FROM CHRONIC OVERNUTRITION TO INSULIN RESISTANCE:
THE ROLE OF FAT-STORING CAPACITY AND INFLAMMATION

L. Lionetti 1, M.P. Mollica 1, A. Lombardi, G. Cavaliere, G. Gifuni, A. Barletta*

Department of Biological Sciences, Section of Physiology, University of Naples "Federico II", Via Mezzocannone 8, 80134 Naples, Italy

Received 14 July 2008; received in revised form 9 October 2008; accepted 31 October 2008

Abstract
Aims: We analyze how the inflammatory state in adipose tissue caused by a condition of chronically positive energy balance can lead to insulin resistance first in adipose tissue, then in all insulin-sensitive tissues.

Data synthesis: Chronic nutrient overload causes an increase in adipose depots that, if adipose tissue expandability is low, are characterized by an increased presence of hypertrophic adipocytes. This adipocyte hypertrophy is a possible stress condition for the endoplasmic reticulum (ER) that would lead to a proinflammatory state in adipose tissue. In this condition, ER stress would activate metabolic pathways that trigger insulin resistance, release of macrophage chemoattractant proteins, and in chronic inflammation, the death of the hypertrophic adipocyte. The infiltrated macrophages in turn release inflammatory proteins causing further recruitment of macrophages to adipose tissue and the release of inflammatory cytokines. Following these events, insulin resistance becomes extended to all adipose tissue. Insulin-resistant adipocytes, characterized by low liposynthetic capacity and high lipolytic capacity, cause increased release of free fatty acids (FFA). FFA released by lipolitic adipocytes may also activate Toll-like receptors 4 and then chemokines and cytokines release amplifying insulin resistance, lipolysis and inflammation in all adipose tissue. Moreover, increased circulating FFA levels, reduced circulating adiponectin levels and leptin resistance lead to decreased lipid oxidation in non-adipose tissues, thereby triggering ectopic accumulation of lipids, lipotoxicity and insulin resistance.

Conclusion: All the conditions that increase circulating fatty acids and cause lipid overloading (obesity, lipoatrophy, lipodystrophy, catabolic states, etc.) induce a lipotoxic state in non-adipose tissues that gives rise to insulin resistance.

© 2008 Elsevier B.V. All rights reserved.

* Corresponding author. Fax: +39 0812535090.
E-mail address: antonio.barletta@unina.it (A. Barletta).
1 These authors contributed equally to the work.

0939-4753/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.numecd.2008.10.010
Fat-storing capacity and insulin resistance

Introduction

Insulin resistance is defined as a decreased response of the peripheral tissues to insulin and occurs in many different contexts. It is well known that increasing adiposity, strongly reduced adiposity, and high catabolic states are all associated with insulin resistance and an increased risk of type II diabetes.

A failure to develop an adequate adipose tissue mass (lipodystrophy) or the absolute lack of adipose tissue mass (lipoatrophy) results in severe insulin resistance and diabetes [1–3]. Also an increase in fat cell size, which is associated with increasing adiposity, results in peripheral insulin resistance and the development of diabetes [4–6]. Increased fat cell size may represent a failure of the adipose tissue mass to expand, and hence to be unable to accommodate an increased energy influx. In fact, a chronically positive energy balance promotes an increased mass of adipose tissue through increases in both cell size (adipocyte hypertrophy) and cell number (adipocyte hyperplasia) [7–9]. It seems likely that a continuous nutritional surplus induces an initial filling of existing fat cells in white adipose tissue (WAT). Only when these fat cells reach a critical cell volume are signals generated that promote the proliferation and recruitment of preadipocytes [10,11]. What is less intuitive is the concept that the expansion of adipose tissue and its capacity to accommodate any excess of nutrient may be genetically prefixed or limited [12]. It is, therefore, not surprising that specific genetic profiles that are able to maintain the storage competence of the adipose tissue fail to cope with excessive demands [12]. The transcriptional control of adipogenesis involves the sequential activation of a transcription factor cascade [8,9]. Unfortunately, it is not yet possible to identify and dissect the factors involved in low adipose tissue expandability. An important role in the inhibition of adipogenesis could be played by the canonical Wnt signaling pathway [13]. However, strategies including increased adipocyte recruitment and facilitating the development of hyperplastic forms of adipose tissue prevent the deleterious effects of obesity-induced lipotoxicity. In fact, these strategies are currently available through the use of thiazolidinediones, activators of the proadipogenic transcription factor peroxisome proliferator-activated receptor γ (PPARγ) [14].

