Aberrant Expression of Perilipins and 11-β-HSD-1 as Molecular Signatures of Metabolic Syndrome X in South East Asians

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Abstract
Metabolic syndrome is common in SE Asian. An hypothesis that aberrant expression of perilipins and 11-β-hydroxysteroid dehydrogenase-1 (11-β-HSD-1) enzyme plays a significant role in the development of metabolic syndrome X in Indians is proposed. Thus, methods designed to target perilipins and 11-β-HSD-1 may form a novel approach in the prevention and management of metabolic syndrome X. ©

INTRODUCTION
Abdominal obesity, atherosclerosis, insulin resistance and hyperinsulinemia, hyperlipidemias, endothelial dysfunction, essential hypertension, type 2 diabetes mellitus, and coronary heart disease (CHD) are components of metabolic syndrome X. Other features of metabolic syndrome X also include: hyperfibrinogenemia, increased plasminogen activator inhibitor-1 (PAI-1), low tissue plasminogen activator, nephropathy, micro-albuminuria, and hyperuricemia. Although genetics could play an important role in the higher prevalence of metabolic syndrome X in certain populations, it is not clear how genetic factors interact with environmental and dietary factors to increase its incidence.

Insulin resistance is a dominant feature of metabolic syndrome X, abdominal obesity, hypertension, type 2 diabetes, hyperlipidemias, CHD, and stroke. Hyperinsulinemia may be a consequence of this. In the early stages of metabolic syndrome X insulin resistance is restricted to muscle tissue whereas adipose tissue is not resistant to insulin. Hence, exercise is beneficial in the prevention and treatment of insulin resistance since it decreases insulin resistance and enhances glucose utilization in the muscles. In addition, exercise is anti-inflammatory in nature. Biochemical and functional differences between adipose tissues of different regions
Adipose tissue distribution is dependent on genetic, environmental, and hormonal factors, and is an important predictor of obesity-associated morbidity and mortality. Females have more subcutaneous and gluteofemoral region adipose tissue compared to males. Males have higher adipose tissue localized intra-abdominally. The gluteofemoral fat cells are enlarged in females that have a higher lipoprotein lipase (LPL) activity. Females do not accumulate fat in visceral depots up to a certain degree of obesity whereas males deposit excess fat in this region parallel with other depots. Gluteal region fat cells from females had higher insulin receptor binding and higher rates of non-insulin-stimulated and maximally insulin-stimulated rates of glucose transport and glucose metabolism. These differences in the distribution and properties of fat between males and females could be attributed to female sex steroid hormones and their interaction with cortisol. Omental adipose tissue contains more number of glucocorticoid receptors (GR) compared to subcutaneous adipose tissue with similar Kd values whereas LPL activity in subcutaneous adipose tissue is lower compared to omental adipose tissue. A positive correlation between LPL activity and glucocorticoid binding exists. Human adipose tissue glucocorticoid binding was higher in omental than in subcutaneous adipose tissue, whereas LPL activity was higher in omental than in subcutaneous adipose tissue. Leptin mRNA expression is higher in abdominal subcutaneous adipocytes compared with omental adipocytes. An inverse relationship exists between adipocytes, PPAR-γ expression and body mass index (BMI). Cellular inhibitor of apoptosis protein-2 (cIAP-2) that regulates tumor necrosis factor-α (TNF-α) signaling was expressed at higher levels in omental than subcutaneous adipocytes implying that depot-specific differences exist in the regulation of adipocyte apoptosis. Subcutaneous adipose tissue produces less interleukin-6 (IL-6) and corticosterone and more TNF-α in...
Comparison to mesenteric adipose tissue, PPAR-γ is involved in adipocyte development and insulin sensitivity and exerts a negative control on TNF-α synthesis, suggesting that a complex but local network of events regulate adipocytes accumulation, metabolism and function. This emphasizes the fact that different depots of fat display distinct characteristics that are unique to each region of the body. In this context, it is relevant to note that increase in intramyocellular lipid content can influence insulin resistance.

**Intramyocellular Lipid Content and Insulin Resistance**

Intramyocellular lipid content in humans can be determined by using proton nuclear magnetic resonance spectroscopy technique, which enables non-invasive and rapid (approximately 45 min) determination of intramyocellular lipid (IMCL) content. Determination of intramyocellular lipid concentration showed an inverse correlation (r = -0.579, p = 0.0037) between intramyocellular lipid content and insulin sensitivity. When the contribution of intramyocellular lipid (IMCL) content to skeletal muscle insulin resistance was compared in insulin-resistant and insulin-sensitive subjects, who were matched for sex, age, BMI, percent body fat, physical fitness, and waist-to-hip ratio, and who were first-degree relatives of type 2 diabetic subjects, it was observed that in soleus muscle: IMCL was increased by 84%, and in tibialis anterior muscle: IMCL was increased by 57% in the insulin-resistant offspring, with no difference in the extramyocellular lipid content and total muscle lipid content was noted. These results indicate that increased IMCL represents an early abnormality in the pathogenesis of insulin resistance and contributes to the defective glucose uptake in skeletal muscle in insulin-resistant subjects. Perseghin et al observed that healthy young lean offspring of type 2 diabetic parents, who are at high-risk of developing diabetes in the future, had increased muscle triglyceride content that correlated with the severity of whole body insulin resistance, and these subjects demonstrated no change in the content of unsaturated/saturated fatty acids in adipose tissue when compared with healthy normal subjects without a family history of diabetes. The increased intramyocellular triglyceride content was noted only in the soleus but not in the tibialis anterior muscle, and this difference has been attributed to the presence of more number of type I fibers, which is more insulin sensitive, in soleus than the tibialis anterior muscle. A similar association of intramyocellular lipid content with insulin sensitivity and obesity in Europeans was reported by Forouchi et al, who noted that such association was absent in South Asians. These results were supported by those of Sinha et al who observed that soleus muscle IMCL content correlated significantly with measures of generalized and abdominal obesity but not with insulin sensitivity or CRP levels in healthy Asian Indian males.

