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From a glucocentric to a lipocentric approach towards metabolic syndrome

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Insulin resistance, the essential component of metabolic syndrome, has traditionally been defined from a glucocentric viewpoint, with glucotoxicity playing a lead role. However, as overabundant circulating fatty acids are now known to be overt contributors, there is a paradigm shift in the understanding of metabolic syndrome acknowledging the importance of lipotoxicity as a major perpetuator of insulin resistance. Ectopic accumulation of fat in liver, adipose, muscle and pancreatic islets, provokes insulin resistance through various mechanisms. Chronic inflammation/adipocytokine generation, endoplasmic reticulum stress and mitochondrial dysfunction/oxidative stress also contribute significantly towards insulin resistance. Targets that can act as counter regulators/master switches at the converging point of all these metabolic pathways are currently under intense development.

Type 2 diabetes mellitus (T2DM) is fast assuming epidemic proportions in the 21st century. The disease is characterized by pre- and post-prandial hyperglycaemia because of impaired suppression of endogenous glucose production in response to elevated plasma glucose and/or insulin levels, as well as impaired glucose uptake in peripheral tissues. An insulin resistant state with increased glucose intolerance can be observed a decade before T2DM is manifested [1]. Though it is clear that T2DM is a polygenic, multifactorial disease, the earliest evidence for the disease is an area of active research. Thus far, the covert insulin resistance that precedes T2DM manifestation is considered the starting point [2]. Traditionally, the interpretation of the pathogenesis of T2DM has been dominated by a glucocentric view wherein overt hyperglycaemia was taken as the starting point of therapeutic intervention [3,4]. In recent years, however, convergence of many cross-sectional studies has resulted in a major paradigm shift. In many studies, inept handling of plasma free fatty acids (FFAs) has been observed before glucose intolerance, leading to a precedence of 'lipid intolerance' over 'glucose intolerance' [5,6].

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From 'Randle's hypothesis' to 'Shulman's hypothesis'

More than 30 years ago Randle *et al.* [7] proposed the FFA–insulin resistance theory: an inversely proportional relationship between plasma non-esterified fatty acids (NEFA) and glucose tolerance. Though the exact mechanism by which this takes place is still debated; studies have confirmed this theory. In a Paris Policemen Prospective study, in subjects who underwent an oral glucose tolerance test (OGTT) it was shown that, along with older age, high NEFA levels were predictors of deterioration from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) [5]. A longitudinal study of PIMA Indians also corroborates the hypothesis that a high NEFA concentration is a predictor of conversion of NGT to IGT and from IGT to T2DM [6]. Young, lean, healthy offspring of T2DM parents can be the ideal candidates for examining the earliest defects leading to insulin resistance. In a cross-sectional study of this insulin resistant subset, an inverse relationship has been reported between fasting plasma fatty acid concentrations and insulin sensitivity [8].

The FFA–insulin resistance theory has been confirmed in healthy humans: high levels of plasma fatty acid 5 h results in a 50% reduction in insulin-stimulated rates of muscle glycogen synthesis, whole body glucose oxidation and inhibition of total body glucose uptake resulting in an insulin resistant state [1]. This

