Ageing, adipose tissue, fatty acids and inflammation

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Abstract

A common feature of ageing is the alteration in tissue distribution and composition, with a shift in fat away from lower body and subcutaneous depots to visceral and ectopic sites. Redistribution of adipose tissue towards an ectopic site can have dramatic effects on metabolic function. In skeletal muscle, increased ectopic adiposity is linked to insulin resistance through lipid mediators such as ceramide or DAG, inhibiting the insulin receptor signalling pathway. Additionally, the risk of developing cardiovascular disease is increased with elevated visceral adipose distribution.

In ageing, adipose tissue becomes dysfunctional, with the pathway of differentiation of preadipocytes to mature adipocytes becoming impaired; this results in dysfunctional adipocytes less able to store fat and subsequent fat redistribution to ectopic sites. Low grade systemic inflammation is commonly observed in ageing, and may drive the adipose tissue dysfunction, as proinflammatory cytokines are capable of inhibiting adipocyte differentiation. Beyond increased ectopic adiposity, the effect of impaired adipose tissue function is an elevation in systemic free fatty acids (FFA), a common feature of many metabolic disorders. Saturated fatty acids can be regarded as the most detrimental of FFA, being capable of inducing insulin resistance and inflammation through lipid mediators such as ceramide, which can increase risk of developing atherosclerosis. Elevated FFA, in particular saturated fatty acids, maybe a driving factor for both the increased insulin resistance, cardiovascular disease risk and inflammation in older adults.

Keywords: Ageing, adipose, ceramide, saturated fatty acids, inflammation, insulin resistance

Introduction

Ageing is associated with many physiological changes which manifest as functional deterioration over time. The most visually apparent of these changes is perhaps the change in body fat distribution. The ageing phenotype is typically associated with a decline in fat-free mass (Sakuma and Yamaguchi 2010), and an altered adipose tissue distribution (Kuk et al. 2005; Kuk et al. 2009) defined by increased central and visceral adiposity. The true state of adiposity in the ageing phenotype is masked somewhat by the fact that fat mass can increase in the absence of total body weight changes (Kuk et al. 2009; Zamboni et al. 2003). In this review, the specific changes in adipose tissue redistribution, the possible causes and the consequences of adipose remodelling for inflammatory responses will be described.

Ageing associated changes in adipose tissue distribution

Much of the age-associated increase in abdominal adiposity is visceral rather than subcutaneous in nature (Fuke et al. 2005; Machann et al. 2005; Zamboni et al. 2003). On the other hand, the lower body subcutaneous stores have a reduced capacity to store fat during ageing, as observed in both reduction of hip circumference and increasing waist to hip ratios (Hughes et al. 2004; Van Pelt et al. 2011). Thus the redistribution of adipose can be summarised as a shift away from lower body subcutaneous to central visceral adipose depots (the reasons behind these changes will be explained later under *Altered adipocyte function in ageing*).

Ageing is also associated with an increase in ectopic adiposity, i.e. storage at sites not normally associated with fat deposition. In skeletal muscles, elevated adipose deposition is observed at both inter- and intra-muscular sites (Miljkovic et al. 2009; Schwenzer et al. 2009). The reported effects of age on hepatic adiposity are mixed; an increase in hepatic adiposity (Frith et al. 2009; Li et al. 2009), no change (Bedogni et al. 2006; Tiikkainen et al. 2002) and negative correlations (Bedogni et al. 2005; Zhou et al. 2007) have been observed with age. The epicardial adipose depot, residing between the myocardium and pericardium surrounding the heart, increases in size (Silaghi et al. 2008; Ueno et al. 2009) and is coupled to an increase in myocardial muscle adiposity (van der Meer et al. 2008) in an age related manner. Beyond organs, ectopic adiposity is also present in the ageing bone marrow, and is often associated with osteoporosis (Liney et al. 2007; Rosen et al. 2009; Rosen and Bouxsein 2006).

