CORRELATION BETWEEN THE SEX HORMONE BINDING GLOBULIN LEVEL AND LEVEL OF INSULIN RESISTANCE IN OBESE PREPUBERTAL CHILDREN

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CERTIFICATE

Certified that this dissertation entitled "**CORRELATION BETWEEN THE SEX HORMONE BINDING GLOBULIN LEVEL AND LEVEL OF INSULIN RESISTANCE IN OBESE PREPUBERTAL CHILDREN"** is a bonafide work done by **Dr.V.SEENIVASAN, M.D.,** Post Graduate Student of Pediatric Medicine, **Institute of Child Health and Hospital for Children,** Egmore, Chennai - 600 008 attached to Madras Medical College, during the academic year 2003 - 2006.

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DECLARATION

I declare that this dissertation entitled **"CORRELATION BETWEEN THE SEX HORMONE BINDING GLOBULIN LEVEL AND LEVEL OF INSULIN RESISTANCE IN OBESE PREPUBERTAL CHILDREN"** has been conducted by me at the Institute of Child Health and Hospital for Children, under the guidance and supervision of my unit chief **Prof.K.R.Ravindran M.D., D.C.H.,** and **Dr. P.G. Sundararaman M.D., DM.,** Lecturer, Department of Endocrinology, Institute of Obstetrics and Gynaecology, Madras Medical College, Chennai. It is submitted in part of fulfillment of the award of the degree of M.D (Pediatrics) for the September 2006 examination to be held under The Tamil Nadu Dr.M.G.R Medical University, Chennai. This has not been submitted previously by me for the award of any degree or diploma from any other university.

(Dr. V.SEENIVASAN)

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CONTENTS

INTRODUCTION

Insulin resistance is defined as an impaired ability of plasma insulin at usual concentrations to adequately promote peripheral glucose disposal, suppress hepatic glucose output and inhibit very low density lipoprotein (VLDL) output.

Insulin resistance results in compensatory increase in insulin secretion. The resulting hyperinsulinemia overcomes the insulin resistance for sometime and keeps the blood sugar in normal range. However when relative beta cell insufficiency (insulin secretion insufficient for the level of hyperglycemia) also sets in, overt diabetes develops.

Metabolic syndrome also known as Syndrome X is associated with increased risk of coronary heart disease and stroke. The features of metabolic syndrome include abdominal obesity, hyperinsulinemia, dyslipidemia, type 2 diabetes mellitus, and hypertension[1]. The core feature is elevated insulin levels which develops early in the course of disease.

It is increasingly being recognized that Indians are an ethnic group at high risk for insulin resistance. In a 1998 publication, WHO has predicted that India would experience the largest increase in type 2 diabetes mellitus in the next 25 years and would have the greatest number of diabetic individuals by the year 2025[3].

According to a large body of epidemiological data from many parts of world, the seeds of insulin resistance are sown during prenatal life itself. Low birth weight and more so intra uterine growth retardation followed by rapid catch-up growth and relative postnatal obesity have been shown to be associated with insulin resistance[4,5]. According to Barker et al, low birth weight acts as a surrogate marker for poor gestational environment and there is inverse relationship between birth weight and insulin resistance later in life.

In addition to insulin resistance, tracking of other cardiovascular risk factors such as obesity, physical inactivity, hyperlipidemia, and hypertension is seen from childhood through adolescence to adulthood. Therefore the steps for the prevention of insulin resistance and other cardiovascular risk factors should ideally be initiated during childhood.

Clinical features of insulin resistance syndrome

The clinical phenotype of insulin resistance includes centrally biased obesity; characteristic skin involvement (acanthosis nigricans, skin tags, striae, acne, hirsutism, and frontal balding); an allergic diathesis, especially as manifested by asthma; hypertension; an atherogenic dyslipidemia increased VLDL with raised triglycerides and reduced levels of the protective high density lipoprotein (HDL) cholesterol; early atherosclerosis, tall stature and pseudoacromegaly (with suppressed growth hormone levels); focal segmental glomerulosclerosis; hepatic steatosis; and adrenal and ovarian hyperandrogenism (Table-1). Importantly, insulin resistance is not infrequent in the absence of obesity, whereas even considerably obese persons can be insulin sensitive.

Obese patients represent heterogeneous subgroups of metabolic and phenotypical expressions of insulin resistance, whereas individuals with the same Body Mass Index (BMI) can have varying degrees of insulin resistance and metabolic compensation. However, most individuals with BMI more than 35–40 kg/m^2 are insulin resistant.

Table -1

Features of IRS	Pediatric features of IRS
• Acanthosis Nigricans • White striae • Centrepetal obesity • Hirsutism, ovarian hyperandrogenism and infertility • Dyslipidemia (↑TG,↓HDL) • Premature atherosclerosis • Hypertension • Hyperuracemia/gout • Allergies/asthma • Fatty liver (NASH) • Chronic pancreatitis • Focal glomerulosclerosis • Glucose intolerance • Type 2 diabetes • Increased cancer risk • Increased incidence of Alzheimer's disease	• Positive family history of diabetes, obesity, hypertension, CHD, and/or stroke • History of maternal gestational diabetes • SGA (mostly) or LGA (less often) • Asthma/allergic rhinitis • Premature pubarche • Red (new) and white (old) striae, from adrenarche onward • Obesity appears or worsens at adrenarche • Decreasing resting energy expenditure • Low resting fat to carbohydrate oxidation rates • Acanthosis Nigricans • Tall stature/pseudoacromegaly • Hirsutism/PCOS with adolescence • Adipomastia/gynecomastia • Acute pancreatitis • Premature atherosclerosis • Hypertension/glomerulonephritis • Type 2 diabetes

Children with BMI higher than the 85th percentile for age and gender are classified as overweight, whereas those that are higher than the 95th percentile are designated obese[6]. Adolescents and adults with BMI of 25 kg/m^2 or more are at risk for adiposity-related morbidity, whereas those with BMI greater than 30 kg/m^2 are obese according to the WHO panel.

PATHOGENESIS

Nature vs. nurture.

The dramatic rise in obesity-associated insulin resistance syndrome reflects environmental increased availability and consumption of food with high carbohydrate and fat contents together with decreased physical activities. Genetic predispositions to obesity favor selection of metabolically advantageous (energy thrifty) traits resulting in an enhanced ability to store excess calories in tissues as fat and to spare protein breakdown for gluconeogenesis, favoring survival in times of hunger. Genotypic factors influence the ability to use food energy efficiently through mechanisms of intra abdominal fat distribution, resting metabolic rate, changes in energy expenditure, body composition to overfeeding, feeding behavior (including food preferences), adipose tissue lipoprotein lipase activity, and the basal rate of lipolysis.

Genes involved in insulin resistance

The pathogenesis of insulin resistance is multifactorial. Thus, several molecular pathways in energy homeostasis, lipid metabolism, insulin receptor signaling pathway, cytokines, hormone-binding proteins including those that are serine protease inhibitors (SERPINS), and other protease regulators are responsible for the development of insulin resistance, obesity, or lipodystrophy. In the energy homeostasis pathway, uncoupling proteins, leptin-proopiomelanocortin (POMC), ghrelin-neuropeptide Y (NPY), and sympathetic nervous system regulation pathways are important. In the insulin-signaling pathway, mutations in insulin receptors, development of insulin receptor auto-antibodies, and defects in plasma cell membrane glycoprotein-1 and glucose transporter 4 (GLUT4) molecules are reported. In the lipid homeostasis pathway, adipocyte-derived hormones, leptin, adiponectin, resistin, peroxisomal proliferator-activated receptor-γ (PPAR- γ) and PPAR-α are variously involved, as are lipoprotein lipase and genes responsible for adipose tissue formation. Increased availability of free fatty acids (FFAs) to muscle provokes insulin resistance. Proteases contributing to the development of diabetes are represented by CAPN 10 and prohormone convertase deficiencies.

Acquired insulin resistance

Insulin receptor antibodies, Cushing's syndrome, glucocorticoid steroid therapy, acromegaly, hyperparathyroidism, and exogenous obesity can all produce insulin resistance. In practice, however, steroid-induced insulin resistance in a person who happens to be genetically prone to insulin resistance is the most commonly encountered, especially when the obese child also has insulin resistance-associated asthma. GH therapy can provoke transient insulin resistance. In the small for gestational age (SGA) disorders without catch-up growth, such as the Russell-Silver syndrome, insulin resistance may develop even before GH is given.

Birth weight and length.

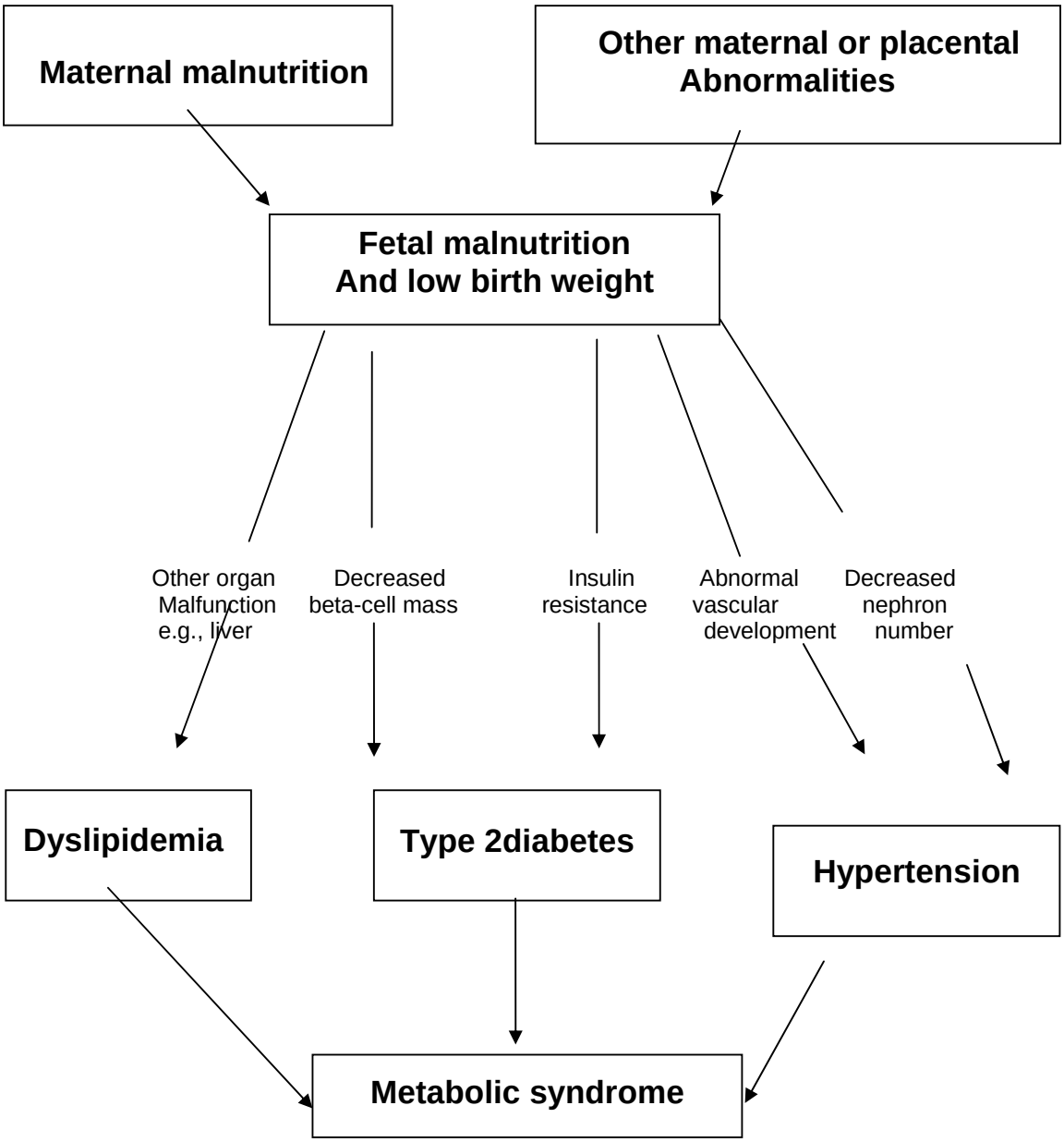
An increased incidence of adulthood diseases, such as cardiovascular diseases, type 2 diabetes, and hypertension, is noticed in small for gestational (SGA) babies. Insulin resistance is particularly apparent when an SGA newborn undergoes rapid postnatal weight gain. The Early Bird Study suggested that insulin resistance at 5yrs was related not to birth weight, but, rather, to weight after catch-up growth, especially in girls[4]. Such growth patterns following fetal growth restraint are associated with maternal-uterine factors such as primiparity,

smoking, restrictions in the maternal diet, maternal insulin resistance, and gestational diabetes (Fig-1). Alternatively, if an inherited insulin resistance state was manifested *in utero*, then diminished fetal growth with SGA might be anticipated, because insulin is a powerful prenatal growth hormone(Fig-2). Curiously, large for gestational age children are also at risk of insulin resistance as well. A U-shaped relation between birth weight and fasting insulin, BMI and fat mass has been established[41,42].