In contrast, in a 4-week intervention study wherein a reduction of dietary glycaemic index was used to manipulate insulin sensitivity in a cohort of healthy volunteers, and the effects of this intervention on IMCL showed that significant improvements in the insulin sensitivity index occurred following the dietary intervention with no changes in IMCL storage levels. This led to the suggestion that, at least, in healthy volunteers insulin sensitivity is independent of IMCL storage and high levels of IMCL found in insulin-resistant subjects may as a consequence rather than the cause of insulin resistance. It was reported that adolescent obese subjects have low plasma adiponectin levels that were positively related to insulin sensitivity with a strong inverse relationship between adiponectin and plasma triglyceride levels. Nicotinic acid-induced decrease in insulin sensitivity occurred with increased availability of circulating fatty acids to muscle with no significant changes in muscle lipid content. Paradoxically, trained endurance athletes are markedly insulin sensitive, despite an elevated mixed muscle lipid content. Mixed muscle lipid content was substantially greater in the endurance athletes compared with the type 2 diabetes patients and the overweight men. More than 40% of the greater mixed muscle lipid content was attributed to a higher proportion type I muscle fibers which contained 2.8 ± 0.3-fold more lipid than the type II fibers. The remaining difference was explained by a significantly greater IMCL content in the type I muscle fibers of the trained athletes. Differences in IMCL content between groups or fiber types were accounted for by differences in lipid droplet density, not lipid droplet size. IMCL distribution showed an exponential increase in lipid content from the central region toward the sarcolemma, which was similar between groups and fiber types. These results suggest that IMCL contents can be substantially greater in trained endurance athletes compared with overweight and/or type 2 diabetes patients. Because structural characteristics and intramyocellular distribution of lipid aggregates seem to be similar between groups, it was suggested that elevated IMCL deposits are unlikely to be directly responsible for inducing insulin resistance. The content of IMCL following bariatric surgery was found to decrease dramatically, and the decrease in IMCL content was related to the improvement in insulin resistance that occurred following bariatric surgery. Conversely, several studies reported that with physical activity, IMCL content typically remains unchanged or increases, even though insulin resistance improves.

In the insulin resistant, obese and sedentary subjects, the level of IMCL is increased and correlates closely and inversely with insulin sensitivity. In these subjects, weight loss achieved by caloric restriction decreases
IMCL and improves insulin sensitivity. On the other hand, endurance trained athletes have high levels of IMCL, and are insulin sensitive. Exercise training not only improves insulin sensitivity but also increases IMCL. In contrast, weight loss achieved by a combination of caloric restriction and exercise improved insulin sensitivity but leaves IMCL unaffected, suggesting that IMCL has no role in insulin sensitivity. However, it was reported that IMCL was dispersed into smaller droplets after caloric restriction and exercise and the decrement in droplet size correlated highly with improved insulin sensitivity.27 In an extension of this study, it was found that 12 wk of exercise training would increase both IMCL and the oxidative capacity of skeletal muscle in older previously sedentary subjects. The increase in maximal aerobic capacity (from 1.65 ± 0.20 to 1.85 ± 0.14 l/min, P < 0.05) was associated with enhanced systemic fat oxidation [\(\dot{V}O_2\max\)] increased from 15.03 ± 4.0 to 19.29 ± 0.80 (micromol.min \(^{-1}\). kg fat-free mass \(^{-1}\), P < 0.05); whereas IMCL measured in vastus lateralis biopsies increased from 22.9 ± 1.9 to 25.9 ± 2.6 arbitrary units, (P < 0.05). The oxidative capacity of muscle, determined by succinate dehydrogenase staining intensity, and the percentage of type I fibers significantly increased, suggesting that exercise training increases IMCL in older persons in parallel with an enhanced capacity for fat oxidation.28 These results coupled with the observation that plasma lipid elevation (induced by lipid infusion) resulted in a significant reduction in whole-body glucose metabolism and lower rise of glucose-6-phosphate without changes in IMCL with a reduction in insulin-stimulated muscle ATP synthase flux in parallel with induction of insulin resistance29 indicates that it is the quality of IMCL that is important rather than the amount of IMCL.

**INTRAMYOCYTOCELLULAR LIPID DROPLETS AND INSULIN RESISTANCE**

How and why, the storage of free fatty acids as triacylglycerol and/or cholesterol esters in lipid droplets in skeletal muscle leads to insulin resistance in sedentary subject but not in those who do endurance exercise? In other words, what is the physiological significance of intramyocellular lipid droplets?

