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Review

Adipose tissue expandability, lipotoxicity and the Metabolic Syndrome — An allostatic perspective

Sam Virtue *, Antonio Vidal-Puig *

Institute of Metabolic Science, Metabolic Research Laboratories, University of Cambridge, Box 289, Level 4, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK

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ABSTRACT

While the link between obesity and type 2 diabetes is clear on an epidemiological level, the underlying mechanism linking these two common disorders is not as clearly understood. One hypothesis linking obesity to type 2 diabetes is the adipose tissue expandability hypothesis. The adipose tissue expandability hypothesis states that a failure in the capacity for adipose tissue expansion, rather than obesity *per se* is the key factor linking positive energy balance and type 2 diabetes. All individuals possess a maximum capacity for adipose expansion which is determined by both genetic and environmental factors. Once the adipose tissue expansion limit is reached, adipose tissue ceases to store energy efficiently and lipids begin to accumulate in other tissues. Ectopic lipid accumulation in non-adipocyte cells causes lipotoxic insults including insulin resistance, apoptosis and inflammation. This article discusses the links between adipokines, inflammation, adipose tissue expandability and lipotoxicity. Finally, we will discuss how considering the concept of allostasis may enable a better understanding of how diabetes develops and allow the rational design of new anti diabetic treatments.

1. The Metabolic Syndrome

The Metabolic Syndrome (MetS), or Syndrome X, was originally described as a set of four pathogenic states that cluster in individuals with a greater frequency than can be expected by chance alone. The four aspects of the Metabolic Syndrome are obesity, insulin resistance, dyslipidaemia and hypertension [1,2]. The fact that these four metabolic complications cluster together suggests that there may be a common pathogenic link between them. In this article we explore how adipose tissue expansion and lipotoxicity can provide a potential mechanism that integrates the diverse manifestations of the MS [3–6]. When adipose tissue cannot expand further to store excess nutrients then lipid accumulates in other tissues. Ectopic lipid deposition can cause insulin resistance, cardiovascular complications and other lipotoxic effects such as apoptosis. It is notable that the idea of lipid overspill (dyslipidaemia), exhausted adipose tissue expansion (usually when an individual is obese), cardiovascular complications and insulin resistance are all components of the Metabolic Syndrome. Here we focuse on the pathways leading to insulin resistance and diabetes, rephrasing the adipose tissue expandability hypothesis in the context of allostasis. An allostatic perspective of insulin resistance and diabetes focus not so much on the pathogenesis of the disease as a series of steps, but considers how each step may represent attempts by the organism to regain control of blood glucose by invoking parallel systems. The chronic usage of alternative systems unsuited to long-term maintenance of blood glucose ultimately leads to a failure of these systems as they become exhausted or overwhelmed. In our opinion considering the progression of weight gain to beta cell failure in terms of allostasis allows a more rational approach to the treatment of obesity, insulin resistance and diabetes. To that end this article will also discuss the different processes involved in the aetiology of insulin resistance and diabetes and whether intervening at the level of these processes is rational from an allostatic perspective.

2. The concept of allostasis and rationalising treatment strategies to combat diabetes

Allostasis is the concept of maintaining stability through change, a change that requires energy and may be associated with unwanted collateral damage. It differs from homeostasis (defined as the stability of physiological parameters) because it incorporates the systems that maintain the homeostatic set point. Indeed, a key concept of allostasis is that the idea of a 'set point' for a metabolic parameter is in fact not necessarily true. Over a 24 h period blood glucose in a healthy individual will fluctuate quite markedly, ranging between 70 and 110 mg/dl [7]. Furthermore to maintain blood glucose levels within even a quite broad range also requires constant adjustment of the systems that are used to maintain blood glucose. To maintain blood glucose at an appropriate level will require very different amounts of insulin shortly after a meal when compared to waking up from a night's sleep. Further to the concept of allostasis is the physiological concept of allostatic load and from a pathophysiological point of view,

* Corresponding authors. Tel.: +44 1223 336792; fax: +44 1223 330598.
E-mail addresses: Sv234@medschl.cam.ac.uk (S. Virtue), ajv22@medschl.cam.ac.uk (A. Vidal-Puig).

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allostatic overload. Considering blood glucose levels again, then allostatic load can be affected by processes such as pregnancy, seasonal changes in body weight or more acutely, as the example given above shows, the daily rhythms of meals. Allostatic overload describes a state that occurs when a system is driven so hard that normal allostatic mechanisms cannot cope with demand. Essentially if allostatic overload is not removed within a relatively short period of time, then the system will fail. In our opinion the driving allostatic loads leading to insulin resistance and diabetes are; a) the demand to store excess nutrients in a state of positive energy balance and b) the subsequent ectopic deposition of lipids in non-adipose organs when the ability of adipose tissue to store lipid is exceeded. The development and causes of obesity have been considered extensively from an allostatic point of view [8,9], however, here we consider how the biological responses to changes in energy balance, which often (though not always) manifest themselves in an obese state, ultimately lead to type 2 diabetes.

BOX 1

Homeostasis is the stability of biological systems to maintain life.
Allostasis is maintenance of stability through change. It includes both the concepts of the mechanisms needed to maintain homeostasis and crucially the idea that the putative 'set point' defended by homeostatic mechanisms is actually continually altered in response to environmental clues.
Allostatic load is the level of demand on systems for correction. For example there is a greater allostatic load on the beta cell after a meal as opposed to during fasting. Allostatic load should be viewed as a normal physiological demand.
Allostatic overload describes a state of prolonged demand on a given allostatic system that will ultimately cause the system to fail if not corrected.
(Adapted from McEwan and Wingfield 2003)

3. What causes insulin resistance in the context of obesity?

It has been suggested that development of insulin resistance may be an early event in the development of common forms of type 2 diabetes. It is a clinical fact that the majority of insulin resistant patients are obese. Furthermore, on an epidemiological level, obesity is clearly associated with insulin resistance and diabetes. In the next section of this article potential mechanisms that connect obesity to insulin resistance will be discussed.

4. The adipose tissue expandability hypothesis

While it is clear that obesity is associated with diabetes based on population studies, there is some controversy as to the mechanisms by which this occurs on an individual level. One hypothesis, which perhaps links many others, is that of limited adipose tissue expandability. The adipose tissue expandability hypothesis can be stated as follows; adipose tissue has a defined limit of expansion for any given individual. As an individual gains weight a point will eventually be reached when their adipose tissue can no longer store more lipid. Once adipose tissue storage capacity is exceeded then net lipid flux to non-adipose organs will increase and lipids will begin to be deposited ectopically. Ectopic lipid accumulation in cells such as myocytes hepatocytes and beta cells then causes toxic effects such as insulin resistance and apoptosis.