Gestational diabetes *per se* significantly increases the subsequent risk of obesity and type 2 diabetes[10].

FIG-1

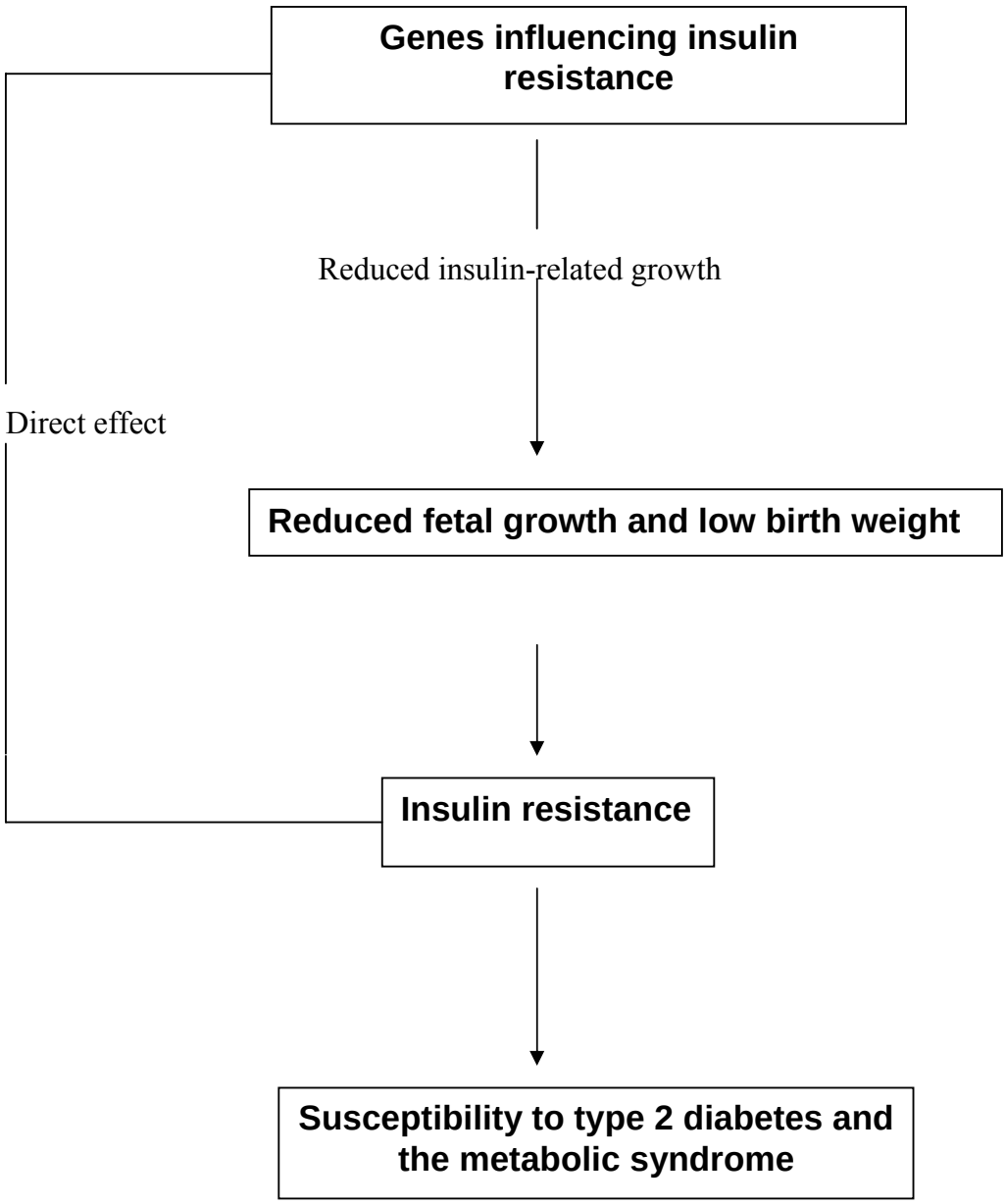
THRIFTY PHENOTYPE HYPOTHESIS



Schematic representation of the thrifty phenotype hypothesis, showing the vital role of fetal nutrition in the development of the metabolic syndrome.

FIG .2.

THE FETAL INSULIN HYPOTHESIS



Insulin resistance, leptin resistance, ghrelin, and satiety

The insulin/leptin-arcuate nucleus of the hypothalamus axis regulates energy homeostasis through control of appetite and energy expenditure. Both hormones rise in direct proportion to adipose mass; they cross the blood-brain barrier and have receptors in the arcuate nucleus. Leptin acts on POMC (pro opio melanocortin) expression and α-MSH (melanocyte stimulating hormone) release. α-MSH in turn, interacts with MC3/4R (melanocortin 4 receptor) to reduce food intake and increase energy expenditure by activating the sympathetic nervous system. Leptin down-regulates anabolic NPY(neuro peptide Y), agouti-related peptide (AGRP), orexins, and melanin-concentrating hormone in the hypothalamus. The central melanocortin system is a key mediator of the catabolic effects of insulin in the brain. Gastric secretion of ghrelin is increased by fasting and increases pituitary GH release, thereby stimulating lipolysis to provide energy substrates. Ghrelin stimulates NPY-AGRP to antagonize α-MSH. The resultant lack of anorexic pressure on MC4Rs results in increased feeding behavior and energy efficiency (with reduced fat oxidation) to store energy as fat. Conversely, in the fed state, insulin and leptin levels are increased, which increases the synthesis and processing of hypothalamic POMC to its component peptides, including α-MSH, which, together with its

colocalized neuromodulator cocaine/amphetamine-regulated transcript, acts at the MC4R to decrease appetite. Insulin and leptin also directly inhibit NPY-AGRP, further limiting feeding and providing for unantagonized MC4R occupancy. Therefore, ghrelin, insulin, and leptin represent afferent hormonal links between peripheral energy metabolism and central feeding behavior and tie together the gut, pancreas, adipocyte, hypothalamus, and pituitary to form a coordinated growth and energy regulatory system[11,12].

Natural history of the clinical insulin resistance syndrome

The natural history of insulin resistance syndrome begins in childhood, from the interplay of genetic and environmental factors. Although it is generally unclear whether a primarily genetically encoded state of insulin resistance and/or satiety disorder appears first, insulin resistance results in hyperinsulinism and precocious development of atherosclerosis and type 2 diabetes[13]. The modern day diet from early childhood replete with large amounts of saturated fats and excess carbohydrates is probably important to the development of hyperinsulinemia and obesity. Dietary carbohydrates (and fats) induce hyperinsulinism, a reduction in fatty acid (FA) oxidation, and hypertryglyceridemia. Diets rich in saturated FAs add a strong insulinotropic effect. In children, obesity and insulin resistance precede the development of hyperinsulinism. The hyperinsulinemia can thus be

seen as a compensatory mechanism for the preexisting, genetically programmed insulin resistance which represents a mechanism for protection against the development of impaired glucose tolerance and diabetes.

Insulin hyper secretion (especially portal) leads to increased free fatty acid synthesis, especially in the liver and adipose tissue. A compensatory increase in glucose oxidation and increased malonyl coenzyme A signaling in the face of abundant fatty acids directs diversion away from ß-oxidation to compensatory increases in long-chain fatty acids and triglycerides synthesis in the liver. Normally appetite can be suppressed by both leptin and insulin; however, diets high in fat stimulate appetite directly. The liver, in turn, becomes insensitive to compensatory leptin signaling to increase ß-oxidation, which is blocked in insulin resistance state, because of high levels of malonyl CoA. Elevated levels of malonyl CoA block fatty acid ß-oxidation, leading to triglyceride accumulation in muscle and liver, with impaired serine phosphorylation of insulin receptor substrate-1, decreased GLUT4 translocation, and thereby decreased glucose oxidation. In the pancreatic islets, these events lead to activation of caspases and increased ceramide levels inducing apoptosis of ß-cells. Type 2 diabetes thus results when there is insufficient insulin secretion to counter preexisting insulin resistance.

FIG.3

CLINICAL FEATURES OF IRS WITH NATURAL HISTORY

Infancy

FH of obesity & type 2 diabetes

SGA & LGA **Childhood**

Gestational diabetes

Acanthosis nigricans **Adolescence**

Premature adrenarche

Obesity, Pseudo acromegaly

Striae, Skin tags,Amenorrhea **Adulthood**

Tall stature, Pseudo acromegaly

Fatty liver, Focal glomerulosclerosis

Hirsutism, ovarian hyperandrogenism, PCOS

Increased carotid wall thickness,Stroke, CHD

Glucose intolerance, type 2 diabetes

FIG. 4

LABORATORY FEATURES OF INSULIN RESISTANCE WITH NATURAL HISTORY

Infancy

Hyperinsulinism & insulin resistance

↓ IGFBP-1, ↓ SHBG ↑Free testosterone **childhood**

↓CBG ↑Free cotrtisol

↑ VLDL, ↑TG, ↓HDL

↑ PAI-1 ↑CRP, ↑FIBRINOGEN **Adolescenc**e

Adhesion molecules and uric acid

Decreased first insulin response

Increased decompensated insulin resistance **Adulthood**

Postprandial hyperglycemia

Fasting hyperglycemia

Diabetes

Loss of first phase insulin response to predict development of diabetes

Children affected by insulin resistance are usually hyperinsulinemic individuals in whom carbohydrates can induce a delayed, but excessive, rise in insulin secretion[14]. This may cause an excessive fall in glucose levels 3–4 h later, of sufficient severity to provoke symptoms of hypoglycemia (late reactive hypoglycemia). As the ability to secrete insulin declines, postprandial glucose intolerance appears, followed by fasting hyperglycemia and diabetes.

Hyperinsulinism and insulin resistance are not benign, even without diabetes

The majority of persons with insulin resistance will not develop type 2 diabetes. Insuin resistant individuals who can compensate by hyperinsulinemia may escape diabetes, but are still prone to other complications, such as early atherosclerosis, progression of obesity (especially central type), acanthosis nigricans, increased skin tags, hypertension, dyslipidemia, hypercoagulation, polycystic ovarian syndrome, fatty liver infiltration, focal segmental glomerulosclerosis, and an increased cancer rate as well[15]. Thus insulin resistant syndrome is not benign even when diabetes does not develop.

HYPERINSULINISM AND INSULIN RESISTANCE ASSOCIATED PATHOLOGIES

Adipose Tissue

It is widely believed that obesity itself, especially increased visceral fat accumulation, can lead to insulin resistance[16]. Genetically induced insulin resistance can be the primary mechanism underling and evoking the progression of obesity. In contrast, non-obese lean individuals can develop insulin resistance also. It has been shown that lean sisters and brothers of patients with obesity complicated by insulin resistance and polycystic ovarian syndrome can have insulin resistance, confirming that insulin resistance can be a primary mechanism.

Visceral fat

Visceral fat is a potent modulator of insulin action on hepatic glucose production[17].Central distribution of body fat (waist/hip ratio, >0.90 in females and >1.0 in males) is associated with an increased risk of stroke, CHD, diabetes, and early mortality and is a more sensitive indicator of impending morbidity than absolute fat mass. Waist circumference correlates with cardiovascular morbidity as well as BMI. Leptin levels are higher in subcutaneous fat and show greater correlations with subcutaneous adiposity than with visceral adiposity[18].