Adiposity phenotype, skeletal muscle and insulin resistance in ageing

An increase in fat infiltration into skeletal muscle has implications for the development of metabolic diseases. Ectopic adiposity within skeletal muscles negatively impacts on muscle strength and power, and is commonly observed in individuals with type-2 diabetes and the obese (Hilton et al. 2008). Two studies by Marcus et al demonstrated the negative effect of increased adiposity in lean tissues on muscle strength and physical activity in older adults (Marcus et al. 2010), where gains in intramuscular adiposity reduced improvements in muscle quality following exercise training (Marcus et al. 2013). In addition to ectopic adiposity, skeletal muscle wasting or sarcopenia is a common feature of ageing and negatively impacts on muscle strength, mobility and power (Marcus et al. 2010).

Skeletal muscle is a major site of glucose uptake and insulin action and as a consequence is the primary influence over global insulin sensitivity. The normal role of insulin is to facilitate cellular glucose metabolism through promoting glucose uptake and storage (Figure 1). There is considerable data to suggest linkage of intramuscular adiposity to the development of insulin resistance in both rodents and humans (Cree et al. 2004; Lim et al. 2009; Perseghin et al. 2008), which suggests that in ageing, an increase in skeletal muscle adiposity could contribute to insulin resistance. Precisely how the increase in the adiposity within this site induces insulin resistance is unclear, with many intermediates and complex lipid metabolites thought to be involved.

The accumulation of fat as triglycerides in individuals with type-2 diabetes and obese subjects are associated with changes in FAT/CD36 and altered fatty acid transport which favours lipid storage in myocytes (Bonen et al. 2004). A one week high fat diet study in humans demonstrated an increase in lipid deposition with a

simultaneous decrease in β-oxidation (Schrauwen-Hinderling et al. 2005) and a similar study also revealed an attenuation of the insulin mediated suppression of pyruvate dehydrogenase kinase 4which contribute to lipid deposition (Tsintzas et al. 2007).

Whilst associations and correlations exist between triglycerides and insulin resistance, there is a growing body of evidence to indicate that complex lipids or effector molecules are responsible. Triglycerides may not induce insulin resistance, for example prolonged insulin infusion can raise intramuscular triglycerides without affecting insulin sensitivity in type 2 diabetics (Anderwald et al. 2002). However, in trained athletes elevated muscle triglycerides are commonly observed despite high insulin sensitivity (Dube et al. 2008; van Loon and Goodpaster 2006).

Other lipid mediators such as diacylglycerol, fatty acyl-CoAs and ceramides have all been demonstrated to mediate negative effects on insulin sensitivity (Figure 2). Long chain fatty acyl-CoAs are the metabolically activated form of long chain fatty acids, and can act as signalling molecules. LCFA-CoA activates AMP kinase and the transcription factor FadR (involved in expression of fatty acid synthesis and metabolism genes) in *E.coli;* the latter reported to have a role in pancreatic beta cells (Cronan 1997; Feng and Cronan 2009). Elevations in LCFA-CoA have been observed in the skeletal muscle of insulin-resistant animals (Kim et al. 2001; Laybutt et al. 1999) and humans (Ellis et al. 2000) and are associated with elevated myocellular lipid. Conversely, weight loss in obese individuals improves insulin sensitivity with a decrease in skeletal muscle LCFA-CoA (Houmard et al. 2002).

Postulated mechanisms of LCFA-CoA-mediated insulin resistance include the inhibition of hexokinase activity, which as the first step in glucose utilisation, reduces the effect of insulin (Thompson and Cooney 2000). Activation of protein kinase C isoforms by LCFA-CoA lead to phosphorylation of serine Ser307 on the insulin receptor substrate 1 (IRS-1) preventing tyrosine phosphorylation of the insulin receptor and subsequent binding and activation of PI 3-kinase required for normal insulin signalling (Itani et al. 2002; Tomas et al. 2002; Yaney et al. 2000; Yu et al. 2002).