When the adipose tissue expandability becomes a limiting factor and nutrients cannot be stored safely in adipocyte, the mean adipocyte size increases [11,15,16]. If the adipose tissue expandability is high, the adipocyte will have a low mean cell volume and a greater capacity than normal to accommodate an increased energy influx. On the other hand, if the adipose tissue expandability is low, the adipocyte will have a high mean cell volume and a consequently reduced capacity to accommodate an increased energy influx [15]. These observations support the concept of “acquired lipodystrophy” as a link between adiposity and peripheral insulin resistance. Peripheral insulin resistance is thought to be the result of increased circulating levels of free fatty acids (FFA) and lipids [17,18]. High catabolic states (sepsis, trauma burns, etc.) are also characterized by increased circulating levels of FFA and lipids, and consequently make peripheral tissues prone to develop insulin resistance.

Increased levels of FFA and lipids in the blood can be due to factors other than a failure of adipose tissue to accommodate an increased energy influx. These factors include: (i) an increased lipolytic character of adipose tissue, once it has become insulin resistant [19], and (ii) a strongly compromised secretion/function of some of the adipocyte hormones (adiponectin and leptin) involved in fat oxidation in all insulin-sensitive tissues [12,20–22]. In this paper, we describe a possible mechanism by which a condition involving a chronically positive energy balance may lead, if the adipose tissue expandability is low, to insulin resistance first in adipose tissue and then in all insulin-sensitive tissues.

Obesity and inflammation of adipose tissue

In order to clarify the link between adiposity and insulin resistance, Weisberg et al. [4] carried out studies to identify the genes whose expression in adipose tissue correlated with adiposity. To this end, they utilized microarray analysis to profile gene expressions in perigonadal adipose tissue from mice in which adiposity varied due to sex, diet, and obesity-related mutations. This analysis identified 1304 transcripts that were significantly correlated with body mass. Of the 100 genes exhibiting the most highly significant correlations, 30% encoded macrophagic proteins. By immunohistochemical analysis of perigonadal, perirenal, mesenteric and subcutaneous adipose tissue, Weisberg et al. confirmed that the source of this expression was adipose tissue-resident macrophages [4]. This study showed that the percentage of macrophages in a given adipose tissue depot is positively correlated with adiposity and adipocyte size. Similar relationships were found in human subcutaneous adipose tissue [4,23]. They also found that adipose tissue macrophages express proinflammatory factors such as tumor necrosis factor α (TNF-α), inducible nitric oxide synthase (iNOS), and interleukin 6 (IL-6). Moreover, Xu et al. [23] while confirming the up-expression of macrophage or inflammation genes in the WAT of mice with genetic or diet-induced obesity also showed that the adipose inflammatory response increases with adiposity (before any increase in the fasting insulin level) and intensifies with the onset of hyperinsulinemia. In fact, in the above study [23], it was shown that in the WAT of obese mice, the expression of monocyte chemoattractant protein-1 (MCP-1), a proinflammatory chemokine involved in macrophage activation and recruitment, was significantly increased by three weeks feeding on a high-fat diet, without any change in the serum insulin level. On the other hand, at 16 weeks on the high-fat diet, WAT MCP-1 expression displayed a very strong increase, concomitantly with a very strong increase in the serum insulin level, supporting a possible causative role of this chemokine in the observed macrophage infiltration [23].