These lipid droplets also called as adiposomes or eicosasomes go by several other names: oil bodies in plants, lipid storage droplets in fruit flies, lipid particles in yeast, and are known as milk fat globules in breast cells that produce milk. These intracellular lipid droplets, encased in a thin phospholipid membrane, contain three proteins: perilipin, adipose differentiation related protein (ADRP or adipophilin) and TIP47. These three proteins together are called as FAT (perilipin/ADRP/TIP47), though several other molecules of similar function have since been recognized. Perilipin was first described in differentiated cultured 3T3-L1 adipocytes but not in their precursor 3T3L1 fibroblasts29,30 in which it was found to be closely associated with the periphery of lipid storage droplets in cultured adipocytes. Because perilipin is found primarily in the adipose cells, it was suggested that it could play a role in lipid deposition and/or lipolysis. Perilipin A increased the triacylglycerol content of cells by forming a barrier that reduced lipolysis, suggesting that perilipin A regulates triacylglycerol storage and lipolysis.31 Perilipins (A, B, and C) are a family of phosphorylated proteins encoded by a single gene and detected in almost all cells that store excess cholesterol and triacylglycerol as cholesterol and triacylglycerol esters in lipid storage droplets. Adipocytes express predominantly perilipin A, with smaller amounts of perilipin B. Under basal conditions, hormone-sensitive lipase (HSL) resides in the cytosol, and unphosphorylated perilipin upon the lipid droplet. Young rats have high rates of lipolysis and showed translocation of HSL to the lipid droplet, and demonstrated no movement of perilipin from the droplet to the cytosol, though phosphorylation of perilipin also occurred. In contrast, mature rats, upon lipolytic stimulation, showed no HSL translocation but perilipin phosphorylation and movement of perilipin away from the lipid droplet was evident. These results suggest that high rates of lipolysis requires translocation of HSL to the lipid droplet whereas low rates of lipolysis is due to movement of phosphorylated perilipin, and translocation of HSL and perilipin occur independent of each other. Since adipocytes from younger rats have markedly greater rates of lipolysis compared to those from the older rats, and translocation of HSL is needed for high rates of lipolysis, it is evident that a loss of the ability to translocate HSL to the lipid droplet whereas decreases with age that ultimately leads to increase in lipid storage. This is so, since perilipins increases triacylglycerol storage by decreasing the rate of triacylglycerol hydrolysis.31 If so, what is the relationship between the activities of perilipins and HSL and insulin resistance and intramyocellular lipid droplets described above?

**INTRAMYOCYTOCELLULAR LIPID DROPLETS, INSULIN RESISTANCE, PERILIPINS AND HSL**

When perilipin A was ectopically and stably expressed in fibroblastic 3T3-L1 pre-adipocytes that normally lack the perilipins, revealed that compared to control cells that showed a few minute and widely dispersed lipid droplets, the transfected cells showed more numerous and widely dispersed larger lipid droplets. In cells stably expressing perilipin A, the lipid droplets were tightly clustered in one or two regions of the cytoplasm, stored 6-30-fold more triacylglycerol
compared to the presence of smaller, few and perinuclear distribution of the lipid droplets seen in the control. The lipolysis of stored triacylglycerol was 5 times slower in lipid-loaded cells expressing perilipin A compared to the control suggesting that perilipin A increases the triacylglycerol content of cells by forming a barrier that reduces the access of soluble lipases to the stored lipids. Thus, perilipins seem to play a major role the regulation of triacylglycerol storage and lipolysis in adipocytes. When these results are extrapolated to those seen with regard to the relationship between IMCL and insulin resistance, it is clear that dispersion of IMCL into smaller droplets after caloric restriction and exercise and the decrement in droplet size that correlated highly with improved insulin sensitivity is akin to the presence of smaller, fewer and perinuclear distribution of the lipid droplets seen in fibroblastic 3T3-L1 pre-adipocytes, whereas more numerous, widely dispersed and larger lipid droplets seen in perilipin A expressing pre-adipocytes are similar to those seen in the insulin resistant, obese and sedentary subjects in whom the larger in size IMCL droplets correlated closely with insulin sensitivity. This implies that in the insulin resistant, obese, and sedentary subjects the levels of perilipins are high and that of HSL will be low leading to accumulation of triacylglycerol and cholesterol esters in the lipid droplets, whereas caloric restriction and exercise decreases the activity of perilipins will be low and that of HSL will be high so that lipolysis is facilitated. This is supported by the observation that perilipin null (per−/−) mice exhibited elevated basal lipolysis, decreased adipose tissue mass (~30%), elevated plasma leptin concentrations despite reduced adipose mass, showed greater lean mass even though they consumed same amount of food as wild-type (per+/+). Perilipin null mice exhibited dramatically attenuated stimulated lipolytic activity, suggesting that perilipin is needed for maximal lipolytic activity, are resistant to diet-induced obesity but not to glucose intolerance, showed increased metabolic rate, an increased tendency to develop glucose intolerance and peripheral insulin resistance. These features are somewhat similar to those seen in lean type 2 diabetics in Southeast Asians especially in the Indian subcontinent, who are lean, show increased tendency for glucose intolerance, peripheral insulin resistance, but relatively increased abdominal obesity and less subcutaneous adipose tissue. But studies performed by Saha et al44 revealed that perilipin-null (plin−/) showed increased β-oxidation in muscle, liver, and adipose tissue, reduced white adipose tissue, resistant to diet-induced obesity, and low or normal plasma concentrations of free fatty acids despite increase in constitutional lipolysis due to an increase in β-oxidation, increase in the expression of the transcripts for uncoupling proteins-2 and −3, increased plasma adiponectin and resistin and normal leptin levels, had normal plasma glucose but reduced basal hepatic glucose production and showed peripheral insulin resistance that was more evident in 42-old mice. Despite increased peripheral insulin resistance, plin+ animals showed normal plasma glucose due to compensated β-oxidation and reduced hepatic glucose production. Although some of the indices reported by these two groups in perilipin null mice are slightly different, in general, these mice showed increased peripheral insulin resistance and diet-induced obesity,35,36 Absence of perilipin produced leanness and reversed obesity in db/db mice. Similar to perilipin knockout mice, even ADRP lacking mice showed lower amounts of triglycerides and less fat in their liver cells, and are lean. Perilipins, ADRP and TIP47 share extensive amino acid sequence similarity, are localized to lipid storage droplets, and related proteins from various species target mammalian lipid droplet surfaces in vivo, suggesting that they have a common function for lipid deposition and/or mobilization and are conserved across several species.30