Visceral fat tissue, through its portal drainage, is an important source of free fatty acids that increase hepatic lipogenesis and decrease glucose oxidation.In comparison with subcutaneous fat,visceral fat has more glucocorticoid receptors and higher local concentrations of glucocorticoids. Omental adipose tissue contains significantly more 11ß-hydroxysteroid dehydrogenase type 1 (11ßHSD1) activity than subcutaneous adipose tissue[19], promoting increased cortisol production from conversion of inactive cortisone. Growth Hormone (and/or IGF-I) was suggested recently to inhibit 11ßHSD1, whereas in obesity, GH levels are decreased, leading to higher 11ßHSD1 activity[20]. 11ßHSD1 activity correlates with insulin resistance[21.] A local increase in glucocorticoid hormone action in visceral fat may contribute to the pathogenesis of key features of the metabolic syndrome. Increased abdominal striae and biochemically increased urinary free cortisol levels in obesity are observed.

Patients with Cushing's syndrome have high levels of serum cortisol, and the patient with insulin resistance syndrome has low to normal levels, though both have increased levels of urinary free cortisol. The explanation lies in the decreased levels of corticosteroid-binding globulin (CBG) found in insulin resistance syndrome, where circulating cortisol is disproportionately free and bioactive, with increased

conversion of cortisone to the metabolically active cortisol. The clinical distinction between patients with Cushing's and IRS is that the former is invariably growth retarded, in contrast to the child with insulin resistance syndrome in whom linear growth is excessive.

Fatty liver or hepatic steatosis.

Hepatic steatosis is another complication of insulin resistance that may progress over years with inflammation and fibrosis (nonalcoholic steatohepatitis). In adult patients with diabetes and obesity, 100% have mild steatosis, 50% have steatohepatitis, and 19% have cirrhosis[22] .The disease is usually silent over many years. Serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and γ-glutamyltransferase are elevated and have been proposed as surrogate markers of hepatic fat accumulation[23] . The ratio of aspartate aminotransferase to alanine aminotransferase is usually less than 1, but this ratio increases as fibrosis advances.

Hypertension

Hyperinsulinemia can increase blood pressure by several mechanisms: via its effect to increase renal sodium absorption, via increased activity of the sympathetic nervous system, and via FFA-

induced sensitivity to adrenergic stimuli and antagonized nitric oxide vasorelaxation[24].

Insulin resistance as an initiator of atherosclerosis.

Studies of adults have shown that there is an association between insulin resistance and atherosclerosis. Increased thickness of the arterial carotid wall and an atherogenic dyslipidemic profile compounded by low SHBG levels are factors for increased risk of atherosclerosis.

Importantly, hyperinsulinemia is an independent cardiovascular risk factor[25].The most predictive childhood risk factor was increased BMI. Coronary artery calcifications were also associated with increased blood pressure and decreased HDL cholesterol levels measured during childhood[26]. Fatty streaks can be found in the aorta in children older than 3 yr of age and in coronary arteries by adolescence[27]. The Pathobiological Determinants of Atherosclerosis in Youth study confirmed the origin of atherosclerosis in childhood, showed that progression toward clinically significant lesions may occur in young adulthood, and demonstrated that the progression of atherosclerosis is strongly influenced by CHD risk factors[28].

The thickness of the carotid wall, a validated surrogate marker for atherosclerosis in teenagers and young adults, is sensitive to the intake

of cholesterol, serum levels of cholesterol and triglycerides, BMI, smoking, hypertension, and fasting glucose[29].

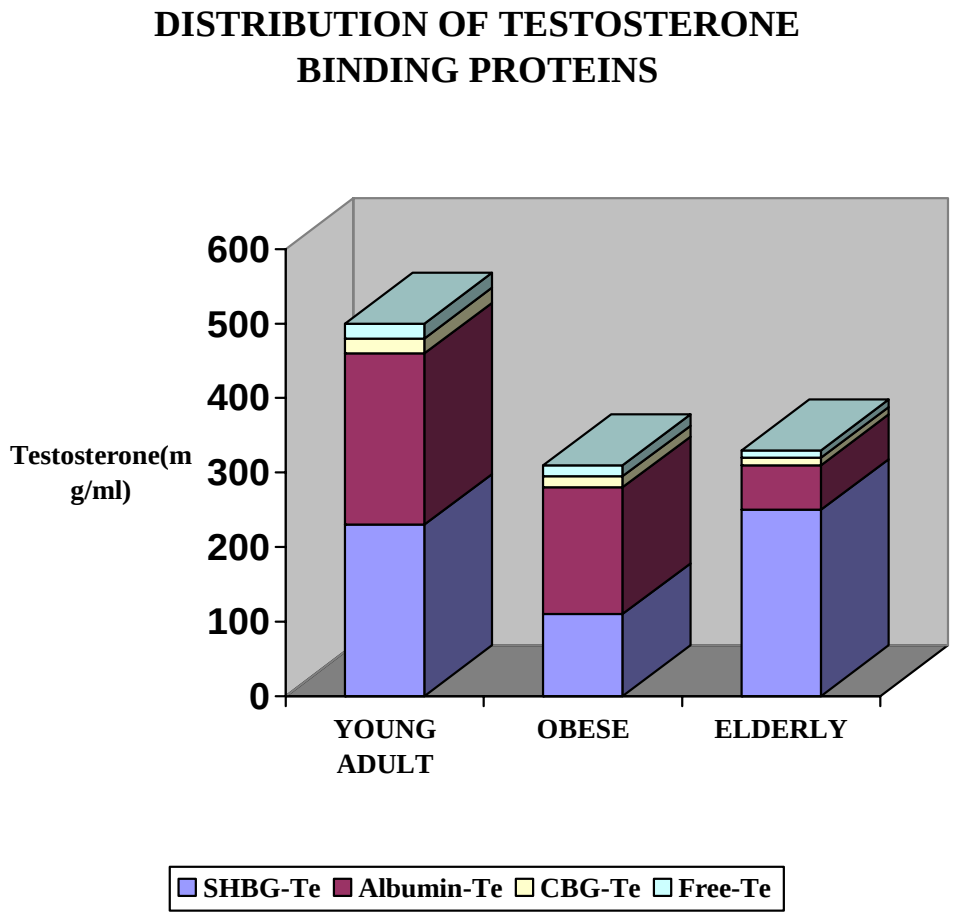
Endothelial dysfunction is an early event preceding the formation of plaques, representing an early disease process of atherosclerosis that begins in childhood and is associated with insulin resistance and hyperinsulinemia[30].

Low hormone-binding proteins and SERPINS.

Sex Hormone Binding Globulin(SHBG) has been found to be negatively correlated with BMI and fasting insulin levels. Decreased SHBG increases testosterone bioavailability, leading to the development of hyperandrogenism, even when serum levels of testosterone are normal.

SHBG is a ß-globulin produced by liver. SHBG is also referred to as testosterone-binding globulin (TeBG) or sex steroid binding protein (SBP). SHBG is present in plasma as 100-kDa heterodimer of variable glycosylated subunits[31]. In normal men about 50% of circulating testosterone is bound to SHBG (Fig.5). The normal range of

FIG. 5



DISTRIBUTIN OF TESTOSTERONE IN BLOOD PLASMA[32]

The free fraction represents 1% to 4% of the total testosterone. When the level of SHBG is reduced , as in obesity, the total testosterone is low, but bioavailanble and free testosterone levels are generally normal.

Sex hormone binding globulin level is 10 to 50 nmol/L, for men, 45 to 90 nmol/L for children, and 30 to 90 nmol/L for women. Aging and various medical disorders alter plasma levels of SHBG.

TABLE-2

CONDITIONS WITH ABNORMAL SEX HORMONE BINDING GLOBULIN CONCENTRATIONS

INCREASED	DECREASED
• Aging	• Hyperinsulinemia
• Androgen deficiency	• Obesity
• Estrogen treatment	• Androgen treatment
• Thyrotoxicosis	• Hypothyroidism
• Alcoholic cirrhosis	• Hypercortisolism
• Hepatitis	• Nephrotic syndrome
• Growth hormone deficiency	• Acromegaly
	• Familial

IGFBP-1 is often strikingly depressed in IRS, producing an excessive of free IGF-I. This can lead to the development of microvascular complications and pseudoacromegaly. It is found that low

levels of IGFBP-1 are associated with the severity of IR, whereas IGFBP-3 correlates directly with the degree of hyperinsulinism.

The low levels of CBG found in IRS lead to disproportionately free and active circulating cortisol. That can lead to clinical and metabolic overlap between Cushing's syndrome and IR. Increased conversion of inactive cortisone to active cortisol by 11ßHSD1 in visceral fat compounds the effect. CBG secretion has been shown to be negatively regulated by both insulin and IL-6[33].

Thyroid binding globulin levels in insulin resistance are often depressed, leading to confusion as to the presence of hypothyroidism. Obese patients are thus often unnecessarily treated for hypothyroidism that they do not have.

Cortisol Binding Globulin (CBG), thyroid binding globulin, and plasminogen activator inhibitor-1 (PAI-1) belong to a family of SERPINS (serine protease inhibitors). These binding proteins (serine protease inhibitors) are substrates for elastase that is expressed at the surface of neutrophils. Increased peripheral white blood cell count and neutrophils are usually found in both obesity and insulin resistance, which might facilitate serine protease availability and binding protein

cleavage. This mechanism is likely to contribute to decreased serum binding globulins levels in obesity and insulin resistance.

Inflammation, asthma, eczema, and impaired immunity.

Insulin resistance syndrome and type 2diabetes have increased markers of inflammation, such as C-reactive protein (CRP), erythrocyte sedimentation rates, and TNF-α levels. BMI correlates with levels of CRP[34], and adiposity has been reported to be the major determinant of CRP levels in children. Leptin has been shown to up-regulate the production of proinflammatory cytokines, including TNF-α and IL-6, to increase phagocytosis by macrophages, and to increase T helper cell type 1 (Th1) levels and suppression of Th2 cytokine production

Significant association between asthma and obesity has been noted, especially during puberty. One of the possible mechanisms is that obesity represents a proinflammatory state, and leptin levels influence Th1 cytokine responses. BMI correlated with the prevalence of asthma in both boys and girls. It was noted that girls who became obese between ages 6–11 yr were 7 times more likely to develop new asthmatic symptoms at ages 11–13 yr[35].

Hypoventilation and sleep apnea.

Excess body fat leads to a decline in the expiratory reserve volume, vital capacity, total lung capacity, and functional residual volume, probably due to the excess body mass, though others implicate excessive leptin levels[36].

Acanthosis Nigricans (AN)

AN is a skin lesion that is widely used as a clinical surrogate of laboratory-documented insulin resistance/hyperinsulinemia, denoting a subgroup with a high risk for type 2 diabetes. The common sites of involvement include axillae, posterior region of the neck, antecubital fossae, and groins. Less commonly, it involves the other flexural areas, umbilicus, submammary region, knuckles, elbows, and, in extreme cases, the entire skin. The severity of AN correlates well with the degree of insulin responses to insulin resistance. AN usually precede insulin resistance documentable by oral or intravenous glucose tolerance test. However, AN also persists into the decompensated phase of insulin resistance where insulin levels may be normal or low.

High levels of insulin acting on both insulin and IGF-1 receptors present on keratinocytes and elevated levels of TNF-α and IFN-γ inducing up-regulation of PPARβ/δ result in keratinocyte proliferation[36].

Hyperandrogenism and reproductive abnormalities

Insulin resistance can present with overt virilization or hirsutism, menstrual irregularity, persistent acne, scalp hair loss, hyperhydrosis, infertility, or precocious adrenarche in childhood. Menstrual irregularity and evidence of hyperandrogenism are associated with the polycystic ovarian disease.Hyperinsulinemia potentiates ovarian hyperandrogenism by enhancing pituitary LH secretion, potentiating ovarian 17-hydroxylase and 17,20-lyase activities, and suppressing blood SHBG and CBG levels and inhibiting both estradiol- and T_4-stimulated SHBG production[37].

Pseudoacromegaly

Linear and acral growth is usually accelerated in insulin resistance and may present as pseudoacromegaly. Hyperinsulinemia promotes linear growth by activating skeletal IGF-I receptors, whereas low levels of IGFBPs can promote IGF-I action by allowing it to be freely and metabolically available. Ghrelin is known to stimulate GH secretion, and in obesity ghrelin levels are decreased. Direct action of leptin on bone growth can predispose to pseudoacromegaly[38]. Pseudoacromegaly is seen in the face of low plasma GH levels secretion so typical for obesity. Leptin decreases GHRH receptor gene transcription, thereby reducing GH levels and reduces responsiveness to GHRH .

