1998 and 2000. Higher consumption of polyunsaturated fat, protein, calcium, and folate and more frequently consumed dairy products found to be in subjects form higher income families. In families with higher levels of education intakes of total fat, saturated fat, monounsaturated fat and cholesterol have been reduced. The recommended daily intake of dairy products, fruits, and vegetables were less likely is consumed by subjects from families with parents who had higher educational attainment.

Islam *et al.*, (2004) studied the influence of socio-economic status on energy intake, anthropometric characteristics and body composition in pre-menopausal women in two locations in Bangladesh. Based on the dietary and anthropometric results, they concluded that malnutrition is a common feature among low-income rural women, which is in contradiction to data from western countries, where obesity is prevalent in low-income groups.

The survey conducted by Rebato *et al.*, (2001) in the town of Bilbao found that all anthropometric variables, except height, were higher in women of low socio-economic status

compared to the local reference and control sample. Low socio-economic males had lower estimated percentage of body fat than the control sample, while females showed the opposite pattern. On the other hand, estimates of food intake in males did not reveal great differences among samples from different socio-economic backgrounds, while low socio-economic status females had greater intakes of food than the better-off control sample. A study by Dastgiri *et al.*, (2006) showed a negative correlation of obesity with education and income for both women and men in Tabriz, one of the major cities in Iran.

These results are in concordance with our study in the combined Indian group, but no correlations were found between anthropometric variables and socio-economic status in the combined African group. In the combined Indian group education had negative correlations with BMI, WC, waist/height and body fat (%), subcutaneous and visceral fat. Household amenities had negative correlations with WHR in the combined Indian group. Furthermore, household amenities and education displayed statistically significant differences in the combined group, the values being higher in the Indian subjects. This suggests that one of the reasons for the higher prevalence of obesity in the African than Indian populations maybe related to lower socio-economic status and educational attainment in the former population; however one must keep in mind the lack of correlation between anthropometry and socio-economic status in the African population.

Socio-economic status may influence the risk of the metabolic syndrome. Park *et al.*, (2007) studied this association in Korean adults and found that lower socio-economic status was associated with a higher risk of the metabolic syndrome in Korean women but not in Korean men. The highest prevalence of type 2 diabetes was observed among the rich, and the lowest prevalence was observed among the poor socio-economic classes of rural and urban populations in Bangladesh (abu Sayeed *et al.*, 1997). Larranaga *et al.*, (2005) who studied people older than 24 years in the Basque Country, Spain, established that the prevalence of known Type 2 diabetes was higher in patients of lower socio-economic status, especially among women. These observations are in concordance with our results where we found that diabetic African patients had significantly lower educational level in comparison with obese non-diabetic subjects. In the Indian group significant differences in education were observed between obese and lean women (higher in lean).

In the present study education and household amenities had positive correlations with insulin (AUC) in the combined African group. Education correlated negatively with proinsulin/insulin ratio (AUC) and had positive correlation with insulinogenic index in the combined African group. In the combined Indian group education had negative correlations with diastolic blood pressure and insulin (AUC) and positive correlation with proinsulin/insulin ratio (AUC). Education was a strong independent predictor of the variance in insulin (AUC) in the combined Indian group. It is interesting that greater educational attainment has opposite influences on markers of insulin secretion in these two population groups. The reasons for this are unknown but highlight the complex interaction between environmental factors and metabolism and how these interactions vary across different ethnic and cultural groups.

CHAPTER-5 CONCLUSIONS AND SHORTCOMINGS

5.1 CONCLUSIONS

The objectives of our study are given below and following each objective are the conclusions from our study that relate to that objective:

1. To assess the body fat distribution in both ethnic groups & to determine how it influences glucose & lipid metabolism.

- The significantly higher level of visceral fat in Indian women in comparison with African women could lead to increased levels of insulin resistance and may be a greater risk for development of type 2 diabetes in the female Indian population.

- Low-density lipoprotein (LDL) was statistically higher in the lean group of Indian subjects, which suggests that Indian population might be predisposed to the development of IHD via raised cholesterol levels.

- Significantly higher triglycerides levels in obese Indian subjects could lead to the higher prevalence of CVD in Indian population.

2. To assess the role of anthropometric, metabolic and hormonal factors in determining insulin resistance in each ethnic group.

- There may be an increased risk of type 2 diabetes in the female Indian population as a result of higher HOMA-IR and visceral fat levels in this group compared to African female subjects.

3. To compare the aetiology of type 2 diabetes between the two ethnic groups, particularly with reference to beta cell function and insulin resistance.

- Higher insulin resistance, which is compensated for by reduced HIE and improved proinsulin processing in the non-diabetic Indians compared to the non-diabetic African

group may predate glucose intolerance and type 2 diabetes in the Indian population.

- Ethnic differences in HOMA-IR could not be explained by differences in adipokine concentrations; however, HOMA-IR was related to visceral fat in the Indian group.

4. To examine the relationship of anthropometric and metabolic indices with blood pressure, in each ethnic group.

- Ethnic differences in the determinants of blood pressure in relationship to anthropometric variables showed the importance of abdominal obesity in the African group whilst in Indian group total body fat was more important.

5. To ascertain the role adipokines play in the aetiology of insulin resistance in each ethnic group.

- Leptin levels correlated positively with systolic BP in the Indian, but not in the African subjects, suggesting that ethnic differences in the aetiology of obesity-related hypertension exist.

- The significance of the higher TNF-α values measured in the African subjects is unknown.

- Lower leptin concentrations observed in the lean African subjects may promote caloric intake and hence a rise in obesity.

6. To determine the impact of socio-economic and dietary status on intermediary metabolism in each ethnic group.

- The higher prevalence of obesity in the African population may be explained by a greater energy intake in this population compared to the Indian population.

- The greater proportional intake of energy from fat, in the Indian diet, may predispose this population to cardiovascular disease.

- The association between socio-economic status with anthropometric variables in the Indian but not in the African group suggest that the higher prevalence of obesity in the African than in the Indian population maybe related to lower socio-economic status and educational attainment in the African population. Data from the literature also demonstrates a negative correlation of obesity prevalence with education and income.

In conclusion, we can state that there are three major findings from this study. First, the higher visceral fat level, higher HOMA-IR and adverse lipid profile may lead to the higher

prevalence of cardio vascular disease and greater risk for development of type 2 diabetes in the female Indian population. Second, the differences in adipokine levels do not fully explain the ethnic differences in the level of insulin resistance in these two groups. And third, the higher prevalence of obesity in female African population may be explained by the higher energy intake in this population which could be related to lower socio-economic status. Furthermore, the low leptin levels observed in the lean African females may underline the higher prevalence of obesity in this population group. This hypothesis must be tested by a longitudinal study in which weight gain in lean Indian and African females is compared and analysed against baseline leptin levels.

5.2 SHORTCOMINGS OF THE CURRENT STUDY

We acknowledge that there are shortcomings of the present study. The N numbers are relatively small; nevertheless, the results of our study are consistent with larger epidemiological studies and were sufficiently high enough to demonstrate ethnic or BMI related differences in a number of variables. The diabetic Indian group had statistically significantly higher age ($p<0.05$) than the diabetic African group and therefore in our statistical analysis it was necessary to make corrections for age.

The CT-scan analysis performed in the current study did not allow us to measure muscle area at the gluteal and mid-thigh level. Such an analysis in future studies may be important for determining whether ethnic differences in insulin sensitivity may be due to differences in muscle mass.

Although ethnic differences in variables reported in our study are robust to statistical control of household amenities and education in multiple regression analysis, this relatively crude adjustment may be insufficient to disentangle "true" race/ethnic group affects from those due explicitly to socio-economic status.

The food questionnaire assessment used in our study experienced higher numbers of low energy reporters particularly in the Indian group. The food intake questionnaire used in our study was validated for the South African black population, but had not been validated for

the South African Indian population. In view of these shortcomings, we recommend that future studies examining the nutritional status of the South African Indian population should develop a more reliable questionnaire, tailored to the dietary intake of this population.

