

Mechanism of Lipid Induced Insulin Resistance: An Overview

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Type 2 diabetes (T2D) is rapidly spreading throughout the world. It's an insidious disease and still treated in an indirect manner without having specific drug target. In majority cases T2D is treated with drugs that address type 1 diabetes, majority of drugs aim to increase insulin release although the root cause for T2D is not the dearth of insulin release, it occurs in the later stage of disease development. T2D silently progressed in the patient; it begins with insulin resistance that takes place due to the loss of insulin sensitivity. Though insulin resistance is the centre of pathogenesis, our treatment of the disease has not yet addressed it. It is now a fact that insulin resistance is manifested by lipid and fatty acids (FAs) play a critical role in blunting insulin sensitivity. Our understanding is still poor in deciphering how lipid impose insulin insensitivity, majority of workers suggest it is because of insulin signaling defects which implements insulin function in inhibiting glucose to the cell from circulation. Number of long chain saturated FA has been shown to produce insulin signaling defects. However, we really need further investigation to find specific target(s) for FA induced damage. In addition to these information, a new dimension of T2D is getting attractive is fetuin-A/ α_2 -Heremans-Schmid Glycoprotein, a secretory protein from liver. Its gene locus has been identified as T2D susceptible. Fetuin-A's excess expression occurs by FA and it disrupts adipocyte function. It has been shown to be associated with T2D especially in obesity. In this review, we briefly discuss the present status on the mechanistic understanding of lipid induced insulin resistance that leads to T2D. More we understand the mechanism; opportunity to fight the battle with T2D will be increasing. Since, this field is now vast; we covered a few major events. (*Endocrinol Metab* 27:12-19, 2012)

Key Words: Alpha-2-HS-Glycoprotein, Fatty acids, HMGAI, Insulin resistance, Lipids, Novel PKCs, Type 2 diabetes

INTRODUCTION

Type 2 diabetes (T2D) is now posing a major threat to human health. World Health Organization has cautioned that it is taking the shape of a global epidemic. We could not solve the riddle about how exactly T2D is related to excess lipid input and have not developed proper understanding of this disease even though there is no dearth of research activities in this direction. Prior to the manifestation of T2D there occurs impaired ability of insulin to maintain glucose homeostasis. This is because of defects in insulin sensitivity which leads to insulin resistance that results responsive tissues and cells suffer from impairment of glycemic regulation. To search the possible cause for the development and pathogenesis of insulin resistance, hyperlipidemia and dyslipidemia have been identified as the leading factors [1,2]. Continuation of this state ultimately manifested in the form of T2D. Several reports indicated that over-

supply of lipid increases circulatory level of free fatty acid (FFA) and that contributes to the development of insulin resistance [3,4]. Patients suffering from insulin resistance often show signs of abnormal lipid metabolism where excess of plasma FFA level is coincided with reduced insulin-stimulated glucose uptake [3,5]. Lowering of circulatory elevated FFA level in diabetic patients improves insulin sensitivity [6]. Forcing their entry through infusion in rats and human impairs insulin stimulated glucose disposal into the skeletal muscle [3,5]. These reports along with several others established that T2D is lipidocentric and not glucocentric as was the impression existed earlier till the time of discovery of insulin. It should be recalled that epidemic diabetes is T2D and not type 1 diabetes (T1D) which is precisely due to the pancreatic β -cell dysfunction. More than 95% are suffering from T2D while only 3-5% are T1D among world diabetic population [7,8]. It is also necessary to remember that in T2D loss of insulin sensitivity is the major event.

