REVIEW ARTICLE

Lipid links inflammation, immunity and insulin resistance to cause epidemic diabetes

Samir Bhattacharya* and Sandip Mukherjee

Cellular and Molecular Endocrinology Laboratory, Centre for Advanced Studies in Zoology, School of Life Science, Visva-Bharati, Santiniketan 731 235, India

Incidence of type 2 diabetes (T2D) is dramatically increasing in the past few decades and presently affecting more than 350 million people all over the globe. Oversupply of lipid is one of the major forces behind T2D. Excess of lipid decreases insulin sensitivity or activity that causes insulin resistance, a stage which occupies the centre of pathogenesis in T2D. Lipid induces adipose tissue inflammation that accompanies certain critical defects in adipocytes, a major cell in abdominal adipose tissue. These include increased population of hypertrophied adipocytes, decline in adipokines secretion, attenuation of adipogenesis, and increased lipotoxicity effecting greater deposition of fat which interferes with glucose uptake by insulin target cells. Inflammation of adipose tissue is further intensified due to the infiltration of macrophages, a member of the innate immune system, and their transformation from anti-inflammatory M2 to pro-inflammatory M1 phenotype. Hence, secretion of pro-inflammatory cytokines from both M1 macrophage and inflamed adipocytes is greatly elevated which adversely causes insulin resistance that leads to T2D. Association between lipid-induced inflammation and insulin resistance makes diabetes a critical disease.

Keywords: Adipose tissue inflammation, FetuinA, insulin resistance, macrophage infiltration, type 2 diabetes.

TYPE 2 DIABETES (T2D) apparently looks to be a simple disease where excess of lipid causes loss of insulin sensitivity which begins with insulin resistance. Initially, this defect in insulin activity could not be perceived by patient as the dysregulation of glycemic level is being prevented by the compensatory increase in insulin secretion from the pancreatic β-cell. Hence, it is hyperinsulinaemia that maintains the non-diabetic state with euglycaemia. This stage is termed as insulin resistance. But, a time comes when β-cell fails to bear this extra burden of hyperinsulinaemia and a decline in plasma insulin level results onset of T2D[1–3]. In contrast, this stage is absent in type 1 diabetes where hyperglycaemia occurs due to the dearth of insulin secretion because of β-cell destruction caused by the autoimmune disorder[1]. But this is not an epidemic disease as only 3–5% of diabetic population belong to this class whereas 95–97% are T2D, which is now posing a threat to global health[4].

Hence, it is lipid or fat which induces loss of insulin sensitivity or inactivation of insulin. Several reports have demonstrated that removal of fat permits regaining of insulin sensitivity[5–7]. More precisely, long chain saturated free fatty acids (FFA) or non-esterified fatty acids (NEFA) have been shown to be the major player affecting the impairment of insulin activity. Last few years of research documented that excess of lipid is associated with chronic inflammation, particularly in the adipose tissue and this causes insulin resistance in different animal models[4,8–9]. In human beings, obesity-induced chronic inflammation is the major factor for insulin resistance. Interestingly, increased macrophage infiltration in adipose tissue and their transformation to proinflammatory subtypes play a significant role in aggravating adipose tissue inflammation as observed with hyperlipidaemic diabetic patients[10–13]. In this review, we will primarily deal with the association between lipid-induced adipose tissue inflammation, participation of immune cells therein and insulin resistance that leads to the development of T2D.