Another limitation is that this study was conducted in one geographic location and results may not apply to women living in other parts of rural or urban South Africa. Subjects were recruited from the Gauteng province with the aim of gathering a sample that accurately represents the urban group in that region.

The gold standard for investigating and quantifying insulin resistance is the "hyperinsulinemic euglycemic clamp". Given the complicated nature of the "clamp" technique and the number of subjects in our study we decided to use HOMA to assess insulin resistance. Another shortcoming in the current study is that subjects were not matched for age or socio-economic status, therefore all results have been age adjusted whereas socio-economic status was not a major predictor for any of the variables in our study. We must also state that the measurement of basal metabolic rate from bioelectrical impedance analysis is crude.

APPENDIX 1

Human Ethics Committee Clearance Certificate

Department of Chemical Pathology

University
of the Witwatersrand,
Johannesburg

Medical School, 7 York Road, Parktown, 2193 South Africa • Telephone: +27 11 717-2521 or 717-2021 • Student Enquiries: +27 11 717-2123
Fax: +27 11 489-8451 or 717-2521
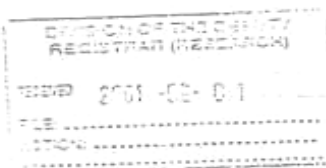
Tel: 489 8559
E-mail: 158njcro@chiron.wits.ac.za

30th January, 2001

To The University of the Witwatersrand Committee for Research on Human Subjects
(Medical):

We would like to make changes to our present research protocol (Protocol Number:
M 970618). The changes are:

1. Study will be extended to obese, lean and diabetic Indian females who will be compared
to African females.
2. The number of subjects per each of these 3 groups will be 20.

These changes do not require that we make alterations to the consent forms.

Yours sincerely,

Dr NJ Crowther

APPROVED
3 1 JAN 2001
PROF. PE CLEATON-JONES

216
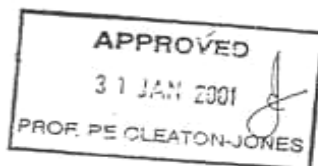
APPENDIX 2

Biochemical Investigations

1. Determination of glucose in plasma

Glucose in plasma samples was analysed on Roche MODULAR, by using a glucose oxidase assay from Roche Diagnostics (used according to method specifications).

Test principle:

Buffer containing Adenosine 5'-Triphosphate (ATP) and Nicotinamide-Adenine Dinucleotide Phosphate (NADP) added to sample, followed by addition of hexokinase (HK) and Glucose-6-Phosphate Dehydrogenase (G-6-PDH).

$$Glucose + ATP \xrightarrow{\text{HK}} G\text{-}6\text{-}P + ADP$$

Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP

$$G\text{-}6\text{-}P + NADP^+ \xrightarrow{\text{G-6-PDH}} gluconate\text{-}6\text{-}P + NADPH + H^+$$
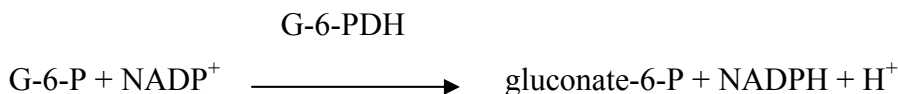
Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH-formation (reduced form of NADP) during the reaction is directly proportional to the glucose concentration and can be measured photometrically.

Measuring/reportable range:

Serum/plasma: 0.11-41.6 mmol/l (2-750 mg/dl)

Precision:

Intra-assay CV= 1 %; Inter-assay CV=1.7 %

Analytical sensitivity:

Detection limit:0.11 mmol/l (2mg/dl)

2. Lactate determination in plasma

Enzymatic in vitro test for the quantitative determination of lactate in plasma was performed on automated Roche/Hitachi MODULAR analyzer (method was used according to the instructions).

Test principle:

The method uses an enzymatic reaction to convert lactate to pyruvate. The hydrogen peroxide produced by this reaction is then used in an enzymatic reaction to generate a coloured dye (Trinder,1969, Barham *et al.,*1972).

Measuring / reportable range: 0.2 – 15.5 mmol/l (2.0- 140 mg/dl)

Precision:

Intra-assay CV = 0.4 %; Inter-assay CV =1.0 %

Analytical sensitivity:

Detection limit: 0.2 mmol/l (2.0 mg/dl)

Limitations – interference:

At high concentrations, Dopamine (10 mg/l), Levadopa (20 mg/l) and Methyldopa (20 mg/l) significantly reduce the lactate results. However, Dopamine at 1 mg/l, Levadopa at 4 mg/l and Methyldopa at 2 mg/l do not significantly affect lactate results. No interference with the assay was found with any of the other drugs tested. Glycolate, a metabolite of ethylene glycol, causes a positive interference, which is variable from lot to lot of reagent.

3. Determination of plasma FFA concentrations

The concentration of FFAs (normal range: fasting 100-600 μM) in the plasma was analysed using an enzymatic colorimetric half-micro test kit from Roche Pharmaceuticals (Mannheim, Germany).

Test principle:

Free fatty acids are, in the presence of the enzyme acyl-CoA synthetase (Acyl CS), converted by adenosine-5'-triphosphate (ATP) and coenzyme A (CoA) into acyl-coenzyme A, (acyl-CoA), adenosine-5'-monophosphate (AMP) and pyrophosphate. Acyl-CoA reacts with oxygen (O_2) in the presence of acyl-CoA oxidase (ACOD) to form 2, 3-enoyl-coenzyme A (enoyl-CoA). Hydrogen peroxide (H_2O_2), which was formed converts 2, 4, 6-tribromo-3-hydroxy-benzoic acid (TBHB) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to a red dye that is measured at 540nm. The intensity of the red dye formed is directly proportional to the amount of FFA in the plasma (Shimizu *et al.,*1980).

$$\text{FFA + CoA + ATP} \xrightarrow{\text{Acyl-CS}} \text{acyl-CoA + AMP + pyrophosphate}$$

$$\text{Acyl-CoA + O2} \xrightarrow{\text{ACOD}} \text{enoyl-CoA} + H_2O_2$$

$$H_2O_2 + \text{4-AA + TBHB} \xrightarrow{\text{POD}} \text{red dye} + 2H_2O + \text{HBr}$$
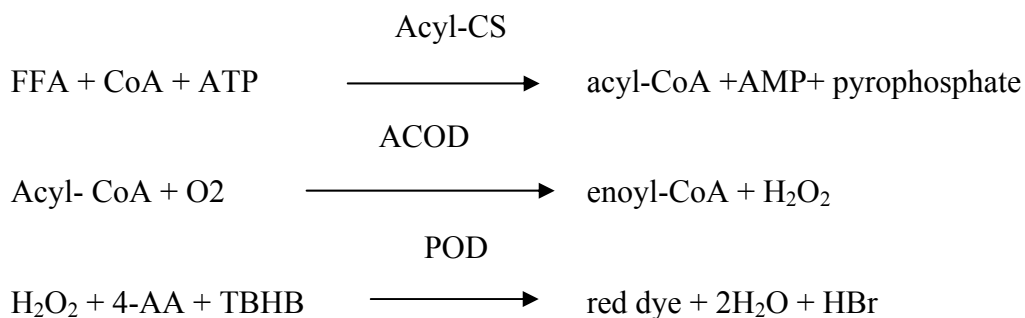
This method is applicable to monocarboxylic FFA with 6-18 carbon atoms. The original method from Roche diagnostics was adapted for use in NUNC 96 micro well plates (Nalge Nunc International, Denmark). This modification was necessary to minimize the costs involved in the sample analysis and to increase sample turnover time. The volumes used in the modified assay were 5 times lower but in the same ratio (1/5) as those used in the original assay protocol. The original wavelength of 546nm was adjusted to a wavelength of 540nm, (which was the closest available wavelength on the plate reader). A new absorption coefficient (ε_{540}) was derived from a comparison of sample absorbance in cuvette at 540nm and 546nm. We verified this coefficient by comparing the absorbance of a freshly prepared palmitic acid standard both in cuvettes and on plates.