Adipose tissue mass by itself is unlikely to be the driving factor linking obesity to diabetes. If there was a simple and direct correlation between adipose tissue mass and insulin sensitivity, all individuals would become diabetic at the same adipose tissue mass. However, on an individual level this is not true, some relatively lean individuals become insulin resistant whereas some very obese individuals do not [10]. Even on population levels the association between obesity and insulin resistance can vary. The WHO is considering revising the definition for the MetS for Asian populations because metabolic complications seem to occur at a lower average BMI in Asian populations than occur for Caucasian populations [11].

Many factors may influence the capacity of adipose tissue to expand and several mouse models and human mutants exist in support of this concept. Relatively few examples exist of models where adipose tissue expansion is relatively unlimited, but these will be mentioned towards the end of this section.

5. Preadipocyte to adipocyte differentiation

Mature adipocytes are derived from preadipocyte precursors. The processes involved in terms of preadipocyte to adipocyte conversion have been extensively studied using a variety of *in vitro* models and genetically modified mice [12,13]. Perhaps the most important transcription factor in the control of adipogenesis is PPARγ. PPARγ is a nuclear hormone receptor (NHR) super-family member that regulates much of the adipogenic program. While at least 4 different mRNAs have been shown to be transcribed from the PPARγ gene, these transcripts encode two separate protein isoforms, PPARγ1 and PPARγ2. While PPARγ1 is expressed widely, PPARγ2 is found almost exclusively in adipose tissue. Lack of both isoforms of PPARγ results in embryonic-lethality, due to a failure in placental development. Tetraploid rescue of PPARγ null mutants and chimeric mice made from *PPARγ−/−* and *PPARγ+/+* blastocysts show that no adipocytes develop from PPARγ null cells [14].

Several murine models with a partial lack of PPARγ function have been generated. PPARγ2 KO mice lack one of the two PPARγ protein isoforms. These mice have no obvious metabolic phenotype until challenged with a genetically obese leptin-deficient (*ob/ob*) background (the POKO mouse). POKO mice possess around 10–20% more adipose tissue mass than wild-type mice but are less than half the mass of an *ob/ob* mouse. Despite being leaner than an *ob/ob* mouse these animals exhibit frank diabetes and severe insulin resistance from an early age [15].

Several humans with mutations in PPARγ have also been characterised [16]. Humans heterozygous for a dominant negative mutation in PPARγ (P467L) have greatly reduced body fat and severe insulin resistance. A murine model of the P467L mutation (corresponding to P465L in mice) was also crossed onto an *ob/ob* background, demonstrating a subtle reduction in fat mass compared to an *ob/ob* mouse model but greatly reduced insulin sensitivity [17].

Both the POKO and P465L DN × *ob/ob* mouse models show states of increased adipose mass when compared to a lean animal but less adipose tissue mass than an *ob/ob* mouse. As such the POKO and P465L DN × *ob/ob* mouse models could be considered analogous to people who were moderately overweight, rather than massively obese. However, in concordance with their reduced capacity for adipose tissue expansion, both these models demonstrate a reduction in insulin sensitivity when compared to the far more obese *ob/ob* mouse. Taken together the POKO and P465L DN × *ob/ob* mice support the concept that a genetic limit on adipose tissue expansion, under conditions of positive energy balance, can lead to insulin resistance and other severe metabolic complications.

6. Mechanical limitations on adipose tissue expansion

Although cell culture systems have been used extensively to investigate the mechanisms of preadipocyte to adipocyte conversion, *in vivo* adipocytes do not exist as a monolayer of identical cells but in a

complex milieu of different cell types embedded within an extracellular matrix (ECM). In order for adipocytes to increase in size (hypertrophy) and number (hyperplasia) the extracelullar matrix must be remodelled, if it is not, then these processes cannot occur. The extracelullar matrix is physically made up of proteins, principally collagen. For the purpose of remodelling, collagen can be secreted from cells including adipocytes. Equally, to remove excess extracellular matrix and allow adipocyte hypertrophy then proteases such as matrix metallo proteases (MMPs) must break down excess matrix. The ECM is by no means simple in composition and many proteins, often with signalling functions, are found within it.

A small number of studies have investigated the role of extracellular matrix proteins in terms of adipose tissue biology, specifically the role of collagen VI and several matrix metallo proteases (MMP). Ablation of collagen VI allowed adipocytes to increase in size dramatically, however without the problems of insulin resistance and increased chemokine production usually associated with hypertrophic adipocytes [18]. It is possible that as adipocytes become hypertrophied that pressure exerted on them by the extracellular matrix contributes to their increased production of chemokines (which attract macrophages) and that pressure from the ECM may lead to rupture of adipocytes, releasing lipids, which in turn can activate macrophages [19]. The collagen VI KO mouse model was, however, unexpectedly lean and had lower food intake and energy expenditure than WT controls. The reason for the altered metabolism in the collagen VI KO mouse model was not clear. Conversely, ablation of MMPs has been shown to limit adipocyte hypertrophy in mice, shown by the MMP14 null mouse model, which has greatly reduced adipose tissue mass and much smaller adipocytes [20]. As MMPs break down collagens to allow remodelling of the ECM they can perhaps be seen as an inverse model of the Collagen VI KO mouse model (mice lacking MMPs would have excess an excess of collagen due to a failure in the processes that remove it).

7. Angiogenesis as a modulator of adipose tissue expansion

Angiogenesis is the process of new blood vessel formation and is required for expansion of adipose tissue. Unsurprisingly, inhibitors of angiogenesis such as TNP-470, angiostatin and endostatin inhibit adipose tissue expansion and by themselves are sufficient to reverse obesity in dietary and genetic models of obesity [21].

Unexpectedly, from the point of view of the adipose tissue expandability hypothesis, the one study which looked at the metabolic consequences of inhibiting angiogenesis (by treating mice with TNP-470) demonstrated that treated mice were more insulin sensitive. However, the reasons for the metabolic improvement in TNP-470 treated mice were not entirely clear and may have been related to non-adipose tissue related effects. Although weight loss in TNP-470 treated animals was associated with decreased food intake, pair-fed controls did not reduce body weight by as much as TNP-470 treated animals. The failure of pair-fed mice to lose as much weight as TNP-470 treated mice suggests that TNP-470 increases energy expenditure [22,23]. The potential hypermetabolic effects of TNP-470 raise the question as to whether improvements in the metabolic profile after TNP-470 treatment are due to effects on white adipose tissue. More research into the effects of blocking adipose tissue angiogenesis on metabolism will be required.