Perilipins In Humans

Since perilipin is associated with adipose tissue and increased adiposity leads to insulin resistance and development of type 2 diabetes, it is reasonable to expect that alterations in the expression of perilipins, ADRP and TIP47 play a significant role in type 2 diabetes mellitus. Perilipin A is the most abundant perilipin present in human adipose tissues. The calculated mass of perilipin per fat cell remained constant in lean vs obese subjects, suggesting that the fat cell increase in volume is not accompanied by a similar or proportionate increase in perilipin concentration. This decrease in perilipin is not seen in other adipocyte proteins such as lipoprotein lipase suggesting that its (perilipin) decrease in obese is specific to perilipin. Basal lipolysis is twice as high in adipocytes of severely obese compared with non-obese. One of the functions of perilipin is to restrain basal lipolysis. Hence, the decreased perilipin concentrations observed in obese could be protective phenomena to control the elevated lipolysis in the obese. Furthermore, phosphorylation of perilipin is essential for adrenergic stimulation of lipolysis implying that the purpose of decreased levels of perilipin in obese subjects could be to limit the magnitude of lipolytic response of adipocytes to catecholamines. Significant differences between the omental and subcutaneous adipocytes in their perilipin content were noted: omental adipose cells had a higher perilipin protein compared to subcutaneous cells lending support to the observation that omental adipose cells have a much lower basal lipolysis compared to adipose cells in other sites. Perilipin protein content was higher in obese men compared with women indicating that basal lipolysis is higher in women than in men. Kern et al43 reported that perilipin A mRNA and protein content were increased in obese who were non-diabetic but showed insulin resistance. However, no correlation between perilipin...
content and plasma non-esterified fatty acid content, insulin resistance, and circulating TNF-α, IL-6 and adiponectin levels was noted. This suggests that elevated perilipin A content could be a compensatory mechanism to limit lipolysis. The reason for this discrepancy in the levels of perilipin between these two studies\(^5\),\(^6\) may reflect differences in the subjects selected for the study, degree of obesity and any other associated condition present in the study subjects. It is possible that altered plasma TNF-α, IL-6 and adiponectin levels (which were not measured) present in the subjects could be responsible for the low concentrations of perilipin A reported by Wang et al\(^4\) since, TNF-α down regulates perilipin expression. Since TNF-α, IL-6, adiponectin, and perilipins are produced by adipocytes, it is expected that there could exist a positive and negative feedback loop among them. It is possible that an increase in TNF-α levels occurs as a result of high perilipin concentrations that, in turn, could suppress perilipin expression leading to a fall in its (perlipin) levels, whereas in the initial stages of an increase in perilipin levels TNF-α levels would remain normal. So, depending at what stage of this feedback loop measurements of perilipin and TNF-α were done, one could find either an increase, decrease or normal levels of perilipin and TNF-α (see Fig. 1). It is possible that studies conducted by Kern et al\(^4\) were in the early phase of increase in perilipin levels and before the feed back changes in the concentrations of TNF-α, IL-6 and adiponectin were yet to set in. This suggests that to understand the relationship between perilipin, TNF-α IL-6 and adiponectin serial measurement of these indices is necessary at periodic intervals and correlate them to changes in BMI (body mass index and/or changes in body weight).

**Factors Regulating the Expression and Action of Perilipin**

The expression of perilipins is restricted mainly to adipocytes and steroidogenic cells, whereas ADRP and TIP-47 are expressed ubiquitously. Even though perilipins consist of four isoforms A, B, C, and D; perilipin A and B are expressed mainly in adipocytes and steroidogenic cells, whereas C and D are present only in the steroidogenic cells.\(^29\),\(^45\),\(^46\) Perilipin A is the most abundant isoform present in adipose tissue and differentiated cultured 3T3-L1 adipocytes and its function appears to be to increase triacylglycerol storage and the size of lipid droplets by decreasing the rate of triacylglycerol hydrolysis. The multiple phosphorylation sites of perilipin can be induced by protein kinase A. During the process of lipolysis, translocation of HSL from cytosol to the surface of lipid droplet occurs. This translocation of HSL requires the phosphorylation of both HSL and perilipin.\(^35\),\(^47\) In contrast, perilipin A prevents lipolysis in the absence of protein kinase A stimulation.

Subjects with severe obesity have hyperplasia and hypertrophy of adipocytes with an increase in the size of lipid droplets. These large size adipocytes increase insulin resistance by releasing increased amounts of TNF-α, IL-6, C-reactive protein (CRP), free fatty acids, resistin, and decreased production of adiponectin, and visfatin.\(^48\)-\(^51\) On the other hand, thiazolidinediones by activating PPAR-γ reduce insulin resistance and increase the number of small adipocytes containing small lipid droplets in white adipose tissues of obese Zucker rats.\(^52\) This is supported by the observation that adipocytes in heterozygous PPAR-γ-deficient mice are smaller than those in wild-type mice.\(^53\) These results imply that it is the size of adipocytes rather than their number that plays an important role in the development of insulin resistance. This idea is reinforced by the observation that IMCL was dispersed into smaller droplets after caloric restriction and exercise and the decrement in droplet size correlated highly with improved insulin sensitivity.\(^35\)

In this context, it is interesting to note that regular exercise suppresses the production of inflammatory markers IL-6, TNF-α, CRP, and intracellular adhesion molecule-1 and enhances the anti-inflammatory indices TGF-β, IL-4, IL-10, and adiponectin (reviewed in 3, 4). Regular exercise stimulates the synthesis of eNO, prostacyclin (PGI₂) from the vascular endothelial cells, and tissue Mn-SOD (manganese superoxide dismutase). Thus, regular exercise and diet control induces weight loss, disperses IMCL into smaller droplets, and improves insulin resistance, enhances anti-inflammatory indices, and thus, protects against the development of hypertension, type 2 DM, and CHD. Since, perilipin and TNF-α play a significant role in insulin resistance it is natural that an interaction exists between them.