Assay protocol:

The reagent bottles and the standard were brought to room temperature prior to performing the assay. Reaction mixtures A consisting of: tribromohydroxybenzoic acid, $MgCl_2$, ATP, coenzyme A, acyl-CoA-synthetase, peroxidase, ascorbate oxidase and 4-aminoantipyrine was prepared according to the original Boehringer assay protocol. Using a SMI pipette 10μl of blank (distilled water), 10μl of sample and 10μl of standard were dispensed into the 96-well plate. Solution A (200μl) was dispensed into each well and incubated on a shaker (~500 rpm) for approximately 10 minutes at 25 ± 1^0C. N-ethyl-maleinimide (10μl) was added and mixed on a shaker for a few seconds and absorbance was measured at 540nm.

The presence of N-ethyl-maleinimide in the test is necessary for the oxidation of the activated fatty acids by acyl-CoA-oxidase-ACOD (ACOD). 10μl of solution B (ACOD) was added to each well and further incubated on a shaker at 25 ± 1^0C for 20-25 minutes until the reaction was completed. Absorbance was measured at 540nm.

Calculation of the molar absorption coefficient at 540nm:

This was achieved by measuring the absorbance of the red dye in cuvettes both at 540nm (A_{540}) and 546nm (A_{546}). Thus, ε_{540} were calculated using the Beer-Lambert law as shown below.

Cuvettes: λ = 546nm $A_{546} = \varepsilon_{546}$ x C x d

Cuvettes: λ = 540nm $A_{540} = \varepsilon_{540}$ x C x d

For a constant concentration (C) and a fixed light path (light path, d=1 cm)

C x d $= A_{546}/\varepsilon_{546} = A_{540}/\varepsilon_{540}$

Therefore, $\varepsilon_{540} = A_{540}$ x $\varepsilon_{546}/ A_{546}$

The ε_{540} for the plate was calculated to be 22.494 mM^{-1} x cm^{-1}

Calculations:

The light path (d) on a 96 well plate was derived using the published well geometry [Equation: Y = 0.028x + 0.2; where Y= height (mm) and x= volume in μl] (Nunc Laboratories, 1985).

Calculation of FFA concentrations on a micro-titre plate (adapted from the original Boehringer package insert)

$C = [V / (\varepsilon_{540} \times d \times v)] \times \Delta A$ (mM)

$C = 1.549 \times \Delta A$

V = final volume (230µl)

v = sample volume (10µl)

d = light path (0.66cm)

ε = molar absorption coefficient of the dye at 540nm

ε_{540} = 22.494 [1 x mM^{-1} x cm^{-1}]

C = molar concentration [mM^{-1}]

A1 = first absorbance after adding N-ethyl-maleinimide

A2 = final absorbance

$\Delta A = \Delta A_s - \Delta A_{b;}$

ΔA_s = absorbance difference of the sample (A_2-A_1)

ΔA_b = absorbance difference of the blank (A_2-A_1)

Precision:

Intra-assay CV = 2.4 %; Inter-assay CV = 4.8 %

Analytical sensitivity for the plate:

The lower limit of detection is 0.005 mM, which represents the lowest measurable FFA concentration that can be distinguished from zero. The upper limit of detection is 1500 mM.

4. Determination of plasma glycerol concentrations

Glycerol concentrations in plasma samples were measured using an UV-enzymatic food analysis method (Boehringer Mannheim / R-Biopharm Enzymatic BioAnalysis/ Food Analysis). The original manual assay method was modified to run on a Roche Modular/Hitachi 917-auto analyser instrument. Automation assisted in reducing the following: analysis time, plasma volume, random errors, costs and sample turnover times. We replicated the manual steps as closely as possible on the auto analyser, and then refined the parameters to

provide a working application. Reagent 1 (buffer/coenzyme) was reconstituted according to the instructions given in the packet insert but the two enzyme suspensions had to be pre-diluted (1 in 31) to provide realistic sample volumes for the autoanalyser. A "2 point end point" reaction was selected for the application. The three reagents were added at the fixed intervals t1, t2 and t3 (t = time). These time intervals corresponded to 4.5 seconds, 90 seconds, and 5 minutes respectively after addition of a sample. At 37^0C, both the pre-reaction and final reaction reached completion within these timed intervals.

Principal of the assay:

Glycerol is phosphorylated with adenosine-5'-triphosphate (ATP) to L-glycerol-3-phosphate in the reaction catalysed by glycerolkinase (GK).

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{L-glycerol-3-phosphate} + \text{ADP}$$
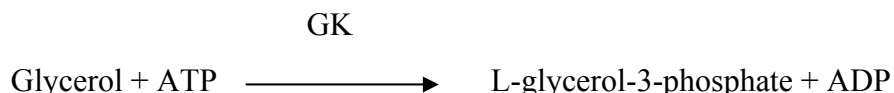
The adenosine-5'-diphosphate (ADP) formed, in the above reaction is converted into ATP by phosphoenolpyruvate (PEP) with the aid of pyruvate kinase (PK) with the formation of pyruvate.

$$\text{ADP} + \text{PEP} \xrightarrow{\text{PK}} \text{ATP} + \text{pyruvate}$$
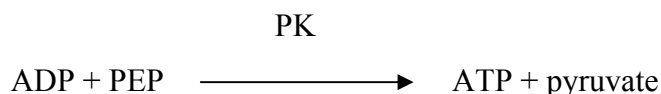
In the presence of the enzyme L-lactate dehydrogenase (L-LDH), pyruvate is reduced to L-lactate by reduced nicotinamide-adenine dinucleotide (NADH) with the oxidation of NADH to NAD.

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{L-LDH}} \text{L-lactate} + \text{NAD}^+$$
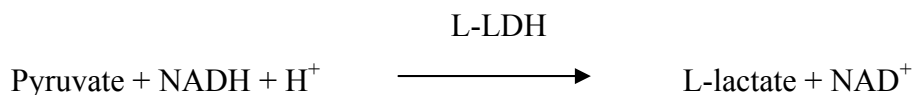
The amount of NADH oxidised in the above reaction is stoichiometric to the amount of glycerol. NADH is determined by means of its light absorption at 334, 340 or 365 nm.

Sample preparation (Eggstein *et al.,*1974, Wieland,1984):

Plasma samples were deproteinized to prevent any artifactual release of glycerol from triglycerides. This was accomplished by a 1 in 3 dilution of the plasma in water followed by

incubation for 7 minutes in a heating block set at 100°C. Samples were centrifuged at 2° C for 25 minutes to obtain the relevant supernatant (0.5 ml). The dilution factor is obtained from the sample volume (1.000ml), the volume of redistilled water (2.00 ml), the specific gravity of the sample material (1.03 g/ml plasma or serum) and the fluid content (0.92 in the case of plasma or serum):

F= (1.000 x 1.03 X0.92 + 2.000) / 1.000=2.95

The samples were then stored at minus 20^0C until future analysis.

Precision:

Intra-assay CV = 2.5 %; Inter-assay CV =2.6 %

Specificity:

This method is specific for glycerol. Dihydroxyacetone is not converted under the given conditions.

Sensitivity and detection limit:

The smallest differentiating absorbance for the procedure is 0.005 absorbance units.

The detection limit of 4.34mmol/l (0.4 mg/l) is derived from the absorbance difference of 0.02 (as measured at 340 nm) and a maximum sample volume v= 2.000ml.

Interference/sources of error: 0.2 mmol/l

The slow hydrolysis of ATP and phosphoenolpyruvate as well as the air oxidation of NADH results in a slow creep reaction which can be taken into account by extrapolation. An extrapolation is not absolutely necessary if the absorbances of blank and sample are measured immediately one after another.

5. Glycosylated haemoglobin (HbA$_{1c}$) measurement in plasma

Plasma levels of HbA$_{1c}$ were measured on Modular autoanalyser from Roche diagnostics (Munich, Germany) using an assay kit from Boehringer Mannheim, (Manheim, Germany).