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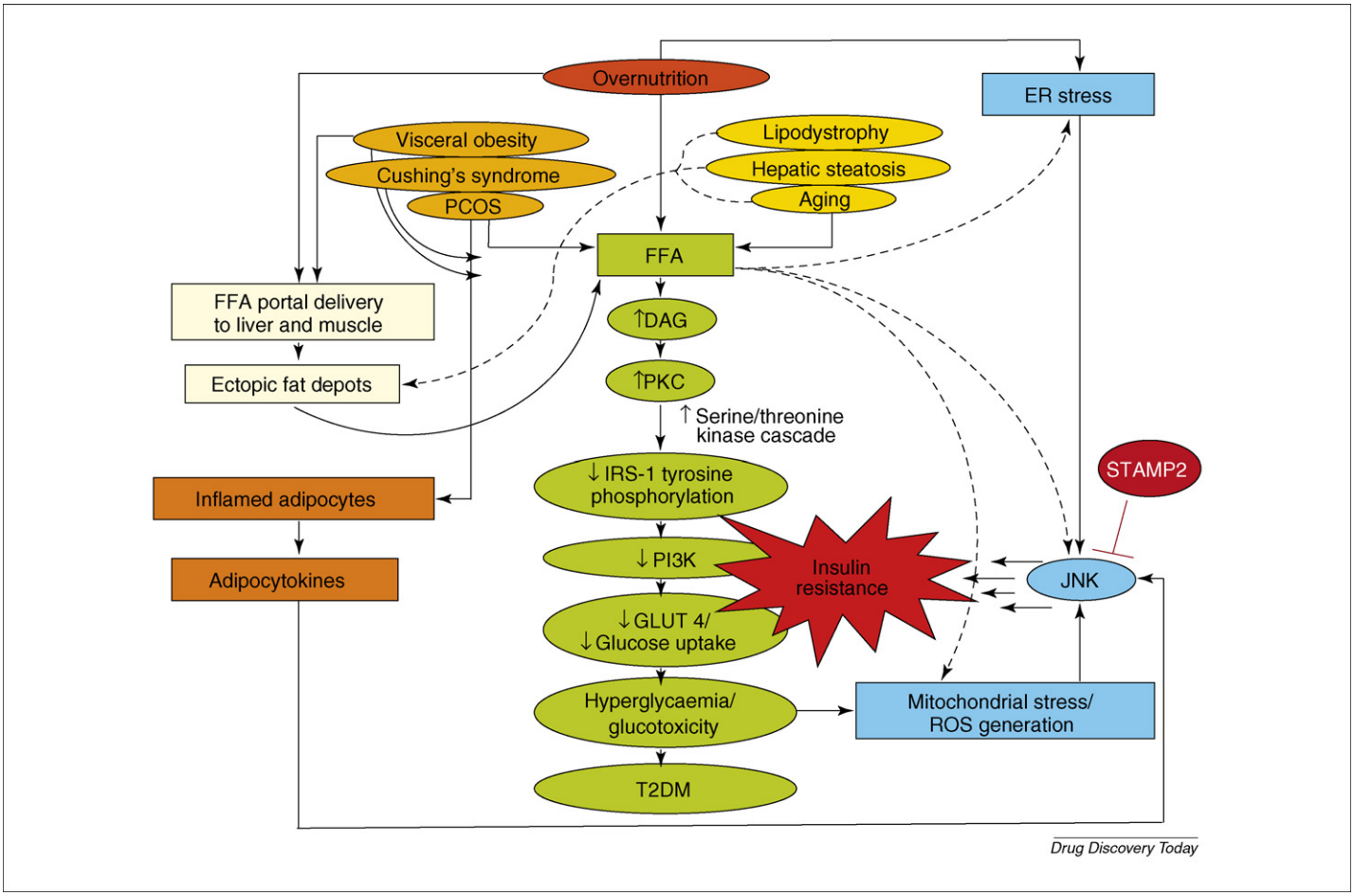

FIGURE 1

Lipocentric approach to insulin resistance: overnutrition as well as visceral obesity spill FFA into liver, muscle and pancreas through portal delivery where ectopic deposition ensues. Lipodystropy, hepatic steatosis and aging also present ectopic deposition of fat in insulin sensitive tissues. These fat depots cause insulin resistance through increase in diacylglycerol (DAG), increase in PKC leading to ↓ IRS-1 tyrosine phosphorylation/↑ IRS-1 serine phosphorylation, further leading to decrease in PI3K and GLUT-4. This results in decreased glucose uptake and subsequent hyperglycaemia. Hyperglycaemia is an overt producer of reactive oxygen species (ROS) that activate JNK. Overnutrition/FFA lead to endoplasmic stress (ER stress) which also converges on JNK. Activated JNK is an avid contributor towards insulin resistance. Adipocytes from obese individuals and from individuals suffering from Cushing's syndrome and polycystic ovarian syndrome (PCOS) are very susceptible to lipolysis and release inflammatory adipocytokines which are again direct activators of JNK. FFA's *per se* are also documented to activate ER stress, mitochondrial stress and JNK. STAMP2 has been recently discovered as a counter regulator of pathological processes set on a roll by JNK activation.

state is similar to that seen in obese individuals [9], T2DM patients [10], and in the lean, normoglycaemic insulin-resistant offspring of T2DM individuals [11].

Randle hypothesized that intracellular fatty acid accumulation eventually leads to an inhibition of hexokinase II, which results in an increase in intracellular glucose concentration and decreased muscle glucose uptake. Shulman and co-workers have challenged the role of hexokinase II and instead have proposed glucose transport as the rate-limiting step for fatty acid-induced insulin resistance [1,12]. In the normal insulin signaling cascade, insulin activates its receptors leading to tyrosine phosphorylation of insulin receptor substrate (IRS)-1 in skeletal muscles and adipose tissue and IRS-2 in the liver which, in turn, activates PI3K and subsequently recruits glucose transporter, GLUT-4, to the cell surface. GLUT-4 internalizes glucose into the cells thereby normalizing glucose levels in the circulation. A unifying hypothesis has been presented for insulin resistance both in skeletal muscle and liver, which states that intracellular fatty acid metabolites (diacyl glycerol (DAG), fatty acyl CoA's and so on) activate a serine/threonine kinase cascade that is inhibited by phosphokinase C_θ (PKC_θ) in rodents and PKC($_\beta$ and $_\lambda$) in humans (Figure 1) [1]. This leads to phosphorylation of IRS-1 at serine-307 and serine-612 that fails to activate PI3K, resulting in decreased trafficking of GLUT-4 from the cell surface. It is now being advocated that even glucotoxicity works through signaling of bioactive lipids (Box 1, [13–15]).