Stimulation of lipolysis in 3T3-L1 adipocytes by TNF-α is associated with a decrease in the expression of perilipin A and B, suggesting that a decrease in perilipin levels is needed for TNF-α-induced lipolysis. In contrast, overexpression of perilipin A or B maintained perilipin protein levels on the lipid droplet and blocked TNF-α-induced lipolysis, but did not inhibit isoproterenol-stimulated lipolysis and did not alter the isoproterenol-induced migration of perilipins from the lipid droplet.\(^54\) On the other hand, thiazolidinediones, which are PPAR-γ agonists, increased perilipin expression in fully differentiated adipocytes\(^55\)-\(^57\) and decreased TNF-α production and show anti-inflammatory actions to a limited extent.\(^56\),\(^59\) Normally, one would expect insulin resistance to increase whenever perilipin expression is enhanced as it happens when PPAR-γ is stimulated by thiazolidinediones. But, contrary to these expectations, PPAR-γ agonists increase perilipin expression and decrease insulin resistance. The ability of PPAR-γ to decrease insulin resistance has been attributed to decrease in TNF-α production. This implies that there exists a close positive and negative feedback regulation
between perilipins, TNF-α, adipocyte size, PPAR-γ, exercise and insulin resistance as shown in Fig. 1.

**Perilipins and inflammation**

Obesity is associated with increase in IMCL, low-grade systemic inflammation, insulin resistance, and perilipin expression. But, it is not clear whether perilipin has pro-inflammatory actions or not. Human mast cells, neutrophils, eosinophils, monocytes, and murine fibroblasts showed the presence of prostaglandin hydroperoxide (PGH) synthase on lipid bodies. It is known that the number and size of lipid droplets increase in cells associated with inflammation, especially in monocytes and macrophages, suggesting that lipid droplets may have a role in inflammation. Recent studies showed co-localization of cytosolic phospholipase A₂ (cPLA₂) and its activating protein kinases, including extracellular signal-regulated kinase 1 and 2 (ERK1 and ERK2) and p85 and p38 MAPKs, on lipid droplets in monocytic U937 cells. These data suggest that lipid droplets could be active sites for arachidonic acid release and eicosanoid formation.

Further studies showed that macrophages and monocytes when stimulated to make lipid droplets by feeding them with free fatty acids, also made eicosanoids such as leukotrienes (LTs) and prostaglandins (PGs). This formation of eicosanoids occurred on the lipid droplet's surface. On the other hand, aspirin, a COX inhibitor, prevented lipid droplet formation independent of its ability to inhibit COX enzyme. These results strongly suggest that lipid droplets play an active role in the formation of PGs and LTs that have pro-inflammatory actions. Since IMCL was dispersed into smaller droplets after caloric restriction and exercise and the decrement in droplet size correlated highly with improved insulin sensitivity and exercise is anti-inflammatory in nature, it is likely that the bigger the size and higher the number of lipid droplets more amounts of pro-inflammatory eicosanoids are formed and when the droplet size and number is decreased the formation of eicosanoids falls. Thus, IMCL have a close relationship to inflammation supporting the proposal that obesity, type 2 diabetes mellitus and metabolic syndrome X are low-grade systemic inflammatory conditions.

**Abdominal obesity and 11β-hydroxysteroid dehydrogenase type-1 activity**

Of all the features of metabolic syndrome X, abdominal obesity is the most common and dominant component. Although obesity is often associated with insulin resistance and a cluster of metabolic disturbances, it is unclear why some obese individuals fail to show traditional risk factors associated with the insulin resistance syndrome. Brochu et al. examined the metabolic characteristics of obese, sedentary postmenopausal women (mean ± SD, 58.0 ± 6.0 yr) who were metabolically normal but obese (MNO) or as metabolically abnormal obese (MAO) based on insulin sensitivity (measured by the hyperinsulinemic/euglycemic clamp technique). MNO subjects displayed high insulin sensitivity (11.2 ± 2.6 mg/min.kg lean body mass) whereas MAO showed lower insulin sensitivity (5.7 ± 1.1 mg/min.kg lean body mass). Despite comparable total body fatness between these two groups (45.2 ± 5.3% vs. 44.8 ± 6.6%; P: = NS), MNO individuals had 49% less visceral adipose tissue than MAO subjects (141 ± 53 vs. 211 ± 85 cm²; P: < 0.01), whereas no difference was noted between groups for abdominal subcutaneous adipose tissue (453 ± 126 vs. 442 ± 144 cm²; P: = NS). MNO subjects had significantly lower fasting plasma glucose and insulin concentrations and lower insulin levels during the oral glucose tolerance test, lower plasma triglycerides and higher high-density lipoprotein cholesterol concentrations than MAO individuals. Stepwise regression analysis showed that visceral adipose tissue and the age-related onset of obesity explained 22% and 13%, respectively, of the variance observed in insulin sensitivity, suggesting that visceral adipose tissue may account for the differences between MNO and MAO. This indicates that visceral adipose tissue accumulation is the main culprit in the development of metabolic syndrome X and insulin resistance. Hence, understanding the aetiopathogenesis of abdominal obesity is important.

Mice over expressing 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) enzyme selectively in adipose tissue develop abdominal obesity and exhibit insulin-resistant diabetes, hyperlipidemia, and hyperphagia despite hyperleptinemia, features that are very similar to those seen in subjects with metabolic syndrome X.
syndrome X. This suggests that abdominal obesity is like localized Cushing’s syndrome.