The source of this increased MCP-1 is not unequivocally established, and in particular it is not clear if the source can change during the dietary treatment. Moreover, the molecular signals triggering macrophage activity in WAT in obese individuals are not yet known. Nevertheless, a relationship between adipose tissue macrophage accumulation
and adipocyte size has been demonstrated in all WAT depots studied [4,23,24]. It is, therefore, possible to hypothesize that an increase in adipocyte size is a necessary condition for adipocytes to synthesize and release certain proteins that play a role of some kind in inflammation and insulin resistance [25]. Although MCP-1 is mainly produced by macrophages and endothelial cells, the MCP-1 gene is also expressed in 3T3-L1 adipocytes and in the adipocytes of obese mice [26–28] and obese humans [29,30]. Moreover, macrophage infiltration is apparent in the adipose tissue of transgenic mice over-expressing MCP-1 mainly in adipocytes [28], while the macrophage accumulation in adipose tissue that is induced by feeding normal mice on a high-fat diet is almost absent in MCP-1 homozygous null mice [31]. Moreover, a deficiency of the chemokine receptor-2 (CCR2; a MCP-1 receptor) reduces both the macrophage content and the inflammatory profile of adipose tissue in null mice fed a high-fat diet [32]. The above results suggest that MCP-1 from adipocytes is important for the recruitment of macrophages to adipose tissue. In addition to MCP-1, it would appear that with increasing adiposity, adipocytes may release other signals such as macrophage migration inhibitory factor (MIF) [33], and m-colony stimulating factor-1 (M-CSF-1) [34] causing an increased influx of macrophages/monocytes and creating a permissive micro-environment for these monocytes to differentiate and survive as mature macrophages within adipose tissue. From the above results, it could be hypothesized that the primary source of chemoattractant proteins, after short-term high-fat diet treatment, is the hypertrophic adipocyte itself [23]. The decisive results by Skurk et al. [35] clearly indicate that adipocyte size plays a really important role in the secretion of proinflammatory adipokine. In fact, the fractionation of the adipocytes according to cell size revealed that the hypertrophic, very large adipocytes show a markedly increased MCP-1 and granulocyte colony-stimulating factor (G-CSF) secretion calculated both per adipocyte and per square micron adipocyte surface [35]. The infiltrated chemoattracted macrophages may in turn secrete a variety of chemokines (MCP-1, etc.) and cytokines (TNF-α, etc.) that promote a further local macrophage accumulation and an inflammatory response, thereby affecting gene expression in adipocytes and resulting in insulin-resistant adipocytes [23]. It can, therefore, be hypothesized that after a longer period on a high-fat diet, the source of chemokines and cytokines, at a time when hyperinsulinemia has developed, could be above all the infiltrated macrophages [23].

Adipocyte stress and inflammation of adipose tissue

Taking all the above results together, it can be hypothesized that an increased adipocyte size is a stress condition for adipocytes [36,37] which triggers the release of macrophage chemoattractant proteins (such as MCP-1 and M-CSF-1) from hypertrophic adipocytes [35], and thereby results in macrophage infiltration. The above hypothesis is supported by the finding that MCP-1 expression is upregulated in 3T3-L1 adipocytes cultured without glucose for 36 h, a stress condition that inhibits protein glycosylation [28].

If the above “stress hypothesis” is correct, then the WAT macrophages present in lean and obese mice and humans should be preferentially localized around hypertrophic adipocytes, because the latter release chemoattractant proteins (MCP-1 and M-CSF-1). In fact, Cinti et al. [24] have demonstrated that the WAT macrophages present in lean and obese mice and humans are indeed localized mainly around individual adipocytes. In fact, more than 90% of all the macrophages in the WAT of obese mice and humans are localized around large dead or dying adipocytes [24]. Significantly, the frequency of adipocyte death is positively correlated with increased adipocyte size in obese mice and humans [24]. Cinti et al., using a model of adipocyte hypertrophy without increased adipose mass (hormone-sensitive lipase K.O. mice), also showed that adipocyte hypertrophy per se, apart from obesity itself, promotes adipocyte death and macrophage aggregation around individual adipocytes [24].

The mechanism by which hypertrophy may promote adipocyte death is not clear, but endoplasmic reticulum (ER)-stress in the adipocyte may be involved [31,36,37]. The ER is a vast network of membranes in which all the secretory and membrane proteins are assembled into their secondary and tertiary structures. The proper folding, maturation, storage and transport of these proteins takes place within this organelle, and unfolded or misfolded proteins are detected, removed from the ER, and degraded by the proteasome system.

An accumulation of unfolded or misfolded proteins could result from an increased demand on the synthetic machinery, inhibition of protein glycosylation, an imbalance of ER calcium levels, or overnutrition. Such an accumulation would give rise to perturbations in the ER lumen, and create stress [38,39]. This would be especially true of adipose tissue which, in conditions of nutrient overload, undergoes severe perturbations that elicit ER stress in adipocytes [36,38,39]. The ER alleviates this stress by initiating a transcriptional program referred to as the unfolded protein response (UPR), which slows protein synthesis and promotes degradation [40]. In addition to a selective inhibition of de novo protein synthesis, the UPR also induces the transcription of chaperones to assist with the unfolded protein load [40]. The UPR has the ultimate aim of cell recovery and survival, but if the ER stress is not relieved the UPR will induce cell death via apoptosis [37,40,41], and so the hypertrophic adipocytes will die [24].