It has been clearly demonstrated that lipid dysregulation is not only found in obesity but also in any condition with increased intramyocellular fatty acid metabolites, such as lipodystrophy, aging, genetic susceptibility of being born to T2DM parents or being a PIMA Indian [12,16–18]. This review briefly discusses these conditions and new pharmacological options available for tackling the metabolic syndrome (MS) through the lipocentric approach.

Visceral fat theory

Abdominal adiposity represents a powerful risk factor for insulin resistance. Adipocytes in the central depot/abdomen are resistant

BOX 1

Glucotoxicity/lipotoxicity?

Chronic glucotoxicity *per se* is documented as an independent factor in the development of insulin resistance [13]. Poitout and Robertson [14] propose that chronic hyperglycaemia, independent of hyperlipidemia can be toxic for β-cell function, whereas chronic hyperlipidemia requires concomitant hyperglycaemia to affect the β- cell function. They propose glucolipotoxicity as the basis of malfunctioning of insulin sensitive tissues. A recent study, however, proposes that many of the intracellular signaling pathways induced by elevated glucose are eventually mediated by bioactive lipids [15]. This indicates a plethora of interactions between glucose and lipids. Chronic glucose elevation increases acyl-CoA levels in rat skeletal muscles; fatty acyl-CoA synthesis being a requirement for DAG synthesis [15]. Elevation of glucose leads to increased levels of malonyl-CoA which inhibits fatty acid β oxidation by inhibiting mitochondrial fatty acyl CoA uptake, thereby channeling increased levels of acyl-CoA's into DAG and triacylglycerols. Elevated glucose levels have also been shown to regulate several arachidonic acid metabolites such as TXA2 and 12(s)-HETE leading to advanced lipoxidation end products and lipid alterations [15]. High glucose levels are also known to cause nutritional burden/metabolic stress in mitochondria leading to excess of ROS production. It also leads to endoplasmic reticulum (ER) stress and formation of misfolded proteins all eventually leading to impaired fatty acid oxidation and induction of stress activated kinases such as JNK and NFKβ.

to the antilipolytic action of insulin and increased lipolysis releases FFA into the hepatic portal vein, exposing the liver to high lipid levels [19]. Also, there is evidence that visceral depots are controlled primarily by the hypothalamic pituitary axis (HPA) and the subcutaneous depots by insulin. The HPA axis controlling the adrenal/cortisol secretion and sympathoadrenergic system [19,20] has recently been receiving attention as a progenitor of insulin resistance and T2DM. Several studies now link visceral adiposity, T2DM and metabolic syndrome (MS) to psychosocial stress [21]. Insulin-resistant subjects seem to be particularly sensitive to sympathetic nervous system and show attenuated responses to parasympathetic nervous system. A recent study by Kuo and coworkers [22] demonstrates that stress and sympathetic nervous system work together towards secretion of abnormal amounts of catecholamines and glucocorticoids that regulate human fat cell lipolysis [23].

As regional variations in adipocyte lipolysis make visceral depots particularly susceptible to more release of FA's and as only visceral fat is linked to the liver (by the portal vein), alterations in visceral adipocyte tissue lipolysis directly affect the liver. This 'portal hypothesis' thus posits an increased delivery of FFAs to the liver via the portal vein [19,24,25]. This surplus fat renders insulin-sensitive tissues, such as liver, pancreas and skeletal muscle insulin-resistant. In contrast to visceral fat, subcutaneous adipose tissue can act as an 'energy sink' and can deal with a large surplus of energy as it is a less lipolytic site with less CNS interaction. Peroxisome proliferator-activated receptor (PPAR) γ agonists, such as glitazones, are known to siphon off visceral and ectopic fat and direct it towards subcutaneous fat deposition, thereby improving insulin sensitivity [26]. Metabolic syndrome and T2DM share many metabolic and morphological features with Cushing's syndrome, a rare disorder caused by systemic glucocorticoid excess. This condition is characterized by insulin resistance, glucose intolerance, central obesity and hypertension [21]. In Cushing's syndrome there is upregulation of 11 β hydroxysteroid dehydrogenase-1 (11β HSD-1) that increases local conversion of cortisone to cortisol and leads to pertubations in lipid and glucose metabolism. Polycystic ovarian syndrome (PCOS), another metabolic disorder, has many similarities to metabolic syndrome/insulin resistance. It is a hyperandrogenic state associated with a male type of fat distribution. PCOS women are prone to upper body obesity and a highly increased risk of developing T2DM [23]. In these women, in spite of normal total body fat content, visceral fat cells increase in size and are exposed to enhanced catecholamine action. PCOS is now regarded as an important risk factor for metabolic syndrome.

