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Adipogenesis and lipotoxicity: role of peroxisome proliferator-activated receptor γ (PPARγ) and PPARγcoactivator-1 (PGC1)

Gema Medina-Gomez*, Sarah Gray and Antonio Vidal-Puig
[1]Department of Clinical Biochemistry and Medicine, University of Cambridge, Box 232, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QR, UK

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Abstract

Obesity is characterised by an increase in the adipose deposits, resulting from an imbalance between food intake and energy expenditure. When expansion of the adipose tissue reaches its maximum limit, as in obesity, fat accumulates in non-adipose tissues such as liver, heart, muscle and pancreas, developing a toxic response known as lipotoxicity, a condition that promotes the development of insulin resistance and other metabolic complications. Thus, the lipotoxic state may contribute to the increased risk of insulin resistance, diabetes, fatty liver and cardiovascular complications associated with obesity.

We are interested in studying adipose tissue, specifically how mechanisms of adipogenesis and remodelling of adipose tissue, in terms of size and function of the adipocytes, could be considered a strategy to increase the capacity for lipid storage and prevent lipotoxicity. The peroxisome proliferator-activated receptors (PPARs) are a family of transcription factors that regulate energy balance by promoting either energy deposition or energy dissipation. Under normal physiological conditions, PPARγ is mainly expressed in adipose tissue and regulates diverse functions such as the development of fat cells and their capacity to store lipids. The generation of PPARγ knockout mice, either tissue specific or isoform specific, has provided new models to study PPARγ's role in adipose tissue differentiation and function and have highlighted the essential role of PPARγ in adipogenesis and lipogenesis.

A second strategy to prevent lipotoxicity is to increase the capacity of tissues to oxidise fatty acids. PPARγcoactivator-1α is a coactivator of PPARγ that induces the expression of genes that promote the differentiation of preadipocytes to brown adipocytes. Recently, it has been implicated in increasing the oxidation of fatty acids via increasing mitochondrial capacity and function, making this co-factor a key candidate for the treatment of lipotoxicity.

Keywords
Peroxisome proliferator activated receptors (PPARs)
Obesity
Lipotoxicity
Adipogenesis
PGC1
β-oxidation

Fat mass is determined by a dynamic equilibrium between food intake and energy expenditure. Disruption of this balance leads to obesity, a multifactorial disease that involves an increase in adipose tissue mass. An adipocentric view considers obesity as a major risk factor for the development of insulin resistance, hyperglycaemia (with or without type 2 diabetes), hyperlipidemia and hypertension, collectively referred to as the metabolic syndrome. These inter-related disorders predispose patients to a variety of cardiovascular conditions that lead to high risk of heart attack and stroke.

The clinical observation that not every obese individual develops these problems suggests that is not a direct effect of the absolute amount of fat accumulated. Many obese patients are remarkably metabolically healthy despite massive accumulation of fat, whereas others who are only moderately obese develop the full metabolic syndrome. Therefore, there are indications that adipose tissue expandability may be an important factor determining the metabolic complications associated with obesity[1,2].

It has been suggested that the link between the expansion of adipose tissue and these co-morbidities is insulin resistance, a state characterised by an impaired response to insulin in peripheral tissues. Two non-exclusive mechanisms have been proposed to explain how expansion of adipose tissue affects insulin sensitivity. The first one suggests that excessive accumulation of fat is associated with a chronic state of inflammation characterised by increased cytokine production by adipocytes and/or the macrophages infiltrating adipose tissue. Cytokines produced by these adipocytes or macrophages may directly antagonise

*Corresponding author: Email mgm28@cam.ac.uk

insulin signalling[3,4]. The second mechanism suggests that metabolic changes in adipocytes decrease their capacity to store lipid, facilitating the outflow of lipid into other organs[5–8]. When the amount of fuel entering these organs exceeds the organs oxidative or storage capacity, a toxic response known as lipotoxicity is induced with the formation of metabolites that inhibit insulin action. This phenomenon of ectopic lipid accumulation, and more specifically reactive lipid species, appears to be an important link between insulin resistance, obesity and possibly other features of the metabolic syndrome.

