

**Ageing, adipose tissue, fatty acids and inflammation**

Chathan Pararasa, Clifford J Bailey, and Helen R Griffiths.

School of Life and Health Science and Aston Research Centre for Healthy Ageing

Aston University, Aston Triangle, Birmingham, B4 7ET, UK

Correspondence: Professor Helen Griffiths, email: [h.r.griffiths@aston.ac.uk](mailto:h.r.griffiths@aston.ac.uk)

Tel: +44 (0) 121 204 3950

Fax: +44(0) 121 359 0357

## **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  $\beta$ -oxidation (Schrauwen-Hinderling et al. 2005) and a similar study also revealed an attenuation of the insulin mediated suppression of pyruvate dehydrogenase kinase 4 which 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  $\theta$  and  $\epsilon$  (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- $\theta$  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- $\zeta$  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 fibrosis, 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  $\alpha$  (C/EBP $\alpha$ ) and peroxisome proliferator activated-receptor  $\gamma$  (PPAR- $\gamma$ ) (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  $\alpha$  (C/EBP $\alpha$ ) and peroxisome proliferator-activated receptor  $\gamma$  (PPAR- $\gamma$ ) (Zuo et al. 2006). The process is initiated with the transient expression of C/EBP isoforms  $\beta$  and  $\delta$ . Simultaneously there is an alleviation of adipogenic suppression by proteins such as C/EBP- $\beta$ -LIP and CHOP. In ageing rat preadipocytes, C/EBP- $\alpha$ , C/EBP- $\delta$  and PPAR- $\gamma$  expression is reduced compared to young rats with increases in the suppressors C/EBP- $\beta$ -LIP and CHOP (Karagiannides et al. 2001; Karagiannides et al. 2006; Tchkonina 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- $\alpha$  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- $\alpha$  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- $\alpha$  (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- $\alpha$  mediated interference with differentiation is achieved through multiple mechanisms including the inhibition of C/EBP $\alpha$  and PPAR $\gamma$  expression and activity (Stephens and Pekala 1992; Zhang et al. 1996). Inhibitors of adipogenesis, CHOP, CUGBP and C/EBP- $\beta$ -LIP are induced by TNF- $\alpha$  serving to reinforce the inhibition of adipogenesis in preadipocytes observed in ageing (Tchkonina 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 (PPAR $\gamma$  and C/EBP $\alpha$ ) 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- $\kappa$ B 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.

#### *Acknowledgements*

We would like to acknowledge the BBSRC Targeted Priority Studentship in Ageing scheme which funded CP.

#### **Legends**

**Figure 1.** Insulin signalling pathway: The insulin receptor is composed of two extracellular  $\alpha$  and two transmembrane  $\beta$  subunits, and on binding of insulin induces a conformational change resulting in the tyrosine autophosphorylation present on the  $\beta$  subunit. The phosphorylated tyrosine residues are detected phosphotyrosine binding domains of adaptor proteins, namely the insulin receptor substrate (IRS). Tyrosine 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<sub>2</sub>) producing Ptd(3, 4, 5)P<sub>3</sub>, 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 PKC $\theta$  which can inhibit PI3K. Ceramides can activate both protein phosphatase 2A (PP2A, which dephosphorylates AKT) and PKC $\zeta$  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. Inflammation mediates an increase in pro-inflammatory cytokines (TNF- $\alpha$ ) 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.

## References

- Aasen G, Fagertun H, Tonstad S, Halse J (2009) Leg fat mass as measured by dual X-ray absorptiometry (DXA) impacts insulin resistance differently in obese women versus men *Scandinavian Journal of Clinical & Laboratory Investigation* 69:181-189 doi:10.1080/00365510802464641
- Adams JM, Pratipanawatr T, Berria R, Wang E, DeFronzo RA, Sullards MC, Mandarino LJ (2004) Ceramide content is increased in skeletal muscle from obese insulin-resistant humans *Diabetes* 53:25-31
- Amati F et al. (2011) Skeletal Muscle Triglycerides, Diacylglycerols, and Ceramides in Insulin Resistance Another Paradox in Endurance-Trained Athletes? *Diabetes* 60:2588-2597 doi:10.2337/db10-1221
- Anderwald C et al. (2002) Effects of insulin treatment in type 2 diabetic patients on intracellular lipid content in liver and skeletal muscle *Diabetes* 51:3025-3032
- Assmann A, Mohlig M, Osterhoff M, Pfeiffer AFH, Spranger J (2008) Fatty acids differentially modify the expression of urokinase type plasminogen activator receptor in monocytes *Biochemical and Biophysical Research Communications* 376:196-199 doi:10.1016/j.bbrc.2008.08.115
- Bartlett DB et al. (2012) The age-related increase in low-grade systemic inflammation (Inflammaging) is not driven by cytomegalovirus infection *Aging Cell* 11:912-915 doi:10.1111/j.1474-9726.2012.00849.x
- Bastie CC, Hajri T, Drover VA, Grimaldi PA, Abumrad NA (2004) CD36 in myocytes channels fatty acids to a lipase-accessible triglyceride pool that is related to cell lipid and insulin responsiveness *Diabetes* 53:2209-2216