8. Intrinsic limits on preadipocyte formation

Recently adipose tissue stem cells have been identified [24,25]. Adipose stem cells appear associated with the vasculature of adipose tissue [25]. At present little is known about the capacity of these stem cells to produce new preadipocytes, or how preadipocyte formation is regulated. If preadipocyte number does differ between individuals this may contribute to an intrinsic limit on adipose tissue expansion. The idea that adipocytes are turned over during the whole lifecycle of humans has been demonstrated by the work of Spalding et al. [26]. Their work showed that there was a steady but maintained formation of new adipocytes in adulthood and that adipocytes had a lifespan of around 10 years on average. Intriguingly, and in support of the adipose tissue expandability hypothesis, the number of adipocytes in the subcutaneous adipose tissue depots of the obese groups fell by 25% between the ages of 45 and 65 [26]. Although the metabolic parameters of the lean and obese groups were not described, it is known that aging is a risk factor for the development of type 2 diabetes. The drop off in adipocyte number observed in the obese group after 45 years of age would be expected to limit adipose tissue's lipid buffering capacity and contribute to increased rates of insulin resistance and then type 2 diabetes in later life.

9. Other hypotheses and how they tie in

Considerable research has focussed on other concepts that can link obesity to diabetes. Perhaps two of the most popular hypotheses are the adipokine hypothesis and the low grade inflammatory state hypothesis. The low grade inflammatory state hypothesis posits that obesity is associated with inflammation in adipose tissue and liver, which leads to an increase in pro-inflammatory cytokine production by immune cells such as macrophages. Pro-inflammatory cytokines can directly impair insulin sensitivity, principally through NFκβ and JNK pathways [27]. There is some evidence that cytokines produced by tissues such as adipose tissue can affect systemic insulin sensitivity, for example administering neutralising antibodies against the inflammatory cytokine TNF-α to obese rodent models can ameliorate their insulin resistance [28]. In humans the evidence for systemic effects of cytokines are perhaps less well established, with some studies showing an association between inflammatory cytokines and others showing no association.

It has also been observed that as obesity progresses the number of macrophages resident in adipose tissue increases [29]. Furthermore, there is considerable evidence that the nature of those macrophages is altered. In the obese state macrophages appear to become polarised toward a more M1 phenotype, whereas in lean animals the macrophage population expresses greater amounts of M2 markers. M1 macrophages are traditionally viewed as being more pro-inflammatory, whereas M2 macrophages generally are viewed as anti-inflammatory, based on the profile of the cytokines these cell types produce. Further support for the importance of the M1/M2 switch has been demonstrated in mouse models where PPARγ was deleted in macrophages and granulocytes using the cre-loxP system under the control of a LysM promoter. Animals lacking PPARγ exhibited a more M1 phenotype in their macrophages and had a greater degree of insulin resistance [30,31].

An obvious question is what drives the accumulation and activation of macrophages in adipose tissue. The adipose tissue expandability hypothesis can explain some of these aspects. Firstly, larger adipocytes have been shown to express and secrete higher levels of chemoattractants, which promote macrophage infiltration [32]. The reasons for the increase in chemoattractant production by hypertrophic adipocytes are not entirely clear. One possible explanation is that hypertrophic adipocytes may seek to signal that they are entering an unhealthy state and release chemokines to attract macrophages ready to clear up cellular remnants after adipocyte apoptosis/necrosis. Although not proved, the idea of adipocytes calling in macrophages to clean up after they have died has some merit and is supported by several lines of evidence. Firstly, as adipocytes grow they may become hypoxic and activate the Hif pathway. Larger fat droplets force nuclei and cytoplasmic compartments of the adipocyte farther from blood vessels and therefore oxygen supply. Hypoxia activates the JNK and IKK/NFκβ pathways, which up-regulate the production of chemokines and pro-inflammatory cytokines in adipocytes [33].

Additionally, physical pressure on adipocytes from the ECM may stimulate chemokine production as shown by the reduction in chemokine levels found in the collagenase VI KO mouse [18]. It is notable that when macrophages are observed in adipose tissue they often form crown like structures around what may be dead or dying adipocytes. [29]. This phenomenon of macrophages 'clearing up' dead adipocytes is perhaps most clearly demonstrated in the FAT-ATTAC mouse where adipocytes are killed by targeted activation of Caspase 8. In this model the vast majority of adipocytes within adipose tissue are killed simultaneously leading to massive macrophage infiltration [34].

In addition to macrophage infiltration macrophage activation can be increased in obese, insulin resistant adipose tissue. When adipose tissue becomes insulin resistant then FFA production is not suppressed in the post-prandial state. In turn, elevated FFA levels can directly activate macrophages via Toll-like receptors, resulting in an increase production of pro-inflammatory cytokines [19]. However, the links between macrophages and adipose tissue expansion do not necessarily flow in only one direction. As mentioned above, macrophages secrete anti-adipogenic (such as TNF-α) cytokines that also inhibit insulin action [35]. The anti-adipogenic and insulin resistance-inducing effects of macrophages suggest a vicious circle. Pro-inflammatory macrophage-released cytokines decrease the formation of 'good' small adipocytes. By reducing new adipocyte formation inflammatory cytokines cause a relative increase in larger insulin resistant adipocytes. The larger adipocytes in turn produce more chemokines secretion and more FFAs. Increased chemokines and FFAs attract and activate yet more macrophages further inhibiting adipocyte function and preadipocyte differentiation (Fig. 1).

A third hypothesis linking obesity and insulin resistance focuses on the endocrine role of adipose tissue. Adipokines are hormones released from adipose tissue. Perhaps the two most studied adipokines in terms of their effects on metabolism are leptin and adiponectin. Leptin is principally viewed as an 'adipostat', informing the body of when it has insufficient adipose stores and regulating energy dependent processes such as food intake, metabolic rate and fertility. Leptin levels fall when adipose tissue mass is low and during fasting conditions. Conversely, adiponectin levels rise during fasting conditions. The role of adiponectin in terms of insulin sensitivity has been extensively investigated in mouse models. Several models of both adiponectin null and adiponectin transgenic mice have been generated. Generally the models demonstrate that adiponectin acts as an insulin sensitizer. Additionally adiponectin levels in serum are strongly inversely-correlated with insulin sensitivity [36].

One particular adiponectin related mouse model is particularly interesting with respect to the adipose tissue expansion hypothesis. The AdTG mouse over expresses adiponectin in adipose tissue. When crossed onto an *ob*/*ob* background the AdTG-*ob*/*ob* mouse has greatly increased adipose tissue mass when compared to an *ob*/*ob* mouse alone. Despite the increase in adiposity of the AdTG-*ob*/*ob* mouse it has considerably improved carbohydrate metabolism compared to *ob*/*ob* controls [37]. The AdTG-*ob*/*ob* mouse model seems to have a near limitless capacity to expand its adipose tissue and does not develop the MetS. This study provides strong support for the adipose tissue expandability hypothesis, as an animal with massively increased fat mass (but with smaller adipocytes and improved lipid profile) can be more insulin sensitive than a relatively leaner animal. It also points to the concept that an adipokine can affect insulin sensitivity by increasing adipose tissue expansion.