Ectopic fat theory

Hepatic and cardiac steatosis

Ectopic deposition of fat in liver causes hepatic steatosis, also called non- alcoholic fatty liver disease (NAFLD), a clinical condition recognized as one of the key risk factors for MS. This condition is characterized by severe hepatic insulin resistance [1,2,12] and is associated with central adiposity and insulin resistance in overweight men. A reversal of severe hepatic steatosis has been observed after a moderate (~8 kg) weight reduction in T2DM patients [1]. This weight loss resulted in depletion of the ectopically deposited fat depots in liver. It is proposed that a relatively small pool of intra-hepatic lipids (hepatic diacyl glycerol; DAG) is responsible for both the cause and the reversal of hepatic steatosis [12]. Myocardial steatosis is now a clinical reality that eventually leads to left ventricular hypertrophy and non-ischaemic dilated cardiomyopathy [27]. With NMR spectroscopy it is possible to quantify the intramyocardial lipid content. The treatment paradigms that reverse ectopic fat deposition can also be helpful in reversing cardiac steatosis.

Lipodystrophy

Congenital generalized lipodystrophy is a rare disorder with a paucity of subcutaneous and visceral fat depots and any fat intake is stored ectopically in susceptible tissues including liver, skeletal muscles and pancreas [28]. Lipodystrophy is associated with severe insulin resistance, hypertriglyceridemia and deficiency of the satiety hormone, leptin. Low-dose *icv* leptin corrects the insulin resistance and fatty liver in lipodystrophic aP2-nSREBP-1c mice [29]. In another transgenic fatless mouse model of lipodystrophy (9A-ZIP/F), insulin resistance and metabolic abnormalities are reversed after adipose tissue is transplanted into the subcutaneous area, which act as a sink for body fat intake [1,2,12]. Recently, the highly active antiretroviral therapy (HAART) has been associated with development of lipodystrophy in 18–83% HIV patients [30]. HAART targets visceral fat and its redistribution precedes insulin resistance and dyslipidemia in these patients.

Aging

Aging is associated with a 'physiological' increase in lipid accumulation in non-adipose tissue, even in lean individuals, that may be compared to lipodystrophic people suffering from mismanagement of lipids. Fat mass, adipocyte size, metabolic respon-

siveness and pre-adipocyte differentiation capabilities decrease between middle and old age [18]. The decline in adipocyte differentiation fails to accommodate the increasing needs of fat storage, leading to ectopic fat storage in muscle, bone marrow and other tissues. Conversely, individuals who maintain adipocyte responsiveness, also retain insulin sensitivity [18]. Aging is also associated with a reduced oxidative capacity in skeletal muscle and inappropriately high lipogenesis in non-adipose tissues and processes such as adaptive thermogenesis, glucose and fatty acid oxidation decrease with age resulting in insulin resistance [15]. Mammals with genetic or acquired defects in insulin signaling pathway are at risk for age-related diseases and increased mortality. Conversely, efficient insulin sensitivity/low IGF-1 levels have been observed to have a profound role in human longevity as evidenced by a study on centenarians [31].

Stress theory

Adipocyte stress

Adipose tissue has been considered an inert mass of stored energy with insulation and mechanical support properties [32]. Recently, it has emerged as a master regulator of whole body energy homeostasis [33]. In 1990s, when Hotamisligil and co-workers, caught fat cells spitting out enormous amounts of TNF-α, adipocyte physiology generated awe and interest [34]. Adipocytes were observed to release a multitude of adipocytokines that could crosstalk with hepatocytes/skeletal muscle cells, initiating insulin resistance [26,35–37]. Kahn and co workers have observed that adipose tissue, by itself, can manage much of the body's response to insulin. Overexpression of GLUT-4 on the adipocyte surface increases insulin-stimulated whole body glucose disposal [38–40]. Knock out of GLUT-4 on the mouse adipocyte surface leads to insulin resistance in muscle and liver cells, pointing towards a