Adipose tissue, an endocrine secretory tissue

Until the discovery of leptin and other hormones in adipocytes, fat tissue was considered a passive storage organ for excess energy[9]. In addition to this function as an energy reservoir, the adipocyte is now considered to be an endocrine cell, secreting many bioactive factors including leptin, tumour necrosis factor-α (TNFα), interleukin-6 (IL-6), adiponectin, resistin and others[10,11].

Leptin is well known for its effects in regulating food intake and energy expenditure. Humans with leptin deficiency or leptin receptor mutations are severely obese[12,13]. Additionally, leptin has been shown to have a direct effect on insulin sensitivity, reverting insulin resistance in mice and patients with congenital lipodystrophy[14].

TNFα is one of the cytokines produced by the adipocyte that may contribute to the development of insulin resistance in obesity. On one hand, TNFα can impair insulin action thus preventing glucose metabolism and increasing lipolysis. And at the molecular level, TNFα increases serine phosphorylation of insulin receptor substrate-1 (IRS-1) and decreases GLUT4 expression levels, therefore contributing to insulin resistance[10].

An adipokine with insulin-sensitising effects is adiponectin. Expression of adiponectin decreases in obesity and its levels correlate with insulin sensitivity[15,16]. Furthermore, adiponectin not only promotes inhibition of hepatic glucose output but also enhances glucose uptake and glucose utilisation in adipose tissue and muscle[16].

It has been shown that resistin decreases insulin-dependent glucose transport *in vitro* and increases fasting blood glucose concentrations and hepatic glucose production *in vivo*[17,18]. Despite the data obtained in cell lines and rodents, the physiological significance of resistin in humans is less clear.

Role of adipose tissue in lipotoxicity and insulin resistance

Insulin resistance is considered to be the main pathophysiological change in type 2 diabetes, a disease that is also characterised by insulin hyposecretion and hyperglycaemia. There is strong evidence that dysfunction of adipose tissue plays a crucial role in the development of insulin resistance and type 2 diabetes. Obesity demonstrates a situation in which there is an increase in fat accumulation, whereas lipodystrophy represents a situation where adipose tissue development is impaired, preventing the accumulation of fat. In both clinical situations, the capacity to retain lipid in adipocytes is impaired, leading to an abnormal accumulation of triglycerides and other lipid species in non-adipose tissues (lipotoxicity), and therefore developing peripheral insulin resistance[19] (Fig. 1).

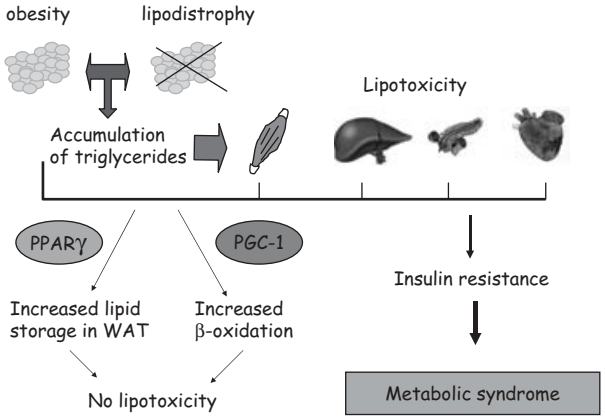

Fig. 1 Adipogenesis and lipotoxicity: role of peroxisome proliferator-activated receptor (PPAR) γ and PPARγcoactivator-1 (PGC1). There is an increase in fat accumulation in obesity; whereas adipose tissue development is impaired in lipodystrophy, preventing the accumulation of fat. In both clinical situations, the capacity to retain lipid in adipocytes is impaired, leading to an abnormal accumulation of triglycerides and other lipid species in non-adipose tissues (lipotoxicity), that contributes to the development of peripheral insulin resistance and metabolic syndrome. Increasing the capacity for lipid storage (PPARγ) and increasing the capacity of tissues to oxidise fatty acids (PGC1) are two possible strategies to prevent lipotoxicity