Impairment of adipose tissue: the major regulator of insulin resistance

Abdominal white adipose tissue (WAT) has been recognized as an important site for storing energy obtained from diet. The predominant cell type in this tissue is adipocytes which store energy in the form of triglyceride (TG) as simple lipid droplets. During caloric need, TG is hydrolysed, resulting in free fatty acids, oxidation of which in the mitochondria, primarily in the skeletal muscle tissue produces energy in the form of adenosine triphosphate (ATP)[14–18]. Adipocytes of WAT are now regarded very important cells, dysfunction of which leads to the extracellular accumulation of lipid, increase in circulatory lipid level and ectopic fat deposition (Figure 1). All these disrupt the balance in energy storage and expenditure, and impair energy homeostasis which is responsible for the decrease in insulin sensitivity that results in insulin resistance[18]. This is because adipocytes perform certain critical functions like uptake of fatty acids, storing them in the form of TG and converting them to free fatty acids (FFAs) through lipolysis during

*For correspondence. (e-mail: bhattacharyasa@gmail.com)

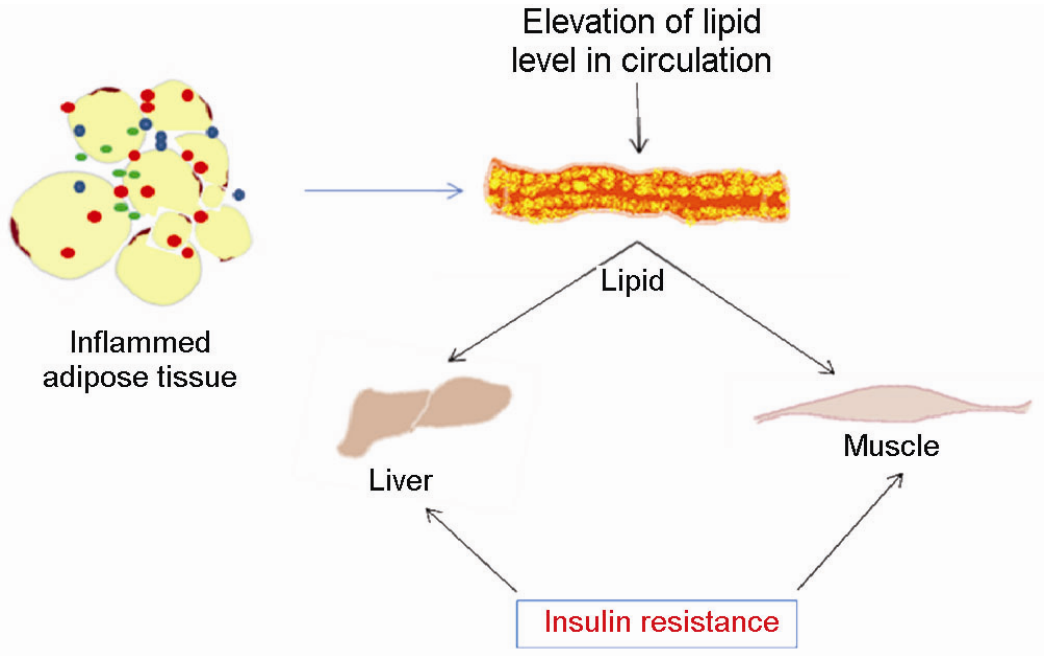

Figure 1. Fatty acid release from inflamed adipose tissue increases lipid level in circulation and effects fat deposition in liver and muscle causing insulin resistance.

energy demand. FFAs released into the circulation from adipocytes are taken up by the muscle, converted into TG and during energy crisis, TGs are hydrolysed to FFAs, and mobilized to mitochondria for β-oxidation to yield energy. It is intriguing to note that the capacity for storage of FFAs is far greater in adipocytes as compared to muscle cells but the ability for oxidation of FFAs in mitochondria is more efficiently operated in muscle cells than in adipocytes[17,18].

Interestingly, morphology of adipocytes varies according to their functional status; for example, populations of preadipocytes and small adipocytes are more in normal condition whereas large or hypertrophied and senescent adipocytes increase in obese diabetic subjects and animals[19,20]. Hypertrophied and senescent adipocytes are considered to be aberrant in function and increase in their number is associated with insulin resistance and T2D[21]. In lean, non-diabetic mice or human beings, adipose tissue is largely occupied by small to middle sized adipocytes which are highly active and do not allow extracellular fat deposition[22,23]. In contrast, during obese diabetic conditions, significant decline in pre- and small adipocytes is observed with a remarkable increase in hypertrophied adipocytes, which are defective cells, unable to uptake glucose and FFA appropriately and in addition, they extrude FFA affecting glucose and insulin intolerance that leads to T2D[17,24]. Senescence in adipocytes, on the other hand, is the product of oxidative stress, sometimes occurring because of over-utilization of adipocytes. They are functionally inert, usually non-dividing but occupy the space in inflamed adipose tissue as observed in diabetic mice and human subjects[21,25].