Whilst there is no direct evidence of LCFA-CoA induced insulin resistance in the elderly, there is perhaps an indirect link supporting a role of this lipid substrate. Impaired mitochondrial oxidation of fatty acids is commonly observed in elderly individuals (Solomon et al. 2008), and therefore may induce an increase in cellular LCFA-CoA levels. Ageing is commonly associated with reductions in metabolic rate (Frisard et al. 2007; Krems et al. 2005; St-Onge and Gallagher 2010), and this perhaps may induce the reduced fatty acid oxidation, possibly inducing the increase in LCFA-CoA.

However, LCFA-CoA are precursors for other lipid metabolites which can, in their own way, induce insulin resistance. The two major culprits are diacylglycerol and ceramide, the former being a metabolite during the formation of triglycerides, and the latter being a sphingolipid.

The disruption of insulin signalling and sensitivity may involve diacylglycerol (DAG), with levels of DAG elevated in skeletal muscle of insulin resistant states. The mechanism underlying the link between DAG and insulin resistance appears to involve PKC isoforms -θ and -ε (Yu et al. 2002). This observation is reinforced by observations in insulin-resistant states where an increase in DAG is common (Qu et al. 1999a; Qu et al. 1999b) and in obese type-2 diabetic individuals where elevated PKC-θ activity is associated with increased DAG mass (Itani et al. 2001; Itani et al. 2002). In ageing, few observations or indeed studies on DAG have been performed, with one study finding plasma elevations of DAG have been recorded, which could reflect elevated tissue DAG (Ishikawa et al. 2014).

Ceramides are a class of neutral lipids requiring saturated LCFA-CoA and serine as precursor molecules for their synthesis. Elevations of this neutral lipid are observed in the obese and diabetics where increased intramyocellular lipid content is seen, an observation often associated with insulin resistance (Adams et al. 2004; Coen et al. 2010; Coen et al. 2013). Conversely exercise training in obese or overweight individuals can improve insulin sensitivity by decreasing DAG and ceramide content (Bruce et al. 2006; Dube et al. 2008). A study of sedentary obese individuals demonstrated an increase in ceramide correlated to insulin resistance

(Amati et al. 2011). One study, however, failed to find a difference in muscle lipid metabolite content between type 2 diabetics, endurance trained athletes, health controls, and those with impaired glucose tolerance, despite a wide variation in insulin sensitivity (Skovbro et al. 2008). Extensive investigation into the mechanism of ceramide-induced insulin resistance indicates a reliance on PKC-ζ through serine phosphorylation of the IRS-1 complex and protein phosphatase 2A, which dephosphorylates the Akt/PKB complex at a separate site preventing downstream effector function (Chavez and Summers 2012).

Age associated elevations in ceramide content are not uncommon. In aged males elevated ceramides in skeletal muscles were postulated to contribute to reduction in function in resistance exercise (Rivas et al. 2012), with further studies in humans supporting an elevation of ceramides with age (Giusto et al. 1992). These findings are reflected in animal studies (Claycombe et al. 2002; Lightle et al. 2000; Ohanian et al. 2014; Perez et al. 2005; Rodriguez-Calvo et al. 2007; Wu et al. 2007; Youm et al. 2012), and in vitro cell culture models of senescence (Venable et al. 2006)

Beyond insulin resistance, accumulation of lipid in skeletal muscle is detrimental to mitochondrial function. This is especially important in insulin-resistant states (Kelley and Mandarino 2000; Petersen et al. 2004) and in older adults (Petersen et al. 2003); reductions in lipid oxidation and hyperglycaemia increase malonyl-CoA accumulation which inhibits carnitine palmitoyl transferase-1, propogating lipid deposition and insulin resistance (Krebs and Roden 2005). Furthermore, intermuscular adipose tissue appears to be a strong and independent risk factor as visceral adipose tissue for cardiovascular disease risk (Yim et al. 2007). Therefore, in an ageing individual the possibility is raised that elevated adiposity within skeletal muscle tissues contributes to both insulin resistance and cardiovascular disease risk.