Although not well established, several mechanisms have been suggested to explain how toxic lipid species are involved in the development of insulin resistance and type 2 diabetes. These involve fatty acid-induced inhibition of glucose entry through inhibition of one or more steps in the insulin-signalling cascade. It has been shown that free fatty acids (FFAs) may impair GLUT4 translocation and/or synthesis in muscle and white adipose tissue[20]. In addition, FFAs may impair steps in insulin-signal transduction, leading to reduced insulin receptor substrate-associated phosphoinositide-3 kinase (PI3 kinase) activity – an important step in triggering the movement of intracellular GLUT4 molecules to the cell surface[21]. Another molecule that may be affected in this process is protein kinase Cθ. There is also evidence that fatty acids decrease glucose conversion into glycogen for storage[22] Accumulation of fatty acids also leads to increased synthesis of ceramides, lipid molecules that impair insulin-stimulated glucose uptake and induce

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apoptosis through activation of nitric oxide synthase. Other mechanisms involve formation of lipid peroxides from cellular oxidative stress[23].

The previously mentioned processes operate simultaneously in a muscle cell; thus, inhibition of several metabolic steps leads to impaired glucose consumption, and thus contributes to hyperglycaemia. Fatty acids may also contribute to the development of hyperglycaemia through effects on the liver, in which impaired glycolysis results in increased hepatic glucose output from gluconeogenesis and glycogenolysis. Fatty acid metabolism also generates adenosine triphosphate and the reduced form of nicotinamide adenine dinucleotide, which favours gluconeogenesis, and thus contributes to the development of hyperglycaemia[24].

The concept of lipotoxicity in the β-cell itself has also been suggested to contribute to the pathology of type 2 diabetes[25]. Accumulation of fatty acids in the β-cell leads to enhanced insulin secretion in acute studies, but when the concentration of fatty acids or the time of exposure to these fatty acids increase, insulin secretion is inhibited.

In our lab, we are interested in adipose tissue, particularly on how peroxisome proliferator-activated receptor γ (PPARγ) mediates mechanisms of adipogenesis and adipose tissue remodelling. Additionally, we are interested in communication between organs during the development of lipotoxicity and the role of specific molecules secreted by the adipocytes in this situation. Finally, we are examining the control of fatty acid oxidation as a mechanism to prevent lipotoxicity by studying the role of PPARγcoactivator-1 (PGC1).

PPARγ, adipogenesis and lipotoxicity

The nuclear receptor PPARγ is critically required for adipogenesis and insulin sensitivity[26–28]. In addition to its effects on preadipocyte differentiation and thus on adipocyte number, activation of PPARγ stimulates storage of fatty acids in mature adipocytes. The finding that the synthetic ligands of PPARγ, the thiazolidinediones (TZDs), act as antidiabetic drugs by improving insulin sensitivity has generated new hope that this receptor will be a key molecular target for the treatment of insulin resistance[29]. Although the precise mechanism of action of TZDs is still not clear, it is likely that they exert their effect on glucose metabolism via adipose tissue and skeletal muscle[30,31]. These pharmacological ligands induce adipocyte differentiation, and thus increase the number of adipocytes expressing GLUT4 glucose transporters and increase lipogenic genes (i.e. CD36 and aP2), reducing circulating FFAs. Also, TZDs are thought to redistribute triglycerides from skeletal muscle and liver to adipose tissue[32].

Studies in mouse models created by tissue-specific genetic engineering have shown the importance of cross talk between tissues in the regulation of energy metabolism. Mice with total and tissue-specific knockout of PPARγ provide tools to dissect tissue-specific roles of PPARγ and demonstrate the importance of inter-tissue communication in the development of the metabolic syndrome. For instance, hypomorphic PPARγ mice[33] and adipose tissue-specific deletion of PPARγ[34] result in congenital and progressive lipodystrophy. This impairment of fat deposition in white adipose tissue (WAT) is associated with lipotoxicity and accumulation of FFAs in non-adipose tissues such as liver, skeletal muscle and pancreas and this is associated with the development of insulin resistance.