In primary cultures of paired omental and subcutaneous human adipose stromal cells, 11β-HSD-1 oxo-reductase activity was significantly higher in omental adipose stromal cells compared with subcutaneous cells despite similar endogenous NADPH/NADP concentrations. Both cortisol and insulin increased the differentiation of adipose stromal cells to adipocytes, but only cortisol increased 11β-HSD-1 activity and messenger RNA levels in a dose-dependent fashion. Cortisone was as effective as cortisol in inducing adipose stromal cells differentiation. The local conversion of cortisone to active cortisol through expression of 11β-HSD-1 was found to be higher in omental human adipose stromal cells compared with subcutaneous cells. This suggests that glucocorticoids have a differential action on different adipose tissue depots, and implies that the increased local metabolism of glucocorticoid may be responsible for abdominal obesity in metabolic syndrome X. These results are supported by the observation that 11β-HSD-1 deficiency protects against the development of high-fat diet induced abdominal obesity and remain insulin sensitive. 11β-HSD-1(-/-) mice expressed lower resistin and TNF-α, but higher PPAR-γ, adiponectin, and uncoupling protein-2 (UCP-2) mRNA levels in adipose tissue, and isolated 11β-HSD-1(-/-) adipocytes exhibited higher basal and insulin-stimulated glucose uptake. 11β-HSD-1(-/-) mice also showed reduced visceral fat accumulation upon high-fat feeding. These data provide in vivo evidence that adipose 11β-HSD-1 deficiency prevents metabolic syndrome X and suggests that increase in 11β-HSD-1 activity may suppress adiponectin, PPAR-α, and UCP-2 activities (see Fig. 1).

Since obesity is frequently associated with insulin resistance and abnormal glucose homeostasis, it is important to note the close interaction between 11β-HSD-1, TNF-α, and insulin. TNF-α plays a significant role in mediating insulin-resistance of obesity through its overexpression in adipose tissue. Adipose tissue cells on treatment with TNF-α increased 11β-HSD-1 activity in a dose dependent fashion from 1.5 to 10-fold. In contrast, insulin had no effect under basal conditions, but inhibited the stimulatory effects of TNF-α on 11β-HSD-1 mRNA suggesting that both TNF-α and insulin are mediating their actions at the levels of gene transcription.

When primary cultures of human hepatocytes and subcutaneous and omental adipose stromal cells (ASC) were treated with TNF-α a dose-dependent increase in 11β-HSD-1 activity was noted only in the subcutaneous and omental adipose cells, but had no effect on 11β-HSD-1 activity in hepatocytes. Insulin-like growth factor I (IGF-I), similar to insulin, caused a dose-dependent inhibition of 11β-HSD-1 activity in subcutaneous and omental stromal cells, but not in human hepatocytes. Both TNF-α and IL-1β enhanced the expression of 11β-HSD-1 activity both in subcutaneous and omental stromal cells in a time- and dose-dependent manner. PPAR-γ ligands significantly increased 11β-HSD-1 activity in omental and subcutaneous adipose cells. These results suggest that tissue-specific regulation of 11β-HSD-1 occurs and the response of omental adipose cells differs from that seen in subcutaneous adipocytes. These results are interesting in the light of the fact that glucocorticoids, which induce abdominal obesity, insulin resistance and possess anti-inflammatory actions, inhibit TNF-α synthesis, whereas in subcutaneous adipocytes from lean subjects, TNF-α inhibited adiponectin release but had no effect on adiponectin release from subcutaneous or omental adipocytes from obese subjects. On the other hand, dexamethasone significantly inhibited adiponectin release. These results suggest a positive and negative feed back regulation exists between glucocorticoids, TNF-α, 11β-HSD-1 activity, adiponectin secretion, insulin, and PPARs that may have relevance to obesity, insulin resistance, and metabolic syndrome.
X (see Fig. 1). In the light of these evidences, it is interesting to note that glucocorticoids influence perilipin expression. There is preliminary evidence to suggest that glucocorticoids may enhance the expression of perilipins. Glucocorticoids and perilipins

Glucocorticoids produce abdominal obesity, cause insulin resistance, and possess anti-inflammatory actions, inhibit TNF-α synthesis, whereas TNF-α inhibited adiponectin release. Dexamethasone significantly inhibited adiponectin release, whereas TNF-α increased 11β-HSD-1 activity in a dose dependent fashion. In contrast, insulin had no effect under basal conditions, but inhibited the stimulatory effects of TNF-α on 11β-HSD-1 mRNA. Insulin suppresses the production of TNF-α, IL-6, IL-1, IL-2, and macrophage migration inhibitory factor (MIF), and enhances the production of anti-inflammatory cytokines: IL-4 and IL-10. Thus, insulin has anti-inflammatory actions. Glucocorticoids and TNF-α have inhibitory action on adiponectin production, an endogenous molecule that enhances the action of insulin and shows anti-inflammatory activity; and glucocorticoids suppress TNF-α synthesis and glucocorticoids and TNF-α have opposite actions on inflammation yet both glucocorticoids and TNF-α induce peripheral insulin resistance. TNF-α down regulates, whereas glucocorticoids enhance perilipin expression. In addition, excess production of TNF-α causes cachexia (as seen in patients with cancer), whereas glucocorticoids produce abdominal obesity suggesting that some of their down stream events could be different and their actions on adiposity are due to their opposite actions on perilipin expression.