Others

Additional complications include focal (IgA type) segmental glomerulosclerosis, uric acid elevation, cholelilthiasis, pseudotumor cerebri, Blount's disease, slipped capital femoral epiphysis, and psychological problems.

MEASURING INSULIN ACTION IN VIVO

Insulin resistance is defined as an impaired ability of plasma insulin at usual concentrations to adequately promote peripheral glucose disposal, suppress hepatic glucose, and inhibit very low density lipoprotein (VLDL) output, but it can be inferred on strong clinical evidence and confirmed by insulin and glucose measurements made by fasting insulin/glucose screening, oral glucose tolerance tests (OGTT), the minimal model frequently sampled iv glucose tolerance test (FSIVGTT), and insulin/glucose clamp studies. Fasting levels of insulin greater than 15 μU/ml, or insulin peak (post-OGTT) levels of more than 150 μU/ml and/or more than 75 μU/ml at 120 min of OGTT are hyperinsulinemic levels, which infer insulin resistance[2,39].

In normal individuals ingestion of a meal increases glucose and other secretogogues, including glucagons-like peptide 1(GLP1), which act in concert to stimulate insulin secretion. Hyperinsulinemia in turn

acts to renormalize the plasma glucose level by suppressing glucose production and increasing glucose utilization. Thus this 'closed loop' feed back system regulates plasma glucose concentration. In states of insulin resistance, the elevated insulin levels will be less able to normalize the glucose , therefore resulting in secondary stimulus to the β-cells and hyperinsulinemia. However, β-cell over secretion could also account for postprandial hyperinsulinism. Because of the closed-loop relationship between insulin secretion and insulin action it is problematic to infer the existence of insulin resistance directly from a 'closed loop' procedure like oral glucose tolerance test (OGTT).

Hyperinsulinemic-euglycemic clamp technique

This technique uses an external feedback control to 'open the loop' between insulin secretion and sensitivity. By maintaining euglycemia endogenous insulin secretion by pancreas is effectively 'clamped'[40].

Procedure: After over night fasting venous catheters are inserted in right and left arms and 3 samples of blood to measure the basal insulin and glucose are taken at 5 min interval. The insulin infusion was started at a rate of 1mU/kg/min and continued for 3 hours. The starting time is marked as '0'. An infusion of 20% glucose was started at time 0. The

plasma glucose level is measured at every 5 minutes and glucose infusion is adjusted to maintain euglycemia at 5.6mmol/L. Insulin levels are measured usually after 2 hours of study at 140, 160, and 180min. Usually it takes at least 2 hours to attain steady state of euglycemia. The rate of glucose infusion is considered a reflection of insulin sensitivity.

The glucose clamp-derived index of insulin sensitivity (SI_{clamp})=M/(G*ΔI)

M- The steady state of glucose infusion (mg/min)

G- The steady state blood glucose concentration (mg/dl)

ΔI- The difference between basal and steady state plasma insulin concentration (microunits/ml)

SI_{clamp} is usually expressed per kg body weight, per unit of surface area, or per unit fat free mass.

Modified minimal frequently sampled intravenous glucose tolerance test:

In the normal 24-h day the insulin-sensitive tissues are never exposed to steady-state conditions. After a meal, glucose and insulin levels are changing, the rate of glucose uptake lags in time behind the time courses of glucose and insulin. It may be preferable to measure insulin action from a dynamic relationship between glucose, the primary nutritive carbohydrate, insulin, the primary anabolic hormone, and the

rate of glucose disposition. One approach to such a measurement is the frequently sampled intravenous glucose tolerance test with 'minimal model'. Minimal model is a computer model that simply represents the plasma dynamics in a compact and accurate package[41].

Procedure : After 12 hours of fasting, 2 vascular accesses obtained by inserting 2 flexible indwelling intravenous catheters through both antecubital veins. One is used for insulin administration; the other is used for glucose administration. Three samples of blood drawn for measuring fasting insulin and glucose at 10 min interval. Glucose in the form of 25% dextrose is administered intravenously over one minute period at a dose of 0.3g/kg. After 20 min insulin is administered in the dose of 0.03u/kg. In next 3 hour period 30 blood samples are drawn for estimation of glucose and insulin at frequent intervals. These values are entered into MINMOAD computer programme (e.g- version 3,Richard N.Bergeman 1994) to evaluate the following:

- First phase insulin release ($AIR_{glucose}$): reflects β cell functionality.
- Insulin sensitivity index (Si) : Reflects ability of insulin to enhance effect of glucose to normalize its own concentration after injection.
- Disposition index (DI): Equals S_i times $AIR_{glucose}$. It reflects insulin sensitivity normalized to the degree of insulin resistance.

On the basis of intravenous glucose tolerance testing, insulin release consists of two phases[14]. In individuals with type 2 diabetes, the second phase response is diminished, and the first phase response is almost absent. However, the first phase response decreases long before the development of type 2 diabetes. AIR predicts the development of diabetes at a time when many subjects still have normal glucose tolerance. The DI is an excellent method to detect latent ß-cell defects, though hyperinsulinism documented by a high AIR is a predictor of the rate of increased fat mass.

Surrogate Measures of Insulin Sensitivity:

Both hyperinsulinemic-euglycemic clamp technique and frequently sampled intravenous glucose tolerance test are time consuming, invasive, expensive, labor intensive, requires experienced personnel, and technically difficult to perform in obese young individuals. Here arises the need for simple but accurate methods for use in large populations. Indices of insulin sensitivity derived from fasting plasma glucose and insulin (HOMA-IR,QUICKI,FGIR, and fasting insulin) correlate strongly with Si assessed by the frequently sampled intravenous glucose tolerance test in obese children and adults[42].

Fasting Insulin:

There is curvilinear relationship between fasting insulin and insulin sensitivity. If insulin sensitivity is low, fasting insulin will be elevated. The fasting insulin concentration will not represent an accurate reflection of insulin sensitivity comparing individuals or groups for whom β- cell function is not identical. When we compare fasting insulin between normal individuals and individuals with impaired glucose tolerance, the latter group is not only insulin resistant, but characterized by a β-cell defect of at least 50%.

Homeostasis model assessment index (HOMA-IR):

This is based on fasting insulin and fasting glucose values .This is calculated by following formula

$$\text{HOMA-IR} = \frac{\text{Fasting glucose (mmol/L)} \times \text{fasting insulin}(\mu\text{mol/ml})}{22.5}$$

In non diabetic individuals plasma glucose differs little compared to fasting plasma insulin levels. In non diabetic subjects HOMA-IR values are proportional to the fasting insulin concentration. HOMA-IR will not reflect insulin sensitivity accurately in nondiabetic subjects with differing β-cell function.

Quantitative Insulin Check Index (QUICKI): It is equal to the inverse of the sum of the logarithms of fasting plasma glucose and fasting plasma insulin.

QUICKI= 1/ (log fasting insulin(micromol/ml)+log fasting glucose(mg/dl))

Despite the logarithmic transformation, QUICKI values exhibit nonlinear proportionality to fasting plasma insulin and may underestimate insulin resistance in a population with a latent decrease in β-cell function.

Fasting Insulin Glucose Ratio (FIGR): The fasting glucose-to-insulin ratio has also been proposed as a useful estimate of insulin sensitivity.

$$\text{FIGR} = \frac{\text{Fasting glucose (mg/dl)}}{\text{Fasting insulin (micromol/ml)}}$$

Insulin Sensitivity Based Upon the OGTT: There are insulin sensitivity indices based on oral glucose tolerance test like Stumvoll index and Matzuda index. It is clear that a correlation between proposed OGTT-derived indices and clamp-derived insulin sensitivity may exist. However, the OGTT-derived indices contain endogenous insulin response in their calculation and it can not be excluded that reported

correlations reflect islet cell response rather than insulin sensitivity. As OGTT-based methods reflect secretion rather than sensitivity, application of the OGTT methods to subjects with impaired β-cell secretory capacity(e.g., those with impaired glucose tolerance,type 2 diabetes) would incorrectly underestimate insulin resistance as post load hyperinsulinemia would be reduced. Another limitation is poor reproducibility[43]. This is due to high day-to-day variability in gastrointestinal function (gastric functioning,absorption and G.I hormones). It is concluded that variations in OGTT can not be readily interpreted to reflect changes in insulin sensitivity but rather changes in gastric emptying, insulin secretion, etc. At this juncture, it may not possible to support the use of the OGTT for assessing insulin resistance.

TREATMENTS

In children, insulin resistant is usually well compensated by hyperinsulinemia, but there may be progressive failure of compensation through puberty with rising glucose and triglyceride levels. Even compensated hyperinsulinemia can lead to numerous complications from fatty liver and atherosclerosis to increased cancer risk. It is thus increasingly obvious that this sequence of events will be most easily interrupted at the earliest phase of life i.e., during childhood. The child with insulin resistant syndrome should be aggressively treated by

involvement in an exercise program, such as walking or swimming for 30–40 min most days of the week, because exercise provokes glucose entry into muscle without the involvement of insulin. Calorie and especially carbohydrate restriction is the key to reduce weight. However, where there is also an increased level of triglycerides, restriction of animal fats should be imposed. Fibrates may be required, especially when trigglycerides levels exceed 500 mg/dl, at which point acute pancreatitis and gall bladder disease become real risks. In this regard, behavioral therapy and metformin have been proven safe and effective in improving insulin sensitivity in pediatric patients[44]. Laparoscopic surgery as well has been shown to be effective in decreasing weight, dyslipidemia, and insulin resistance in adults.

Family-based behavioral interventions for obese children are safe and useful treatments for pediatric obesity. These interventions have been associated with reductions in total cholesterol, increases in HDL cholesterol, reductions in insulin resistance, and return of ovulatory cycles[45].

Metformin is the drug of choice for insulin resistance syndrome. Metformin has various mechanisms of action in insulin resistance. It enhances insulin binding to insulin receptor with augmented phosphorylation and tyrosine kinase activity of the receptor[46]. It is

effective even in cases of insulin receptor mutations. It increases peripheral utilization of glucose through potentiating the phosphoinositol 3-kinase after engagement of the insulin receptor, increasing translocation of the glucose transporters GLUT1 and GLUT4 isoforms to cell membrane in different tissues; increases the activity of adenosine monophosphate kinase in muscle and liver. It increases IGFBP-1; decreases endothelin-1, a marker of vasculopathy; and decreases hepatic glucose output. Metformin down-regulates TNF-α expression and uncoupling protein-2 mRNA concentrations in liver, thus decreasing hepatic lipid biosynthesis. Metformin is safe for the treatment of insulin resistance syndrome in pediatric patients[47].

The PPAR-γ (thiazolidenediones) agonists are a group of ligand-activated transcription factors that govern energy metabolism, cell proliferation, and inflammation[48]. PPAR-γ agonists are effective at insulin sensitization, but are less useful in supporting weight loss.

Lipid-lowering agents

Fibrates lower triglyceride levels, as mediated through the PPAR-γ transcription factor, mainly in liver, where it has an important role in FA oxidation, gluconeogenesis, and amino acid metabolism. Pretreatment of endothelial cells with a PPAR-γ agonist (fenofibrate) reduced markers of

inflammation such as vascular cell adhesion molecule-1 expression, CRP, fibrinogen, PAI-1, and IL-6. Statins inhibit 3-hydroxy-3-methylglutaryl-CoA reductase, the rate-limiting enzyme synthesis of cholesterol. To compensate for decreased synthesis and to maintain cholesterol homeostasis, hepatocytes increase the expression of LDL receptors, which increases the uptake of plasma LDL, the main carrier of extracellular cholesterol, resulting in lower plasma LDL concentrations. Decreased plasma LDL levels reduce the progression of atherosclerosis and may even lead to the regression of preexisting atherosclerotic lesions. Statins have important immunomodulatory effects as well and are able to decrease the recruitment of monocytes and T cells into the arterial wall and inhibit T cell activation and proliferation[49].

Low doses of aspirin inactivate the enzyme cyclooxygenase, which catalyzes the conversion of arachidonic acid to prostaglandins G_2 and H_2. These prostaglandins are precursors of thromboxane, a potent platelet proaggregant and vasoconstrictor. Low doses of enteric coated aspirin is preferred. Aspirin may have a place in dyslipidemic children with insulin resistance syndrome prone to pancreatitis.