Alternatively, many adipokines that have been implicated in causing insulin resistance, such as RBP4 and resistin, and their serum-levels are increased during obesity. The role of resistin in terms of insulin sensitivity has been controversial for several reasons. In rodents resistin is produced predominantly by adipocytes, whereas in humans the major site of production is monocytes and macrophages. Furthermore, circulating resistin levels have been negatively associated with insulin sensitivity in humans in some studies, whereas no correlation has been found in others. In attempt to clear up some of the controversy surrounding resistin, a recent study looked to 'humanise' a mouse with respect to resistin. To do this, the human resistin gene was expressed under the control of a monocyte specific CD68 promoter. The Cd68-resistin TG mouse was then crossed onto a resistin null mouse. This study demonstrated that human resistin, expressed in the same cell type as it is found in humans (though admittedly under a non-native promoter) was able to cause insulin resistance [38]. Overall resistin highlights the complications in distinguishing the differences between 'adipokines' and cytokines. In humans, resistin is principally not found in adipocytes, but is produced from adipose tissue. Regardless it does appear to link inflamed adipose tissue and insulin resistance in both mice and men.

Interleukin-6 is another controversial 'adipokine' in terms of its function on insulin sensitivity. Circulating levels of Il-6 can be increased during obesity and insulin resistance, however acute alterations in circulating Il-6 levels occur during exercise. The increase in Il-6 levels found during exercise is caused by Il-6 production by muscle and may actually promote insulin sensitivity [39]. The fact Il-6 can promote insulin sensitivity when produced from muscle suggests that the term 'adipokine' must be treated with some caution when applied to Il-6.

10. Evidence to support the adipose tissue expandability hypothesis from humans

While the above sections have summarised much of the experimental data from rodent models that support the concept of adipose tissue expansion, there is some intriguing evidence from human studies that supports the adipose tissue expansion hypothesis.

Firstly, as mentioned briefly above, a number of human mutations that cause lipodystrophy (a lack of adipose tissue) result in severe insulin resistance. Mutations in multiple different genes have been found to cause congenital lipodystrophy, including PPARγ, Sepin/BCSL2, Lamin A and AKT2 [61]. These diseases are characterised by substantial reductions in adipose tissue mass, associated with severe insulin resistance and often hepatosteatosis [62].

Based on the adipose tissue expandability hypothesis it would be expected that metabolically normal obese individuals would have improved adipose tissue function than metabolically compromised individuals. Obese individuals who are metabolically normal have elevated levels of proadipogenic factors such as the proadipogenic Wnt signalling regulator Dapper1 [63] and increased levels of lipid droplet proteins such a Perilipin, Cidea and FSP27, [64] compared to obese metabolically compromised individuals.

Further support for a failure in adipose tissue lipid buffering capacity comes from work showing that in insulin resistant individuals, insulin does not appropriately suppress lipolysis from adipose tissue [65]. The failure in type 2 diabetes of insulin to suppress lipolysis from adipose tissue would promote delivery of lipids to non-adipose organs.

While studies looking at adipose tissue function in mice and humans can show that failures in adipose tissue lead to insulin resistance, it still raises a question as to where adipose tissue failure fits in the time line of obesity to type 2 diabetes. The adipose tissue expandability hypothesis posits that adipose tissue fails *first* leading to lipid accumulation in other organs. The lipid accumulation in other organs leads to metabolic complications caused by lipotoxic mechanisms (see below). If this is true then fat accumulation in non-adipose organs should show a stepwise increment between, lean, obese, obese impaired fasting-glucose and finally obese diabetic subjects. Several recent studies have indeed demonstrated that in pancreas and myocardium lipid accumulation increases between lean, obese IGT and DM-2 subjects [66,67]. Crucially these studies demonstrate that ectopic lipid accumulation precedes metabolic complications. In the next section of this review we will discuss how these increasing lipid levels cause metabolic complications.