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When it is well accepted that lipid is causing insulin resistance and T2D, questioning about how lipid is involved therein becomes obvious. Many authors have shown that it is the fatty acid (FA) of lipid which is mostly associated with insulin resistance [3,5]. Some authors indicated that long chain saturated FAs are major culprit [9]. The list of FAs later has been shortened to 3-5 FAs and these are palmitate, myristate, stearate (saturated FAs) and oleate (unsaturated FA) [10,11]. There may be others, but reports on circulatory lipid profile in diabetic patients showed prevalence of these FAs and research area in the field of insulin resistance has predominantly used these FAs to blunt insulin sensitivity [6,12,13]. Apparently, next query would be raised on how FAs could adversely affect insulin sensitivity causing insulin resistance. To search this, number of molecules in insulin signaling pathway has been implicated. That insulin resistance is primarily due to insulin signaling defects, raises no doubt. These defects, in fact, may occur at various places from upstream to downstream and that include 1) insulin receptor (IR), 2) phosphatidylinositol 3-kinase (PI3K), phosphoinositide-dependent kinase1 (PDK1) and Akt mediated glucose transporter 4 (Glut4) activation, and 3) interplay of novel protein kinase Cs (nPKCs). Besides insulin signaling pathway another area of research is getting prominence which is adipocyte function. This is because any defect in the functioning of adipocyte would be reflected in lipid imbalance which is the root cause for insulin resistance.

Insulin resistance, the centre of pathogenesis in T2D, is indeed a vast area with umpteen number of research publications in every year. In this review we tried to briefly cover the advancement made so far in the above three areas to understand the mechanism of lipid induced insulin resistance.

LIPID INDUCED INSULIN SIGNALING DEFECTS

Insulin signaling is initiated when insulin binds to its receptor. IR is a $\alpha 2\beta 2$ heterodimeric transmembrane protein that possesses intrinsic protein tyrosine kinase activity. Insulin binding to specific regions of the α -subunit leads to a rapid conformational change in the receptor that eventuates in autophosphorylation of specific tyrosine residues of the β -subunits through a transphosphorylation mechanism, i.e., C-terminal of $\beta 1$ subunit phosphorylates tyrosine residues of $\beta 2$ and vice-versa because of their kinase property. However, it is not yet clear about how insulin brings about mutual phosphorylation of the two tyrosine protein kinases. Autophosphorylation of tyrosine residues stimulates the catalytic activity of receptor tyrosine kinase and creates recruitment sites for insulin receptor substrate (IRS) proteins [14-16] which dock on pTyr moiety of IR when phosphate is transferred to IRS. PI3K is a target of IRS proteins, which phosphorylates specific phosphoinositides to form phosphatidylinositol 3,4,5 tri-phosphate from phosphatidylinositol 3,4 bis-phosphate that in turn activates serine/threonine kinases PDK1 which phosphorylates Akt [16,17]. Akt plays an important role by linking Glut4 to insulin signal transduction pathway [18]. This insulin signaling pathway is schematically represented in Fig. 1. FA has been shown to disrupt this pathway. Various authors described FA induced defects in different signaling molecules starting from IR to Glut4 [3,5,19-23].

Several evidences have been accumulated that hold FAs responsible for insulin inaction. Elevated FAs in circulation is associated with impaired insulin function and is commonly linked with obesity and T2D [5,24,25]. Increase of plasma FFA concentrations through lipid