In an older population of male and female adults (aged 70-79 years old), intramuscular lipid content is associated with the metabolic syndrome (Goodpaster et al. 2005), with weight loss in older women (aged 58-83 years old) reportedly mediating reductions in low density lean tissue mass, adipocytes present in muscle, leading to improvements in insulin resistance (Mazzali et al. 2006). Data concerning the link between muscle adiposity in older adults and adverse metabolic changes in humans is scarce, however, the effect of age per se on muscle in animal models decreased Glut4 mRNA levels (Lin et al. 1991), decreased Glut4 protein (Houmard et al. 1995) and reduced glucose oxidation (Gumbiner et al. 1992).

Effect of ageing of hepatic adiposity

Non-alcoholic fatty liver disease is a disorder defined by fatty infiltration of hepatic tissues with accompanying inflammation, with the range of severity moving from mild infiltration (steatosis) to steatohepatitis and fribrosis, which can ultimately lead to the development of hepatocarcinoma.

There is a very strong correlation between metabolic syndrome and the subsequent development of non-alcoholic fatty liver disease, with a reciprocal relationship of hepatic steatosis being predictive for the development of metabolic syndrome with age (Fan and Farrell 2009; Liu et al. 2010). Conversely, the age-related elevation in hepatic adiposity and insulin resistance can cause liver dysfunction, with associations between fatty liver with reduced insulin sensitivity and insulin resistance (Gaggini et al. 2013; Takamura et al. 2012).

Investigations into the causes or effects of fatty liver in are limited. A study by Flannery et al. indicated that skeletal muscle insulin resistance induces non-alcoholic fatty liver disease in older individuals through promotion of hepatic de novo lipogenesis and hyperlipidaemia (Flannery et al. 2012). In a study of middle aged and older Chinese individuals (adults aged 45 years or above), liver fat content was closely associated to carotid atherosclerosis (Li et al. 2012). Previously, Kagansky et al. described the prevalence of non-alcoholic fatty liver disease in older individuals that was not associated with metabolic syndrome suggesting that NAFLD is a common occurrence in older patients (Kagansky et al. 2004).

Altered metabolic outcome associated with age-related redistribution of adipose depots

The age-related redistribution of adipose away from lower body and subcutaneous depots to central, visceral and ectopic sites in close proximity to the heart can increase risk of developing type-2 diabetes, metabolic syndrome cardiovascular disease risk. Age-related elevations in visceral adiposity were weakly associated to the decrease in insulin sensitivity, with unfavourable changes in lipid profile i.e. increased total cholesterol and LDL in non-obese women which may enhance cardiovascular disease risk (DeNino et al. 2001). Further associations have been described in older individuals between visceral adiposity and metabolic risk factors such as aortic stiffness (Sutton-Tyrrell et al. 2001), myocardial infarction (Hansen et al. 2009; Racette et al. 2006), and chronic heart failure (Nicklas et al. 2006). However, central adiposity did not predict atherosclerotic plaque severity in older adults (Kim et al. 2008).

Whilst the elevated risk of developing metabolic complications such as cardiovascular disease and type- 2 diabetes is strongly associated with visceral adipose tissue, the inverse is true of lower body and subcutaneous adiposity. Lower body adiposity or gynoid adiposity is associated with reduced cardiovascular disease, or metabolic risk factors, in a range of populations including type 2 diabetics (Park et al. 2012), older individuals (Fantin et al. 2013; Van Pelt et al. 2011; Wu et al. 2010), men (Hu et al. 2011; Lee et al. 2012a) and women (Aasen et al. 2009; Heitmann and Frederiksen 2009). Similarly, healthy, overweight, and obese individuals (McLaughlin et al. 2011) show a positive association between thigh fat and insulin sensitivity and on the other hand increased visceral adiposity correlated with reduced lower body and gynoidal adiposity (Lee et al. 2012b), and diabetes (Heshka et al. 2008; Li et al. 2010; Shay et al. 2010).