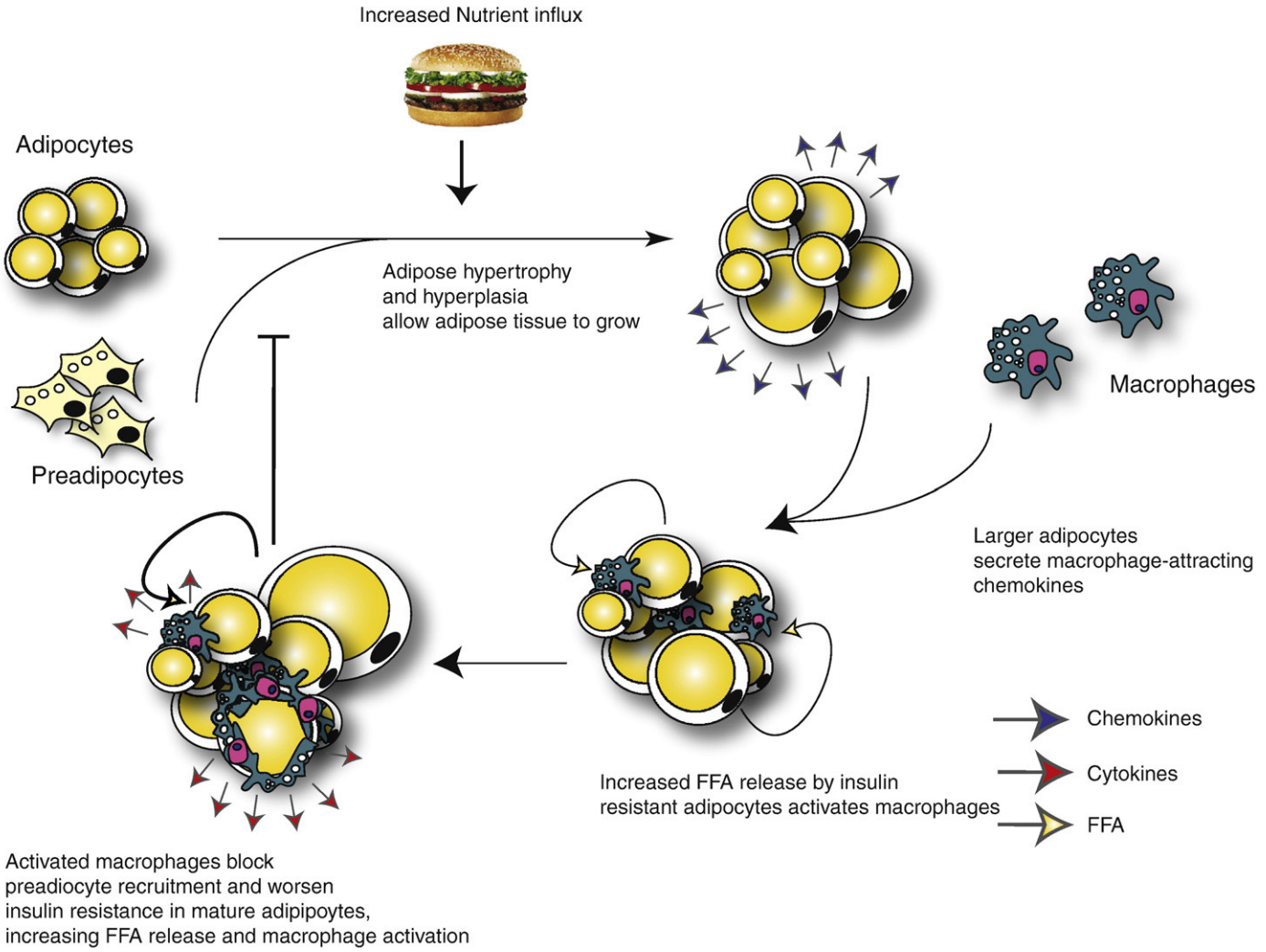

Fig. 1. Diagram showing the vicious circle of adipocyte hypertrophy, macrophage recruitment and activation. Adipocyte hypertrophy leads to macrophage recruitment. In turn hypertrophied adipocytes release elevated amounts of FFA and activate macrophages. Activated macrophages secrete cytokines which a) worsen adipocyte insulin sensitivity leading to yet more FFA release and b) block preadipocyte recruitment, thus leading to yet larger adipocytes.

11. Lipotoxicity and how insulin resistance occurs

In the next section of this article the reasons why a failure in adipose tissue expansion leads to insulin resistance will be discussed. A failure in adipose tissue expansion can be observed when there is a slowing in the rate of adipose tissue mass expansion, coupled with insulin resistance within adipose tissue. Under these conditions adipocytes tend to hypertrophy and the appropriate physiological functions of adipose tissue are diminished. Adipose tissue no longer appropriately buffers lipid flux. In the post-prandial state there is a reduction in the ability of insulin to suppress adipose tissue lipolysis and a failure in the capacity of adipose tissue to take up lipids from the circulation. These processes lead to elevated serum FFA and TG and a net flux of lipids to tissues other than adipose tissue [40].

Elevated circulating FFA levels lead to an increased disposal of these lipids into peripheral organs such as muscle and liver. In the obese the increase in FFA uptake by liver and muscle occurs in the absence of a significant increase in fatty acid oxidation rates [41]. The excess non-oxidised fatty acids that enter liver or muscle are targeted to several different cellular fates. Crucially from the point of view of lipotoxicity, many of the pathways that excess NEFA can be channelled down are implicated in causing insulin resistance and/or apoptosis. For example, palmitate can be channelled via serine palmitoyl-transferase into the ceramide biosynthetic pathway. While ceramides are essential for normal cellular function, in excess they have been shown to cause insulin resistance and or apoptosis. In terms of insulin resistance ceramides are perhaps best defined as activators of protein phosphatase 2a, which acts at the level of PKB to limit insulin signalling. Alternatively ceramides have been suggested to activate multiple pro-inflammatory signalling kinases including JNK, IKK/NFκβ, mTOR and PKC θ [42]. Pro-inflammatory kinases are able to phosphorylate multiple components of the insulin signalling cascade usually down-regulating insulin signalling and therefore causing *de facto* insulin resistance. Alternatively palmitate can also be esterified to form triglyceride. The pathway from FFA to triglyceride is also fraught with danger from an insulin sensitivity point of view. FFAs are first converted to lysophosphatidic acid (LPA), then phosphatidic acid (PA) and then diacylglycerol (DAG) and finally triacylglycerol (TAG). Of note LPA, PA and DAG can all activate some or all of the same pro-inflammatory kinases activated by ceramides [43]. Intriguingly, altering the balance of intermediates to end points in the TAG synthesis pathway by over expressing DGAT, which converts DAG to TAG, actually protects muscle from insulin resistance [44].

12. Specific lipid species and how they cause lipotoxicity in specific organs

Muscle is the major site of insulin-stimulated glucose disposal in humans and rodents. Several studies have looked at the effects of intramyocellular lipid deposition and found that increased levels of lipid accumulation correlate with insulin resistance [45–48]. This would seem to agree well with the concept of lipotoxicity induced insulin resistance. However, a substantial and outstanding question was raised by the 'athletes paradox'. The athletes paradox is the fact that trained athletes have very high levels of intramyocellular lipid but are very insulin sensitive [49].

Recently, several papers have provided evidence that allows us to restate the lipotoxicity hypothesis in a manner that includes lipid types rather than simply lipid amount. In 2007 Liu et al. published a study over expressing the enzyme DGAT1 in muscle. DGAT1 esterifies FFAs to DAGs. Increasing expression of DGAT1 in muscle raised intramyocellular lipid levels, however the increase was not uniform across all lipid species. While mTG-DGAT1 mice had increased intramyocellular TAG levels they also had reduced levels of Diacylglycerols (DAG) and ceramides. In accordance with the presence of less 'toxic' lipid species the mTG-DGAT1 mice had improved muscle and whole-organism insulin sensitivity, in spite of the elevated total intramyocellular lipid content. The mechanisms for the decreased levels of DAG and TAG were not investigated, however in the case of DAGs this was presumably a direct effect of DAG being converted to TAG by DGAT1. In the case of ceramide this may either have been caused by depletion of palmitate (the substrate for serine palmitoyl-transferase) by feed-forward from the increased TAG synthesis or secondary to the increased insulin sensitivity in the muscle of mTG-DGAT1 mice [44].

A second key aspect when considering muscle and lipid levels is the concept of oxidation of lipids. Both genetic and environmental methods to increase fatty acid oxidation in muscle can improve lipid profiles. In humans exercise training of obese humans led to decreases in DAGs and Ceramides without decreasing TAG stores. These changes were associated with improved glucose tolerance [50]. Equally, a mouse over expressing UCP3 in muscle demonstrated greatly improved insulin sensitivity. UCP3 TG mice overexpress the protein UCP3 which is an inner mitochondrial membrane protein. The effect over expressing UCP3 is to cause the inner mitochondrial membrane to become 'leaky' allowing protons to flow across the inner mitochondrial membrane without passing though ATP synthase. The energy lost due to proton leakage is dissipated as heat and results in a very low oxidative phosphorylation to ATP conversion rate (metabolic efficiency) in muscle. As a result of this elevated proton leak, additional calories are required to maintain ATP levels in muscle. The increased oxidative rate of the UCP3 TG mice requires higher rates of fatty acid oxidation in muscle and a higher metabolic rate in the animal as a whole. The improvements in insulin sensitivity due to over expression of UCP3 in muscle were seen even after only 10 days of high fat diet when comparing these animals to WT controls matched for percentage fat mass. Under 10 day HFD conditions, UCP3 muscle-TG mice had lower DAG and ceramide levels when compared to WT controls but had equal levels of TAG [51]. The short-term nature of this study is particularly important as it removes the confounding effect of body weight on insulin sensitivity. Overall this study suggests that increased muscle fatty acid oxidation can improve insulin sensitivity independently of effects on weight loss.