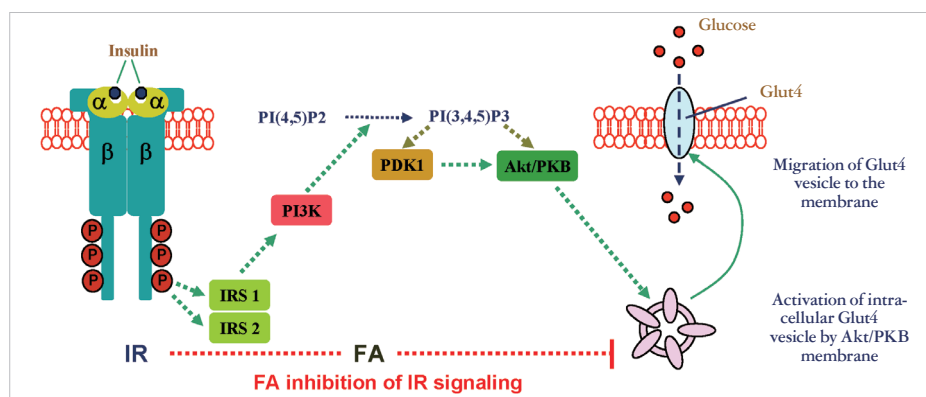


Fig. 1. Schematic representation of insulin signaling pathway. Fatty acid (FA) has been reported to impair this pathway by producing defects on insulin receptor (IR), insulin receptor substrate (IRS1), phosphatidylinositol 3-kinase (PI3K), phosphoinositide-dependent kinase1 (PDK1), Akt/protein kinase B (PKB), and glucose transporter 4 (Glut4).

infusion causes insulin resistance in rat and human skeletal muscle [25,26]. Incubation of isolated muscle strips or cultured muscle cells with FFAs or lipoprotein lipase expression in skeletal muscle reduces insulin-mediated glucose uptake [3,27,28]. All these reports suggest that greater deposition of lipid in insulin sensitive tissues promotes insulin inaction and resistance. Lowering of glucose transport by FA is linked to inhibition of insulin-stimulated IRS-1 phosphorylation and IRS-1 associated PI3K activation indicating impairment of insulin activity due to insulin signaling defects [19-22,29,30]. These reports drive our attention towards FFAs as the major compounds causing insulin resistance and T2D. Many workers show palmitate as the most potent FA in inhibiting insulin activity [3,21,30,31] and the question confronting us is how palmitate imposes such defects. FA inhibits insulin activation of IRS-1-associated PI3K activity in muscle. Reduction of insulin-stimulated IRS-1 tyrosine phosphorylation and IRS-1-associated PI3K activity by FFA has been shown to be associated with an increase in IRS-1 serine phosphorylation. This in turn decreases IRS-1 tyrosine phosphorylation, impairing downstream effectors [20]. FA can disrupt further downstream insulin signals. Administration of saturated fat blocks insulin activation of Akt/protein kinase B with a concomitant increase in the amount of ceramide and diacylglycerol in cultured muscle cells [30]. Insulin actively sequesters Glut4 at an intracellular location which increases the rate of Glut4 trafficking to the membrane [32]. Glut4 is the penultimate molecule in the signal and its translocation to the membrane by insulin is the ultimate step in signaling, as only then glucose is transported into the cell. Lipid-associated insulin resistance has also been shown to be linked to Glut4 translocation defects [33]. Previous reports from our laboratory have shown that palmitate incubation of skeletal muscle cells and adipocytes, two major insulin target cells, suppressed insulin stimulated phosphorylation of receptor tyrosine kinase, PI3K, PDK1, and Akt. This inhibition of insulin signaling caused an impairment of Glut4 translocation to the membrane causing a significant deficiency in glucose uptake [21, 22,34].

ROLE OF NPKCS IN FA MEDIATED INSULIN RESISTANCE

From the above description it would be evident that FAs are involved in insulin resistance. However, the precise mechanism is unclear. Interestingly, interplay of PKC especially nPKCs in lipid induced insulin sensitivity defects has been indicated by some authors [28,34,35]. Lipid infusion in rats and human has been shown to impair insulin stimulated glucose disposal in muscles and con-