Test principle:

The HbA$_{1c}$ determination is based on the turbidimetric inhibition immunoassay (TINIA) for haemolysed whole blood. Glycated haemoglobin (HbA$_{1c}$) reacts with the anti-HbA$_{1c}$

antibodies to give a soluble immunocomplex. Polyhaptens from reagent R2 then bind the excess antibodies, and the resulting agglutinated complex is measured turbidimetrically.
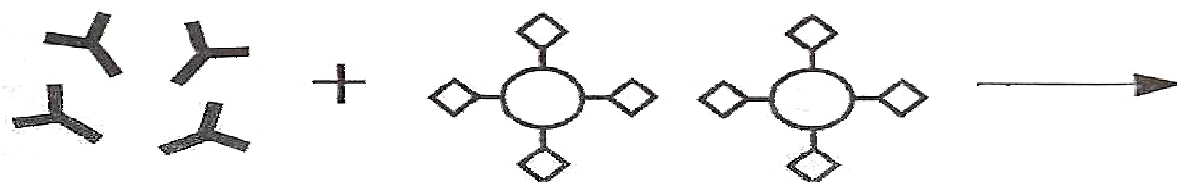
Excess anti-HbA$_{1c}$ antibody   Polyhaptens
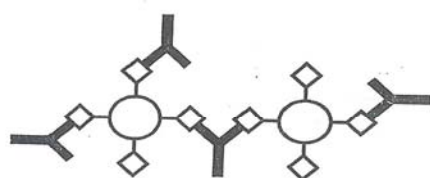
Antibody / Polyhapten complex   Turbidimetric Measurement
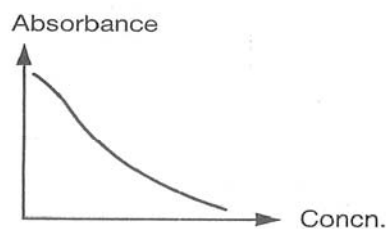
Precision:

Intra-assay CV = 2.3 %; Inter-assay CV =3.2 %

Analytical sensitivity (lower detection limit) (Data on file at Roche): HbA$_{1c}$: 0.2 g/dl

Specificity (Data on file at Roche):

No cross-reactivity with HbA$_0$, HbA$_{1a}$, HbA$_{1b}$, acetylated hemoglobin, carbamylated hemoglobin, glycated albumin, labile HbA$_{1c}$ and HbA$_{1d}$ was observed for the anti-HbA$_{1c}$ antibodies used in this kit.

6. Insulin determination in serum

MEDGENIX INS-EASIA kit from BIOSOURCE was used to analyze insulin levels in samples. It is a solid phase Enzyme Amplified Sensitivity Immunoassay performed in microtiter plates (used according to method specifications).

<u>Principle of the test:</u>

This assay is set up on the oligoclonal system in which several monoclonal antibodies (Mabs) directed against distinct epitopes of insulin are used. The use of several distinct Mabs avoids hyperspecificity and allows highly sensitive assays with extended standard range and short incubation time. Standards or samples containing insulin react with capture antibodies (Mabs 1) coated on a plastic well and with monoclonal antibodies (Mabs 2) labeled with horseradish peroxidase (HRP). The microtiter plate is washed to remove unbound enzyme- labelled antibodies after an incubation period allowing the formation of a sandwich coated Mabs 1- INS- Mabs2-HRP. The revelation solution [tetramethylbenzydine (TMB) – H_2O_2] is added and incubated. The reaction is stopped with H_2SO_4 and microtiter plate is read at 450nm. The amount of substrate turnover is determined colorimetrically by measuring the absorbance that is proportional to the insulin concentration.

<u>Precision:</u>

Intra-assay CV = 4.2 %; Inter-assay CV =7.0 %

<u>Sensitivity:</u>

The detection limit was 1.05 pmol/l (0.15 μIU/ml).

<u>Specificity:</u>

The cross-reactivity was determined by the kit manufactures by addition of different analytes to a serum containing 700 pmol/l (100μIU/ml) insulin and measuring the apparent insulin concentration. As shown hereafter, animal insulin (except rat insulin) cross-react whereas human, pork and beef proinsulin present no cross-reaction. A 3% cross-reaction was observed with 32-33 split proinsulin.

7. C-peptide, Total proinsulin & Intact proinsulin

C-peptide, Total Proinsulin and Intact proinsulin were measured using DakoCytomation enzyme-linked immunosorbent assays (ELISA) and used according to method specifications.

7.1 C-peptide measurements in serum

The assay principle:

DAKO C-peptide is an ELISA based on two monoclonal antibodies. Simultaneous incubation of sample and enzyme-labelled antibody in a microplate well coated with specific anti-C-peptide antibody forms a complex. A simple washing step removes unbound enzyme-labelled antibody. The bound conjugate is detected by reaction with the substrate 3, 3', 5, 5'-tetramethylbenzidine (TMB). Adding acid gives a colorimetric endpoint that is read spectrophotometrically and stops the reaction. The inclusion of calibrators of known C-peptide concentration in the assay allows a calibration curve to be constructed from which the level of C-peptide in samples can be determined.

Detection limit: 17pmol/l (0.05ng/ml) (Data obtained at DakoCytomation Limited).

Precision:
Intra-assay CV = 5.4 %; Inter-assay CV = 5.5 %

Cross reactivity:

The specificity of the assay was assessed by the kit manufactures by observing cross-reaction with structurally related peptides and hormones. Cross reactivity (%) was calculated from the mass of C-peptide and the mass of cross reactant giving the same assay signal.

Analyte	Cross reactivity (%)
Human insulin	0*
Intact human proinsulin (biosynthetic)	63
32-33 split proinsulin	75
65-66 split proinsulin	71
des 64-65 split proinsulin	87
des 31-32 split proinsulin	82

*Insulin at concentration of 2000pmol/l was below the detection limit of the C-peptide assay. Fasting concentrations of intact and split proinsulin are typically only 1-2% of C-peptide concentrations. Cross-reactivity with these molecules, therefore, is not clinically significant.

7.2 Total Proinsulin estimation in serum

Proinsulin, the precursor of insulin and C-peptide, is made by the beta cells of the pancreas. The DakoCytomation Total Proinsulin assay measures biologically active proinsulin along with all the intermediates formed during the processing of proinsulin to insulin and C-peptide with a high degree of specificity, using a pair of mouse monoclonal antibodies. These antibodies and prototypes of the assay have been described previously (Kjems *et al.,*1993, Sobey *et al.,*1991).

Principle of the test:

Total Proinsulin is an ELISA based on two monoclonal antibodies. Sequential incubation of sample and enzyme-labelled antibody in a microplate well coated with a specific anti-

proinsulin antibody forms a complex. Two washing steps remove unbound sample and enzyme-labelled antibody. The bound conjugate is detected by reaction with DakoCytomation amplification system (Fig 2.5). Adding acid gives a colorimetric endpoint that is read spectrophotometrically and stops the reaction. The inclusion of calibrators of known proinsulin concentration in the assay allows a calibration curve to be constructed from which the level of TOTAL proinsulin in patient samples can be determined.
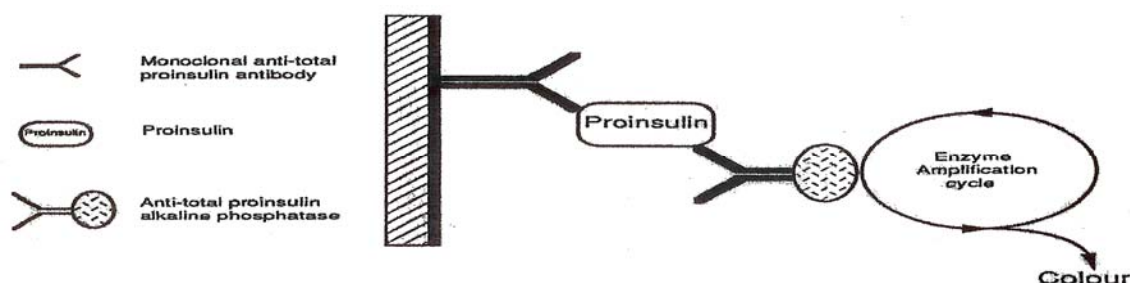
DAKO Proinsulin assay principle

Detection limit: < 0.5pmol/l

Precision:

Intra-assay CV = 3.4 %; Inter-assay CV = 3.3 %

Cross reactivity:

The specificity of the assay was assessed by observing cross reaction with structurally related peptides and hormones

Analyte	Cross reactivity (%)
Human insulin	0 (tested up to 240 nmol/l)
C-peptide	0 (tested up to 1000 nmol/l)
32-33 split proinsulin	100%
64-65 split proinsulin	100%

Insulin cross reactivity:

Total Proinsulin shows no cross reactivity with insulin therefore insulin-secreting tumours may not be detected.