- Bays HE et al. (2008) Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity Expert review of cardiovascular therapy 6:343-368 doi:10.1586/14779072.6.3.343
- Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C (2006) The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population BMC Gastroenterology 6 doi:33  
10.1186/1471-230x-6-33
- Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S (2005) Prevalence of and risk factors for nonalcoholic fatty liver disease: The Dionysos Nutrition and Liver Study Hepatology 42:44-52
- Berneburg M (2010) Research in practice: More than skin deep -aging of subcutaneous fat tissue Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology : JDDG 8:776-778 doi:10.1111/j.1610-0387.2010.07480.x
- Bethel M, Chitteti BR, Srour EF, Kacena MA (2013) The changing balance between osteoblastogenesis and adipogenesis in aging and its impact on hematopoiesis Current osteoporosis reports 11:99-106 doi:10.1007/s11914-013-0135-6
- Bjorntorp P (1990) PORTAL ADIPOSE-TISSUE AS A GENERATOR OF RISK-FACTORS FOR CARDIOVASCULAR-DISEASE AND DIABETES Arteriosclerosis 10:493-496
- Bonen A et al. (2004) Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36 FASEB Journal 18:1144-+
- Bruce CR, Thrush AB, Mertz VA, Bezaire V, Chabowski A, Heigenhauser GJF, Dyck DJ (2006) Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content American Journal of Physiology-Endocrinology and Metabolism 291:E99-E107 doi:10.1152/ajpendo.00587.2005
- Cartwright MJ, Tchkonja T, Kirkland JL (2007) Aging in adipocytes: Potential impact of inherent, depot-specific mechanisms Experimental Gerontology 42:463-471
- Chavez JA, Summers SA (2003) Characterizing the effects of saturated fatty acids on insulin signaling and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes Arch Biochem Biophys 419:101-109 doi:10.1016/j.abb.2003.08.020
- Chavez JA, Summers SA (2012) A Ceramide-Centric View of Insulin Resistance Cell Metabolism 15:585-594 doi:10.1016/j.cmet.2012.04.002
- Cinti S et al. (2005) Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans Journal of Lipid Research 46:2347-2355 doi:10.1194/jlr.M500294-JLR200
- Claycombe KJ, Wu DY, Nikolova-Karakashian M, Palmer H, Beharka A, Paulson KE, Meydani SN (2002) Ceramide mediates age-associated increase in macrophage cyclooxygenase-2 expression Journal of Biological Chemistry 277:30784-30791 doi:10.1074/jbc.M204463200
- Coen PM, Dube JJ, Amati F, Stefanovic-Racic M, Ferrell RE, Toledo FGS, Goodpaster BH (2010) Insulin Resistance Is Associated With Higher Intramyocellular Triglycerides in Type I but Not Type II Myocytes Concomitant With Higher Ceramide Content Diabetes 59:80-88 doi:10.2337/db09-0988
- Coen PM et al. (2013) Reduced skeletal muscle oxidative capacity and elevated ceramide but not diacylglycerol content in severe obesity Obesity (Silver Spring, Md) doi:10.1002/oby.20381
- Cree MG et al. (2004) Intramuscular and liver triglycerides are increased in the elderly Journal of Clinical Endocrinology & Metabolism 89:3864-3871 doi:10.1210/jc.2003-031986
- Cronan JE (1997) In vivo evidence that acyl coenzyme A regulates DNA binding by the Escherichia coli FadR global transcription factor Journal of Bacteriology 179:1819-1823
- DeFronzo RA (1981) GLUCOSE-INTOLERANCE AND AGING Diabetes Care 4:493-501 doi:10.2337/diacare.4.4.493