While increasing fatty acid oxidation in muscle is attractive therapeutically it does not demonstrate whether alterations in fatty acid oxidation actually lead to diabetes. Interestingly, evidence from humans suggests that reduced mitochondrial capacity for lipid oxidation may be causative of insulin resistance and subsequently type 2 diabetes. In 2005 Ritov et al. demonstrated a reduction in mitochondrial DNA in obese and diabetic subjects [52]. Further to this, it was subsequently shown that first degree relatives of type 2 diabetics, who were themselves metabolically healthy, had reduced mtDNA content, suggesting that reduced mitochondrial capacity may be causative rather than consequential [53].

13. The role of specific fatty acids in liver

In liver, analogous to muscle, there has generally been observed a strong correlation between hepatosteatosis (macroscopic lipid accumulation) and insulin resistance. Rates of hepatosteatosis in type 2 diabetics are much higher than those found in the general population or non-diabetic obese subjects. Equally, several mouse models have shown that insulin sensitivity can be improved by reducing hepatosteatosis. However, as with muscle, it may be the case that TAG accumulation (seen as hepatosteatosis) serves as a marker of increases in the concentration of other lipids rather than itself being directly causal of pathologies. Analogous to the DGAT1 over expression in muscle model discussed above, over expressing DGAT2 in liver led to an increase in hepatic triglyceride levels. In the case of DGAT2 over expressing livers there was no actual improvement in insulin resistance, but notably the mice had both normal whole body insulin sensitivity and normal hepatic insulin sensitivity despite the increased lipid levels [54]. Conversely, knocking down DGAT2 in liver using antisense oligonucleotides (ASO)

affected non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH) conversion. Hepatosteatosis, in addition to being associated with hepatic insulin resistance, is also a risk factor for NAFLD to NASH transition. Treating *db*/*db* mice with a methionine–choline deficient diet induces fibrosis, mimicking the transition from NAFLD to NASH. Mice which were treated with DGAT2 ASOs had reduced steatosis compared to control *db*/*db* mice, but had worsened fibrosis progression [55]. These data point to a concept of triglycerides being relatively 'inert' storage lipids, with lipids such as DAGs and ceramides actually causing insulin resistance and other lipotoxic pathologies such as NAFLD to NASH conversion.

14. Allostasis, energy balance and insulin resistance — an historical perspective

The term allostasis was first coined in the 1980s. Allostasis encompasses both the processes that control homeostasis and the concept of stability through change. A key aspect of allostasis is that it includes a predictive element to the body's responses to the environment with the brain setting new 'homeostatic' set points based on environmental cues. Examples of cues that can affect allostatic set points in a predictive manner are the circadian clock, which will drive a rise in blood pressure immediately *prior* to people waking and getting out of bed.

Describing diabetes in allostatic terms is in no way new. The original observations that drove the description of allostasis were based on the prevalence of stroke and other cardiometabolic diseases in poor communities [68]. These early studies led to the key observation that diseases such as obesity, hypertension and type 2 diabetes were difficult to explain from a homeostatic perspective, a concept that becomes clearer when considering obesity. It has been noted by many obesity researchers that in the majority of the obese population there is only a very subtle dysregulation of energy balance, amounting to perhaps as little as 9000 kcal per year on average. Given that 9000 kcal represents only 1% of the yearly calorific intake, it is clear that the systems that regulate energy balance are not grossly impaired. However a crucial question is why homeostatic mechanisms for energy balance can fail in this subtle manner. Allostasis attempted to answer this question by focussing heavily on the integration of external environmental cues with the more traditionally recognised cues from feeding circuitry principally located in the hypothalamus. To this end, a large portion of the work on allostasis in both the setting of human society and animal behaviour has focussed on circuits that regulate stress. The central idea behind applying allostasis to metabolic disease is that that our modern lifestyle of high-stress environments leads to dysregulation of the hypothalamic pituitary adrenal axis (HPA axis). This stress in turn leads to a state of hyposatisfaction. The concept of hyposatisfaction suggests that people in stressful environments derive inappropriately low reward from processes such as salt, sugar or fat consumption. This in turn leads them to consume more in effort to be satisfied. The same concept is applied to explain the increased susceptibility of individuals in poor environments to addictions to alcohol and drugs [8].

The idea that the HPA axis can regulate physical attributes such as visceral obesity is very well supported by evidence such as patients with Cushing's syndrome as well as correlations between free cortisol levels and visceral obesity. The connection between dysregulation in the HPA axis caused by environmental factors (such as the stress of modern life) and it being causative of obesity and diabetes should, however, be considered as only one factor leading to the increase in the MetS and obesity. Many other environmental factors correlate very well with the increase in obesity and the MetS, such as free availability of calorie dense processed food and reduction in physical activity. Further evidence that it is an oversimplification to consider only external environmental stress as the driving factor behind obesity comes from comparing mono and dyzygotic twins. Concordance rates for obesity are far higher in monozygotic than dyzygotic twins with about 80% of variance in BMI being accounted for by genetic factors. Of course this argument could be countered by claiming that all twin studies into obesity do is show the underlying genetic susceptibility to the modern environment, i.e. how susceptible to stress you are. Therefore the concordance rates for obesity would actually be concordant with rates for susceptibility to stress. However the heritability for other disorders that allostasis attempts to explain via the same mechanism, such as drug addiction and alcoholism, are much lower (30–50%) [69]. Overall, while it is likely that stress is important, the role of the HPA axis to the development of obesity in the western world should not be overstated. A final point on the relevance of the HPA axis is that most of the data supporting a link between it and the MetS tends to be clinical and correlative. An interesting preclinical study in mice shows that social stress can affect body weight gain in animals [56].

In this article, rather than take the view of considering allostasis in its historical manner we aim to use the concepts of allostasis to consider how physiological systems attempt to rectify metabolic parameters. Allostasis is valuable from this perspective because there is a tendency to think of systems as either 'redundant' or 'compensatory'. In biological terms, redundancy and compensation are problematic ideas. Redundancy is often cited in studies of murine genetic models to explain unexpectedly mild phenotypes. The term redundancy suggests that there are multiple systems that respond to the same physiological cues in the same manner. It is extremely hard to envisage how two systems could evolve that both have exactly the same function. Natural selection would presumable favour one or the other. Instead a 'redundant' system may be one that fulfils a similar role to another but may be designed to operate under different physiological conditions (i.e. chronic vs. acute regulation of a process). This 'redundant' system can perform the same job with similar if not identical efficiency to the original, however, as discussed below this may come at a price. The concept of overlapping but subtly different roles leads onto the second idea of 'compensation'. 'Compensation' suggests that the organism is in some way consciously employing a system to account for reduced function in another. Instead, it is more likely that the apparent compensatory mechanisms that are employed represent systems that are more normally employed in other processes. In pathological states when a normal system is compromised, then a 'compensatory' system may be activated in response to a pathogenic stimulus that mimics another physiological state.