7.3 Intact Proinsulin estimation in serum

The DAKO Cytomation kit measures only the intact form of human proinsulin. It is suggested that term 'intact proinsulin' may be used for the 86-amino-acid hormone (Clark, 1999). Pure intact proinsulin has little, if any, insulin activity. In normal individuals intact proinsulin present in the circulation in very low concentrations, and in type 2 diabetic patients, especially obese ones; it can be significantly elevated and especially high in rare cases of proinsulinoma.

Principle of the test:
The same principle as for the Total Proinsulin applies to the Intact Proinsulin assay.

Detection Limit: < 0.5 pmol/L

Precision:
Intra-assay CV = 3.1 %; Inter-assay CV = 4.7 %

Cross reactivity:

Analyte	Cross reactivity (%)
Human insulin	0 (tested up to 240 nmol/l)
C-peptide	0 (tested up to 1000 nmol/l)
32-33 split proinsulin	0 (tested up to 24 nmol/l)
64-65 split proinsulin	100%

7.4 Calculated des 31, 32 split Proinsulin

There is evidence that the des rather than the split forms are the major circulating form of the partially processed proinsulins, with the des 31, 32 split Proinsulin being present in higher concentrations in human plasma than the des 64, 65 split Proinsulin. Data are accumulating that elevations of intact and partially processed proinsulins are markers of beta cell dysfunction and may in some populations predict progression to type 2 diabetes (Clark, 1999). The levels of des 31, 32 split Proinsulin in our study were calculated by subtraction of intact proinsulin levels from the total proinsulin levels.

8. Determination of Cortisol in serum

(ADVIA ® Centaur System, used according to method specifications)

Assay principle:

The ADVIA Centaur Cortisol assay is a competitive immunoassay using direct chemiluminescent technology. Cortisol in the patient sample competes with acridinium ester-labeled cortisol for binding to polyclonal rabbit anti-cortisol antibody on the solid phase. The polyclonal rabbit anti-cortisol antibody is bound to monoclonal mouse anti-rabbit antibody, which is covalently coupled to paramagnetic particles.

Limitations:

Structurally related steroids such as prednisolone & prednisone could cross-react with the assay leading to falsely elevated results. Prednisolone at concentration of 100 μg/dL has 27 % cross-reactivity and prednisone (1000 μg/dL) has 6.6 % cross-reactivity with the assay.

Expected results:

Based on a central 95% interval, the following reference ranges were established:

Sample Category	Cortisol Range (nmol/l)	Cortisol Range (μg/dl)
Serum (7-9 a.m.)	118.6 - 618.0	4.30 - 22.40
Serum (3-5 p.m.)	85.30 - 459.6	3.09 - 16.66

Precision:

Intra-assay CV = 2.89 %; Inter-assay CV = 3.07 %

Sensitivity and Assay Range: 5.5-2069 nmol/l (0.20 – 75 μg/dl)

Specificity:

The ADVIA Centaur Cortisol assay is highly specific for cortisol. Cross-reactivity by structurally related compounds and pharmaceuticals were determined by spiking each compound into separate human samples to a final level of 1000 μg/dL, unless otherwise noted.

% Cross-reactivity = (cortisol in spiked sample, μg/dL-cortisol in unspiked sample, μg/dL) x 100

$$\overline{\hspace{7cm}}$$

(Concentration of compound added, μg/dL)

9. Determination of Cholesterol in Serum

Cholesterol CHOD-PAP, ROCHE/Boehringer Mannheim Systems (used according to method specifications) and measured on a MODULAR Autoanalyser.

Test principle:

This is an enzymatic colorimetric test. Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids.

Cholesterol esterase

Cholesterol esters + H_2O $\longrightarrow$ cholesterol + RCOOH
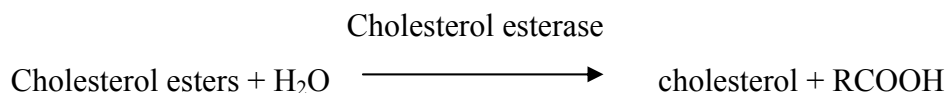
Cholesterol is converted by oxygen with the aid of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide.

Cholesterol oxidase

Cholesterol + O_2 $\longrightarrow$ cholest-4-en-3-one + H_2O_2
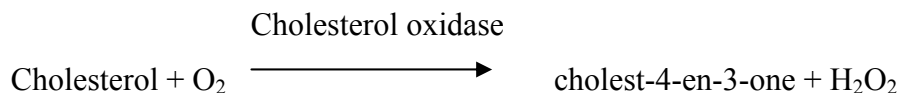
The hydrogen peroxide created, forms a red color by reacting with 4-aminophenazone and phenol under the catalytic action of peroxidase. The color intensity is directly proportional to the concentration of cholesterol and can be determined photometric

peroxidase

2 H_2O_2 + 4-aminophenazone + phenol $\longrightarrow$

4-(p-benzoquinone-monoimino) phenazone + 4 H_2O
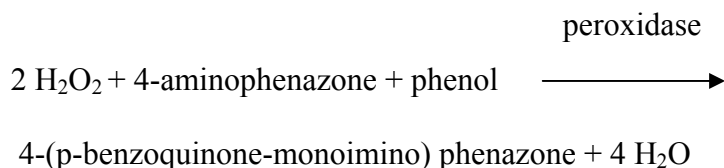
Precision:

Intra-assay CV = 0.8 %; Inter-assay CV =1.7 %

Analytical sensitivity (Data on File at Boehringer Mannheim):0.08 mmol/l (3 mg/dl)

10. Measurement of high-density lipoprotein (HDL) cholesterol in serum

HDL-Cholesterol was measured using the Boehringer Mannheim direct HDL cholesterol assay (used according to method specifications). This automated method for direct determination of HDL-cholesterol in serum and plasma uses polyethylene glycol (PEG)-modified enzymes and dextran sulfate. When cholesterol esterase and cholesterol oxidase enzymes are modified by PEG, they show selective catalytic activities toward lipoprotein fractions, with the reactivity increasing in the order: LDL< VLDL ≈ chylomicrons < HDL. The Boehringer Mannheim

direct HDL cholesterol assay meets the 1998 National Institute of Health (NIH)/ National Cholesterol Education Program (NCEP) goals for acceptable performance (Kimberly *et al.,*1999).

<u>Test principle</u> (Sugiuchi *et al.*, 1995, Matsuzaki *et al.*, 1996):

This is a homogeneous enzymatic colorimetric test. In the presence of magnesium sulphate, dextran sulfate selectively forms water-soluble complexes with LDL, VLDL and chylomicrons, which are resistant to PEG-modified enzymes. The cholesterol concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups.