- DeNino WF, Tchernof A, Dionne IJ, Toth MJ, Ades PA, Sites CK, Poehlman ET (2001) Contribution of abdominal adiposity to age-related differences in insulin sensitivity and plasma lipids in healthy nonobese women *Diabetes Care* 24:925-932 doi:10.2337/diacare.24.5.925
- Dube JJ, Amati F, Stefanovic-Racic M, Toledo FGS, Sauers SE, Goodpaster BH (2008) Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete's paradox revisited *American Journal of Physiology-Endocrinology and Metabolism* 294:E882-E888 doi:10.1152/ajpendo.00769.2007
- Ellis BA, Poynten A, Lowy AJ, Furler SM, Chisholm DJ, Kraegen EW, Cooney GJ (2000) Long-chain acyl-CoA esters as indicators of lipid metabolism and insulin sensitivity in rat and human muscle *American Journal of Physiology-Endocrinology and Metabolism* 279:E554-E560
- Fan J-G, Farrell GC (2009) Epidemiology of non-alcoholic fatty liver disease in China *Journal of Hepatology* 50:204-210 doi:10.1016/j.jhep.2008.10.010
- Fantin F et al. (2013) Central and peripheral fat and subclinical vascular damage in older women *Age and Ageing* 42:359-365 doi:10.1093/ageing/aft005
- Feng Y, Cronan JE (2009) A New Member of the Escherichia coli fad Regulon: Transcriptional Regulation of fadM (ybaW) *Journal of Bacteriology* 191:6320-6328 doi:10.1128/jb.00835-09
- Flannery C, Dufour S, Rabol R, Shulman GI, Petersen KF (2012) Skeletal Muscle Insulin Resistance Promotes Increased Hepatic De Novo Lipogenesis, Hyperlipidemia, and Hepatic Steatosis in the Elderly *Diabetes* 61:2711-2717 doi:10.2337/db12-0206
- Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G (2000) Inflamm-aging - An evolutionary perspective on immunosenescence *Molecular and Cellular Gerontology* 908:244-254
- Frisard MI et al. (2007) Aging, resting metabolic rate, and oxidative damage; Results from the Louisiana healthy aging study *Journals of Gerontology Series a-Biological Sciences and Medical Sciences* 62:752-759
- Frith J, Day CP, Henderson E, Burt AD, Newton JL (2009) Non-Alcoholic Fatty Liver Disease in Older People *Gerontology* 55:607-613 doi:10.1159/000235677
- Fuke Y, Okabe S, Kajiwara N, Suastika K, Budhiarta AAG, Maehata S, Taniguchi H (2007) Increase of visceral fat area in Indonesians and Japanese with normal BMI. In: 13th Korea-Japan Symposium on Diabetes Mellitus, Seoul, SOUTH KOREA, Nov 11-12
- Sep 2005. pp S224-S227. doi:10.1016/j.diabres.2007.01.062
- Gaggini M, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E, Gastaldelli A (2013) Non-Alcoholic Fatty Liver Disease (NAFLD) and Its Connection with Insulin Resistance, Dyslipidemia, Atherosclerosis and Coronary Heart Disease *Nutrients* 5:1544-1560 doi:10.3390/nu5051544
- Gao D, Bailey CJ, Griffiths HR (2009) Metabolic memory effect of the saturated fatty acid, palmitate, in monocytes *Biochemical and Biophysical Research Communications* 388:278-282 doi:10.1016/j.bbrc.2009.07.160
- Gao D, Pararasa C, Dunston CR, Bailey CJ, Griffiths HR (2012) Palmitate promotes monocyte atherogenicity via de novo ceramide synthesis *Free Radic Biol Med* 53:796-806 doi:10.1016/j.freeradbiomed.2012.05.026
- Giusto NM, Roque ME, DeBoschero MGI (1992) EFFECTS OF AGING ON THE CONTENT, COMPOSITION AND SYNTHESIS OF SPHINGOMYELIN IN THE CENTRAL-NERVOUS-SYSTEM *Lipids* 27:835-839 doi:10.1007/bf02535859
- Goodpaster BH et al. (2005) Obesity, regional body fat distribution, and the metabolic syndrome in older men and women *Archives of Internal Medicine* 165:777-783
- Gumbiner B, Thorburn AW, Ditzler TM, Bulacan F, Henry RR (1992) ROLE OF IMPAIRED INTRACELLULAR GLUCOSE-METABOLISM IN THE INSULIN RESISTANCE OF AGING *Metabolism-Clinical and Experimental* 41:1115-1121
- Guo W et al. (2007a) Aging results in paradoxical susceptibility of fat cell progenitors to lipotoxicity *American Journal of Physiology-Endocrinology and Metabolism* 292:E1041-E1051 doi:10.1152/ajpendo.00557.2006