Whilst the increase in visceral adiposity and decrease in gynoidal adiposity associated with ageing may elevate risk of developing metabolic complications, other factors associated with ageing come into play such as genetics, and other age related diseases which could explain why we do not see a higher level of type-2 diabetes mellitus compared to younger obese individuals

Altered adipocyte function in ageing

Beyond these key differences in fat distribution, adipocyte function and the processes which govern their maturation from stem cells appear to be altered during of ageing. Adipogenesis is reported to be accelerated in a murine model of ageing using the senescence accelerated mice-P6 (SAMP6) (Kajkenova et al. 1997), and a similar finding was observed also in human subjects biopsies(Justesen et al. 2001). Increased adipogenesis has been linked to age-related increases in oxidative stress, low levels of vitamin D and loss of estrogen during menopause (Bethel et al. 2013). However, a decline in adipose depot size is observed which is due to a reduction in cell size and impaired differentiation from preadipocytes to mature adipocytes (Kirkland et al. 2002).

Therefore, there is also an age-related increase in the extent of lipotoxicity due to the reduced capacity of adipose depots to store free fatty acids (FFA) (Slawik and Vidal-Puig 2006). The underlying cause of this age associated elevation of FFA can be explained by altered maturation process of adipocytes. Replication and maturation into adipocytes depends on the activity of CCAAT/enhancer binding protein α (C/EBPα) and peroxisome proliferator activated-receptor γ (PPAR-γ) (Wu et al. 1999; Zuo et al. 2006) which are diminished in ageing (Cartwright et al. 2007; Karagiannides et al. 2001; Karagiannides et al. 2006). The process of differentiation and maturation of preadipocyte into an adipocyte depends on the activity of CCAAT/enhancer binding protein α (C/EBPα) and peroxisome proliferator-activated receptor γ (PPAR-γ) (Zuo et al. 2006). The process is initiated with the transient expression of C/EBP isoforms β and δ. Simultaneously there is an alleviation of adipogenic suppression by proteins such as C/EBP-β-LIP and CHOP. In ageing rat preadipocytes, C/EBP-α, C/EBP-δ and PPAR-γ expression is reduced compared to young rats with increases in the suppressors C/EBP-β-LIP and CHOP (Karagiannides et al. 2001; Karagiannides et al. 2006; Tchkonia et al. 2001).

Moreover, age is negatively correlated to subcutaneous stromal preadipocyte cell differentiation (van Harmelen et al. 2004) possibly due to a loss in nucleotide excision repair enzymes Cockayne syndrome A and B, enhanced mutation rate and subsequent cell loss by apoptosis diminishing depot size (Berneburg 2010). The changes in maturation and differentiation of both the visceral and subcutaneous adipose depots, i.e. the reduced capacity to store lipids, leads to an elevation in systemic FFA with age.

Inflammation and age-related adipose tissue dysfunction

The mechanisms underlying the dysfunction of adipose tissue in ageing are unclear, however, inflammation is thought to play a major role. Ageing is associated with a chronic systemic low grade elevation in inflammation, with the cytokine milieu favouring the proinflammatory TNF-α and IL-6 rather than the anti-inflammatory e.g. IL-10 (Bartlett et al. 2012; Franceschi et al. 2000; Lio et al. 2002).

The low grade increase in inflammation is also observed in adipose tissue, with increased levels of TNF-α and IL-6 recorded in rodent models of ageing (Morin et al. 1998; Morin et al. 1997; Starr et al. 2009). Under the culture conditions preadipocytes were deemed to be the source of TNF-α (Kern et al. 1995). In fat cells this cytokine appears to decrease cell size, increase lipolysis and interfere with insulin responsiveness (Hube and Hauner 1999), with the additional effect of impeding differentiation of preadipocytes.