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

$$\text{HDL-cholesterol esters} + H_2O \xrightarrow{\text{PEG-cholesterol esterase}} \text{cholesterol} + RCOOH$$
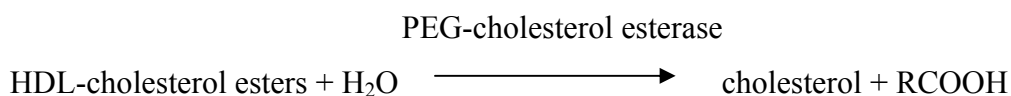
In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4-cholestenone and hydrogen peroxide.

$$\text{Cholesterol} + O_2 \xrightarrow{\text{PEG-cholesterol oxidase}} \Delta^4\text{-cholestenone} + H_2O_2$$
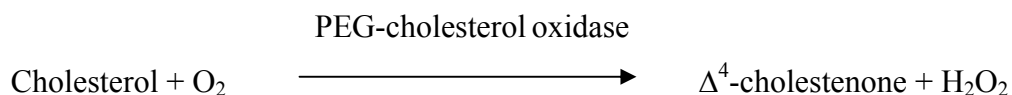
In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminophenazone and sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HSDA) to form a purple blue dye. The colour intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

$$2\,H_2O_2 + \text{4-aminophenazone} + HSDA + H + H_2O \xrightarrow{\text{peroxidase}} \text{purple blue pigment} + 5\,H_2O$$
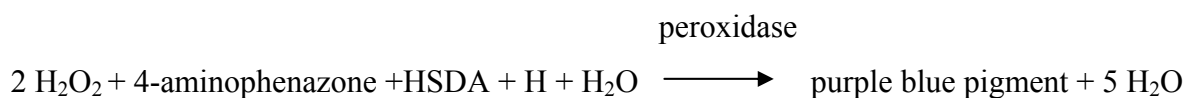
<u>Measuring range:</u> 0.08-3.12 mmol/l (3-120 mg/dl).

<u>Precision:</u>

Intra-assay CV = 1.6 %; Inter-assay CV = 2.7 %

<u>Analytical sensitivity</u> (Data on file at Roche): 0.08 mmol/l (3 mg/dl)

11. The Friedewald Equation for calculating low-density lipoprotein (LDL) in serum

The ultracentrifuge measurement of LDL is time consuming and expensive and requires specialist equipment. For this reason, LDL-cholesterol is most commonly estimated from quantitative measurements of total and HDL-cholesterol and plasma triglycerides (TG) using the Friedewald (1972) equation:

[LDL-chol] = [Total chol] - [HDL-chol] - ([TG]/2.2))

Where all concentrations are given in mmol/L and if calculated using all concentrations in mg/dL then the equation is:

[LDL-chol] = [Total chol] - [HDL-chol] - ([TG]/5))

The quotient ([TG]/5) is used as an estimate of VLDL-cholesterol concentration. It assumes, first, that virtually all of the plasma TG is carried on VLDL, and second, that the

TG: cholesterol ratio of VLDL is constant at about 5:1.

<u>Limitations of the Friedewald equation:</u>

The Friedewald equation should not be used under the following circumstances: when chylomicrons are present, and when plasma triglyceride concentration exceeds 400 mg/dL (4.52 mmol/L) samples generally are turbid, and in patients with dysbetalipoproteinemia (type III hyperlipoproteinemia). In circumstances in which these conditions apply, LDL-cholesterol should be measured directly. Chylomicrons are visible as a floating "cream" layer when the specimen is allowed to stand undisturbed at 4°C overnight. The recognition of dysbetalipoproteinemia (type III hyperlipoproteinemia), however, requires the identification of b-VLDL. Since b-VLDL contains proportionately more cholesterol than normal VLDL, the use of the factor [TG] / 5 underestimates the amount of cholesterol in the VLDL fraction, and consequently the Friedewald equation overestimates LDL-cholesterol. Use of the Friedewald equation in this case will result in the misidentification of a dysbetalipoproteinemic (type III) patient as having hyperbetalipoproteinemia (type II hyperlipoproteinemia).

12. Measurement of triglycerides in serum

Triglycerides were measured using the GPO-PAP triglyceride assay by Roche (used according to method specifications).

Test principle (Siedel *et al.,* 1993):

This is an enzymatic colorimetric test based on the work by Wahlefeld using a lipoprotein lipase (LPL) from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red color (Trinder endpoint). Solution R1 (buffer/4-chlorophenol/enzymes) is added to the sample to start the reaction:

$$\text{Triglycerides} + 3\ H_2O \xrightarrow{\text{LPL}} \text{glycerol} + 3\ RCOOH$$

$$\text{glycerol} + ATP \xrightarrow[Mg^{2+}]{GK*} \text{glycerol -3- phosphate} + ADP$$

$$\text{glycerol-3- phosphate} + O_2 \xrightarrow{GPO**} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{peroxidase}}$$

4-(p-benzoquinone-monoimino)-phenazone + 2 H_2O + HC
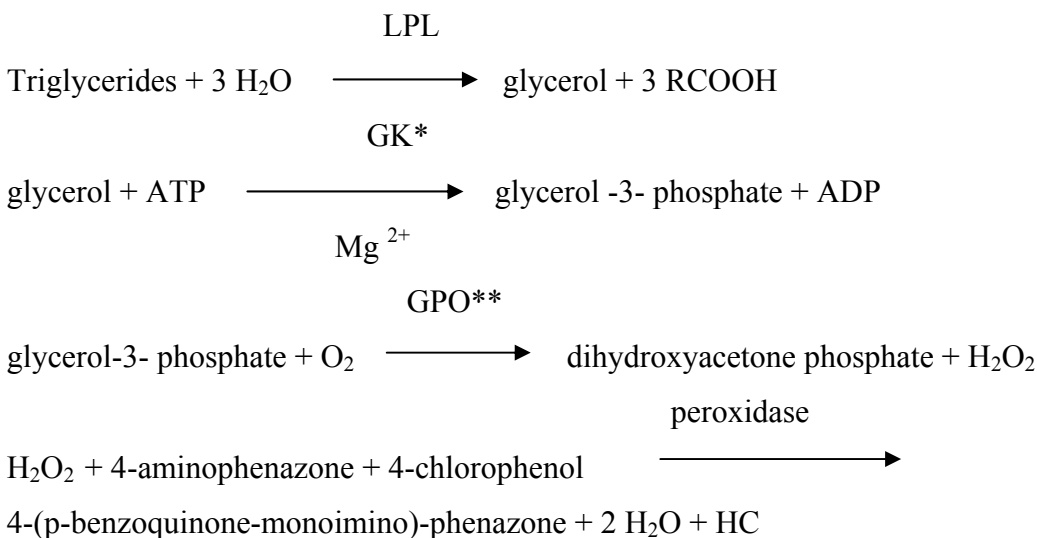
Abbreviations : * GK – glycerol kinase; ** GPO- glycerol phosphate oxidase

Precision:

Intra-assay CV = 1.5 %; Inter-assay CV = 1.8 %

Analytical sensitivity: (Data on file at Roche): 0.05mmol/l (4 mg/dl)

13. Determination of Leptin and Human Leptin Receptor in serum

Human Leptin and Human Leptin Receptor ELISAs were obtained from BioVendor Laboratory Medicine, Inc. (used according to method specifications).

Principle of the test:

Standards, quality controls, and samples are incubated in microtitration wells coated with polyclonal anti-human leptin or leptin receptor antibody. After a thorough wash, anti-human leptin (receptor) antibody labelled with horseradish peroxidase (HRP) is added to the wells and incubated with the immobilized antibody-leptin (receptor) complex. Following another washing step, the remaining HRP-conjugated antibody is allowed to react with the substrate H_2O_2 -tetramethylbenzidine. The reaction is stopped by addition of acidic solution, and absorbance of the resulting yellow product is measured spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of leptin (receptor). A standard curve is constructed by plotting absorbance values versus leptin (receptor) concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

Precision:

Intra-assay CV -human leptin = 3.93 %; human leptin receptor = 5.03 %

Inter-assay CV - human leptin 4.1 %; human leptin receptor = 3.68 %

Sensitivity:

The limit of detection of human leptin is 0.5 ng/ml.

The limit of detection of human leptin receptor is 0.4 unit recombinant leptin receptor / 1ml sample.