Allostasis is an excellent intellectual framework to consider the concepts of redundancy and compensation, as it already builds in the idea of altered physiological loads and multiple systems. For example, pregnancy dramatically alters many facets of metabolism including, a substantive increase in beta cell function. This means that when the body becomes insulin resistant systems do exist that can allow elevated beta cell function to occur for at least several months. From a pathophysiological point of view allostasis also brings in the concept that using systems that are not deigned to cope with a chronically elevated allostatic load comes at a cost. If an unsuitable system is pressed into service because of a failure in another then, unless the load causing the failure is removed, the system will ultimately fail.

15. At what stage does it make sense to intervene? An allostatic perspective

In this section we will discuss how applying the concept of allostasis may help to inform where it is of benefit to intervene in the treatment of type 2 diabetes, paying particular regard to the concept of lipotoxicity.

In general terms, when trying to intervene in any disease state the fundamental question that arises is at which level should it be targeted. It is possible to envisage treating specific signs of a disease (such as elevated blood glucose in the case of diabetes) or alternatively to try to treat the underlying cause of the disease, which may do little to directly target the principal signs. While not targeting the

principal signs may seem counterintuitive, this approach may ultimately be effective by removing the load driving the disorder. Traditionally, treatments to target type 2 diabetes have only had to fulfil the requirement of treating the cardinal sign of diabetes, that of hyperglycaemia. However, recent evidence has suggested that solely trying to maintain control of glycaemia does not necessarily result in the prevention of cardiovascular outcomes associated with type 2 diabetes. To that end the US Federal Drug Administration has issued new guidelines to drug companies for antidiabetic drugs, requiring them to show improvements in cardiovascular end points (Nature Reviews Drug discovery, February 2009, p. 1). In our opinion, this suggests that therapeutic strategies for diabetes that only target hyperglycaemia are not actually targeting the main underlying pathogenic factors leading to both diabetes and cardiovascular disease (factors including insulin resistance, defective lipid homeostasis and inflammation). With treating underlying causes in mind, any strategy which aims to combat or reverse insulin resistance and type 2 diabetes should consider also the concept of allostasis.

In terms of the development of obesity and its subsequent metabolic complications then there are several potential points for intervention. Clearly the ideal point would be to prevent the positive energy balance that leads to obesity. However, so far, all treatments to try to combat obesity have been ineffectual. With this in mind we now discuss if there are other points in the progression from obesity to type 2 diabetes that may be suitable for intervention and discuss their suitability in terms of allostasis.

When considering the adipose tissue expandability/lipotoxicity hypothesis for type 2 diabetes then we must define the allostatic loads in question. In our opinion, the principle allostatic load that leads to insulin resistance in the context of obesity is a failure in adipose tissue expansion. This in turn causes increased lipid flux to non-adipose organs and leads to pathogenic accumulation of toxic lipids in non-adipocyte cell types, which then causes insulin resistance. To that end any strategy that reduces the availability of metabolically toxic lipids should have therapeutic benefit. Fig. 2 describes the steps leading from positive energy balance to type 2 diabetes and shows where the following potential points of intervention beyond the manipulation of energy balance can be considered to be acting:

1) To prevent a failure in adipose tissue expansion
2) Once adipose tissue expansion has failed to oxidise lipids that spill over into the circulation, preventing ectopic deposition.
3) Preventing the accumulation of lipids in non-adipose organs
4) To promote storage of ectopic lipids in a less damaging form within cells
5) In the event of insulin resistance occurring to prevent glucolipotoxic effects on beta cells and maintain elevated beta cell function.

15.1. Prevention of a failure in adipose tissue expansion

In theory a dramatic and substantial increase in the capacity for adipose tissue to expand may prevent the development of metabolic complications. Surprisingly, there is a mouse model representative of such a state, the adiponectin-TG *ob*/*ob* mouse. The AdTG mouse possesses nearly twice the adipose tissue mass of a normal mouse, but in spite of this is far more insulin sensitive and does not develop fatty liver. In the case of the AdTG mouse model and as a treatment strategy in general, the allostatic load on cellular function caused by having to deal with toxic lipids is ameliorated by storing lipids in a safe and physiologically appropriate location — adipose tissue [37]. This strategy has obvious benefits in terms of metabolic health and is, on a more limited level, one of the principal modes of action of the thiazolidinedione class of insulin-sensitizing drugs [70]. From an allostatic perspective, increasing adipose tissue storage capacity is