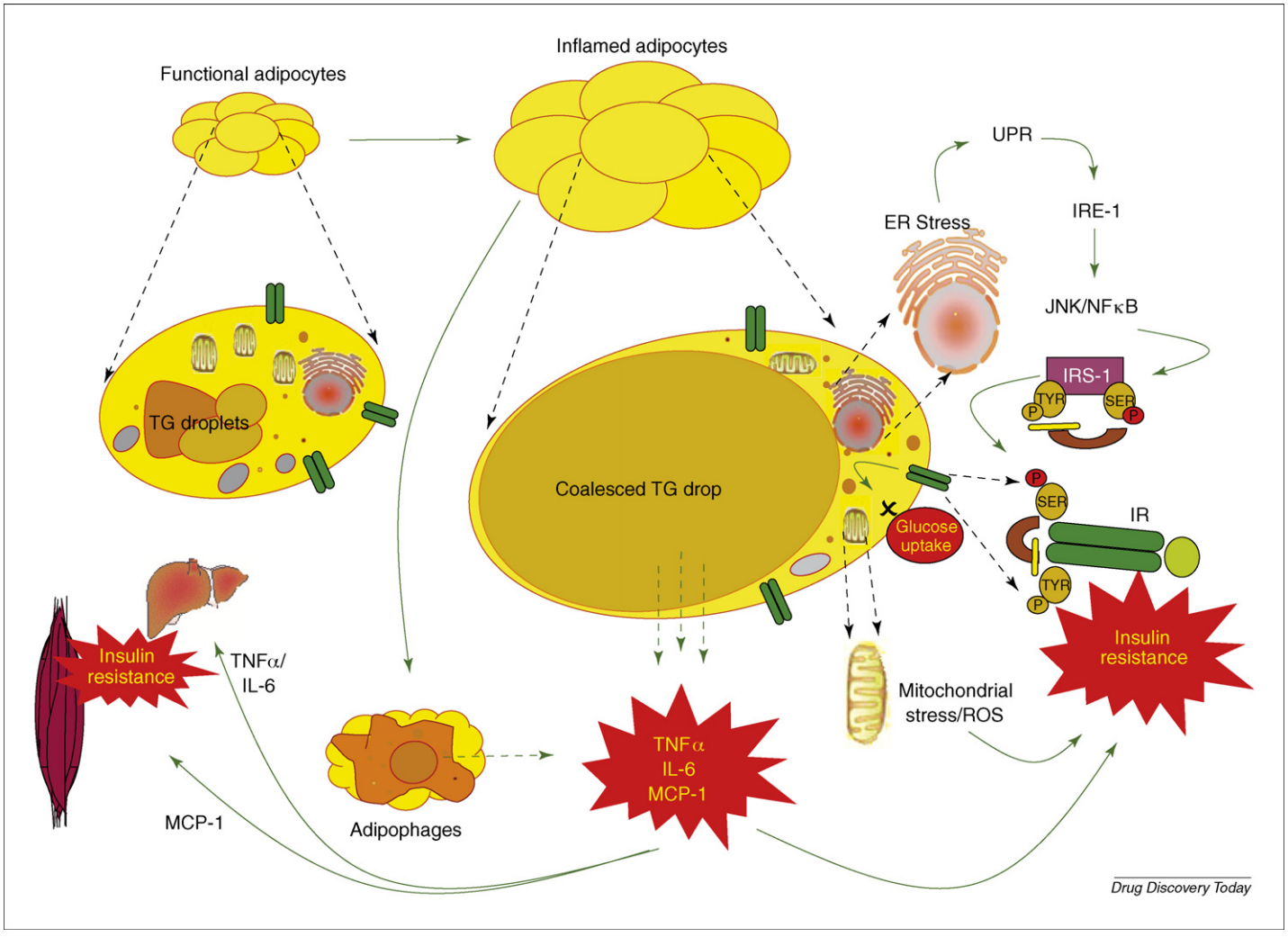

FIGURE 2

'Lean' fat cells versus 'Fat' fat cells: functional adipocytes are master regulators of glucose homeostasis with ample provision of insulin receptors and GLUT-4 receptors on their cell surface. They also store TG droplets as an energy source to be used as and when required. However, when surplus TG is stored the adipocytes surpass their critical mass of expansion and become a good source of adipocytokines such as TNF-α, IL-6 and MCP-1. In addition the lipid overload initiates ER and mitochondrial stress/ROS generation. This leads to more adipocytokine production converging on JNK and thereby eliciting increased insulin resistance and decreased glucose uptake. Large, inflamed adipocytes attract macrophages that infiltrate them and start functioning as adipophages further adding to the vicious release of cytokines. Adipocytokines also crosstalk with hepatocytes and skeletal myocytes to cause insulin resistance in these insulin sensitive tissues.

circulating factor that crosstalks between adipocytes and other cells. In 2005 this molecule was identified as retinol binding protein 4 (RBP4) [41,42].

Hell hath no fury like a fat cell ignored

Adipocytes pump out more than 50 adipokines, chemokines, proteins and growth factors that crosstalk with the rest of the body [26]. Inflammatory adipocytokines, such as TNFα, IL-6, IL-8, macrophage inflammatory protein- 1α/β, monocyte chemotactic protein-1 (MCP-1), are emerging as key regulators of insulin sensitivity [26]. Human adipocytokines that induce insulin resistance are still elusive, as the role of TNFα, that is upregulated in obesity and T2DM in animal models, in humans is still controversial [43]. In a co-culture of human adipocytes with skeletal muscles, MCP-1 was observed to play a key role in signaling between the two leading to impaired insulin signaling and glucose uptake at physiological concentrations [44,45].