14. Determination of human tumor necrosis factor alpha (TNF-α) in serum

The Quantikine high sensitivity (HS) TNF-α immunoassay (R&D system used to assay specifications) is a 6.5-hour solid phase ELISA designed to measure TNF-α in serum and plasma. It contains E. coli-derived recombinant human TNF-α and antibodies raised against the recombinant factor. Results obtained with naturally occurring TNF-α samples showed linear curves that were parallel to the standard curves obtained using the recombinant kit standards suggesting that Quantikine HS Immunoassay kit can be used to determine relative mass values for natural TNF-α. Since the measurement of TNF-α is insensitive to the addition of recombinant forms of either of the two types of soluble receptors, it is probable that this measurement detects the total amount of TNF-α in samples, i.e., the total amount of free TNF-α plus the amount of TNF-α bound to soluble receptors.

Principle of the assay:

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TNF-α has been pre-coated onto a microplate. The Quantikine TNF-α HS Immunoassay kit uses an amplification system in which the alkaline phosphatase reaction provides a cofactor that activates a redox cycle leading to the formation of a coloured product (Self, 1985, Stanley *et al.*,1985, Johansson *et al.*,1986). The alkaline phosphatase is covalently linked to an anti- TNF-α detector antibody.

In this amplification system, alkaline phosphatase dephosphorylates the reduced form of nicotinamide adenine dinucleotide phosphate, NADPH (Substrate), to reduced nicotinamide adenine dinucleotide, NADH. The NADH subsequently serves as a specific cofactor that activates a redox cycle driven by the secondary enzyme system consisting of alcohol dehydrogenase and diaphorase (Amplifier). In the reaction catalysed by diaphorase, NADH reduces a tetrazolium salt (INT-violet or iodonitrotetrazolium violet) to produce an intensely coloured formazan dye and NAD^+. NAD^+ in turn is reduced by ethanol, in an alcohol dehydrogenase-catalysed reaction, to regenerate NADH, which can then re-enter the redox cycle. The rate of reduction of the tetrazolium salt and thus the amount of coloured product formed are directly proportional to the amount of TNF-α bound in the initial step.

Precision:

Intra-assay CV = 6.7 %; Inter-assay CV = 13.4 %

Sensitivity:

The minimum detectable dose (MDD) of TNF-α ranges from 0.06 pg/ml to 0.32 pg/ml.
The mean MDD is 0.12 pg/ml.

Specificity:

This assay recognizes recombinant and natural human TNF-α. The factors related to or associated with TNF-α such as soluble TNF receptor I, soluble TNF receptor II and TNF-β does not cross react.

15. Method for measurements of IL-6 in serum

The Biotrak high sensitivity (HS) human interleukin-6 ELISA system from Amersham Pharmacia Biotech provides determination of IL-6 in cell culture supernatants, urine, plasma, and serum.

Principle of the assay:

The assay system is based on a solid phase ELISA, which utilizes an antibody for human IL-6 bound to the wells of a microtitre plate together with a biotinylated antibody to human IL-6 and Amdex amplification reagent. The Amdex amplification reagent is a high performance conjugate that utilizes a hydrophilic straight chain dextran backbone to which many hundreds of horseradish peroxidase molecules are covalently coupled, together with, on average, ten streptavidin molecules. The result is a multifunctional conjugate with a significantly enhanced activity and with well-controlled non-specific binding properties. Although the Biotrak IL-6 immunoassay contains recombinant IL-6 and antibodies raised against recombinant IL-6, it has been shown to quantitate accurately both natural IL-6 and recombinant IL-6. In addition to the samples to be tested, a series of wells is prepared using known concentrations of the human IL-6 standard. A curve, plotting the optical density versus the concentration of the standard well, is prepared. By comparing the optical density of the samples to this curve, the concentration of the IL-6 in the unknown samples is then determined.

Precision:

Intra-assay CV <10 %; Inter-assay CV <10 %

Specificity:

This assay recognizes both natural and recombinant (h) IL-6. It does not cross react with human IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-7, IL-8, TNF-a, IFN-γ, GM-CSF, mouse IL-6 or rat IL-6.

Sensitivity: The minimum detectable dose of human IL-6 is 0.1pg/ml (0.01pg/well).

16. Determination of CRP in serum

CRP (Latex) high sensitivity (HS) assay is manufactured by Roche and performed on a MODULAR Autoanalyser according to assay specifications.

Test principle:
This is a particle-enhanced immunoturbidimetric assay.
Sample and addition of R1 (buffer), is followed by addition of R2 (anti- CRP antibody-latex) and start of reaction:

CRP antigen   anti-CRP antibody

Antigen / antibody complex   Turbidimetric measurement
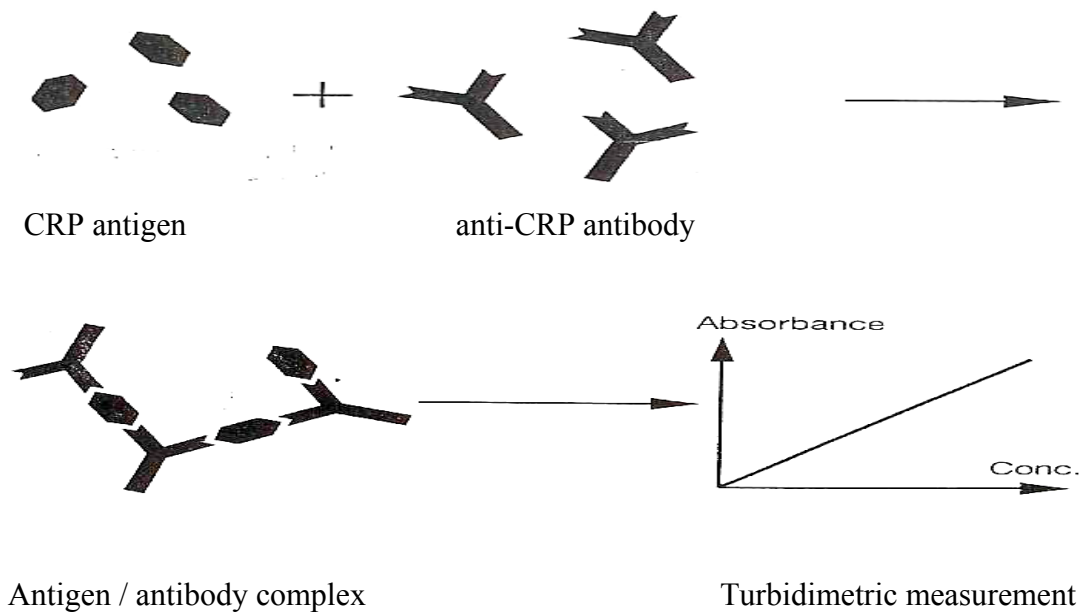
Anti-CRP antibodies coupled to latex microparticles react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically. Addition of PEG allows the reaction to progress rapidly to the end point, increases sensitivity, and reduces the risk of samples containing excess antigen producing false negative results.

Limitations-interference:

A high- dose hook effect does not occur at CRP concentrations below 1000mg/l. In very rare cases gammopathy, in particular type IgM (Waldenstrom's macroglobulinemia) may cause unreliable results.

Measuring range: 0.1-20 mg/l (0.0001-0.02 g/l)

Precision:

Intra-assay CV = 1.34 %; Inter-assay CV = 5.7 %

Analytical sensitivity (lower detection limit): 0.03 mg/l:

Functional sensitivity: 0.11mg/l. This functional sensitivity is the lowest CRP concentration that can be reproducibly measured with an inter-assay coefficient of variation of <10%.

APPENDIX 3

Food Frequency Questionnaire

NAME --

DATE OF BIRTH ------------------------- AGE (YEAR AND MONTHS) -----------------

SEX -----------------------

ETHNIC GROUP ------------------------

DAY OF INTERVIEW -------------------

DATE OF INTERVIEW -----------------

INSTRUCTIONS

The following questions are about the foods you **USUALLY** eat during an average week.