Increase in hypertrophied adipocyte population is one of the markers for the loss of insulin sensitivity which is related to impairment of PPARγ (peroxisome proliferator activator receptor γ) expression and activity. PPARγ is the key factor in improving lipid-induced insulin resistance[26–28]. However, PPARγ itself is not involved in insulin sensitization but it improves insulin action through the transcription of its target genes such as adiponectin, CD36 and aP2 (refs 27, 29, 30). Adiponectin, although secreted from adipocytes, regulates fatty acid mobilization and oxidation predominantly in muscle and therefore is an important adipokine for maintaining insulin sensitivity in muscle[16]. CD36 uptake fatty acid and also act as translocase; and aP2 is a fatty acid binding protein and regulates its intracellular mobilization[31,32]. Negative regulation of PPARγ and its target genes in adipocytes due to excess lipid therefore affects loss of insulin sensitivity causing insulin resistance. From this description, it would be evident that dysregulation of adipocyte function because of excess fat is a key factor in insulin resistance[16,17].

Fatty acid impairs insulin signalling that contributes to insulin resistance

Among some previously described information which demonstrated a direct association of FFA with insulin resistance, the most convincing evidence was available from the report of Santomauro *et al.*[5]. By performing some elegant experiments, they showed that lowering of FFA in T2D subjects significantly reduces insulin

resistance and strikingly improves oral glucose tolerance. Again, forcing FFA entry through infusion in rats and humans has been found to damage insulin sensitivity and produces insulin resistance[23,33]. Blocking of FFA action or reduction of FFA level has been shown to improve insulin sensitivity. There are several studies which show that FFAs are responsible for insulin resistance which ultimately results T2D[2,4,34–37]. FFA is now known to cause defects on insulin activity, which is contributed by several factors those adversely affect insulin responsive tissues such as muscles, liver and adipose tissues. One of such factors is insulin receptor (IR). Binding of insulin with IR initiates insulin signalling cascades which through downstream signalling components such as IRS1, PI3K, PDK1 and Akt activates Glut4 in adipocytes or skeletal muscle cells to uptake glucose from circulation into the cell. Failure of insulin stimulated signals therefore causes hyperglycaemia or increase in blood glucose level. There are both quantitative and qualitative defects of IR in T2D patients as the number of IR per adipocytes is significantly reduced in obese diabetic subjects, and decrease in IR gene expression has been detected in insulin-resistant patients[38–42]. Reduced expression of IR in insulin responsive tissues of animals has also been shown to be affected due to FFAs, mainly by long chain saturated fatty acids. Decrease in IR protein and mRNA expression in hepatocytes and skeletal muscle cells occur due to FFAs such as palmitate which impairs insulin signalling[41,43]. Palmitate, through the inhibition of HMGA1 (high mobility group protein A1), an architectural transcription factor of IR gene, inhibits IR transcriptional activity that downregulates IR gene expression in muscle cells which compromises insulin activity[44].