Glucocorticoids, TNF-α, and inflammation

Glucocorticoids bring about their anti-inflammatory actions by (i) the induction and activation of annexin 1 (also called as lipocortin-1), (ii) the induction of mitogen-activated protein kinase (MAPK) phosphatase 1, and (iii) the inhibition of cyclo-oxygenase-2 (COX-2). Annexin 1 or Lipocortin-1 physically interacts with and inhibits cytosolic phospholipase A2 (cPLA2) so that arachidonic acid (AA) is not released in adequate amounts to form precursor to various pro-inflammatory eicosanoids. Increased expression of cPLA2 is necessary to give rise to anti-inflammatory molecules such as prostaglandin D2 (PGD2) and 15deoxy-12,14-PGJ2, and lipoxins (LXs). Thus, the timing of expression (perhaps a pulsatile expression) of cPLA2 and the local concentrations of glucocorticoids could be one important factor that determines the progression and/or resolution of inflammation. The selective inhibition of COX-2 and inducible nitric oxide synthase (iNOS) expression by glucocorticoids could explain their potent anti-inflammatory actions. Glucocorticoids also inhibit the production of pro-inflammatory cytokines such as IL-1, IL-6, TNF-α, and macrophage migration inhibitory factor (MIF). Glucocorticoids mediate their inhibitory action on iNOS and COX enzymes through lipocortin-1 (annexin1). On the other hand, eNO activates constitutive COX-1 resulting in optimal release of PGE2, whereas iNO activates COX-2 resulting in markedly increased release of PGE2 that results in inflammation. This implies that constitutive production of NO and PGE2 are anti-inflammatory, simply because the quantities of NO and PGE2 are extremely high in the later instance. Low concentrations of glucocorticoids enhance MIF synthesis that, in turn, overrides glucocorticoid-mediated inhibition of secretion of other pro-inflammatory cytokines. MIF induces the production of TNF-α and vice versa.

Glucocorticoids accelerate the catabolism of LTC4 (leukotriene C4), a pro-inflammatory molecule. 15HPETE, an anti-inflammatory eicosanoid formed via lipoxygenase pathway, causes a significant increase in the rate of TNF degradation, an action that may also be seen with LXs. LXA4 inhibited not only the secretion of TNF-α but also prevented TNF-α-induced production of IL-1β, IL-6, cyclin E expression, and NF-κB activation. Thus, glucocorticoids and lipoxins have similar actions on inflammation, both are anti-inflammatory, but their mechanisms of action seem to be different.

In this context, it is noteworthy that both TNF-α and glucocorticoids have opposite actions on PLA2: the former stimulates while the later inhibits. There is evidence to suggest that activation of cPLA2 is crucial to the actions of TNF-α. This indicates that cPLA2 and other PLA2s play a central role in the pathobiology of inflammation and its resolution that could be attributed to the fact that long-chain polyunsaturated fatty acids (LCPUFAs) such as arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) released by PLA2, form precursors to several pro- and anti-inflammatory compounds. On the other hand, glucocorticoids inhibit the production of TNF-α and thus, bring about some of its anti-inflammatory actions, whereas TNF-α increased 11β-HSD-1 activity leading to the formation of increased amounts of cortisol that, in turn, inhibits TNF-α formation and restores normalcy. These results imply that there is close positive and negative interaction between TNF-α, glucocorticoids and the inflammatory process.

Perilipins, 11β-HSD-1, and metabolic syndrome X in Indians

It is evident from the preceding discussion that increased expression of perilipins and 11β-HSD-1 in adipose cells, especially in the omental and mesenteric adipose tissue, could lead to insulin resistance, and low-grade systemic inflammation. Increased expression of perilipins in the mesenteric/ omental adipose cells leads to insulin resistance and increased production of pro-
inflammatory eicosanoids due to the activation of PLA₂. This ushers in low-grade systemic inflammation seen in metabolic syndrome X.

Consumption of even normal food; high calorie diet rich in fats (saturated and trans-fats) or protein and glucose challenge enhances generation of reactive oxygen species by leukocytes and decreases vitamin E levels.109-112 Oxidative stress and pro-inflammatory process induces insulin resistance.113 This increase in reactive oxygen species could be due to increasing production of IL-6, TNF-α, and IL-18, and CRP. IL-6 and TNF-α activate NADPH oxidase and enhance the generation of reactive oxygen species.114 Thus, consumption of energy dense diets induce a state of oxidative stress that is toxic to pancreatic β cells and also produce long-term complications seen in diabetes, hypertension, and CHD. Continued consumption of energy dense diet from childhood effectively abrogates the anti-oxidant defenses of various cells and tissues and leads to the development of obesity, hypertension, type 2 diabetes mellitus, CHD, and metabolic syndrome X. This explains why and how low-grade systemic inflammation occurs in these conditions.

Previously, I proposed that physiological response to even normal food intake (containing carbohydrates, proteins, and fats and mixed meals) includes an increase in the production of TNF-α and IL-6 and consequent increase in plasma CRP and decrease of anti-inflammatory cytokines IL-4 and IL-10, and adiponectin. TNF-α and IL-6 induce oxidative stress and activate NF-κB, which induces insulin resistance and consequent hyperinsulinemia. Insulin secreted in response to food intake is not only intended to normalize plasma glucose, lipid and amino acid concentrations but also to suppress TNF-α and IL-6 and enhance IL-4 and IL-10 synthesis. Insulin stimulates the synthesis of LCPUFAs that, in turn, enhance insulin action.115 Increased production of TNF-α and IL-6 following food intake activate phospholipase A₂ (PLA₂) and induce the release of LCPUFAs from the membrane lipid pool. LCPUFAs thus released, if adequate, suppress the synthesis and release of TNF-α and IL-6 resulting in the restoration of balance between pro- and anti-inflammatory cytokines and suppression of oxidative stress. Continued consumption of energy rich diet and/or saturated and trans-fatty acids and/or sub-optimal intake of LCPUFAs lead to a state of low-grade systemic inflammation and chronic oxidative stress. In contrast, dietary restriction, exercise, and weight loss suppress free radical generation and oxidative stress,109 decrease the production of TNF-α and IL-6 and enhance IL-4 and IL-10, and adiponectin synthesis, and dispersed IMCL into smaller droplets leading to improved insulin sensitivity. Saturated and trans-fats and hyperglycemia interfere with the synthesis of LCPUFAs, and hence, normal inhibitory control exerted by LCPUFAs on TNF-α and IL-6 will be defective or sub-optimal. This derives support from the observation that adequate intake of EPA and DHA but not ALA was inversely associated with plasma levels of sTNF-R1 and sTNF-R2 (soluble tumor necrosis factor receptors 1 and 2) and CRP whereas ω-6 fatty acids did not inhibit the anti-inflammatory effects of ω-3 fatty acids.110 A combined intake of ω-3 and ω-6 fatty acids produced lowest levels of inflammation (see Fig. 1).