comitant activation of PKC θ and PKC δ . PKC δ and θ has been reported to be a negative regulator in the insulin signaling pathway. C1 domain of both these nPKCs is a tandem repeat of classical PKCs (cPKCs) that serves as a diacylglycerol sensor; C2 domain in nPKCs does not function as a Ca²⁺ regulated phospholipid binding module, a characteristics of cPKCs [36]. PKC δ has been shown to be a possible candidate for phosphorylating the IR on serine residues that could decrease tyrosine phosphorylation of IR affecting its routing [37]. Over expression of PKC δ in cultured myotubes induces serine phosphorylation of IR in response to insulin. The serine phosphorylation of IR changes IR distribution on the cell membrane; a rich component of it is localized on the internal membrane components thus causing attenuation of insulin induced tyrosine phosphorylation of IR [38]. Defective phosphorylation state of IR thus impedes downstream signaling cascade in the insulin signaling pathway. Interestingly, activation of PKC δ and θ occurs along with insulin resistance in muscle cells due to FA. What is still not clear is about the mechanism involved in PKC δ mediated downregulation of IR activity and whether it is direct or indirect. It has been demonstrated that PKC δ can directly inhibit IR kinase stimulated IRS1 associated tyrosine phosphorylation [39]. The same thing has also been suggested in lipid induced PKC θ activation in muscle [19,40]. All these information suggests a link of nPKCs in FA induced insulin resistance.

Reduced IR expression has been observed in mice and also in human diabetic patients [41,42] and PKC ϵ has been implicated in IR damage. IR expression is inversely correlated to PKC ϵ in diabetic obese rats [43] and lipid mediated hepatic insulin resistance has been shown to be prevented by knocking down of PKC ϵ [44]. There are several reports on PKC ϵ association with insulin resistance [22,45,46]. However, the most impressive work in relation to PKC ϵ and IR degradation has been demonstrated by Ikeda et al. [43] when they observed in a well characterized diabetic animal model that overexpression of PKC ϵ followed by its higher activity is inversely linked to IR expression which impaired insulin downstream signaling. We have reported that FA initiates disruption of insulin signal by phosphorylating PKC ϵ in a PDK1 independent manner [21,22,47]. Palmitate and myristate phosphorylated PKC ϵ (pPKC ϵ) which then moves to nuclear region. Since PKC ϵ lacks nuclear localization signal (NLS), we found it is F-actin, which has 13 NLS recognizes pPKC ϵ and chaperoned it to nuclear region which coincided with reduced IR expression in skeletal muscle cells [47]. Question is how pPKC ϵ is involved in IR downregulation. *IR* gene is regulated by an architectural tran-