Surgery

Restrictive surgical procedures based on an adjustable silicone band placement around a stomach fundal pouch can create a functional partition of the stomach. Whereas restrictive procedures are effective in reducing intake of solid foods, high consumption of more liquid high calorie foods may prevent weight loss[50]. Intestinal bypass surgery in children should probably only be used only in cases of potentially life-threatening complications such as sleep apnea.

AIM OF THE STUDY

To correlate the level of sex hormone binding globulin with level of insulin resistance and (or) hyperinsulinemia in obese prepubertal children.

REVIEW OF LITERATURE

David E.Laaksonen et al[51] assessed the association of low levels of testosterone and sex hormone binding globulin level with development of metabolic syndrome and diabetes in men. They followed 702 middle-aged Finnish men participating in a population – based cohort study. After 11 years of follow-up, 147 men had developed the metabolic syndrome and 57 men developed diabetes. Men with total testosterone, calculated free testosterone and SHBG levels in the lower fourth had several fold increased risk of developing the metabolic syndrome and diabetes. They concluded that low total testosterone and SHBG levels independently predict development of the metabolic syndrome and diabetes in middle aged men.

V.Jeya Gopal et al[52] tried to assess the biological variability of total testosterone and SHBG in polycystic ovarian syndrome and to determine the use of SHBG as a surrogate marker of insulin resistance in PCOS. The PCOS group had higher testosterone, lower SHBG and greater HOMA-IR than controls. In contrast to HOMA – IR, the intra individual variation in SHBG was lower in PCOS group. This study showed that for patients with PCOS, SHBG is an integrated marker of insulin resistance that may be of use to identity insulin resistant individuals for targeted treatment with insulin sensitizing agents.

Involving diabetic and non diabetic obese population, in a case-contrtol study, V Jeya Gopal et al[53] demonstrated inverse relationship between SHBG concentration and HOMA-IR in the group with type 2 diabetes and in control subjects. In type 2 diabetic group, SHBG concentrations were lower than those in control subjects. The intra individual variation of SHBG for the group with type 2 diabetes was similar to that seen in the control group. In contrast, the mean intra individual variation of serum insulin and HOMA-IR was significantly greater in the group with type 2 diabetes than in the control subjects. Although SHBG levels differed significantly between those with or without diabetes, the absolute mean difference was small; indicating that measurement of SHBG can not be used as a simple test for insulin resistance in diabetics. They concluded that a much larger study is required to investigate whether diagnostic cut off values for low SHBG concentrations and insulin resistance in type 2diabetes can be established. Without these parameters, the utility of a low SHBG concentration as a reflection of insulin resistance in type 2 diabetes will be for serial monitoring of insulin resistance in individuals on treatment after the presence of insulin resistance has been established by conventional means.

Thus a relationship between hyperinsulinemia and decreased serum sex hormone binding globulin has been described in adults. Felix Gascon et al[54] evaluated usefulness of SHBG as an index of hypertsulinemia and/or insulin resistance in obese children (aged 6-9 yrs) of both sexes. They carried out a cross-sectional study of cases and controls. The obese group presented significantly elevated levels of insulin, and insulin / glucose ratio compared with control group. SHBG and testosterone levels were significantly lower than those in the non-obese group. Fasting insulin, BMI and testosterone were inversely correlated with SHBG concentration. Multivariate analysis revealed insulin was the only independent predicting factor for serum SHBG concentration in obese group. They concluded that there is strong relationship between insulin and SHBG. Their data supported the role of insulin in the regulation of serum SHBG level.

PJ Galloway et al[55] assessed the validity of SHBG as a potential marker for hyperinsulinemia / insulin resistance in prepubertal obese children. 25 obese children were studied; 14 Children were found to be hyperinsulinemic. The SHBG concentrations were below the sex related reference range in the hyperinsulinemic group. They concluded that a subnormal SHBG concentration in a prepubertal child is strongly predictive of hyperinsulinemia. By measuring the circulating SHBG concentration, it might be possible to identity those at risk of metabolic syndrome targeting them for lifestyle changes.

STUDY JUSTIFICATION

Traditionally, hyperinsulinemic – euglycemic clamp technique and modified minimal model frequently sampled intravenous glucose tolerance test (FSIVGTT) are used to document the insulin sensitivity or resistance accurately. Both the procedures are time consuming, invasive, expensive, labour intensive, require experienced personnel and are technically difficult to perform in obese young people.

Simple indices based on fasting insulin and glucose concentration have been developed. Homeostasis model assessment for insulin resistance (HOMA-IR), Quantitative insulin sensitivity check index (QUICKI), and fasting glucose-to-insulin ratio (FGIR), are epidemiologically useful insulin sensitivity indices. These indexes derived from fasting samples appear to be a valid tool for estimating insulin sensitivity on obese children adolescents[42].

There are certain limitations in using these fasting indices. In patients with impaired glucose tolerance and overt DM, the utility of these indices decrease especially that of HOMA-IR[56]. In overt type 2 DM; large amounts of proinsulin relative to insulin are secreted. Routine insulin assays will yield falsely high values of insulin and thus make fasting indices less useful. After therapeutic intervention, on serial follow up of children, they are subject to repeated fasting to assess

insulin resistance. Even slight exertion or consumption of single toffee which are quiet unavoidable in young children at times can dramatically alter values fasting insulin indices.

Hence there is a need for a surrogate marker of insulin resistance, which is reproducible, stable and easily measured. It would be invaluable for both research and clinical practice, particularly for following insulin-sensitizing therapy such as metformin and thiazolidinediones.

There is evidence that insulin is an important modulator of SHBG concentration. It is potent inhibitor of SHBG production and also reduces the stimulatory effect of 17 B-estradiol and thyroxine on SHBG production.

Reduced serum SHBG levels have been described in different insulin resistance states with hyperinsulinemia such as polycystic ovary syndrome[52].

Some studies have proved that low SHBG concentration is an independent risk factor for non-insulin-dependent diabetes mellitus[57].

In insulin resistant states unlike insulin and glucose concentrations, SHBG concentrations are stable and will not undergo short-term fluctuations.

MATERIALS AND METHODS

Study Design:

Descriptive study.

Study period :

Aug 2004 to March 2006

Study place:

Pediatric Endocrine Division, Institute of Child Health & Hospital for Children, Chennai – 8.

Inclusion Criteria:

Prepubertal obese children (Tanner stage I) aged move than 5 years with Body Mass Index (BMI) falling above 95th percentile.

Exclusion Criteria:

Secondary causes of obesity (e.g., Genetic syndrome Cushing syndrome, hypothyroidism, etc) Children on long term drug therapy especially with those drugs which are going to alter the body

composition (e.g.,, amphetamine, methylphenidate, growth hormone, etc).

Sample Size

Based on two previous European studies conducted on children, for an α error of 0.05 and the power of study being 0.8 with 95% confidence interval, calculated sample size is 45 that was approximated to 50. The anticipated mean difference in SHBG is 15nmol/L. The anticipated standard deviation is 20nmol/L.

Maneuver

The subjects who were included in the study were admitted in General pediatric medical ward. After 12 hours of fast and bed rest, the following biochemical parameters were measured.

1) Fasting plasma glucose
2) Fasting insulin
3) Sex hormone binding globulin

Plasma glucose was determined by glucose oxidase – peroxidase method in a automated analyzer. Plasma insulin levels were measured by ELISA immunoassay. Sex hormone binding globulin levels were estimated by chemiluminenece immunoassay.

The HOMA-IR, QUICKI, and FGIR were derived as estimates of insulin resistance. The HOMA index was calculated as fasting insulin concentration (μu/ ml) x fasting glucose concentration (mmol/L) / 22.5. The QUICKI was calculated as1/ [log fasting insulin concentration (μu/ ml) + log glucose concentration (mg/dL)]. FGIR is calculated as fasting glucose (mg/dl)/fasting insulin (μu/ ml).

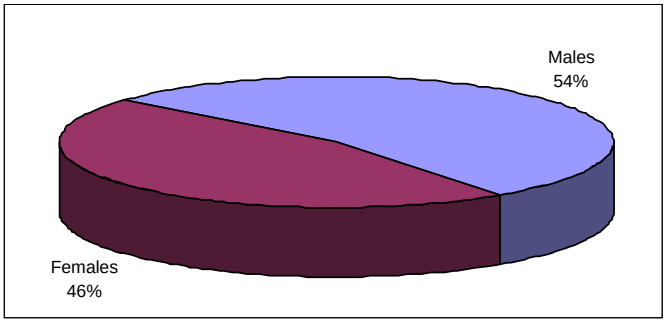
Pearson correlation coefficient was calculated between SHBG and insulin resistant indices. Simple linear regression was constructed taking insulin resistant index as dependent variable and SHBG as independent variable.

RESULTS

50 obese prepubertal children were included in the study based on statistically calculated sample size. Though there was skewed distribution of fasting insulin level, none of them was overtly diabetic.

FIG.6

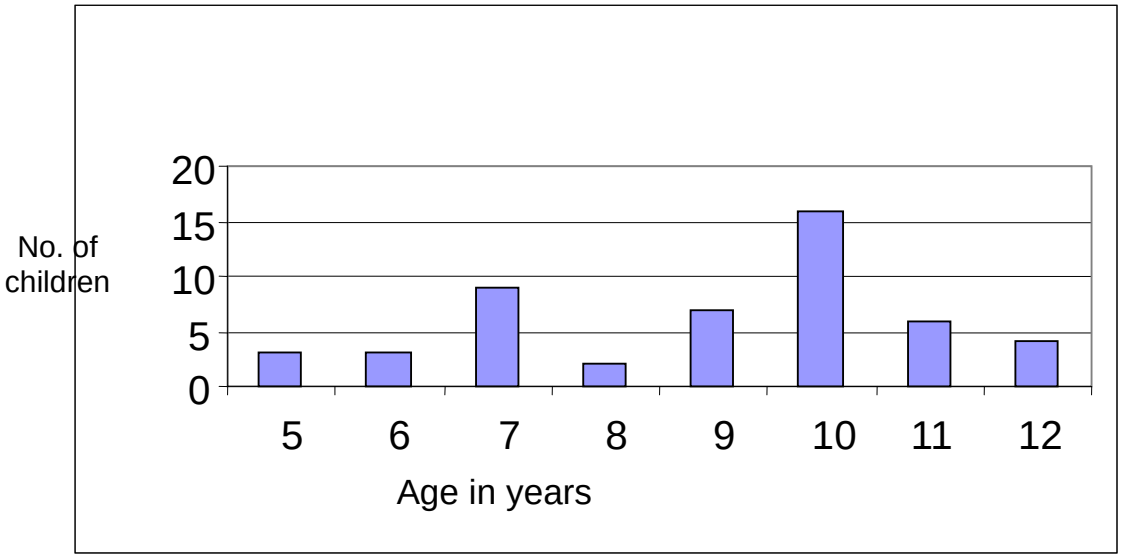
Sex distribution



Of the total of 50 children studied, there were 27 males and 23 females.

FIG.7

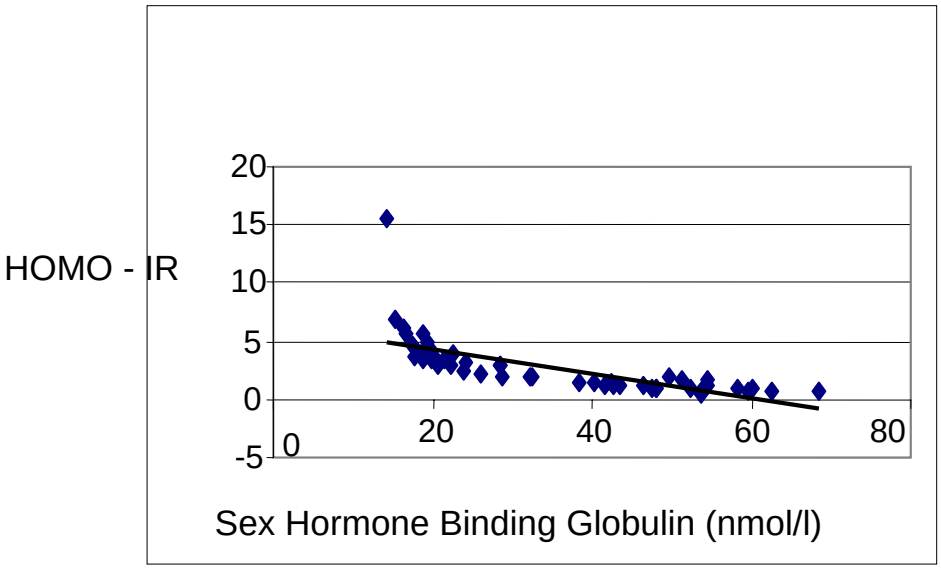
Age distribution



Number of cases registered in our obesity clinic increases along with increasing age. This diagram reflects inclusion criteria of the study whereby older children with signs of pubertal progression are excluded.