- Guo W, Wong S, Xie W, Lei T, Luo Z (2007b) Palmitate modulates intracellular signaling, induces endoplasmic reticulum stress, and causes apoptosis in mouse 3T3-L1 and rat primary preadipocytes *American Journal of Physiology-Endocrinology and Metabolism* 293:E576-E586 doi:10.1152/ajpendo.00523.2006
- Hansen T, Ahlstrom H, Soderberg S, Hulthe J, Wikstrom J, Lind L, Johansson L (2009) Visceral adipose tissue, adiponectin levels and insulin resistance are related to atherosclerosis as assessed by whole-body magnetic resonance angiography in an elderly population *Atherosclerosis* 205:163-167 doi:10.1016/j.atherosclerosis.2008.11.007
- Harman-Boehm I et al. (2007) Macrophage infiltration into omental versus subcutaneous fat across different populations: Effect of regional adiposity and the comorbidities of obesity *J Clin Endocrinol Metab* 92:2240-2247 doi:10.1210/jc.2006-1811
- Heitmann BL, Frederiksen P (2009) Thigh circumference and risk of heart disease and premature death: prospective cohort study *British Medical Journal* 339 doi:b3292  
10.1136/bmj.b3292
- Heshka S et al. (2008) Altered body composition in type 2 diabetes mellitus *International Journal of Obesity* 32:780-787 doi:10.1038/sj.ijo.0803802
- Hilton TN, Tuttle LJ, Bohnert KL, Mueller MJ, Sinacore DR (2008) Excessive Adipose Tissue Infiltration in Skeletal Muscle in Individuals With Obesity, Diabetes Mellitus, and Peripheral Neuropathy: Association With Performance and Function *Physical Therapy* 88:1336-1344 doi:10.2522/ptj.20080079
- Houmard JA, Tanner CJ, Yu CL, Cunningham PG, Pories WJ, MacDonald KG, Shulman GI (2002) Effect of weight loss on insulin sensitivity and intramuscular long-chain fatty Acyl-CoAs in morbidly obese subjects *Diabetes* 51:2959-2963
- Houmard JA et al. (1995) SKELETAL-MUSCLE GLUT4 PROTEIN-CONCENTRATION AND AGING IN HUMANS *Diabetes* 44:555-560
- Hu G et al. (2011) Trunk Versus Extremity Adiposity and Cardiometabolic Risk Factors in White and African American Adults *Diabetes Care* 34:1415-1418 doi:10.2337/dc10-2019
- Huang S et al. (2012) Saturated fatty acids activate TLR-mediated proinflammatory signaling pathways. In: *J Lipid Res*, vol 53. vol 9. United States, pp 2002-2013. doi:10.1194/jlr.D029546
- Hube F, Hauner H (1999) The role of TNF-alpha in human adipose tissue: Prevention of weight gain at the expense of insulin resistance? *Hormone and Metabolic Research* 31:626-631
- Hughes VA, Roubenoff R, Wood M, Frontera WR, Evans WJ, Singh MAF (2004) Anthropometric assessment of 10-y changes in body composition in the elderly *American Journal of Clinical Nutrition* 80:475-482
- Ishikawa M et al. (2014) Plasma and Serum Lipidomics of Healthy White Adults Shows Characteristic Profiles by Subjects' Gender and Age *Plos One* 9 doi:10.1371/journal.pone.0091806
- Itani SI, Pories WJ, MacDonald KG, Dohm GL (2001) Increased protein kinase C theta in skeletal muscle of diabetic patients *Metabolism-Clinical and Experimental* 50:553-557
- Itani SI, Ruderman NB, Schmieder F, Boden G (2002) Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and I kappa B-alpha *Diabetes* 51:2005-2011
- Jerschow E, Anwar S, Barzilai N, Rosenstreich D (2007) Macrophages accumulation in visceral and subcutaneous adipose tissue correlates with age *Journal of Allergy and Clinical Immunology* 119:S179-S179 doi:10.1016/j.jaci.2006.12.066
- Justesen J, Stenderup K, Ebbesen EN, Mosekilde L, Steiniche T, Kassem M (2001) Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis *Biogerontology* 2:165-171
- Kagansky N et al. (2004) Non-alcoholic fatty liver disease - a common and benign finding in octogenarian patients *Liver International* 24:588-594 doi:10.1111/j.1478-3231.2004.0969.x

- Kajkenova O et al. (1997) Increased adipogenesis and myelopoiesis in the bone marrow of SAMP6, a murine model of defective osteoblastogenesis and low turnover osteopenia *Journal of Bone and Mineral Research* 12:1772-1779
- Karagiannides I et al. (2001) Altered expression of C/EBP family members results in decreased adipogenesis with aging *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 280:R1772-R1780
- Karagiannides I et al. (2006) Increased CUG triplet repeat-binding protein-1 predisposes to impaired adipogenesis with aging *Journal of Biological Chemistry* 281:23025-23033  
doi:10.1074/jbc.M513187200
- Kelley DE, Mandarino LJ (2000) Fuel selection in human skeletal muscle in insulin resistance - A reexamination *Diabetes* 49:677-683
- Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB (1995) THE EXPRESSION OF TUMOR-NECROSIS-FACTOR IN HUMAN ADIPOSE-TISSUE - REGULATION BY OBESITY, WEIGHT-LOSS, AND RELATIONSHIP TO LIPOPROTEIN-LIPASE *Journal of Clinical Investigation* 95:2111-2119  
doi:10.1172/jci117899
- Kim DJ, Bergstrom J, Barrett-Connor E, Laughlin GA (2008) Visceral adiposity and subclinical coronary artery disease in elderly adults: Rancho Bernardo study *Obesity* 16:853-858  
doi:10.1038/oby.2008.15
- Kim JK et al. (2001) Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance *Proceedings of the National Academy of Sciences of the United States of America* 98:7522-7527
- Kirkland JL, Tchkonja T, Pirtskhalava T, Han JR, Karagiannides I (2002) Adipogenesis and aging: does aging make fat go MAD? *Experimental Gerontology* 37:757-767
- Krebs M, Roden M (2005) Molecular mechanisms of lipid-induced insulin resistance in muscle, liver and vasculature *Diabetes Obesity & Metabolism* 7:621-632 doi:10.1111/j.1463-1326.2004.00439.x
- Krems C, Luhrmann PM, Strassburg A, Hartmann B, Neuhauser-Berthold M (2005) Lower resting metabolic rate in the elderly may not be entirely due to changes in body composition *European Journal of Clinical Nutrition* 59:255-262 doi:10.1038/sj.ejcn.1602066
- Kuk JL, Lee S, Heymsfield SB, Ross R (2005) Waist circumference and abdominal adipose tissue distribution: influence of age and sex *American Journal of Clinical Nutrition* 81:1330-1334
- Kuk JL, Saunders TJ, Davidson LE, Ross R (2009) Age-related changes in total and regional fat distribution *Ageing Research Reviews* 8:339-348
- Laybutt DR, Schmitz-Peiffer C, Saha AK, Ruderman NB, Biden TJ, Kraegen EW (1999) Muscle lipid accumulation and protein kinase C activation in the insulin-resistant chronically glucose-infused rat *American Journal of Physiology-Endocrinology and Metabolism* 277:E1070-E1076
- Lee JY, Sohn KH, Rhee SH, Hwang D (2001) Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4 *Journal of Biological Chemistry* 276:16683-16689 doi:10.1074/jbc.M011695200
- Lee M, Choh AC, Demerath EW, Towne B, Siervogel RM, Czerwinski SA (2012a) Associations Between Trunk, Leg and Total Body Adiposity with Arterial Stiffness *American Journal of Hypertension* 25:1131-1137 doi:10.1038/ajh.2012.92
- Lee Y et al. (2012b) Comparison of regional body composition and its relation with cardiometabolic risk between BMI-matched young and old subjects *Atherosclerosis* 224:258-265  
doi:10.1016/j.atherosclerosis.2012.07.013
- Li CY, Ford ES, Zhao GX, Kahn HS, Mokdad AH (2010) Waist-to-thigh ratio and diabetes among US adults: The Third National Health and Nutrition Examination Survey *Diabetes Research and Clinical Practice* 89:79-87 doi:10.1016/j.diabetes.2010.02.014
- Li H et al. (2009) Prevalence and risk factors of fatty liver disease in Chengdu, Southwest China *Hepatobiliary & Pancreatic Diseases International* 8:377-382