An entirely different molecular signaling ensues once a functional 'lean' fat cell stores excess fat to become a 'fat' fat cell (Figure 2). The 'lean' fat cell constantly exposed to overnutrition, as in the Western diet, cannot store and expand beyond a 'critical size'. PPARγ activation promotes pre-adipocyte differentiation but, with its downregulation in T2DM, little or no differentiation takes place and hypertrophy of existing adipocytes ensues. These hyperplastic adipocytes brim with 'low grade' inflammatory reactions [46]. This low-grade inflammation precedes the war-scale factory production of adipocytokines and is thought to result from a chronic activation of the innate immune system [47]. Patients with chronic inflammatory conditions, such as chronic hepatitis C, rheumatoid arthritis and inflammatory lung diseases are predisposed to diabetes and metabolic syndrome [36]. Treatment with anti-inflammatory drugs, such as salicylates, has been observed to improve insulin sensitivity [36]. Shoelson and co-workers [33] are collecting clinical evidence to establish this link by treating T2DM patients with salicylates for 14 weeks following an 1876 work, way back before the discovery of insulin, wherein salicylates ameliorated diabetes [33]. Atherogenics Inc., has just announced significant improvement in glycaemic control in T2DM patients in its Phase III clinical trial with its lead oral anti diabetic drug candidate, AG-1067, an anti inflammatory/antioxidant molecule (http://www.atherogenics.com/ press/index.html).

Macrophage infiltration can be clearly observed in expanding adipocytes and co-localization is observed in obesity [36]. Macrophages express many 'adipocyte' gene products, such as FABP/aP2 and PPARγ, while adipocytes can express many 'macrophage' proteins, such as TNFα, IL-6 and MMP's. Functional capability of these two cell types can overlap, if required, and macrophages can take up lipids to become atherosclerotic foam cells, and preadipocytes under some conditions can exhibit phagocytic properties [36].

Endoplasmic reticulum (ER) stress

ER is a specialized cytosolic organelle regulating lipid, glucose, cholesterol and protein metabolism [36,48–50]. Specialized secretory cells, including hepatocytes, pancreatic β cells and adipocytes can adapt their ER capabilities to meet with increased demand of protein synthesis. In conditions of over nutrition, the adipocyte ER breaks down after a critical point and starts delivering unfolded/misfolded proteins. To contain this damage, ER generates an unfolded protein response (UPR) that works towards damage control by selective inhibition of *de novo* protein synthesis, transcription of chaperones to assist with the unfolded protein load leading to cell death/apoptosis through activation of IRE 1α and eventually c-Jun N-terminal kinase (JNK) activation. JNK activity leads to a variety of downstream effects, such as apoptosis, inflammation and insulin resistance. In the nucleus, JNK upregulates the expression of inflammatory genes through activation of AP-1 transcription factor complexes. JNK deficient mice display decreased levels of TNFα, IL-6 and MCP-1 as compared to wild type mice on a high fat diet. ER stress is now hypothesized to be caused by an insufficiency of XBP-1 transcription factor. On a high fat diet XBP-$1^{-/-}$ mice develop hyperinsulinaemia, hyperglycaemia, impaired glucose and insulin tolerance. XBP-1 inhibitors and ER chaperone molecules dramatically improve glucose tolerance, lower blood glucose and insulin levels and increase systemic insulin sensitivity [50]. Whether through direct activation of inflammatory pathways or indirectly through adipocytokines, ER stress seems to be the most plausible cause of obesity-induced inflammation.

Mitochondrial stress and ROS

Physiological levels of ROS are necessary for normal cell functioning, but excess ROS lead to oxidative stress [51]. Defective oxidative phosphorylation is correlated with a reduced number of functional mitochondria as observed in insulin-resistant patients [52]. Clinical conditions associated with increased ROS levels also show increased insulin resistance. Houstis *et al.* [53] identified human diseases with primary defects that affect ROS balance, namely, familial amyotrophic lateral sclerosis, Friedrich's ataxia, and ataxia telangiectasia and, in all these, significant insulin resistance was observed. Shulman and colleagues [54] have observed impaired mitochondrial substrate oxidation in muscles of an insulin-resistant subset of young, healthy and lean offsprings from T2DM patients. Decreased mitochondrial ATP synthesis, and decreased mitochondrial density were observed in this subset along with decreased lipid oxidation and accumulation of intramyocellular fatty acid metabolites. Obesity/nutritional overload also increase production of ROS through release of inflammatory cytokines that stimulate mitochondrial ROS production via ceramide formation [21]. As ROS accumulation seems to be a mitochondrial sensor of energy overflow into cells, both FFA and increased glucose levels lead to increased substrate oxidation and a secondary increase in ROS formation. Hyperglycaemia, corticosteroids, neuroendocrine activation, angiotensin II are all observed to enhance ROS in target tissues [21,55]. Angiotensin receptor blockers attenuate oxidative stress and prevent progression from pre-diabetes to frank T2DM [56].