Based on these results, I propose that Indians are genetically programmed to have increased expression of perilipins and 11β-HSD-1 (especially in the mesenteric/omental adipose cells) that predisposes them to develop abdominal obesity and metabolic syndrome X. This genetic predisposition coupled with lack of adequate exercise and consumption of energy rich diets renders them highly susceptible to develop all the features of metabolic syndrome X. This explains how the interaction between genetic predisposition (in the form of constitutionally increased expression of perilipins and 11β-HSD-1) interact with environmental factors (in the form of lack of exercise and consumption of energy rich diets) could lead to an explosion in the incidence of metabolic syndrome X as is seen in the Indian subcontinent at present.

**Testing The Proposal**

The proposal presented here can be verified by studying the plasma levels of CRP, TNF-α, IL-6, MIF, IL-4, IL-10, adiponectin, resistin, visfatin, EPA, DHA, and AA; and adipose tissue content and expression of 11β-HSD-1 and perilipins, and adipose tissue levels of TNF-α, IL-6, MIF, IL-4, IL-10, adiponectin, resistin, EPA, DHA, and AA and IMCL size and number and comparing them with those seen in normal subjects. It is expected that subjects who have metabolic syndrome X and those at high-risk of developing the same have increased levels of these indices. It is particularly emphasized that the expression and activity of perilipins, 11β-HSD-1, and size and number of IMCL will be high in Indians compared to the Western population, even when compared among normal. In addition, if the this proposal is true, it implies that

1. Indians, in general, will have increased expression of perilipins and 11β-HSD-1 in their mesenteric and omental adipose cells and the IMCL size and number will be higher compared to the Western population, who have much less predisposition to develop metabolic syndrome X.

2. Subjects of Indian subcontinent are likely to have high circulating levels of CRP, IL-6, TNF-α, and resistin, and decreased levels of IL-4, IL-10, adiponectin and visfatin compared to the Western population.

3. Indians who are at high risk of developing metabolic syndrome X, even when they are apparently normal at the time of evaluation (such as those with strong
family history of hypertension, type 2 diabetes mellitus, CHD, and hyperlipidemias), are likely to show an increase in the size and number of IMCL, low plasma concentrations of EPA, DHA, and AA in their phospholipid fraction and adipose tissue (such as mesenteric, subcutaneous, etc.), and elevated CRP, IL-6, TNF-α, MIF, resistin and low IL-4, IL-10, adiponectin, and visfatin in their plasma and elevated adipose tissue content of 11β-HSD-1 and perilipins compared to normal.

4. Increased plasma concentrations of CRP, TNF-α, IL-6, and MIF, and resistin and elevated abdominal adipose tissue content of 11β-HSD-1 and perilipins and enhanced IMCL size and number with a simultaneous decrease in the plasma levels of adiponectin, visfatin, IL-4 and IL-10, and EPA, DHA, and AA are expected even in those who do not have a strong family history of metabolic syndrome X but are at high risk of developing the same.

5. Children with strong family history of metabolic syndrome X may show an increase in size and number of IMCL, enhanced plasma levels of CRP, TNF-α, IL-6, MIF, resistin, and a decrease in those of IL-4, IL-10, adiponectin, visfatin, EPA, DHA, and AA; and adipose tissue 11β-HSD-1, perilipins, EPA, DHA, and AA (both of content and expression).

6. I also propose that serial measurement of these biological markers (perhaps, once every year) may give clues whether a particular individual is a potential candidate to develop metabolic syndrome X or not. When these markers become abnormal it suggests that they are likely to develop metabolic syndrome X in future. In such an instance, adopting preventive measures in the form of diet control and adequate exercise to reduce weight, abdominal obesity, and normalize plasma concentrations of CRP, TNF-α, IL-6, MIF, IL-4, IL-10, adiponectin, visfatin, resistin, EPA, DHA, and AA; and adipose tissue 11β-HSD-1, perilipins, EPA, DHA, and AA content and expression and IMCL size and number will prevent and/or postpone the development of metabolic syndrome X.

7. In contrast, those in whom these indices fail to reach normal values even after diet, exercise and medication indicates that more aggressive measures are needed to prevent or postpone the development of metabolic syndrome X. One such aggressive measure could include Roux-en-Y gastric bypass (RYGB) surgery to decrease food intake and absorption of the digested food and to reduce body weight. Thus, measurement of plasma concentrations of CRP, TNF-α, IL-6, MIF, IL-4, IL-10, adiponectin, visfatin, resistin, EPA, DHA, and AA; and adipose tissue content and expression of 11β-HSD-1, perilipins; and IMCL, EPA, DHA, and AA of adipose cells could be used to monitor the effectiveness of the life style modifications advised or adopted.

8. It is likely that all the indices may not be abnormal at the time of screening in all who are at high risk of developing metabolic syndrome X. These indices need to be studied periodically and in more detail to know which of these indices have a high predictive value since all indices may not have the same predictive value.

If this proposal is true, it raises the possibility that developing methods to inhibit the expression and action of perilipins and 11β-HSD-1 could be useful in the prevention and management of metabolic syndrome X.

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