- Li X et al. (2012) Liver fat content is associated with increased carotid atherosclerosis in a Chinese middle-aged and elderly population: The Shanghai Changfeng study *Atherosclerosis* 224:480-485 doi:10.1016/j.atherosclerosis.2012.07.002
- Lightle SA, Oakley JI, Nikolova-Karakashian MN (2000) Activation of sphingolipid turnover and chronic generation of ceramide and sphingosine in liver during aging *Mechanisms of Ageing and Development* 120:111-125 doi:10.1016/s0047-6374(00)00191-3
- Lim S et al. (2009) Fat in Liver/Muscle Correlates More Strongly With Insulin Sensitivity in Rats Than Abdominal Fat *Obesity* 17:188-195
- Lin JL et al. (1991) ALTERED EXPRESSION OF GLUCOSE TRANSPORTER ISOFORMS WITH AGING IN RATS - SELECTIVE DECREASE IN GLUT4 IN THE FAT TISSUE AND SKELETAL-MUSCLE *Diabetologia* 34:477-482
- Liney GP, Bernard CP, Manton DJ, Turnbull LW, Langton CM (2007) Age, gender, and skeletal variation in bone marrow composition: A preliminary study at 3.0 Tesla *Journal of Magnetic Resonance Imaging* 26:787-793 doi:Doi 10.1002/Jmri.21072
- Lio D et al. (2002) Gender-specific association between-1082 IL-10 promoter polymorphism and longevity *Genes and Immunity* 3:30-33 doi:10.1038/sj/gene/6363827
- Little JP, Madeira JM, Klegeris A (2012) The Saturated Fatty Acid Palmitate Induces Human Monocytic Cell Toxicity Toward Neuronal Cells: Exploring a Possible Link Between Obesity-Related Metabolic Impairments and Neuroinflammation *J Alzheimers Dis* 30:S179-S183 doi:10.3233/jad-2011-111262
- Liu C-C et al. (2010) Age-related Differences in the Clinical Presentation, Associated Metabolic Abnormality, and Estimated Cardiovascular Risks from Nonalcoholic Fatty Liver Disease: A Cross-sectional Study from Health Evaluation Center in Taiwan *International Journal of Gerontology* 4:184-191 doi:10.1016/j.ijge.2010.11.005
- Machann J et al. (2005) Age and gender related effects on adipose tissue compartments of subjects with increased risk for type 2 diabetes: a whole body MRI/MRS study *Magnetic Resonance Materials in Physics Biology and Medicine* 18:128-137 doi:10.1007/s10334-005-0401-x
- Marcus RL, Addison O, Kidde JP, Dibble LE, Lastayo PC (2010) Skeletal muscle fat infiltration: Impact of age, inactivity, and exercise *J Nutr Health Aging* 14:362-366 doi:10.1007/s12603-010-0081-2
- Marcus RL, Addison O, LaStayo PC (2013) Intramuscular adipose tissue attenuates gains in muscle quality in older adults at high risk for falling. A brief report *J Nutr Health Aging* 17:215-218 doi:10.1007/s12603-012-0377-5
- Mata P et al. (1996) Effect of dietary fat saturation on LDL oxidation and monocyte adhesion to human endothelial cells in vitro *Arteriosclerosis Thrombosis and Vascular Biology* 16:1347-1355
- Mazzali G et al. (2006) Interrelations between fat distribution, muscle lipid content, adipocytokines, and insulin resistance: effect of moderate weight loss in older women *American Journal of Clinical Nutrition* 84:1193-1199
- McLaughlin T, Lamendola C, Liu A, Abbasi F (2011) Preferential Fat Deposition in Subcutaneous Versus Visceral Depots Is Associated with Insulin Sensitivity *J Clin Endocrinol Metab* 96:E1756-E1760 doi:10.1210/jc.2011-0615
- Miljkovic I et al. (2009) Greater Adipose Tissue Infiltration in Skeletal Muscle among Older Men of African Ancestry *Journal of Clinical Endocrinology & Metabolism* 94:2735-2742 doi:10.1210/jc.2008-2541
- Montell E et al. (2001) DAG accumulation from saturated fatty acids desensitizes insulin stimulation of glucose uptake in muscle cells *American Journal of Physiology-Endocrinology and Metabolism* 280:E229-E237
- Morin CL, Gayles EC, Podolin DA, Wei YR, Xu MM, Pagliassotti MJ (1998) Adipose tissue-derived tumor necrosis factor activity correlates with fat cell size but not insulin action in aging rats *Endocrinology* 139:4998-5005