Targeting metabolic syndrome through the lipocentric approach

Strategic therapeutic targets catering to integrated and overlapping pathophysiologies of inflammation, lipid dysregulation and insulin resistance are the need of the hour. This approach has been well validated pharmacologically with Avandia® (rosiglitazone) and Actos® (pioglitazone), belonging to the glitazone group of PPARγ agonists, wherein excess plasma fatty acids and ectopic fat

deposits are siphoned off and stored in subcutaneous adipose tissue. Rosiglitazone, however, has recently received an FDA black box warning for use in T2DM [57]. In the current therapeutic scene there is no singularly successful drug that deals with MS through correctional approaches of lipid excess/dysregulation. There are, however, several targets under development that may provide us with the silver bullet for MS.

(i) Transcriptional regulation of lipogenesis by nuclear receptors

Metabolic nuclear receptors act as lipid sensors and upon activation by endogenous and dietary lipids modulate the expression of genes involved in lipid regulation. Pharmaceutical control of metabolic 'lipid' receptors, such as PPARs, retinoid X receptor (RXR), farnesoid X receptors (FXR), and liver X receptors (LXR) by synthetic ligands is a powerful tool for management of MS [26,58–60]. Were it not for their adverse effects, PPARγ agonists were doing a splendid job on correcting lipid mediated insulin resistance. Dual/partial PPAR agonists have not delivered on their theoretical promise until now. 'Selective PPARγ modulators' (SPPARγMs; FMOC-L-leucine) have been recently validated to separate insulin sensitization from adipogenesis as all said and done, weight gain by glitazones is a major side effect [26]. To become transcriptionally active, PPARγ depends upon an indispensable partner, RXR, another probable target for MS. LG101506, a selective modulator of RXR maintains the desirable glucose-lowering, insulin-sensitizing and anti-obesity effects of rexinoids while minimizing the associated hypertriglyceridaemia [58]. FXRs, intracellular regulators of bile acids, are observed to directly regulate adipocyte function. FXR deficiency induces a lipodystrophic phenotype by impairing the ability of adipocytes to accommodate FFA and causing *de novo* hepatic lipogenesis [61]. *In vivo* validation of FXRs as modulators of adiposity and, hence, T2DM was observed when the FXR specific agonist, GW4064, improved insulin resistance in ob/ob mice [61]. LXRs are nuclear receptors involved in transcriptional control of both lipid/glucose metabolism, as well as inflammatory signaling. Following the setback observed with first generation LXR agonists, isoform-specific LXRβ ligands are awaited to avoid increased hepatic lipogenesis and TG levels that are attributable to hepatic LXRα /SREBP-1c activation [60]. Pre clinical proof of concept (POC) with specific LXR agonists, such as T1317 and GW3965, has been established in murine diabetic models wherein induction of glucokinase led to decreased hepatic glucose output and increased glucose utilization [60]. What makes LXR-targeting particularly attractive is that it also regulates macrophage inflammatory pathways and macrophage cholesterol/lipid management, thus offering a unique target that combats even the inflammatory component of MS.

(ii) Regulation of adipokines

Leptin, the adipocyte hormone suffering the dubious title of the wonder anti-obesity drug that did not work, as a result of leptin resistance, is still being given to morbidly obese children, lipodystrophic patients and people with steatohepatitis [62]. Adiponectin, an endogenous insulin sensitizer working through AMPK activation, normalizes dyslipidemia, improves insulin sensitivity and causes weight loss in addition to having anti-inflammatory and anti-atherogenic properties [62]. Development of adiponectin receptor agonists can be a powerful treatment tool for MS. Specific inhibitors of resistin, plasminogen activator inhibitor (PAI-1), MCP-1 and RBP4 can combat MS but druggability issues need to be addressed. Serum RBP4 levels are high in diabetics and antidiabetic therapies are aiming for lower serum RBP4 levels as clinically elevated levels are observed to be normalized with rosiglitazone. Specific overexpression of RPB4 has been observed in human visceral adipose tissue and it is inversely correlated with adipocyte GLUT4 protein [63]. Fenretinide, a synthetic retinoid that increases urinary excretion of RBP4 has been observed to abolish insulin resistance in DIO mice [42]. Small molecule inhibitors of RBP4 are awaited.