Lipid-induced adipose tissue inflammation and insulin resistance

FFAs, especially long chain fatty acids, could trigger cellular proinflammatory pathways as demonstrated by several studies[12,45–47]. FFAs mediate their proinflammatory effect primarily through the activation of toll-like receptors (TLRs)-dependent mechanisms which show that excess of lipid or lipotoxicity is associated with chronic inflammation of adipose tissue that causes decrease in insulin sensitivity[12,45,48–50]. In the absence of TLR4, FFA-mediated inflammatory signalling is suppressed in adipocytes[49]. Moreover, insulin activity remains protected in TLR4 knockout mice against high fat diet[51]. FFA-induced activation of TLR4, a pattern recognition receptor, mediates inflammation through NFκB pathways[47,50,52–54] and it also stimulates JNK and IKK which suppresses insulin signalling through serine phosphorylation of IRS I (ref. 12). This indicates that FFA could act as a ligand of TLR4, and their binding causes insulin resistance in adipocytes. However, it has been shown that fatty acid has no direct association with TLR4 (ref. 55), hence it is critically important to know how FFA can activate TLR4 mediated inflammatory signal without binding to TLR4. It has been reported that excess FFA induces Fetuin A or α_2 Heremans–Schmid glycoprotein (AHSG) expression primarily in liver[56]. In obesity-induced diabetic humans and mice, serum Fetuin A (FetA) level significantly increases as compared to non-diabetic subjects or mice[57–61]. Interestingly, FetA directly binds to TLR4 with high affinity and it also binds to FFA very strongly. It has been found that FetA binds to FFA more avidly than albumin and then presents it to TLR4. FFA–FetA–TLR4 form a ternary complex which activates TLR4–NFkB pathways to produce proinflammatory cytokines from inflamed adipocytes which exacerbate insulin resistance[62].

However, *FetA* gene is not only expressed in liver but also in adipocytes. Its expression is induced by fat and greater accumulation of lipid is correlated with the amount of *FetA* gene expression[63]. FetA knockout mice are shown to be protected from high fat diet-induced insulin resistance[64] and in FetA knockdown mice, high fat diet fails to induce adipose tissue inflammation and loss of insulin sensitivity[62]. FetA's contribution to the development of metabolic disorders leading to insulin resistance and T2D including other complications has recently been dealt in detail by Trepanowski *et al.*[61].

Adipocytes dictate impairment of insulin sensitivity in muscle and liver

Insulin resistance in muscle is largely controlled through adipocyte dysfunction because greater supply of lipid and attenuation of adiponectin secretion adversely affects insulin sensitivity of muscle tissue. Larger amounts of post-prandial glucose in blood after a meal is being deposited (~75%) in skeletal muscle tissues[65–67]. Therefore, loss of insulin sensitivity in the muscles of human subject and mice produces significant effects on glucose homeostasis. Recent studies demonstrated that hyperlipidemic condition during obesity is correlated with enhanced expression of proinflammatory genes in the muscles of mice and human subjects[13,68]. In addition, due to dysfunction of adipocytes, increased level of serum fatty acids markedly enhances FFA influx into the muscle of obese diabetic individuals which significantly attenuates fatty acid oxidation. This further contributes to the accumulation of fatty acid intermediates in muscle that activates JNK, IKK or nPKC signalling pathways causing insulin resistance[44,69–71].

Contribution of adipose tissue for developing insulin resistance in muscle and liver involves adipokines. Adipocyte synthesizes 347 proteins, of which 263 are predicted to be secretory and adipokine in nature[72]. Major adipokines secreted from it (Figure 2) create a microenvironment through interconnected network which enable