ER stress and insulin resistance of hypertrophic adipocytes and adipose tissue

To date, there are many unexplored areas of adipocyte function with regards to ER stress caused by nutrient overload. Many important questions remain, including the mechanism of ER stress on adipocyte insulin signaling, the role of ER stress in the inflammatory response and the origins of ER stress. ER stress and UPR activation may be induced by the increased demand for protein and lipid synthesis under nutrient excess and adipocyte expansion [36,38,39]. Furthermore, it has been shown that ER stress activates the two principal inflammatory pathways that impair the action of insulin (namely, JNK-AP-1 and...
Ikbbb–NF-kB) promoting phosphorylation of the serine 307 residue in insulin receptor substrate 1 (IRS-1) [36,42–45]. Finally, Özcan et al [38] demonstrated that in both dietary and genetic obesity, UPR is indeed increased in adipose and liver tissues, and leads to activation of both JNK and Ikbbb. They observed elevations of several biochemical indicators of ER stress in the liver and adipose tissues of obese animals (versus their lean counterparts), as well as a significant increase in the JNK-mediated serine 307 phosphorylation of IRS-1 [38].

Activation of the JNK-AP-1 and Ikbbb–NF-kB pathways may lead to a variety of downstream effects depending on the cellular context (such as apoptosis, inflammation, etc.) as well as insulin resistance [45–48]. As for as the origins of ER stress, excess nutrients may serve as signals for inducing stress. Studies have shown that saturated fatty acids (FA) induce ER stress and apoptosis in hepatocytes, pancreatic β cells and macrophages [49–52]. The effect of free fatty acids (FFA) on ER function in the adipocyte are yet to be investigated. Nevertheless it has been shown [53] that FFA can induce JNK activation and subsequent insulin resistance in 3T3-L1 adipocytes by promoting phosphorylation of the serine 307 residue in IRS-1 providing a possible link to ER stress if the UPR is responsible for activating JNK.

In addition to inducing ER stress, it has been shown that saturated fatty acids may also activate Toll-like receptors 4 (TLR 4) and then a complex signaling pathway that stimulates, through Ikbbb NF-kB, releases of chemokines and cytokines [54–56]. Adipocytes express Toll-like receptors [57,58] and such expressions are increased in obese mice and hypertrophic adipocytes [59–61]. The above results suggest that saturated FFA, released in large quantities from hypertrophic adipocytes become lipolytic because of insulin resistance caused by ER stress, could activate TLR4 on

![Figure 1](https://example.com/Figure1.png)

**Figure 1** Proposed mechanism linking adipose tissue inflammation to insulin resistance. Under conditions of chronic nutrient overload, ER stressed and insulin-resistant hypertrophic adipocytes release chemoattractant proteins (MCP-1, M-CSF-1, MIF) causing an increased influx of resident macrophages around hypertrophic adipocytes. The resident macrophages around apoptotic hypertrophic adipocytes release chemokines and cytokines that amplify the inflammatory response through the recruitment of further macrophages to adipose tissue. The activated infiltrated macrophages, in turn, release chemoattractant proteins (MCPs) and cytokines (TNF-α, IL-6, IL-1β, etc.) and induce iNOS, further amplifying the inflammatory response in all adipose tissue. Activation of serine kinases (Ikbbb and JNK) by TNF-α will reduce the IRS-signaling ability of all adipocytes. In addition, the NO produced by iNOS will reduce PI3K/PKB activity by s-nitrosylation of PKB. These events lead to the extension of insulin resistance to all adipose tissue, with a consequent increase in lipolysis and decrease in liposynthesis. FFA released by lipolitic adipocytes may also activate Toll-like receptors 4 and then chemokines and cytokines release amplyfying insulin resistance, lipolysis and inflammation in all adipose tissue. The subsequent increase in the serum levels of FFA, lipids and leptin, together with the decrease in the serum adiponectin level, lead to lipid accumulation, lipotoxicity and insulin resistance in non-adipose tissue.