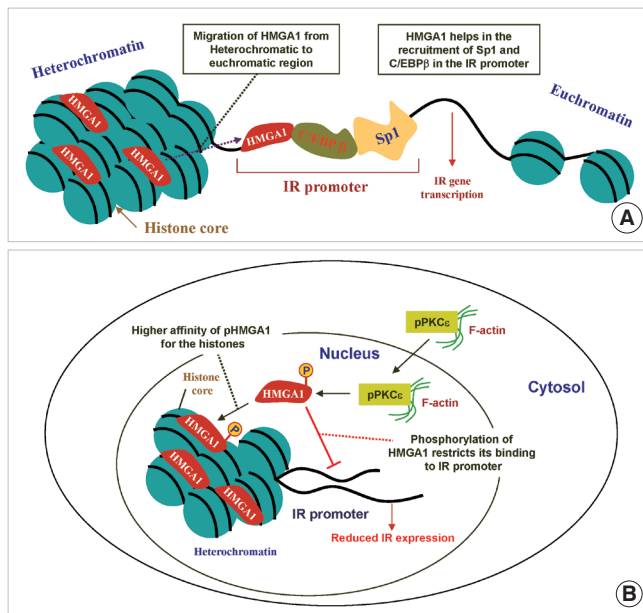


Fig. 2. Fatty acid (FA) causes insulin receptor (IR) downregulation through pPKC ϵ . FA affected a kinase independent phosphorylation of PKC ϵ through palmitoylation in case of palmitate, pPKC ϵ is translocated to nucleus by F-actin where it impairs high mobility group A1 (HMGA1) which regulates IR gene transcription by permitting Sp1 and CCAAT/enhancer-binding protein beta (C/EBP β), the transcription factors of IR gene [48,49]. We have found that FA induced pPKC ϵ impairs HMGA1 [34]. During IR gene transcription HMGA1 migrates from heterochromatin to euchromatin region and activates IR promoter [48,50]. Interestingly, pPKC ϵ phosphorylates HMGA1 which restricted its mobility and therefore unable to activate IR promoter resulting reduced IR expression. In addition, pPKC ϵ also deactivates transcription factor Sp1 which regulates HMGA1 promoter activation. Hence, FAs phosphorylation of pPKC ϵ seriously damaged HMGA1 affecting decreased IR expression [47]. This is a novel dimension of FAs derogatory effect where pPKC ϵ plays a key role in disrupting insulin-IR association that inflicted insulin resistance due to the loss of its sensitivity which is schematically represented in Fig. 2.

sorption factor high mobility group A1 (HMGA1), it is called as architectural because it modulates IR promoter conformation to recruit Sp1 and CCAAT/enhancer-binding protein beta (C/EBP β), the transcription factors of IR gene [48,49]. We have found that FA induced pPKC ϵ impairs HMGA1 [34]. During IR gene transcription HMGA1 migrates from heterochromatin to euchromatin region and activates IR promoter [48,50]. Interestingly, pPKC ϵ phosphorylates HMGA1 which restricted its mobility and therefore unable to activate IR promoter resulting reduced IR expression. In addition, pPKC ϵ also deactivates transcription factor Sp1 which regulates HMGA1 promoter activation. Hence, FAs phosphorylation of pPKC ϵ seriously damaged HMGA1 affecting decreased IR expression [47]. This is a novel dimension of FAs derogatory effect where pPKC ϵ plays a key role in disrupting insulin-IR association that inflicted insulin resistance due to the loss of its sensitivity which is schematically represented in Fig. 2.

ASSOCIATION OF FETUIN-A IN INSULIN RESISTANCE AND T2D

Fetuin-A ($\alpha 2$ Heremans-Schmid glycoprotein) has long been impli-

cated in obesity induced insulin resistance and diabetes. The human *fetuin-A* gene has been mapped on chromosome 3q27 which is identified as T2D susceptible locus [51]. Fetuin-A is synthesized in human liver and secreted into the serum and shown to be linked to insulin resistance and T2D as it inhibits IR kinase phosphorylation that adversely affects insulin downstream signals [52-58]. In fact, consistent elevated levels of circulatory fetuin-A have been observed in high fat fed animals and obese diabetic patients [59,60]. Although fetuin-A appears to be potential in affecting insulin resistance and T2D, number of regulatory processes related to its synthesis, secretion and implementation of insulin resistance function requires a clear understanding. Knockout of *fetuin-A* gene (fetuin-A KO) in high fat diet (HFD) mice showed increased phosphorylation of IR and stimulation of insulin downstream signaling molecules in liver and skeletal muscle in response to insulin. In fetuin-A KO, HFD mice there is an enhanced glucose clearance and improved insulin sensitivity [54]. This implicates that fetuin-A KO mice are resistant to weight gain due to HFD.

Fetuin-A indeed requires a special attention because of its consistent association with insulin resistant and T2D on one hand and our little understanding about its role in the manifestation of this disease on the other. It appears that despite fetuin-A's classical inhibitory effect on insulin resistance by inhibiting IR tyrosine kinase activity it could have some additional target(s). A few recently available reports indicate a coincidence between hyperlipidemia and dyslipidemia and fetuin-A in human being [57,58], this would lead to assume that fetuin-A has a link to lipid. One of such target therefore may be adiposity because human visceral adiposity is associated with incident diabetes in aged persons as a consequence of higher fetuin-A level [57]. In addition, a polymorphism of human *fetuin-A* gene affects adversely insulin action on adiposity and adiponectin production is suppressed due to fetuin-A in animals and humans [61,62]. All these imply that fetuin-A is poised to be an important factor in attenuating insulin activity by adversely affecting adipocyte function. Our work has explained two important aspects of fetuin-A. Although it is known that *fetuin-A* gene is expressed in liver and the protein is synthesized and secreted from there into the circulation in diabetic obese patients [55,60,63], what triggers its elevated synthesis remained unclear until recently. Obviously association of lipid with the excess production of fetuin-A is a clue that lipid is involved, if so, how its expression in liver is controlled by lipid/FA requires to be investigated. We have demonstrated that fetuin-A is overexpressed in *db/db* mice which is an ideal animal model in re-