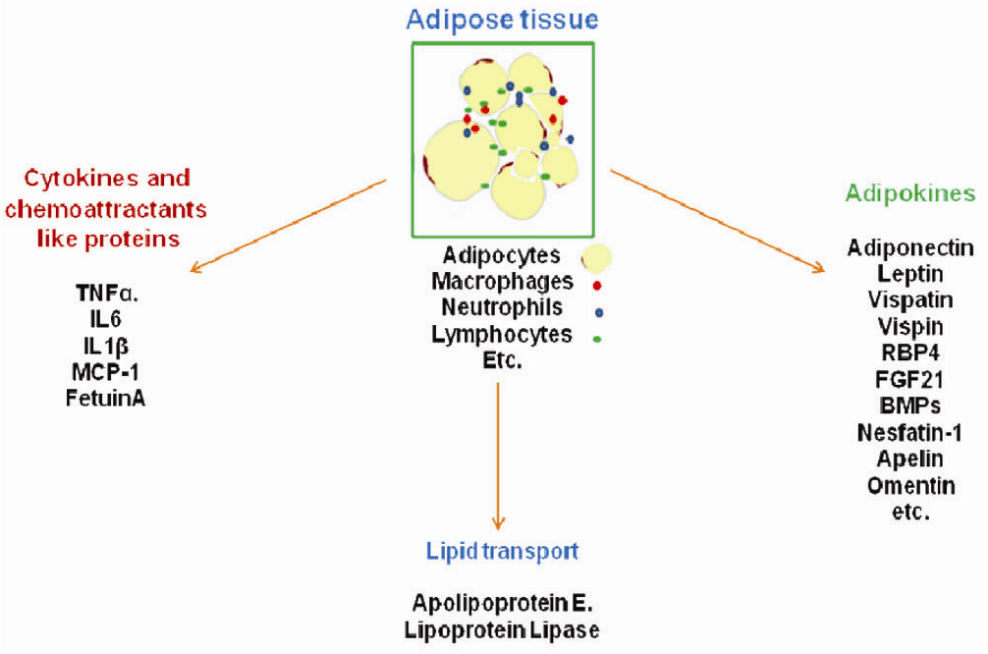

Figure 2. Adipose tissue secretes several proteins such as chemoattractants like proteins, apolipoproteins, adipokines, etc. crucial for maintaining energy homeostasis.

adipocytes to communicate with skeletal muscles and liver[72,73]. During obese diabetic conditions, aberrant adipokine secretion from inflamed adipose tissue worsens the situation in muscle and liver. The most crucial reason behind such abnormality appears to be the excess efflux of FFA by hypertrophied adipocytes of obese diabetic mice and human subjects leading to considerable increase in circulatory FFA level and extracellular lipid accumulation. This causes insulin resistance in adipose tissue, muscle and liver[3,6,7,34–37] and lowering of FFA increases insulin sensitivity in these tissues. A significant increase in muscle triglyceride (TG) concentration and hepatic steatosis are commonly occurring abnormalities observed during insulin resistance[74]. FFA efflux from hypertrophied adipocytes when accumulated in liver and muscle may generate bioactive lipid products which can inhibit insulin signalling[75]. In skeletal muscle cells, fatty acid derived diacylglycerides (DAGs) can augment protein kinase C (PKC) activity which inhibits serine phosphorylation of insulin receptor substrate (IRS) or FFA can directly stimulate PKC activation which leads to the downregulation of insulin receptor – both causing insulin resistance[44]. From these studies, it is evident that abnormalities in adipocytes are transmitted to liver and muscle tissue via the excess lipid load which greatly contribute to the development of insulin resistance and T2D.

Another important dimension of excess FFA in skeletal muscle tissues is its effect on mitochondrial dysfunction which impairs insulin sensitivity. Decreased mitochondrial density and oxidative capacity of muscle have been detected in T2D patients[76–78]. Even in healthy animals and humans, high fat diet or lipid infusion markedly decreases oxidative phosphorylation and ATP synthesis in mitochondria[79–81]. This indicates that FFAs can directly cause dysfunction of skeletal muscle mitochondria which is related to insulin resistance. Several reports also suggest that intramuscular increase of FFAs and triglyceride levels may produce defects in mitochondrial β-oxidation which is associated with insulin resistance[82–84].