TNF-α mediated interference with differentiation is achieved through multiple mechanisms including the inhibition of C/EBPα and PPARγ expression and activity (Stephens and Pekala 1992; Zhang et al. 1996). Inhibitors of adipogenesis, CHOP, CUGBP and C/EBP-β-LIP are induced by TNF-α serving to reinforce the inhibition of adipogenesis in preadipocytes observed in ageing (Tchkonia et al. 2007).

Effects of elevated systemic FFA

A consequence of impaired adipogenesis is a reduction in storage of fat within adipose tissue depots, consequently there is an increase in FFA flux which leads to ectopic fat storage. Storage of fat within these sites leads to metabolic disturbances (Bays et al. 2008), including insulin resistance and elevated risk of developing atherosclerosis.

A common observation in ageing and metabolic disorders is an elevation in circulating FFA (Pilz et al. 2006; Samuel et al. 2010) which has been related to an increase release of free fatty acids from adipose tissue (Bjorntorp 1990). Of the fatty acids released, perhaps the most detrimental are the saturated fatty acids which include palmitate and stearate, contrasted by the unsaturated fatty acids e.g. oleate, which appear protective.

The key differences in effects between the saturated and unsaturated fatty acids can be explained by the differing rates of metabolism and storage. Studies utilising radiolabelled oleate and palmitate in cultured muscle cells demonstrated a preference for saturated fatty acids to be stored in monoacylglycerol (Bastie et al. 2004), diacylglycerol (Chavez and Summers 2003; Montell et al. 2001) and ceramides (Pickersgill et al. 2007), but the monounsaturated fatty acid is preferentially stored in triglyceride. DAG and ceramide are important mediators of saturated fatty acid mediated insulin resistance, the latter a common feature of ageing (Defronzo 1981), and may be responsible for metabolic disturbance within older adults.

Beyond the induction of insulin resistance by saturated fatty acid derived metabolites, this class of fatty acids are also capable of inducing lipotoxicity (Guo et al. 2007b; Takahashi et al. 2008). With ageing, preadipocytes show increased susceptibility toward lipotoxicity (Guo et al. 2007a), and with the increase in systemic FFA the levels of saturated fatty acids are similarly elevated, consequently a vicious circle of adipose tissue and preadipocyte dysfunction ensues. Saturated fatty acids are capable of inducing the production of proinflammatory cytokines from adipocytes (Permana et al. 2006; Suganami et al. 2005; Suganami et al. 2007). Coupled to their lipotoxic effect on preadipocytes, this suggests that saturated fatty acids are extremely deleterious to adipose tissue function.

In obesity, adipose tissue is characterised by infiltration of monocytes and macrophages (Cinti et al. 2005; Weisberg et al. 2003), and the lipid content of macrophages amongst adipose tissue appears to correlate strongly with the extent of obesity and with ageing (Harman-Boehm et al. 2007)(Jerschow et al. 2007), which can be related to preadipocyte derived inflammation and apoptotic signals.

In ageing, dysfunctional preadipocytes are capable of releasing proinflammatory cytokines which have two effects; (i) to inhibit adipogenesis by inhibiting the necessary transcription factors (PPARγ and C/EBPα) and increasing adipogenic inhibitors CHOP, LIP and CUGBP, and (ii) to recruit and activate macrophages. The

combination of preadipocyte dysfunction and apoptosis leads to the release of further FFA establishing a vicious cycle of lipotoxicity.

Elevated FFA on macrophages and monocytes

Increased systemic FFA, in particular the saturated fatty acids, can have a detrimental effect on immune cell behaviour, especially monocytes the circulating precursors of infiltrating macrophages. A high saturated fat diet increased the binding of monocytes to endothelial cells in part by an increase in the expression of adhesion molecules (Mata et al. 1996), and the monocyte cell surface integrin CD11b (Zhang et al. 2006) which has been shown by our research group to rely on the formation of ceramides (Gao et al. 2012). Elevated FFA, namely the saturated fatty acids can increase expression of urokinase type plasminogen activator receptor, which is involved in migration (Assmann et al. 2008), increase CD36 expression (Gao et al. 2012) and induce insulin resistance (Gao et al. 2009). These effects may contribute to increased risk of developing atherosclerosis, a metabolic disease whose risk increases with age.