FIG. 8

CORRELATION BETWEEN SHBG & HOMO - IR



$y = -0.1069x + 6.4796$

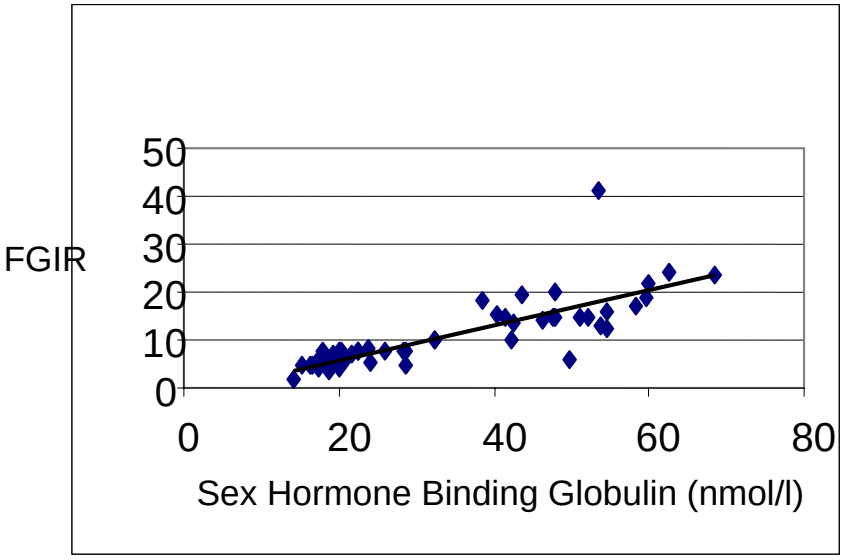
$R^2 = 0.4889$

$r = -0.699214$ $p<0.01$

There is significant negative correlation between HOMA-IR values and Sex Hormone Binding Globulin levels exhibiting non-linear relationship at hyperinsulinemic levels.

FIG. 9

CORRELATION BETWEEN SHBG & FGIR



$y = 0.3627x - 1.3856$

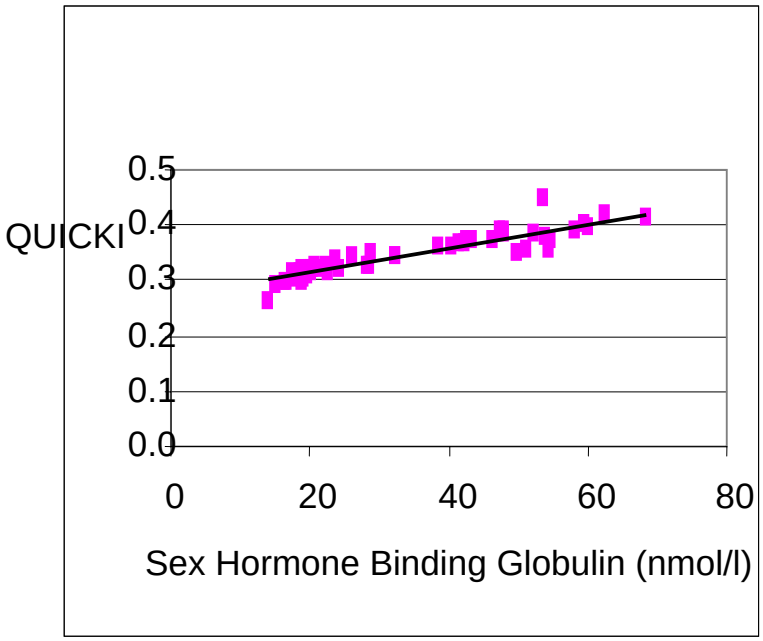
$R^2 = 0.6491$

$r = 0.805667$ $p<0.01$

There is significant positive correlation between Fasting Glucose-Insulin Ratio and Sex Hormone Binding Globulin level.

FIG. 10

CORRELATION BETWEEN SHBG & QUICKI



$y = 0.0022x + 0.268$

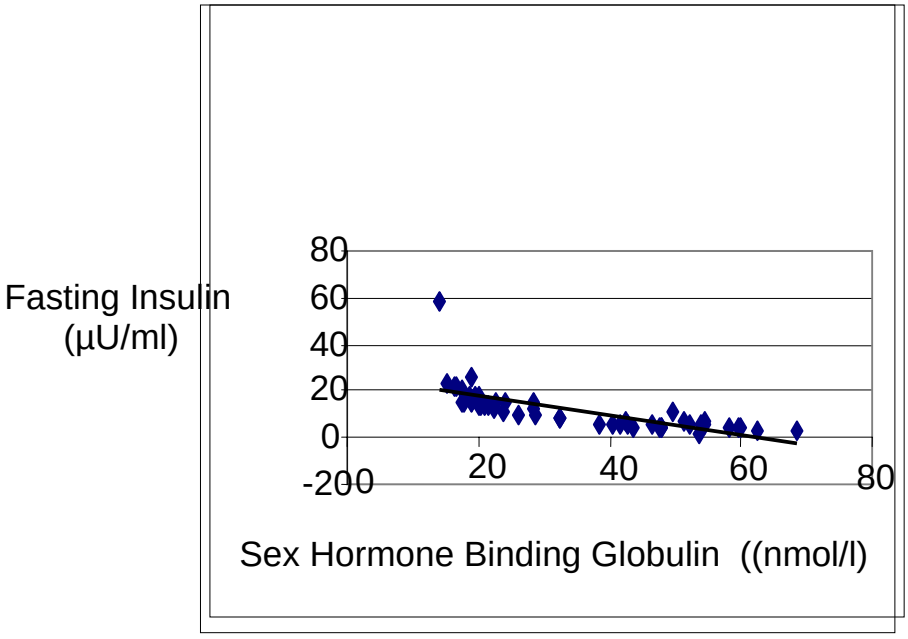
$R^2 = 0.8515$

$r = 0.922768$ $p<0.01$

There is positive healthy correlation between QUICKI values and Sex Hormone Binding Globulins.

FIG.11

CORRELATION BETWEEN SHBG & FASTING INSULIN



$y = -0.409x + 26.07$

$R^2 = 0.5165$

$r = -0.718679$ $p<0.01$

There is significant negative correlation between fasting insulin levels and Sex Hormone Binding Globulin levels which is nearly equivalent to the correlation established between HOMA-IR and SHBG.

DISCUSSION

The plasma SHBG concentration, originally considered to be under the direct regulation of sex hormone levels, is now considered to be under multi factorial regulation. Indeed, the basal production of SHBG seems to be steroid independent and more related to general metabolic factors and nutritional status[58,59]. In this sense some hormones such as growth hormone, insulin like growth factor (IGF)-I and insulin may play a more active role in the regulation of SHBG levels[60]. Recently, there has been increased interest in the role played by insulin in this multi regulation. It is now known that insulin has an inhibitory effect on synthesis of SHBG.

By using cultured HepG2 cells, it has been found that insulin is potent inhibitor of basal production of SHBG. This action may by a direct mechanism or by indirectly increasing free fraction of IGF1. Any way, the relationship between insulin and SHBG is widespread phenomenon in adults[61] and is present even in neonates[62]. Therefore, evidence suggests that it is a more physiological process than was originally thought[63]. Thus SHBG level could be a general marker for hyperinsulinemic insulin resistance. This would explain the observation that a low serum SHBG level is a strong predictor for development of NIDDM.

Though numerous adult studies have already established negative correlation between insulin resistance and sex hormone binding globulin level, prepubertal period is ideal time to test the relationship. During pubertal progression, there are dynamic changes in the level of insulin resistance. Insulin resistance increased immediately at the onset of puberty (T2) but returns to near prepubertal level by the end of puberty (T5). Its peak occurs at T3 in both sexes[64]. Girls are more insulin resistant than boys at all pubertal stages.

More over SHBG level is also influenced by sex hormones. Androgens decrease SHBG level: estrogen increase SHBG level. Pregnancy, oral contraception and liver disease adversely affect the normal physiological levels of SHBG. Because of absence of sex hormone influence on SHBG, insulin alone has greatest impact on its secretion in prepubertal period. More over prepubertal children aged more than 5 years are more prone to develop simple obesity. Hence prepubertal period offers excellent opportunity to study correlation between SHBG and insulin resistant status among all age groups.

David E.Laaksonon[51] et al showed that after adjustment for age, men with concentration of SHBG in the lower fourth were 1.7 – 2.8 times more likely to develop the metabolic syndrome than other men during their 11 years follow up study of 705 adult males. They also

showed that after adjusting for age, men with SHBG concentration in lower fourth had increased risk of incidence of diabetes by 1.7 to 4.3 fold.

Vijay JeyaGopal et al[52] established an inverse relationship between SHBG concentration and HOMA-IR in the adult population of type 2 diabetes (r = -0.32, P – 0.001) and in control subjects (r = - 0.28, P – 0.003).

Vijay JeyaGopal et al[53], in their study of 12 polycystic ovarian cases showed higher HOMA-IR in the PCOS group than in controls and established inverse relationship between SHBG concentration and both serum insulin (r = -0.24, P – 0.001) and HOMA – IR (r = -0.21, P= 0.001).

Very few studies have been conducted so far in children correlating SHBG level and Insulin resistance. Felix Gascon et al[54] in 61 obese children, showed the negative correlation between SHBG level and insulin (r = -0.4512, P - < 0.001). There was positive correlation between FGIR and SHBG (r = 0.4573, P < 0.001).

It is increasingly being recognized that Indians and other South Asians are an ethnic group at high risk for insulin resistance[65]. This is further compounded by large visceral fat, manifested by truncal obesity.

Euglycemic – hyperinsulinemic clamp studies, the gold standard for insulin resistance experiments have shown South Asian men have shown to have lower glucose disposal rates per kg lean body mass as compared to Caucasian American[66]. For a similar BMI, Indian had higher truncal fat than Caucasian. International Diabetic Epidemiology group estimated that DM prevalence in Indians start increasing at BMI of 15 – 20 Kg/m^2 compared with greater than $25kg/m^2$ in Chinese, Japanese and European Population. Thus Indians are at high risk for type 2 DM and metabolic syndrome. So there is a need to validate every conclusion of obesity research in Indian scenario.

Our effort is probably first in India to study the correlation between Insulin resistance and sex hormone binding globulin level in children. Our aim is restricted to find correlation between SHBG and insulin resistant indices only. If healthy correlation is found, it may be useful in our obesity clinic to serially follow the obese children without subjecting them into repeated fasting to determine insulin resistant status.

To fix cut off value for SHBG to determine insulin resistance, a study involving large sample size with cases and controls may be needed.

We not only correlated SHBG level with fasting insulin level but also with epidemiological important derived indexes of insulin resistance like FGIR, HOMA-IR and QUICKI.

a) There is significant negative correlation found between sex hormone binding globulin levels and fasting insulin values.($r = -0.718679$, $p<0.01$)

b) The correlation between SHBG and fasting glucose insulin ratio is positive and significant ($r =0.805667$, $p<0.01$)

c) There is negative correlation between HOMA-IR and SHBG ($r =-0.699214$, $p<0.01$)

d) There is positive healthy correlation between SHBG and QUICKI which is better than correlation of SHBG with any other indices($r =0.922768$, $p<0.01$).

Mehmet Keskin et al[67] evaluated the usefulness of IR indexes in insulin resistant obese children. They conclude that a measure of insulin resistance among children and adolescents the HOMA-IR is more reliable than FGIR and QUICKI.