Macrophage participation in adipose tissue inflammation: a critical stage in insulin resistance

It is now increasingly recognized that the immune system and metabolic disorder leading to insulin resistance and T2D are highly integrated. High energy diet and accumulation of excess lipid are affecting lipotoxicity, triggers a number of metabolic pathways responsible for the development of insulin resistance. One of the critically important pathogenic inputs in insulin resistance is macrophage infiltration into inflamed adipose tissue which produces proinflammatory cytokines that further intensify inflammation and worsen insulin sensitivity[9–12,45]. It is interesting to note that inflammation of adipose tissue due to excess lipid attracts macrophage. Migration of macrophage has been reported to be mediated by Monocyte Chemoattractant Protein1 (MCP1)[46], which is found to be highly expressed in adipose tissue and secreted in HFD mice[13,85]. In addition, MCP1 and MCP1 receptor (CCR2) have shown to be important in the recruitment of macrophages into the adipose tissue.

The most remarkable dimension of adipose tissue inflammation is related to resident adipose tissue macrophage (ATM). In obesity-induced inflammation, accumulation of macrophage in the adipose tissue is a characteristic feature of chronic inflammation which causes insulin resistance and T2D[10–12]. Why inflamed adipose tissue is an attractive site for macrophage infiltration is yet unclear. No doubt MCP1 is a chemoattractant for macrophage migration but CCR2 deficient mice have been shown to be protected from HFD-induced insulin resistance and macrophage accumulation[85,86]. Moreover, a few reports indicate that MCP1 is not sufficient for the amount of macrophage that infiltrates during adipose tissue inflammation[87]. Hence there appears to be other chemoattractant(s) necessary for such purpose. Most conspicuous lacunae in this field is in the understanding of the transformation of M2 or classically activated macrophage (CAM) to M1 or alternative activated macrophage (AAM) which contributes to severe inflammation that significantly reduce insulin sensitivity[13]. It has been recently reported that FetA released from adipocytes is also acting as chemoattractant for macrophage infiltration to the adipose tissue and most interestingly, FetA also contributes to the polarization of M2 to M1 phenotypes[63]. Till date, no other factor has been detected to influence M2 to M1 transformation. The microenvironment thus created by inflamed adipocytes and pro-inflammatory M1 macrophage cohabitation could produce aberrant metabolic functions that result in an unusual increase in the release of proinflammatory cytokines such as TNFα and IL6 (refs 12, 13). In this complex inflammatory condition, migration and accumulation of macrophages in the adipose tissue are reported to be the principal source of inflammatory mediators through the expression and release of TNFα[10,11,45]. Excess secretion of TNFα from both adipose tissue and macrophages is a strong negative regulator of insulin sensitivity, potent enough to cause insulin resistance. Moreover, TNF α augments lipolysis in inflamed adipocytes thus elevating serum FFA level which in turn adversely affects insulin sensitivity[88–90]. Several recent reports emphasize the pathophysiological association between macrophages and adipose tissue inflammation where excess lipid plays the key role. The link between immunity and adipose tissue inflammation that cause T2D poses challenge for developing novel therapeutic interventions that can cover all these intricate complications involved in this insidious disease.

Conclusion

Research during the past few decades has identified adipose tissue as the dominant regulator of energy homeostasis. Its inflammation due to over-supply of lipid not only leads to insulin resistance where uptake and tolerance to glucose is attenuated but also adversely affects lipid uptake, storage and mobilization. FetA is a novel linker between saturated fatty acid and TLR4. When FetA presents fatty acids to TLR4, it mediates inflammation through NFkB dependent pathway and starts secreting proinflammatory cytokines, TNFα and IL6, which in turn cause insulin resistance. This is further aggravated by the mobilization of macrophage into inflamed adipose tissue, which are transformed to pro-inflammatory M1 phenotype and release proinflammatory cytokines. These together lead to an intense inflammatory state which culminates in insulin resistance reaching a critical stage that leads to the onset of type 2 diabetes.

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ACKNOWLEDGEMENTS. S.B. gratefully acknowledges the Indian National Science Academy for 'INSA Golden Jubilee Research Professorship' and S.M. thanks DBT, New Delhi for a Research Associateship to him under a research project (File No. BT/367/NE/TBP/2012).

Received 27 January 2016; accepted 24 March 2016

doi: 10.18520/cs/v110/i10/1922-1928