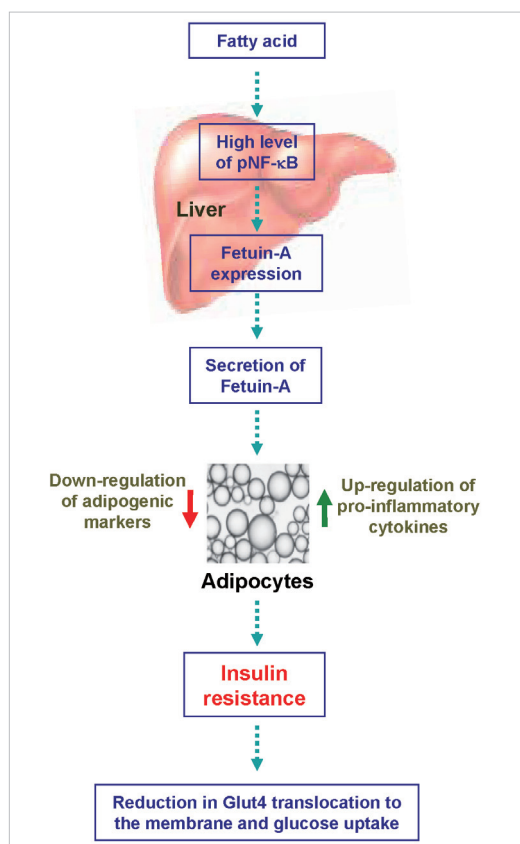


Fig. 3. Fatty acid induced elevated expression of fetuin-A is mediated through NF- κ B. Increase of pNF- κ B level effected enhanced expression of fetuin-A. Since fetuin-A is a secretory protein, it immediately released into the circulation and reaches adipocytes. This causes adipocyte dysfunction as marked by increase of inflammatory cytokines and suppression of adipogenic factors. All together lead to insulin resistance which is evident by decreased glucose uptake along with impaired glucose transporter 4 (Glut4) translocation.

lation to hyperlipidemia and T2D. It was observed that increase in circulatory levels of FA coexisted with higher levels of fetuin-A in this mice pointing out the existing notion to be a possibility. We found that FA could significantly augment fetuin-A release from liver and this effect of FA is not direct but mediated through NF- κ B [63]. NF- κ B is known to play a major role in insulin resistance and lipid induced overexpression of NF- κ B has been earlier demonstrated by us [64]. Lipid induced expression of fetuin-A and subsequent activation of NF- κ B leading to insulin resistance is schematically represented in Fig. 3. We found that FA increased NF- κ B binding to fetuin-A promoter and enhanced fetuin-A expression [63]. These results confirmed earlier reports on the increased level of fetuin-A in diabetic patients and animals [55,57,59]. On studying the detailed mechanism behind fetuin-A regulation it has been observed that there are six NF- κ B binding sites on fetuin-A promoter of which

three are responsible for fetuin-A promoter activation. In both diabetic human adipocyte and 3T3-L1 cell line fetuin-A has been found to abrogate adipogenic function of PPAR γ , adiponectin, FAT/CD36 and aP2. Dysfunction of adipocyte by fetuin-A opened a new opportunity to deal insulin resistance and T2D.