Arie Katz, Sridhar S, Nambi et al[68] performed Hyperinsulinemic euglycemic glucose clamp and minimal model FSIVGIT in 13 obese, 28 non obese and 15 type 2 diabetic subjects. They correlated both HOMA-

IR and QUICKI with the glucose clamp derived index of insulin sensitivity (SI_{clamp}). The correlation between QUICKI and SI clamp is significantly better than correlation between HOMA and SI_{clamp} ($r = 0.78$ Vs $r = - 0.60$).

There is difference between adults and children in choosing better insulin index to assess insulin resistance. This may be partly explained by the fact that HOMA-IR is not linear over wide range of insulin sensitivity. In adult population, as type2 diabetes mellitus and glucose intolerance are common with elevated fasting insulin and glucose level, HOMA-IR is likely to correlate poorly with insulin resistance. Better correlation is found in children, as glucose intolerance and type2 diabetes mellitus are rare.

When the distribution fasting insulin values is skewed, QUICKI correlates well with insulin resistance as it transforms the data by taking both logarithm and reciprocal of glucose – insulin product.

In our study we never conducted oral glucose tolerance test. There were no overt diabetic children. But there is skewed distribution of fasting insulin values with some children showing steep rise in fasting insulin levels without significant increase in fasting glucose level. This

may be the reason for better correlation between SHBG and QUICKI, when compared to one between SHBG and HOMA-IR.

Whatever may be the index used, there exists significant negative correlation between SHBG and hyperinsulinemia which reflects the physiological role played by insulin on SHBG secretion.

CONCLUSION

There is a strong negative correlation between SHBG and insulin resistant status.

Low SHBG concentration is a stable integrated marker of insulin resistance and therefore has the characteristics to be potentially used as a surrogate measure of insulin resistance.

Currently it is cost effective and also avoids the ordeals of measuring fasting insulin indices.

For the time being the utility of SHBG estimation is limited to serial monitoring of insulin resistance in individuals on treatment after the presence of insulin resistance is established by conventional means.

An extensive study with large sample size with cases and control is needed to determine the cut off value of SHBG for insulin resistance.

With ethnic predisposition and drastic changes in life style there is spiralling incidence of metabolic syndrome in Indian population. Studies in the direction of searching such a useful surrogate marker of insulin resistance syndrome will have great impact in combating this epidemic.

BIBLIOGRAPHY

1. Reaven GM 1988 banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37: 1595-1607.
2. Svetlana Ten and Noel Maclaren 2004. Insulin Resistance Syndrome in Children. The Journal of Clinical Endocrinology & Metabolism 89(6):2526-2539.
3. King H, Aubert RE, Herman WH. Global burden of diseases, 1995-2025. Diabetes Care 1998; 21:1-18.
4. Wilkin TJ,Metcalf BS, Murphy MJ, Kirkby J, Jeffrey AN, Voss LD 2002 The relative contributions of birth weight, weight change, and current weight to insulin resistance in contemporary 5-year –olds: the Early Bird Study.Diabetes 51:3648-3472.
5. Bavdekhar A,Yajnik CS,Fall CH,Bapat S,Pandit AN,Deshphande V,Bhave S, Kellingray SD, Joglekar C. Insulin Resistance Syndrome in 8 year old Indian children: small at birth, big at 8 years, or both?. Diabetes 1999 Dec 48:2422-9.
6. Rosner B, Prineas R, Loggie J, Daniels SR 1998 Percentiles for body mass index in US children 5 to 17 years of age. J Pediatr 132:211–222
7. Barker DJ 2000. In utero programming of cardiovascular disease. Theriogenology 53:555–574.

8. Dabelea D, Pettitt DJ, Hanson RL, Imperatore G, Bennett PH, Knowler WC 1999 Birth weight, type 2 diabetes, and insulin resistance in Pima Indian children and young adults. Diabetes Care 22:944–950
9. Murtaugh MA, Jacobs DR, Moran A, Steinberger J, Sinaiko AR 2003 Relation of birth weight to fasting insulin, insulin resistance, and body size in adolescence. Diabetes Care 26:187–192.
10. Dabelea D, Knowler WC, Pettitt DJ 2000 Effect of diabetes in pregnancy on offspring: follow-up research in the Pima Indians. J Matern Fetal Med 9:83–88.
11. Lustig RH 2001 The neuroendocrinology of childhood obesity. Pediatr Clin North Am 48:909–930.
12. Farooqi IS, Keogh JM, Yeo GS, Lank EJ, Cheetham T, O'Rahilly S 2003 Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N Engl J Med 348:1085–1095.
13. McGarry JD, Dobbins RL 1999 Fatty acids, lipotoxicity and insulin secretion. Diabetologia 42:128–138.
14. Daniel S, Noda M, Straub SG, Sharp GW 1999 Identification of the docked granule pool responsible for the first phase of glucose-stimulated insulin secretion. Diabetes 48:1686–1690.

15. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ 2003 Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. N Engl J Med 348:1625–1638.
16. St-Pierre J, Lemieux I, Miller-Felix I, Prud'homme D, Bergeron J, Gaudet D, Nadeau A, Despres JP, Vohl MC 2002 Visceral obesity and hyperinsulinemia modulate the impact of the microsomal triglyceride transfer protein-493G/T polymorphism on plasma lipoprotein levels in men. Atherosclerosis 160:317–324.
17. Barzilai N, She L, Liu BQ, Vuguin P, Cohen P, Wang J, Rossetti L 1999 Surgical removal of visceral fat reverses hepatic insulin resistance. Diabetes 48:94–98.
18. Montague CT, O'Rahilly S 2000 The perils of portliness: causes and consequences of visceral adiposity. Diabetes 49:883–888 .
19. Bujalska IJ, Kumar S, Stewart PM 1997 Does central obesity reflect "Cushing's disease of the omentum?" Lancet 349:1210–1213.
20. Stewart PM, Toogood AA, Tomlinson JW 2001 Growth hormone, insulin-like growth factor-I and the cortisol-cortisone shuttle. Horm Res 56:1–6.
21. Walker BR, Best R 1995 Clinical investigation of 11ß-hydroxysteroid dehydrogenase. Endocr Res 21:379–387.

22. Silverman JF, Pories WJ, Caro JF 1989 Liver pathology in diabetes mellitus and morbid obesity. Clinical, pathological, and biochemical considerations. Pathol Annu 24:275–302.
23. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N 2001 Metformin in non-alcoholic steatohepatitis. Lancet 358:893–894.
24. Despres JP, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, Lupien PJ 1996 Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 334:952–957.
25. Despres JP, Lamarche B, Mauriege P, Cantin B, Lupien PJ, Dagenais GR 1996 Risk factors for ischaemic heart disease: is it time to measure insulin? Eur Heart J 17:1453–1454 (Editorial).
26. Davis PH, Dawson JD, Riley WA, Lauer RM 2001 Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: The Muscatine Study. Circulation 104:2815–2819.
27. Berenson GS 2002 Childhood risk factors predict adult risk associated with subclinical cardiovascular disease. The Bogalusa Heart Study. Am J Cardiol 90:3L–7L.
28. Strong JP, Malcom GT, McMahan CA, Tracy RE, Newman WP, Herderick EE, Cornhill JF 1999 Prevalence and extent of atherosclerosis in adolescents and young adults: implications for

prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. JAMA 281:727–735.

29. Sorof JM, Alexandrov AV, Cardwell G, Portman RJ 2003 Carotid artery intimal-medial thickness and left ventricular hypertrophy in children with elevated blood pressure. Pediatrics 111:61–66.

30. Paradisi G, Steinberg HO, Shepard MK, Hook G, Baron AD 2003 Troglitazone therapy improves endothelial function to near normal levels in women with polycystic ovary syndrome. J Clin Endocrinol Metab 88:576–580.

31. Petra PH. The plasma sex steroid binding protein(SHBG). A critical review of recent developments on the structure,molecular biology and function. J Steroid Biochem Molec Biol 1991:40:735.

32. Dunn JF, Nisula BC, Roadbard D. Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid binding globulin in human plasma. J Clin Endocrinol Metab 1981;53-58.

33. Fernandez-Real JM, Pugeat M, Grasa M, Broch M, Vendrell J, Brun J, Ricart W 2002 Serum corticosteroid-binding globulin concentration and insulin resistance syndrome: a population study. J Clin Endocrinol Metab 87:4686–4690.

34. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB 1999 Elevated C-reactive protein levels in overweight and obese adults. JAMA 282:2131–2135.

35. Castro-Rodriguez JA, Holberg CJ, Morgan WJ, Wright AL, Martinez FD 2001 Increased incidence of asthmalike symptoms in girls who become overweight or obese during the school years. Am J Respir Crit Care Med 163:1344–1349 .

36. Phipps PR, Starritt E, Caterson I, Grunstein RR 2002 Association of serum leptin with hypoventilation in human obesity. Thorax 57:75–76. Tan NS, Michalik L, Noy N, Yasmin R, Pacot C, Heim M, Fluhmann B, Desvergne B, Wahli W 2001 Critical roles of PPARß/δ in keratinocyte response to inflammation. Genes Dev 15:3263–3277.

37. Dunaif A 1999 Insulin action in the polycystic ovary syndrome. Endocrinol Metab Clin North Am 28:341–359.

38. Steppan CM, Crawford DT, Chidsey-Frink KL, Ke H, Swick AG 2000 Leptin is a potent stimulator of bone growth in *ob/ob* mice. Regul Pept 92:73–78.

39. Reaven GM, Chen YD, Hollenbeck CB, Sheu WH, Ostrega D, Polonsky KS 1993 Plasma insulin, C-peptide, and proinsulin concentrations in obese and nonobese individuals with varying degrees of glucose tolerance. J Clin Endocrinol Metab 76:44–48.

40. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214-23.

41. Bergeman RN, Finegood DT, Ader M. Assessment of insulin sensitivity in vivo. Endocr Rev 1985:6:45-86.

42. Louise SC, Wendy JB, Stewart GT, Jennifer AB. Indexes of insulin resistance and secretion in obese children and adolescents .Diabetes Care 27:314-319, 2004.

43. Hucking K, Bergeman RN, Watanabe R, OGTT –derived measures of insulin sensitivity may be confounded by other factors. Diabetes 3:52(suppl 1):1242-p.

44. Jones KL, Arslanian S, Peterokova VA, Park JS, Tomlinson MJ 2002 Effect of metformin in pediatric patients with type 2 diabetes: a randomized controlled trial. Diabetes Care 25:89–94.

45. Pasquali R, Antenucci D, Casimirri F, Venturoli S, Paradisi R, Fabbri R, Balestra V, Melchionda N, Barbara L 1989 Clinical and hormonal characteristics of obese amenorrheic hyperandrogenic women before and after weight loss. J Clin Endocrinol Metab 68:173–179.

46. Matthaei S, Hamann A, Klein HH, Benecke H, Kreymann G, Flier JS, Greten H 1991 Association of metformin's effect to increase insulin-stimulated glucose transport with potentiation of insulin-induced translocation of glucose transporters from intracellular pool to plasma membrane in rat adipocytes. Diabetes 40:850–857.

47. Freemark M, Bursey D 2001 The effects of metformin on body mass index and glucose tolerance in obese adolescents with fasting hyperinsulinemia and a family history of type 2 diabetes. Pediatrics 107:E55

48. Kersten S 2002 Peroxisome proliferator activated receptors and obesity. Eur J Pharmacol 440:223–234.

49. Palinski W, Tsimikas S 2002 Immunomodulatory effects of statins: mechanisms and potential impact on arteriosclerosis. J Am Soc Nephrol 13:1673–1681.

50. Mason EE 1992 Gastric surgery for morbid obesity. Surg Clin North Am 72:501–513.

51. David EL,Tomi-Pekka T, Leo N, Veli-Pekka V, Kari P, Ritta S. Testosterone and Sex Hormone Binding Globulin predict the metabolic syndrome and Diabetes in middle aged men. Diabetes Care 27:1036-1041, 2004.

52. Jeyagopal V, Kilpatric ES, Jennings PE, Holding S, Hepburn DA, Atkin SL.The boil;ogical variation of testosterone and Sex Hormone Binding Globulin in polycystic ovarian syndrome: Implications for SHBG as a surrogate marker of insulin resistance.J clinical endocrine and metab 88((4):1528-1533, 2003.