Lipotoxic accumulation of lipid in peripheral tissues also occurs under conditions of positive energy balance when adipose tissue is challenged to accommodate excess lipids. This can occur by formation of hypertrophic adipocytes that enlarge to accumulate excess lipid. Large hypertrophic adipocytes are thought to be more insulin resistant, and secrete adipokines that promote the development of insulin resistance[35,36]. Furthermore, these adipocytes are unable to hold on to stored FFAs, resulting in the spillover of lipid that leads to elevate circulating FFAs and lipotoxicity in peripheral tissues that thus facilitate the development of the metabolic syndrome.

However, the expansion of adipose tissue associated with obesity may be based on a hyperplasic response of the adipose tissue rather than just on hypertrophy of the mature adipocytes, thus resulting in an adipose tissue with smaller but more numerous adipocytes. These smaller adipocytes retain insulin sensitivity with the secretion of insulin-sensitising adipokines[37]. This is the case in the mouse model that is heterozygous for PPARγ[29,38,39], which shows improved insulin sensitivity and protection from lipotoxicity despite increased fat mass.

PPARγ is expressed as two isoforms. One of the isoforms, PPARγ1, is expressed in many tissues and cell types, including white and brown adipose tissue, skeletal muscle, liver, pancreatic beta cells, macrophages, colon, bone and placenta[40]. The expression of the other splice variant, PPARγ2, is restricted to white and brown adipose tissue under physiological conditions[40,41]. PPARγ2 is not only the more adipogenic isoform *in vitro* but is also the only PPARγ isoform regulated at the transcriptional level by nutrition [41–44]. Furthermore, PPARγ2 is the isoform that is ectopically induced in the liver and skeletal muscle in response to overnutrition or genetic obesity[1,43]. Ectopic expression of PPARγ2 in the liver and muscle in the obese state suggests that PPARγ2 may have a role in insulin resistance and lipotoxicity in these tissues. Recently, it has been shown that overexpression of PPARγ2 in the liver induces acute hepatic steatosis while markedly decreasing peripheral adiposity, accompanied by increasing energy expenditure and improved systemic insulin sensitivity[45].

Our laboratory[1] and Zhang *et al.*[46] have reported selective disruption of PPARγ2 in mouse. Metabolic evaluation of these models showed that PPARγ2-null mice were insulin resistant; however, Zhang's model presented lipodistrophic changes. Animals with impaired adipose tissue accumulation develop insulin resistance, hence it is unclear whether the insulin resistance observed in Zhang's model is secondary to the lipodystrophy or related to independent effects of PPARγ2 on insulin sensitivity.

We generated a mouse model of selective PPARγ2 deficiency, which develops morphologically normal brown and white adipose tissue under normal nutritional conditions. Despite similar weight, body composition, food intake and energy balance, male PPARγ2 knockout mice were more insulin resistant on normal diet than the wild-type animals.

Despite the normal appearance of WAT *in vivo*, PPARγ target genes involved in adipogenesis are decreased and insulin resistance develops. Therefore, we studied the lipid composition in the WAT of PPARγ2KO mice. PPARγ2KO mice had decreased levels of long-chain triglycerides in WAT, although the total lipid mass was conserved. This effect resulted in increased accumulation of other lipid species such as short-chain triglycerides, diacylglycerols, phospholipids and rare ceramide species.

Under conditions of high-fat diet (HFD), the PPARγ2KO mice accumulate similar amounts of excess fat in more hypertrophied adipocytes compared to wild-type animals. However, the insulin resistance already present in the PPARγ2KO mice on chow diet was not worse on this hypercaloric diet despite marked adipocytes hypertrophy and decreased expression of PPARγ target genes.

Also, we showed that this model had decreased levels of plasma adiponectin on a normal chow diet, but levels were similarly low as in wild-type mice during high-fat feeding. These data suggest that PPARγ2 may be involved in the mechanisms mediating HFD-induced insulin resistance through its effect on the regulation of adiponectin.