- Morin CL, Pagliassotti MJ, Windmiller D, Eckel RH (1997) Adipose tissue-derived tumor necrosis factor- $\alpha$  activity is elevated in older rats *Journals of Gerontology Series a-Biological Sciences and Medical Sciences* 52:B190-B195
- Nicklas BJ et al. (2006) Abdominal obesity is an independent risk factor for chronic heart failure in older people *Journal of the American Geriatrics Society* 54:413-420 doi:10.1111/j.1532-5415.2005.00624.x
- Ohanian J, Liao A, Forman SP, Ohanian V (2014) Age-related remodeling of small arteries is accompanied by increased sphingomyelinase activity and accumulation of long-chain ceramides *Physiological reports* 2 doi:10.14814/phy2.12015
- Park JS, Cho MH, Ahn CW, Kim KR, Huh KB (2012) The association of insulin resistance and carotid atherosclerosis with thigh and calf circumference in patients with type 2 diabetes *Cardiovascular Diabetology* 11:8 doi:10.1186/1475-2840-11-62
- Perez GI et al. (2005) A central role for ceramide in the age-related acceleration of apoptosis in the female germline *Faseb Journal* 19:860-+ doi:10.1096/fj.04-2903fje
- Permana PA, Menge C, Reaven PD (2006) Macrophage-secreted factors induce adipocyte inflammation and insulin resistance *Biochemical and Biophysical Research Communications* 341:507-514 doi:10.1016/j.bbrc.2006.01.012
- Perseghin G et al. (2008) Increased mediastinal fat and impaired left ventricular energy metabolism in young men with newly found fatty liver *Hepatology* 47:51-58
- Petersen KF et al. (2003) Mitochondrial dysfunction in the elderly: Possible role in insulin resistance *Science* 300:1140-1142
- Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI (2004) Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes *New England Journal of Medicine* 350:664-671
- Pickersgill L, Litherland GJ, Greenberg AS, Walker M, Yeaman SJ (2007) Key role for ceramides in mediating insulin resistance in human muscle cells *Journal of Biological Chemistry* 282:12583-12589 doi:10.1074/jbc.M611157200
- Pilz S et al. (2006) Free fatty acids are independently associated with all-cause and cardiovascular mortality in subjects with coronary artery disease *J Clin Endocrinol Metab* 91:2542-2547 doi:10.1210/jc.2006-0195
- Qu X, Seale JP, Donnelly R (1999a) Tissue and isoform-selective activation of protein kinase C in insulin-resistant obese Zucker rats - effects of feeding *Journal of Endocrinology* 162:207-214
- Qu XQ, Seale JP, Donnelly R (1999b) Tissue- and isoform-specific effects of aging in rats on protein kinase C in insulin-sensitive tissues *Clinical Science* 97:355-361
- Racette SB, Evans EM, Weiss EP, Hagberg JM, Holloszy JO (2006) Abdominal adiposity is a stronger predictor of insulin resistance than fitness among 50-95 year olds *Diabetes Care* 29:673-678
- Rivas DA et al. (2012) Increased ceramide content and NF kappa B signaling may contribute to the attenuation of anabolic signaling after resistance exercise in aged males *Journal of Applied Physiology* 113:1727-1736 doi:10.1152/jappphysiol.00412.2012
- Rodriguez-Calvo R et al. (2007) Peroxisome proliferator-activated receptor alpha down-regulation is associated with enhanced ceramide levels in age-associated cardiac hypertrophy *Journals of Gerontology Series a-Biological Sciences and Medical Sciences* 62:1326-1336
- Rosen CJ, Ackert-Bicknell C, Rodriguez JP, Pino AM (2009) Marrow Fat and the Bone Microenvironment: Developmental, Functional, and Pathological Implications *Crit Rev Eukaryot Gene Expr* 19:109-124
- Rosen CJ, Bouxsein ML (2006) Mechanisms of disease: is osteoporosis the obesity of bone? *Nature Clinical Practice Rheumatology* 2:35-43
- Sakuma K, Yamaguchi A (2010) Molecular mechanisms in aging and current strategies to counteract sarcopenia *Curr Aging Sci* 3:90-101
- Samuel VT, Petersen KF, Shulman GI (2010) Lipid-induced insulin resistance: unravelling the mechanism *Lancet* 375:2267-2277