53. Jeyagopal V, Kilpatric ES, Jennings PE, Holding S, Hepburn DA, Atkin SL. The biological variation of SHBG in type 2 Diabetes. Diabetes Care, vol-27, num-1,pp-278-280, 2004.

54. Gascon F, Valle M, Marto R, Ruz FJ, Rios R, Montiila P,Canete R. Sex hormone binding globulin as a marker for hyperinsulinemia and/or insulin resistance in obese children. European journal of Endocrinology 143, 85-89, 2000.

55. Galloway PG, Donaldson MDC, Wallace AM. Sex hormone binding globulin concentration as a pre pubertal marker for hyperinsulinemia in obesity.Arch Dis Chlild:2001;85:489-491.

56. Ferrera CM, Goldberg AP: Limited value of the homeostasis model assessment to predict insulin resistance in older men with impaired glucose tolerance. Diabetes Care 2001; 24:245-249.

57. Lindstedt G, Lundberg PA, Lapidus L, Lundgren H, Bengtsson C & Bjorntorp P. Low sex hormone-binding globulin concentrations as independent risk factor for development of NIDDM. 12 year follow of population study of women in Gothenburg, Swedenm. Diabetes 1991; 40:123-128.

58. Schoultz B and Calstrom K. On the regulation of sex hormone-binding globulin: a challenge of an old dogma and outlines of an alternative mechanism. Journal of Steroid Biochemistry and Molecular Biology 1989; 32:327-334.

59. Toscano V, Balducci R, Bianchi P,Guglielmio R, Mangiantini A and Sciarra F. Steroidal and non-steroidal factors in plasma sex-hormone binding globular regulation. Journal of Steroid Biochemistry and Molecular Biology 1992; 43:431-437.

60. Holly JM, Cotterill AM, Jemmotr RC et al. Interrelationships between growth hormone, insulin, insulin like growth factor-1, IGF-binding proteins SHBG in acromegaly.Clinical Endocrinology 1991 34:275-280.

61. Haffner AM. Sex hormone binding globulin, hyperinsulinemia, insulin resistance and NIDDM. Hormone Research 1996 45, 233-237.

62. Simmons D. Interrelation between umbilical cord SHBG , sex steroids, IGF-1,and insulin in neonates from normal pregnancy and pregnancy complicated by Diabetes.J clincal endocrinology and metabolism 1995 ,80:2217-2221.

63. Editorial. SHBG: a maker for hyperinsulinemia/insulin resistance.J clinical endocrinology and metabolism 1993. 76:273-274

64. Antoniette Moran, David R, Jacobs jr, Steinberger j, Hong C, Prineas R,.Insulin resistance during puberty. Results from clamp studies in 357 children. Diabetes, vlo,48.oct 99.

65. Ramachandran A, Snehalatha C, Kapur A, Vijay A, Mohan V, et al.High prevalence of diabetes and impaired glucose tolerance in India: National urban diabetes survey. Diabetologia 2001:2001;44:1094-1101.

66. Chandalia M, Abate N, Garg A, Stray-Gunderson J, Grundy S. Relationship between generalized and upper body obesity to insulin resistance in Asian Indian men. J Clin Endocrinol Metab 1999;84: 2329-2335.

67. Keskin M, Kurtoglu S, Kendirci M, Atabeck ME, Yazici C.Homeostasis Model Assessment is more reliable than the Fasting Glucose-Iinsulin Rratio and Quantitative Insulin Check Index for assessing insulin resistance among obese children and adolescents. Pediatrics 2005;115;500-503.

68. Katz A,Srithar S, Nambi, Mather K, Baron AD, Follman DA, Sullivan G, Quon J. Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrin and Metab(2005) vol. 85, no.7 pp-2402-2410

DATASHEET

Serial no: Endo no:
Date:

Name: Age:
Sex:

Address:

ANTHROPOMETRY

Weight (kg): Height (m): Body Mass Index (kg/m^2):

BIOCHEMICAL PARAMETERS

Fasting Plasma Glucose(mmol/l) :

Fasting Plasma Insulin(µu/ml) :

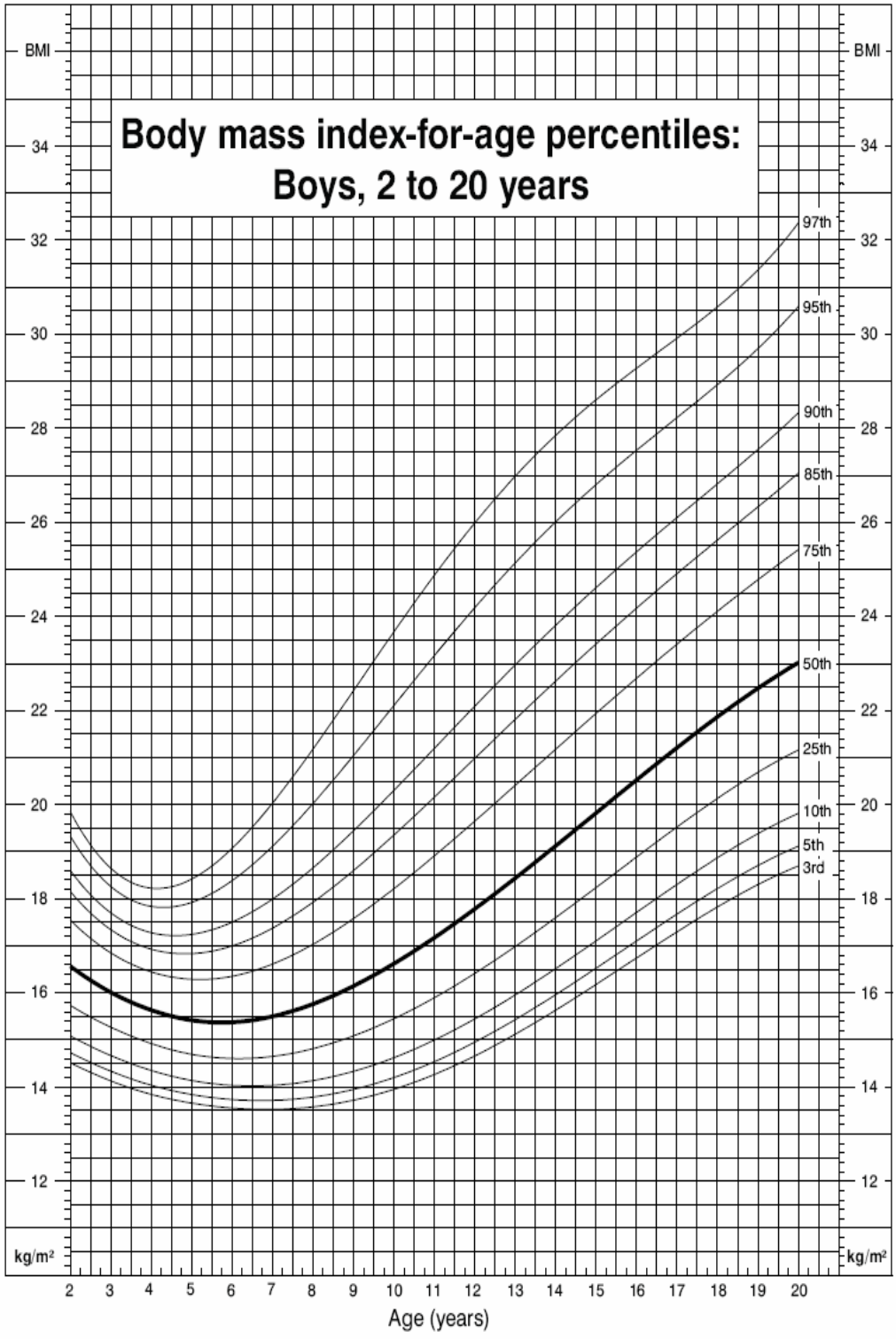
Sex Hormone Binding Globulin(nmol/l) :

FASTING INSULIN INDICES

$$\text{HOMA-IR:} \quad \frac{\text{(Fasting Plasma Glucose(mmol/l) X Fasting Plasma Insulin(µu/ml)}}{22.5}$$

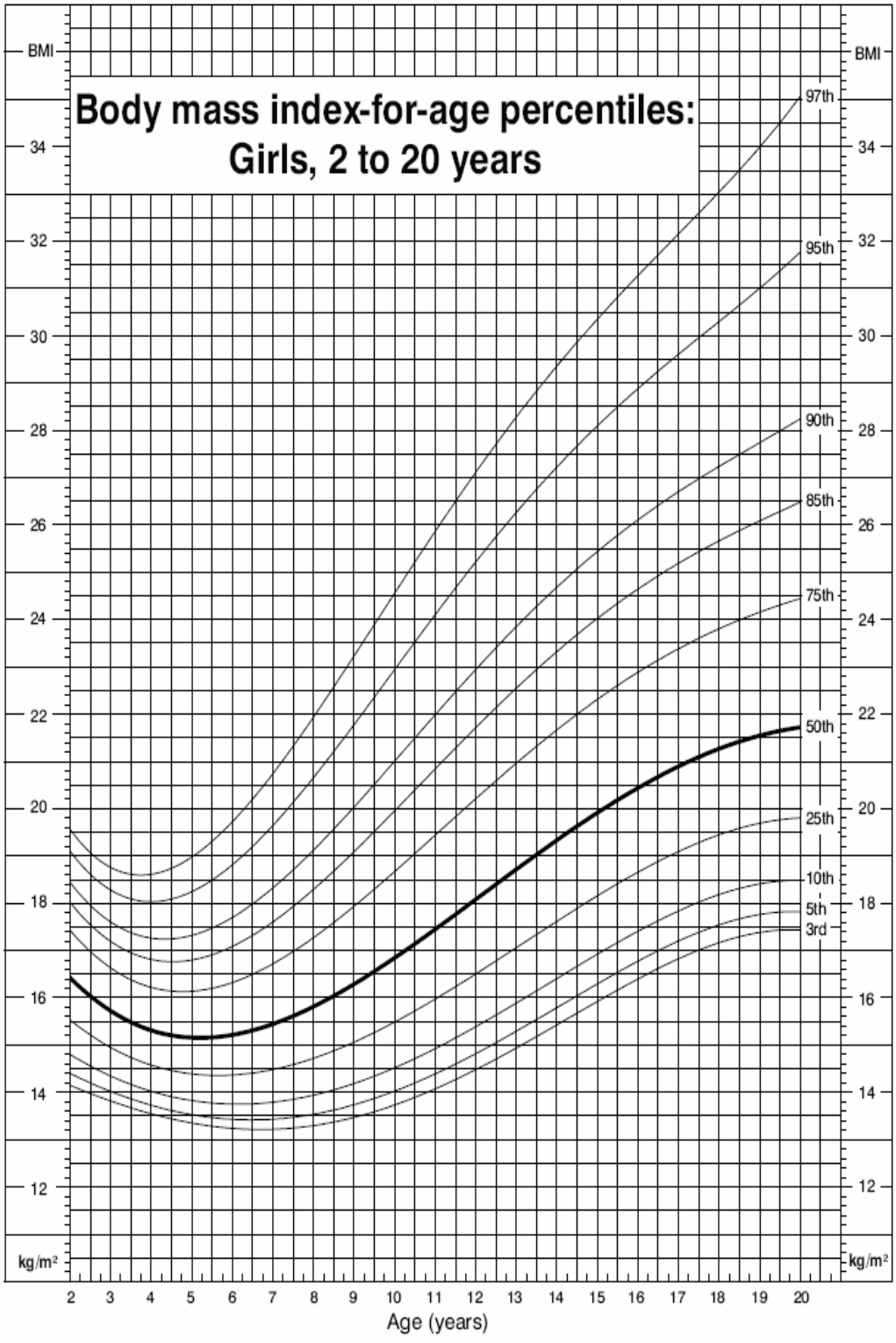
$$\text{QUICKI} : \quad \frac{1}{\text{(log fasting insulin(micromol/ml)+log fasting glucose(mg/dl)}}$$

$$\text{FIGR} : \quad \frac{\text{Fasting glucose (mg/dl)}}{\text{Fasting Plasma Insulin (µu/ml)}} .$$



SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).

Figure 15. Body mass index-for-age percentiles, boys, 2 to 20 years, CDC growth charts: United States



SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).

CDC

Figure 16. Body mass index-for-age percentiles, girls, 2 to 20 years, CDC growth charts: United States