It has been shown that PPARγ2 is induced in the liver and muscle under conditions of high-fat feeding[1,43]. In our PPARγ2 knockout model, the absence of PPARγ2 induction in skeletal muscle with high-fat feeding resulted in upregulation of the PPARα/δ target gene expression programme of fatty acid oxidation, which may contribute to prevent lipotoxicity-induced insulin resistance in these animals.

Role of PGC1 in preventing lipotoxicity

As mentioned previously, lipotoxicity can be prevented by increasing fatty acid storage capacity in adipose tissue, and also by increasing the capacity of fatty acid oxidation in peripheral tissues (Fig. 1). PGC1α is a transcription factor that has been shown to participate in pathways controlling glucose homoeostasis and promoting fatty acid oxidation via increasing mitochondrial function and activity[47]. Therefore, PGC1α may be a critical link in the pathogenesis of type 2 diabetes by preventing lipotoxicity.

PGC1α integrates metabolic pathways that support mammalian survival during starvation or hibernation by promoting hepatic gluconeogesis, β-oxidation and increasing overall mitochondrial function, which increases insulin-independent glucose uptake and metabolism in muscle[48–50].

In the muscle, PGC1α not only facilitates glucose entry by activating MEF2C-dependent transcription of GLUT4[51] but also promotes glucose utilisation, coactivating genes involved in oxidative phosphorylation. Furthermore, overexpression of PGC1α induces a fibre-type switch from fast-twitch type II muscle fibres to slow-twitch type I fibres[52], making the muscle of these mice resistant to contraction-induced fatigue and acquiring more oxidative capacities.

In the liver, PGC1α integrates the metabolic adaptation of the rodent liver to fasting by inducing gluconeogenic enzymes[50,53], thereby enhancing glucose output. It has been shown that hepatic PGC1α expression and gluconeogenesis are induced in mouse models of insulin resistance and type 2 diabetes.

Ectopic expression of PGC1α in white adipocytes increases the expression of uncoupling protein 1 (UCP-1), genes encoding respiratory chain proteins (cytochrome *c*-oxidase subunits COX II and IV) and enzymes of fatty acid oxidation and causing white adipocytes to acquire features of brown adipocytes[47,54].

In ob/ob mice, the expression of transcripts encoding mitochondrial proteins decreases with the development of obesity. TZD treatment in ob/ob mice increases PGC1α expression and increases mitochondrial mass and energy expenditure. Also, PGC1α has an insulin-sensitising role in adipose tissue increasing the expression of glycerol kinase by releasing co-repressors.

PGC1α was initially identified as a PPARγ co-factor and has been shown to co-ordinately regulate the programme of mitochondrial biogenesis and adaptive thermogenesis in brown adipose tissue and skeletal muscle.

Two models of PGC1α KO mice have been generated and shown to exhibit cold intolerance. While one model showed an age-related increase in body fat, the other model was lean.

PGC1α expression is also upregulated in β-cells from animal models of type 2 diabetes. Although this upregulation is not well understood, it is known that fatty acids enhance PGC1α expression and impair β-cell function in rat islets.

PGC1β is the closet homologue of PGC1α and is highly expressed in tissues with high mitochondrial content such as skeletal muscle and heart[55,56]. Unlike PGC1α, PGC1β

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expression is unaltered in rat liver by fasting or cold exposure[55]. Mainly, PGC1β has been shown to control hepatic lipid synthesis and lipoprotein production. Ectopic expression of PGC1β in skeletal muscle induces mitochondrial biogenesis and increases mitochondrial oxygen consumption. But recently it has been shown that PGC1β is also involved in the regulation of skeletal muscle fibre transition and metabolism, demonstrating that PGC1β has overlapping and distinct effects from PGC1α[57]. Both upregulate oxidative metabolism, with no apparent effect on glycolytic metabolism, and both confer a switch towards a slow myofibre type. But only PGC1α upregulates the cellular glycogen content.

More work is needed to further elucidate the biological role of PGC1β, and how together with PGC1α, it coordinately regulates metabolic pathways and biological processes in a tissue-specific manner. Understanding the intricate details of PGC1α and PGC1β expression and function will identify new opportunities for the development of novel therapeutics to treat obesity and type 2 diabetes.

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