ASSOCIATION OF TOLL-LIKE RECEPTOR (TLR) IN FA INDUCED INSULIN RESISTANCE

TLRs are pattern recognition receptors. TLR was originally identified in *Drosophila* where it is required to establish dorso-ventral pattern in developing embryo [65]. Surprisingly, TLR-mutant flies were found to be highly susceptible to fungal infection [66]. Subsequently, mammalian homologues of TLRs were detected in macrophage and found to be associated with the activation of innate immune system through the recognition of bacterial liposaccharide [67]. When macrophage accumulation in adipose tissue is observed during obese condition [68,69], it becomes a matter to consider seriously whether TLR could be linked to insulin resistance [70]. Among TLRs, TLR4 is found to be associated with obesity induced insulin resistance. TLR4 deficiency protected against insulin resistance implemented by diet containing high amount of saturated FA [71]. This suggests TLR4 participation in lipid induced insulin resistance.

This has been further investigated by using C3H/HeJ mice where loss-of-function mutation of TLR4 appears to prevent diet induced obesity and insulin resistance. It has been observed that high-fat diet induced defects in insulin signaling with increased adiposity while in TLR4 mutated mice, these were significantly reversed suggesting that TLR4 may be acting as a mediator in lipid induced insulin resistance [72-74]. However, the research activity on the TLR4 participation to deteriorate insulin sensitivity due to FA has just been initiated and therefore could not achieve the understandable status. This definitely requires a special attention because TLR4 sufficiently expressed in adipocyte, skeletal muscles and liver, the three major insulin target tissues. The mechanism, by which TLR4 can sense the presence of lipid in cellular ambience and then dictate it towards the loss of insulin sensitivity, is an obscure area where future input would provide a clear and relevant information.

CONCLUSION

Mechanism of lipid induced insulin resistance that leads to T2D requires a better understanding. There are number of shaded areas

and questions that require to be addressed. No doubt, last few years of contributions have sharpened our insight on the impairment of insulin sensitivity; understanding of precise mechanism yet appears to be a long way. Although consensus has reached on at least in one issue, i.e., loss of insulin sensitivity is due to insulin signaling defect, we really know little about how lipid can influence it. In this overview, we have described the current status of this subject. Important signaling molecules responsible for Glut4 transporter activation and migration to the membrane making glucose entry into the skeletal muscle and adipocyte are opposed by FA and position of IR could be most crucial in this connection. Defect in IR is shown to be due to its reduced expression because of its gene suppression by FA that imposes defects on HMGA1. HMGA1 is an important regulator of IR gene expression, it shapes IR promoter in such a way so that recruitment of IR transcription factors, Sp1 and C/EBP β , could be possible. This damaging effect of FA is not direct, it is mediated through kinase independent phosphorylation of PKC ϵ . This implies a far reaching destructing ability as it means that if lipid entry in to the insulin target cells continues or its duration is for a longer spell, a persistent availability of pPKC ϵ will allow it to harm HMGA1 affecting a sustained downregulation of IR. Obvious outcome of this would be deficient insulin sensitivity. Another significant area highlighted in this overview is on fetuin-A. Association of fetuin-A with T2D has been reported by several authors and its gene locus has been identified as T2D susceptible. However, it is only recently fetuin-A's involvement in insulin resistance and T2D has been indicated by a few authors. Doubtlessly, there are some impressive information, but that only emphasize the need for additional research, deficiency in understanding fetuin-A's role still exists prominently. However, it is intriguing to know that fetuin-A, whose gene expression in liver cell is upregulated by FA through NF- κ B, which is released into the circulation and can stimulate inflammatory cytokines in adipocyte and at the same time inhibit lipid uptake by adipocyte, will expectedly play a vital role to produce dyslipidemia. Most likely, when such defects occur, lipid induced damage gets magnified and may lead to the pathogenicity to a critical stage.

There are several other pathways and other regulatory genes and proteins those are associated with insulin resistance. In this overview, with limitation of space along with the requirement for specific focus in the subject we attempted to briefly cover some areas where considerable progress has been made in recent years.

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