Please indicate the number of **days per week** that you eat each item on average. Ring the answer as in the example:

If you eat the food every day, ring 7   ----------------------------7 6 5 4 3 2 1 M R

If you the food 3 days / week, ring 3 ---------------------------- 7 6 5 4 3 2 1 M R

If you eat the food only monthly, ring M ----------------------- 7 6 5 4 3 2 1 M R

If you never or rarely eat the food, ring R ---------------------- 7 6 5 4 3 2 1 M R

GRAIN AND CEREAL GROUP

                                           No. Days / week

Bread --- 7 6 5 4 3 2 1 M R

Provide/ cream crackers etc. -------------------------------------7 6 5 4 3 2 1 M R

Do you mainly eat white, brown or whole wheat bread? _____

How many slices of bread do you have per day? _____

BREAKFAST CEREALS AND PORRIDGES

                                           No. Days / week

Cereals (Rice Krispies, Cornflakes) -------------------------------7 6 5 4 3 2 1 M R

Sugar coated cereals (Coco Pops, Frosties) ----------------------7 6 5 4 3 2 1 M R

W/wheat cereals (All Bran, Weetbix) ----------------------------7 6 5 4 3 2 1 M R

Porridge (Oats, Maltabella, Maize meal)------------------------7 6 5 4 3 2 1 M R

Muesli --------------------------------------- -----------------------7 6 5 4 3 2 1 M R

Pronutro -- 7 6 5 4 3 2 1 M R

OTHER STARCHES

                                           No. Days / week

Rice, pasta -- 7 6 5 4 3 2 1 M R

Stiff maize meal - with Amazi (sour milk) ----------------------7 6 5 4 3 2 1 M R

                  - Without ---------------------- ----------- 7 6 5 4 3 2 1 M R

Samp / mielie rice - with beans----------------------------------7 6 5 4 3 2 1 M R

                  - Without ---------------------------------7 6 5 4 3 2 1 M R

MEATS AND MEATS SUBSTITUTES

No. Days / week

Red meat (beef, lamb, pork or mince) --------------------------7 6 5 4 3 2 1 M R

Processed meat (bacon, sausages, polony) --------------------7 6 5 4 3 2 1 M R

Chicken -- --7 6 5 4 3 2 1 M R

Fish -- 7 6 5 4 3 2 1 M R

Eggs and egg dishes --7 6 5 4 3 2 1 M R

Cheese, cheese spread and cheese dishes ---------------------7 6 5 4 3 2 1 M R

Nuts, including peanut butter -----------------------------------7 6 5 4 3 2 1 M R

Dried peas, beans, baked beans or legumes -------------------7 6 5 4 3 2 1 M R

VEGETABLES AND FRUIT

No. Days / week

Green and / or yellow vegetables -------------------------------7 6 5 4 3 2 1 M R

Potatoes --- 7 6 5 4 3 2 1 M R

Fresh fruit--- 7 6 5 4 3 2 1 M R

Canned fruit --- 7 6 5 4 3 2 1 M R

Dried fruit (raisins, prunes, dates) ------------------------------7 6 5 4 3 2 1 M R

Fresh fruit juice (Ceres, Liquifruit) ----------------------------7 6 5 4 3 2 1 M R

Name some of the vegetables you have eaten this past week

_____

Do you eat your vegetables mostly coked or raw? _____

Do you mainly eat your potatoes as boiled, baked in jacket, mashed, roasted or as chips?

_____

What types of fresh fruit have you eaten this past week?

_____

FATS AND OILS

<div align="right">No. Days / week</div>

Oil / butter / margarine---7 6 5 4 3 2 1 M R

Salad dressing / mayonnaise ------------------ ----------------7 6 5 4 3 2 1 M R

Cream -- --------------- 7 6 5 4 3 2 1 M R

Non-dairy creamers (Cremora) ------------------------------- 7 6 5 4 3 2 1 M R

Ice-cream -- 7 6 5 4 3 2 1 M R

Please estimate, on average, the total amount of fat (oil/butter/margarine/dressing/cream/non-dairy creamers) you use per day in teaspoons.

_____tsp. (includes the fat on bread, in cooking and frying and on vegetables /salads)

<div align="right">No. Days / week</div>

How often do you fry your food? ----------------------------- 7 6 5 4 3 2 1 M R

MILK AND MILK PRODUCTS

<div align="right">No. Days / week</div>

Milk --7 6 5 4 3 2 1 M R

Yoghurt - Plain ---7 6 5 4 3 2 1 M R

        - Flavoured ---7 6 5 4 3 2 1 M R

Milo / Nesquick /Cocoa / Horlicks-------------------------- 7 6 5 4 3 2 1 M R

Please estimate on average, how much milk you have per day, including that on cereal, in tea or coffee and milk drinks

_____ml or _____cups

What type of milk do you use?

Full cream _____

Low-fat (2% fat) _____

Skimmed   _____

Condensed _____

Powdered _____

MISCILLANEOUS

Please estimate how many teaspoons of sugar you have in total per day? _____ tsp.

(In tea / coffee/ milk drinks, on cereal / porridge, added to vegetables).

                                                              No. Days / week

Sweets (sucking / jelly type / fudge / toffee) ---------------------- 7 6 5 4 3 2 1 M R

Chocolates / chocolate bars --- 7 6 5 4 3 2 1 M R

Jam, syrup, honey --- 7 6 5 4 3 2 1 M R

Jelly --7 6 5 4 3 2 1 M R

Sweet biscuits / cakes / pastries /doughnuts /

tarts /scones / pancakes ---7 6 5 4 3 2 1 M R

Crisps---7 6 5 4 3 2 1 M R

Popcorn (plain or candied) --7 6 5 4 3 2 1 M R

Puddings (trifle, baked puddings etc.) ------------------------------7 6 5 4 3 2 1 M R

Cold drinks (Coke, Fanta etc) or cordials---------------------------7 6 5 4 3 2 1 M R

                              - Sweetened------------------------- 7 6 5 4 3 2 1 M R

                              - Diet---------------------------------7 6 5 4 3 2 1 M R

Tea ---7 6 5 4 3 2 1 M R

Coffee--7 6 5 4 3 2 1 M R

Spreads (Bovril, marmite, fish paste, sandwich) -------------------7 6 5 4 3 2 1 M R

How many meals do you have per day i.e. Breakfast, lunch and / or dinner _____

How many in between snacks do you have per day i.e. Mid-morning, mid-afternoon and or

late evening? _____

Please indicate how many portions of the following food groups you ate yesterday by circling the appropriate number.

E.g. If you had 1 egg for breakfast, cheese for lunch and chicken for dinner, ring 3 for number 2.

If you have cereal and 1 slice of toast for breakfast, a sandwich for lunch (2 slices of bread) and rice for dinner, ring 5 for number 6.

1. Milk, yoghurt (1 portion = 1 cup / 200 ml) ------------------------ 1 2 3 4 5 6 7 8 9 10

2. Meat / fish / chicken / cheese / eggs / nuts / legumes--------------1 2 3 4 5 6 7 8 9 10

3. Fruit / fruit juice --1 2 3 4 5 6 7 8 9 10

4. Vegetables (green and yellow) ---------------------------------------1 2 3 4 5 6 7 8 9 10

5. Potatoes --- 1 2 3 4 5 6 7 8 9 10

6. Bread / cereal/ porridge / rice / pasta / maize meal / samp/

   mielie rice --1 2 3 4 5 6 7 8 9 10

7. Oil / butter / margarine / cream/ non dairy creamers/

   salad dressings (1 portion = 1 tsp.)---------------------------------1 2 3 4 5 6 7 8 9 10

Were these numbers of portions typical of what you would normally consume on an average day?

Yes _____ No _____

If answer No, was the difference due to:

Illness _____

A party _____

Eating out _____

Other reasons _____

APPENDIX 4

Questionnaire for Assessment of Socio-Economic status

1. Which of the following do you have in your household at the present time?

Electricity --------------Y/N

Television --------------Y/N

Radio -------------------Y/N

Motor vehicle----------Y/N

Fridge -------------------Y/N

Washing machine------Y/N

Telephone --------------Y/N

Video machine --------Y/N

Microwave -------------Y/N

2. Education – last standard passed

No formal education

Grade 1-2

Std   1-3

Std   4-5

Std   6-7

Std   8

Std   9

Matric

Degree

Postgraduate degree

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