- Schrauwen-Hinderling VB et al. (2005) Iramyocellular lipid content and molecular adaptations in response to a 1-week high-fat diet *Obesity Research* 13:2088-2094
- Schwartz EA et al. (2010) Nutrient Modification of the Innate Immune Response A Novel Mechanism by Which Saturated Fatty Acids Greatly Amplify Monocyte Inflammation *Arteriosclerosis Thrombosis and Vascular Biology* 30:802-U372 doi:10.1161/atvbaha.109.201681
- Schwenzer NF, Martirosian P, Machann J, Schraml C, Steidle G, Claussen CD, Schick F (2009) Aging Effects on Human Calf Muscle Properties Assessed by MRI at 3 Tesla *Journal of Magnetic Resonance Imaging* 29:1346-1354 doi:10.1002/jmri.21789
- Shay CM, Secrest AM, Goodpaster BH, Kelsey SF, Strotmeyer ES, Orchard TJ (2010) Regional adiposity and risk for coronary artery disease in type 1 diabetes: Does having greater amounts of gluteal-femoral adiposity lower the risk? *Diabetes Research and Clinical Practice* 89:288-295 doi:10.1016/j.diabres.2010.03.028
- Silaghi A et al. (2008) Epicardial Adipose Tissue Extent: Relationship With Age, Body Fat Distribution, and Coronaropathy *Obesity* 16:2424-2430 doi:10.1038/oby.2008.379
- Skovbro M, Baranowski M, Skov-Jensen C, Flint A, Dela F, Gorski J, Helge JW (2008) Human skeletal muscle ceramide content is not a major factor in muscle insulin sensitivity *Diabetologia* 51:1253-1260 doi:10.1007/s00125-008-1014-z
- Slawik M, Vidal-Puig AJ (2006) Lipotoxicity, overnutrition and energy metabolism in aging *Ageing Research Reviews* 5:144-164 doi:10.1016/j.arr.2006.03.004
- Solomon TPJ, Marchetti CM, Krishnan RK, Gonzalez F, Kirwan JP (2008) Effects of aging on basal fat oxidation in obese humans *Metabolism-Clinical and Experimental* 57:1141-1147 doi:10.1016/j.metabol.2008.03.021
- St-Onge MP, Gallagher D (2010) Body composition changes with aging: The cause or the result of alterations in metabolic rate and macronutrient oxidation? *Nutrition* 26:152-155 doi:10.1016/j.nut.2009.07.004
- Starr ME, Evers BM, Saito H (2009) Age-Associated Increase in Cytokine Production During Systemic Inflammation: Adipose Tissue as a Major Source of IL-6 *Journals of Gerontology Series a-Biological Sciences and Medical Sciences* 64:723-730 doi:10.1093/gerona/glp046
- Stephens JM, Pekala PH (1992) TRANSCRIPTIONAL REPRESSION OF THE C/EBP-ALPHA AND GLUT4 GENES IN 3T3-L1 ADIPOCYTES BY TUMOR-NECROSIS-FACTOR-ALPHA - REGULATION IS COORDINATE AND INDEPENDENT OF PROTEIN-SYNTHESIS *Journal of Biological Chemistry* 267:13580-13584
- Suganami T, Nishida J, Ogawa Y (2005) A paracrine loop between adipocytes and macrophages aggravates inflammatory changes - Role of free fatty acids and tumor necrosis factor alpha *Arteriosclerosis Thrombosis and Vascular Biology* 25:2062-2068 doi:10.1161/01.atv.0000183883.72263.13
- Suganami T et al. (2007) Role of the Toll-like receptor 4/NF-kappa B pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages *Arteriosclerosis Thrombosis and Vascular Biology* 27:84-91 doi:10.1161/01.ATV.0000251608.09329.9a
- Sutton-Tyrrell K et al. (2001) Aortic stiffness is associated with visceral adiposity in older adults enrolled in the study of health, aging, and body composition *Hypertension* 38:429-433
- Takahashi K et al. (2008) JNK- and I kappa B-dependent pathways regulate MCP-1 but not adiponectin release from artificially hypertrophied 3T3-L1 adipocytes preloaded with palmitate in vitro *American Journal of Physiology-Endocrinology and Metabolism* 294:E898-E909 doi:10.1152/ajpendo.00131.2007
- Takamura T, Misu H, Ota T, Kaneko S (2012) Fatty liver as a consequence and cause of insulin resistance: Lessons from type 2 diabetic liver *Endocrine Journal* 59:745-763
- Tchkonina T et al. (2007) Increased TNF alpha and CCAAT/enhancer-binding protein homologous protein with aging predispose preadipocytes to resist adipogenesis *American Journal of*