Fat-storing capacity and insulin resistance 149
adipocytes and macrophage plasma membranes. The TLR4 activation could then induce the inflammatory changes in both adipocytes and macrophages through NF-kB activation amplifying insulin resistance, lipolysis and inflammation in all adipose tissue. The participation of TLR4 in the cross-talk between inflammatory and metabolic signals is supported by finding that mice with a loss-of-function mutation in TLR4 are protected against the development of diet-induced obesity and insulin resistance [62].

Conclusions

On the basis of the evidence summarized in this review, we can describe a process of gradual progression to insulin resistance in obese states (Fig. 1). In this model, nutrient overload causes an increase in adipose depots that, in individuals with low adipose tissue expandability, which is genetically prefixed, is characterized by an increased presence of hypertrophic adipocytes. The increased subcutaneous abdominal adipocyte size is strongly predictive of type II diabetes [5,63]. A less expandable subcutaneous adipose tissue causes an increasing ratio of visceral/subcutaneous abdominal fat [64] and also a percentage increase of hypertrophic adipocytes in the visceral fat [4,24]. Following a caloric restriction, visceral fat is reduced significantly in the early phase of diet therapy more than subcutaneous abdominal fat [65].

We have seen that adipocyte hypertrophy is a possible stress condition of the endoplasmic reticulum (ER) that may activate a proinflammatory state in adipose tissue. Actually, in the hypertrophic adipocyte, ER stress activates JNK-AP1 and Ikbkb—NF-kB pathways which trigger insulin resistance, the release of macrophage chemoattractant proteins (MCP-1, M-CSF-1, MIF), and then, in conditions involving chronic inflammation, leads to hypertrophic adipocyte death. The infiltrated macrophages release inflammatory proteins that cause further recruitment of macrophages into adipose tissue as well as the release of inflammatory cytokines (such as TNF-α, IL-6, IL-1β), together with the induction of iNOS, thereby triggering insulin resistance in all adipose tissues (Fig. 1). The participation of TLR 4 in the cross-talk between inflammatory and metabolic signals amplifies insulin resistance, lipolysis and inflammation in all adipose tissue [59–61]. Insulin-resistant adipocytes, which are characterized by low liposynthetic capacity and high lipolytic capacity, induce, among other changes, increases in circulating FFA and lipids (Fig. 1). FFA induce ER stress and apoptosis in beta cells and then impaired insulin secretion [51,52]. The reduced circulating adiponectin level and the leptin resistance act together to decrease lipid oxidation in non-adipose tissues, and this causes an ectopic accumulation of lipid, a lipotoxic state, and insulin resistance (Fig. 1). However, an ectopic expansion of the lipid stores per se, essentially as triglycerides (TG) may not be harmful to the cells making up the lean body mass. On the contrary, the accumulated intracellular TG may exert a protective role against the insulin resistance induced by FFA [17,18]. Only when the capacity of this buffer has been exceeded, can the harmful effects of fatty acids and their lipid derivatives, such as ceramides [66] and diacylglycerols (DAG) [67], become manifest. Thus, DAG activate IRSs serine kinases such as protein kinases C (PKCs), which elicit phosphorylation of serine 24 in IRSs, thereby first impairing lipid—protein interactions involving the IRSs pleckstrin homology domain and then reducing insulin signaling [67]. Ceramides, too, have been shown to induce insulin resistance by impairing insulin-stimulated protein kinase B (PKB) phosphorylation [68–72]. From the above findings, it appears that DAG and ceramides may play key roles in the lipotoxicity induced by the overloading of various tissues with serum FFA.

In conclusion, all the conditions that increase the circulating FFA and lipid levels (obesity, lipatrophy, lipodystrophy, catabolic states, etc.) and strongly compromise secretion/function of adiponectin and leptin, exacerbate the lipotoxic state in lean tissues and consequently increase their insulin resistance [73–76]. Although much progress has been made in this area, further studies are needed to clarify the underlying initial events that trigger the proinflammatory state in obese WAT.

Acknowledgements

The authors are grateful to the contributors to the studies cited in this review and apologize for the omission of many relevant references due to space limitations.

References


reticulum apoptosis, which may contribute to INS-1 pancreatic beta-cell apoptosis. Endocrinology 2006;147:3398–407.