(iii) Regulation of master switches

If overnutrition can lead to such a wide spectrum of pathophysiologies, there must be some built-in counter-regulatory mechanisms. JNK appears to be a common denominator for free fatty acids, chronic inflammation, adipocytokines and ER/ROS stress. Recent attention has been focused on JNK1 inhibitors as a promising drug target for MS [36,64]. Sustained JNK activation has been observed in both dietary and genetic models of obesity, whereas mice with a targeted mutation at the *Jnk1* locus are resistant to obesity-induced insulin resistance. JNK activation leads to phosphorylation of IRS-1 on Ser307, thus impairing insulin action [65]. Celgene's JNK1 inhibitor, CC105, though not taken forward, showed excellent *in vivo* efficacy in db/db mice acting as an insulin secretagogue/insulin sensitizer along with having protective effects on pancreatic beta cells, anti-dyslipidemic and anti-obesity activities [64]. Therapy with cell-permeable JNK inhibitory peptides is reported to increase glucose utilization and decrease hepatic glucose production [66]. Hotamisligil and co-workers have helped establish early links between metabolic disease and inflammatory processes, and have recently identified STAMP2 as a novel counter-regulator at the interface of inflammation and metabolism in adipocytes having a regulatory hold on JNK activation [67]. Overnutrition and calorie restriction (CR) are opposite extremes of the MS spectrum. CR mimetics provide another interesting therapeutic approach towards MS. Recent studies have linked CR to *SIR2* gene family. *SIR2* and its mammalian orthologue SIRT1 are NAD^+-dependent deacetylases that deacetylate important transcription factors including p53, FOXO proteins etc leading to increased stress resistance and longevity [68]. SIRT1 is linked to lipid, glucose and inflammatory homeostasis. Sirtris Pharmaceutical has recently come up with small molecule inhibitors of SIRT1 that are 1000-fold more potent than resveratrol, a SIRT1 activator isolated from red wine [69]. Another major regulator of energy homeostasis is AMP-activated protein kinase (AMPK) which senses AMP/ATP ratio in cells. AMPK modulation is considered to be a master switch for metabolic homeostasis and is a prime target for treatment of MS [70]. AMPK phosphorylates and inhibits acetyl-CoA carboxylase (ACC), which prevents conversion of acetyl CoA to malonyl

CoA leading to decreased lipogenesis in liver and increased β-oxidation in muscles. Human POC with small molecule activators of AMPK is being eagerly awaited [71].

(iv) Regulators of lipid metabolism

Recent advances have been made in understanding the druggability of metabolic enzymes involved in lipid digestion, absorption, transport, storage and oxidation. Fatty acid binding protein (FABP/aP2) is a powerful target for MS as it integrates both the metabolic and inflammatory pathways [72,73]. FABPs are cytosolic fatty acid chaperones that show a robust impact on lipid/glucose metabolism. $FABP^{-/-}$ mice exhibit a striking phenotype which is protected from diet-induced obese (DIO), insulin resistance, T2DM and steatohepatitis. These mice show increased insulin receptor signaling, increased AMPK activation, decreased SCD-1 and SREBP1c expression [72]. Small molecule inhibitors of adipocyte FABP (aFABP), with nanomolar potency and up to 1000-fold selectivity against muscle/epidermal FABP, have been recently reported [74]. Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, the principle inhibitor of fatty acid entry into mitochondrion for β-oxidation. Mammalian ACC exists as ACC1 and ACC2 that are present as principal isoforms in lipogenic and oxidative tissues respectively. AMPK mediated phosphorylation of ACC inhibits its activity and leads to decreased hepatic lipogenesis and increased fatty acid oxidation in muscles. $ACC2^{-/-}$ mice are resistant to DIO and T2DM and have reduced plasma FFA levels [75]. Specific inhibitors of ACC, such as Pfizer's CP-640186, are currently under development. Pharmacological inhibition of stearoyl-CoA desaturase-1 (SCD-1), diacylglycerol acyltransferase-1 (DGAT-1) and fatty acid synthase (FAS), all acting on the lipogenesis pathway, are also attractive approaches towards controlling lipid induced insulin resistance.

Conclusion

The traditional 'glucocentric' viewpoint of diabetes has shifted towards a 'lipocentric' approach with respect to the insulin-resistance pathophysiology. Randle's hypothesis sensitized our mindset towards the role of FFA in insulin resistance followed by the 'visceral' and 'ectopic' fat theories of insulin resistance. Parallel emergence of Dr Jekyll/Mr Hyde attitude of adipocyte towards regulation of energy homeostasis has put the role of adipocytokines and inflammation on the forefront of insulin resistance. Also, the role of stressors, namely, psychosocial stress and over nutrition, leading to ER stress and mitochondrial stress/ROS generation is fast gaining importance. A pleiotropic effector that can reverse the lipid-induced MS phenotype is eagerly awaited.

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