- Physiology-Endocrinology and Metabolism 293:E1810-E1819  
doi:10.1152/ajpendo.00295.2007
- Tchkonia T, Wise B, Chan G, Karagiannides I, Kirkland JL (2001) Aging, CHOP expression, and preadipocyte differentiation Obesity Research 9:147S-147S
- Thompson AL, Cooney GJ (2000) Acyl-CoA inhibition of hexokinase in rat and human skeletal muscle is a potential mechanism of lipid-induced insulin resistance Diabetes 49:1761-1765
- Tiikkainen M et al. (2002) Liver-fat accumulation and insulin resistance in obese women with previous gestational diabetes Obesity Research 10:859-867
- Tomas E et al. (2002) Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: Acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation Proceedings of the National Academy of Sciences of the United States of America 99:16309-16313 doi:10.1073/pnas.222657499
- Tsintzas K, Chokkalingam K, Jewell K, Norton L, Macdonald IA, Constantin-Teodosiu D (2007) Elevated free fatty acids attenuate the insulin-induced suppression of PDK4 gene expression in human skeletal muscle: Potential role of intramuscular long-chain acyl-coenzyme a Journal of Clinical Endocrinology & Metabolism 92:3967-3972 doi:10.1210/jc.2007-1104
- Ueno K et al. (2009) Increased Epicardial Fat Volume Quantified by 64-Multidetector Computed Tomography is Associated With Coronary Atherosclerosis and Totally Occlusive Lesions Circulation Journal 73:1927-1933
- van der Meer RW et al. (2008) The ageing male heart: myocardial triglyceride content as independent predictor of diastolic function European Heart Journal 29:1516-1522 doi:10.1093/eurheartj/ehn207
- van Harmelen V, Rohrig K, Hauner H (2004) Comparison of proliferation and differentiation capacity of human adipocyte precursor cells from the omental and subcutaneous adipose tissue depot of obese subjects Metabolism-Clinical and Experimental 53:632-637 doi:10.1016/j.metabol.2003.11.012
- van Loon LJC, Goodpaster BH (2006) Increased intramuscular lipid storage in the insulin-resistant and endurance-trained state Pflugers Archiv-European Journal of Physiology 451:606-616 doi:10.1007/s00424-005-1509-0
- Van Pelt RE, Jankowski CM, Gozansky WS, Wolfe P, Schwartz RS, Kohrt WM (2011) Sex Differences in the Association of Thigh Fat and Metabolic Risk in Older Adults Obesity 19:422-428 doi:10.1038/oby.2010.140
- Venable ME, Webb-Froehlich LM, Sloan EF, Thomley JE (2006) Shift in sphingolipid metabolism leads to an accumulation of ceramide in senescence Mechanisms of Ageing and Development 127:473-480 doi:10.1016/j.mad.2006.01.003
- Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW (2003) Obesity is associated with macrophage accumulation in adipose tissue Journal of Clinical Investigation 112:1796-1808 doi:10.1172/jci2000319246
- Wu D, Ren Z, Pae M, Guo W, Cui X, Merrill AH, Meydani SN (2007) Aging up-regulates expression of inflammatory mediators in mouse adipose tissue J Immunol 179:4829-4839
- Wu HY et al. (2010) Independent and Opposite Associations of Trunk and Leg Fat Depots with Adipokines, Inflammatory Markers, and Metabolic Syndrome in Middle-Aged and Older Chinese Men and Women J Clin Endocrinol Metab 95:4389-4398 doi:10.1210/jc.2010-0181
- Wu ZD et al. (1999) Cross-regulation of C/EBP alpha and PPAR gamma controls the transcriptional pathway of adipogenesis and insulin sensitivity Molecular Cell 3:151-158
- Yaney GC, Korchak HM, Corkey BE (2000) Long-chain acyl CoA regulation of protein kinase C and fatty acid potentiation of glucose-stimulated insulin secretion in clonal beta-cells Endocrinology 141:1989-1998

- Yim JE, Heshka S, Albu J, Heymsfield S, Kuznia P, Harris T, Gallagher D (2007) Intermuscular adipose tissue rivals visceral adipose tissue in independent associations with cardiovascular risk *International Journal of Obesity* 31:1400-1405 doi:10.1038/sj.ijo.0803621
- Youm Y-H et al. (2012) The NLRP3 Inflammasome Promotes Age-Related Thymic Demise and Immunosenescence *Cell Reports* 1:56-68 doi:10.1016/j.celrep.2011.11.005
- Yu CL et al. (2002) Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle *Journal of Biological Chemistry* 277:50230-50236
- Zamboni M et al. (2003) Body composition changes in stable-weight elderly subjects: The effect of sex Aging *Clinical and Experimental Research* 15:321-327
- Zhang B, Berger J, Hu EI, Szalkowski D, WhiteCarrington S, Spiegelman BM, Moller DE (1996) Negative regulation of peroxisome proliferator-activated receptor-gamma gene expression contributes to the antiadipogenic effects of tumor necrosis factor-alpha *Molecular Endocrinology* 10:1457-1466
- Zhang WY, Schwartz E, Wang YJ, Attrep J, Li Z, Reaven P (2006) Elevated concentrations of nonesterified fatty acids increase monocyte expression of CD11b and adhesion to endothelial cells *Arteriosclerosis Thrombosis and Vascular Biology* 26:514-519 doi:10.1161/01.atv.0000200226.53994.09
- Zhou Q, Du J, Hu Z, Walsh K, Wang XH (2007) Evidence for adipose-muscle cross talk: Opposing regulation of muscle proteolysis by adiponectin and fatty acids *Endocrinology* 148:5696-5705 doi:10.1210/en.2007-0183
- Zuo Y, Qiang L, Farmer SR (2006) Activation of CCAAT/enhancer-binding protein (C/EBP) alpha expression by C/EBP beta during adipogenesis requires a peroxisome proliferator-activated receptor-gamma-associated repression of HDAC1 at the C/ebp alpha gene promoter *Journal of Biological Chemistry* 281:7960-7967 doi:10.1074/jbc.M510682200