The effects of saturated fatty acids are not limited to cell surface phenotype changes, with the induction or promotion of inflammatory cytokine production a feature of the effects of saturated fatty acids. In murine monocytes saturated fatty acids induce the expression of the proinflammatory COX-2 enzyme (Lee et al. 2001), but in human monocytes palmitate can enhance LPS mediated proinflammatory cytokine expression (Schwartz et al. 2010), can activate the inflammatory NF-κB pathway (Huang et al. 2012) and proinflammatory cytokine production e.g. by palmitate in THP-1 monocytes (Little et al. 2012). The induction of inflammation and promotion of proatherogenic cell surface antigen expression may increase risk of atherosclerosis.

Concluding hypothesis

The dysregulation in adipose tissue function during ageing leads to an elevation in both inflammation and FFA which act in a vicious circle to propagate adipocyte dysfunction. The subsequent release of fatty acids and increased production of proinflammatory cytokines (Figure 3) increases circulating FFA. The elevated systemic FFA may underlie age-related inflammation, increase in cardiovascular disease risk and insulin resistance associated with ageing. This elevated risk maybe achieved through an increase in the circulating pool of saturated fatty acids leading to insulin resistance through the formation of lipid mediators such as ceramide and DAG, and through induction of inflammatory responses in monocytes and macrophages.

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Legends

Figure 1.Insulin signalling pathway: The insulin receptor is composed of two extracellular α and two transmembrane β subunits, and on binding of insulin induces a conformational change resulting in the tyrosine autophosphorylation present on the β subunit. The phosphorylated tyrosine residues are detected phosphotyrosine binding domains of adaptor proteins, namely the insulin receptor substrate (IRS). Tyosine residues present on this substrate are phosphorylated, which can be recognised by Src homology 2 (SH2) domain of the p85 regulatory subunit of PI 3-kinase (PI3K). The catalytic subunit p110 phosphorylates phosphatidylinositol (4, 5) bisphosphate (PtdIns (4, 5) P_2) producing Ptd(3, 4, 5)P_3, which leads to the activation of AKT via PDK by phosphorylation. AKT induces the phosphorylation and inactivation of glycogen synthase kinase 3, and enzyme which inhibits glycogen synthase, an enzyme those catalyses the final step in glycogen synthase, consequently with the inhibition removed glucose can be stored as glycogen. Furthermore, insulin prevents gluconeogenesis and lipolysis, favours the synthesis of fatty acids from carbohydrate sources (malonyl-CoA), via the activation of steroid regulatory element binding protein 1c (SREBP-1c), and promotes protein synthesis.

Figure 2.Inhibition of insulin resistance: A consequence of increased ectopic adiposity is an increase in systemic free fatty acids (FFA), and following the saturation of mitochondrial oxidation and triglyceride pathways, fatty acids are converted to diacylglycerol (DAG), an intermediate in triglyceride formation and saturated fatty acids are also converted to ceramide, a precursor in the production of sphingolipids required in

cellular membranes. DAG is capable of inducing the activation of PKCθ which can inhibit PI3K. Ceramides can activate both protein phosphatase 2A (PP2A, which dephosphorylates AKT) and PKCζ which can inhibit insulin signalling through serine phosphorylation.

Figure 3: Schematic representation of the age-related dysfunction of adipose tissue and the resultant systemic metabolic effects. Inflammageing mediates an increase in pro-inflammatory cytokines (TNF-α) capable of suppressing adipocyte differentiation. Poorly differentiated adipocytes less capable of storing fat mediate an increase in systemic FFA flux. The elevation in FFA, with an increase in saturated fatty acids, can modulate metabolic disease risk through promoting atherosclerotic changes in monocytes and macrophages and induce insulin resistance through the formation of complex lipids such as ceramides and DAG.

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