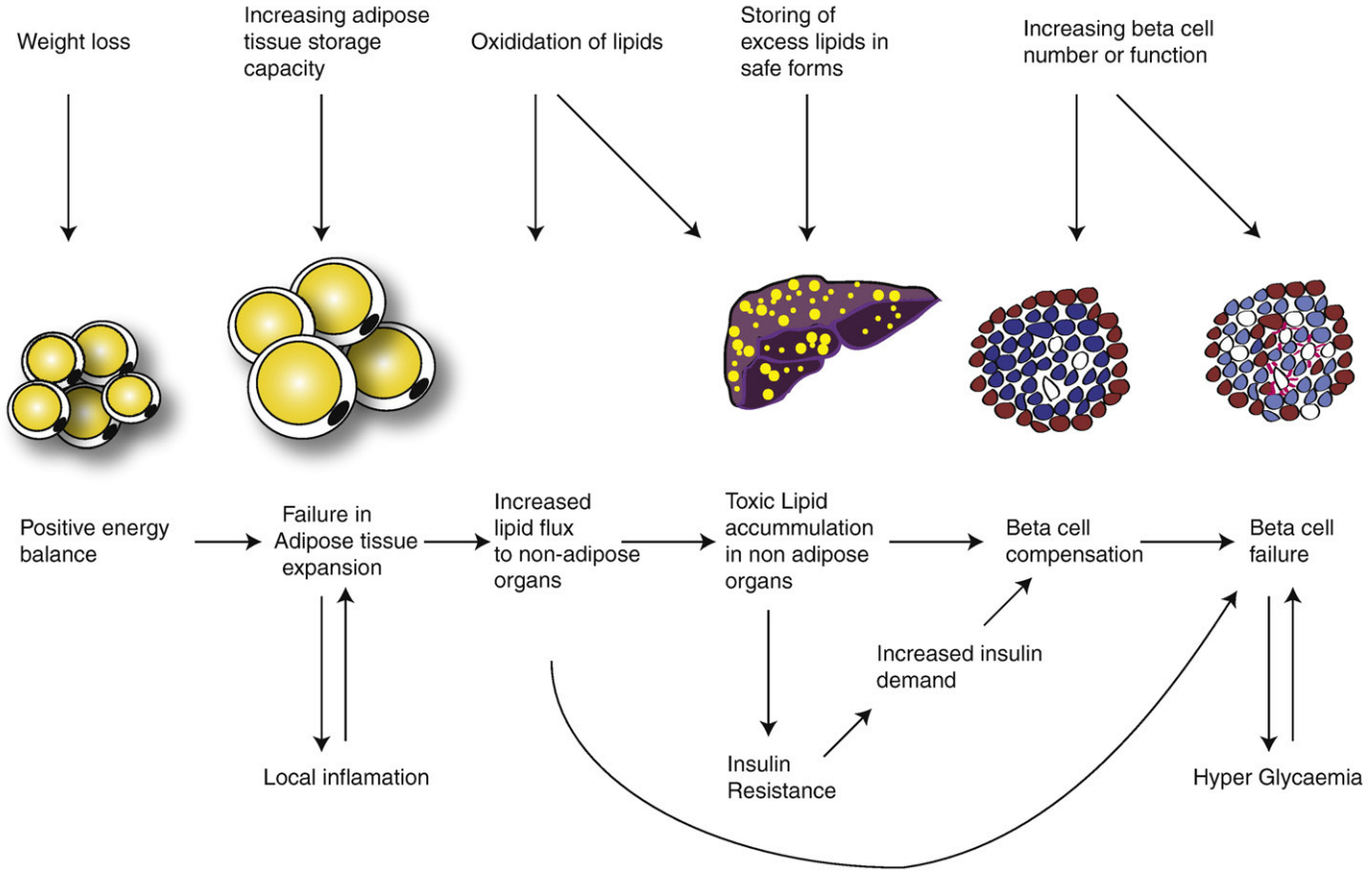

Fig. 2. Diagram outlining how positive energy balance leads to type 2 diabetes, with the principal points for drug intervention indicated above.

attractive as it removes delivery of lipids to non-adipose organs, which we believe is the principal driving factor behind multiple metabolic complications including insulin resistance, NAFLD, adipose tissue inflammation and demand on beta cell expansion. However, one caveat regarding expanding adipose tissue indefinitely is that there would be both aesthetic and mechanical considerations to being morbidly obese.

15.2. Removing excess circulating lipid

From an allostatic perspective the strategy of removing excess circulating lipids is particularly attractive. When adipose tissue expansion fails then insulin can no longer suppress lipolysis from adipose tissue or appropriately promote lipid uptake [17]. If lipids can be eliminated before they are able to accumulate in other organs then it should be possible to prevent lipotoxic effects. The only realistic method of eliminating excess lipids will be to oxidise them. Increasing rates of beta oxidation in rodent models has been highly successful for conferring protection against diet induced obesity and metabolic complications. Most hypermetabolic models tend to be lean, which leads to a question as to whether metabolic improvements from increased b-oxidation rates are primary or secondary to reduced fat mass. One recent study used weight matched animals over expressing mitochondrial uncoupling protein 3 (UCP3) in muscle and demonstrated that these animals had improved insulin sensitivity, suggesting the higher metabolic rate was affecting insulin sensitivity independently of body mass [51]. It is worth noting here that increasing metabolic rate seems to result in weight loss in almost all animal models with this phenotype. Why animals with higher metabolic rates do not simply eat more to increase their body weight back to normal is unclear, but maybe an added benefit of increasing metabolic rate. Overall, increasing metabolic rate has great value from an allostatic point of view. Removing toxic lipids from circulation directly prevents their accumulation in non-adipose organs. Secondly increasing metabolic rate reduces body weight. Reducing obesity, which is the driving factor behind the failure in adipose tissue expansion and thus the increased ectopic lipid load, should allow adipose tissue to regain its appropriate role in maintaining daily lipid homeostasis. Finally activating oxidation does not lead to aesthetic complications associated with increasing adipose tissue expansion alone.

In humans exercise training has been shown to lead to improvements in a vast array of metabolic parameters. Longitudinal studies of patients have shown in large cohorts that exercise and diet are more effective than drugs such as metformin alone. These studies are in part confounded by a weight loss element present in most of these studies, as lifestyle interventions usually include a component of diet as well as exercise. Regardless, exercise and diet has been shown to be more effective than metformin in terms of progression from IFG to diabetes [71]. Short-term exercise, without a significant weight loss component has also been shown to improve insulin sensitivity [50,72]. Furthermore the effects on improving insulin sensitivity may not be apparent as simply a reduction in circulating lipid levels, but instead manifest themselves in a reduction in insulin resistance-inducing lipids accumulated in muscle.

Pharmacologically, there is some evidence for increasing mitochondrial oxidation being beneficially, at least for weight loss. In the 1930s the antiobesity drug Dintrophenol (DNP) was used with high efficacy. DNP works by artificially uncoupling mitochondrial oxidative phosphorylation from ATP production. DNP use was discontinued over safety fears due to potential for fatal overdoses and the fact it caused cataracts in some patients.

Recently a new focus on the role of brown adipose tissue in humans has highlighted a potential safer alternative to chemical uncouplers. Brown adipose tissue expresses UCP1, an uncoupling protein that acts much as DNP does. However UCP1 on a molecular level and brown adipose tissue on a physiological level are subject to high degrees of regulation and therefore drugs that increase BAT mass/activity should be a safer alternative to chemical uncouplers.

16. Preventing lipid accumulation in non-adipose organs

In theory preventing organs such as liver from accumulating lipid could have some value. The issue would be what would be the fate of the lipid that was not accumulated in non-adipose organs? An example of the issues that could occur can be seen in hepatocyte-specific PPARγ KO mice crossed onto an *ob*/*ob* background. Mice lacking PPARγ in liver on an *ob*/*ob* background do not accumulate fat in their livers and as a result have improved hepatic insulin sensitivity. However, on a whole-organism level the mice have worsened insulin sensitivity, suggesting that the ectopic lipids that would have accumulated in liver may have been redirected elsewhere, worsening insulin resistance in other organs [57]. From an allostatic perspective, ectopic lipid accumulation in non-adipose organs may actually be a mechanism to cope with the lipid overload. It seems likely that not all organs respond to excess lipid equally well. As described above, improving hepatic insulin sensitivity by blocking lipid accumulation actually worsened whole-organism insulin sensitivity. This suggests that lipid accumulation in liver may be protective and actually be an allostatic mechanism that acts to protect organs such as muscle and pancreatic islets. It is also important to note that blocking ectopic lipid accumulation in non-adipose organs will not reduce the overall allostatic load and by removing a system that allows the body to cope with excess lipids may actually accelerate the aetiology of the disease.

17. Storage of ectopic lipids in less harmful forms

There is increasing evidence that the type of ectopic lipid accumulated in tissues is more important than the amount of lipid accumulated. To that end it is possible to envisage treating insulin resistance by driving lipids in ectopic depots into safe storage forms (such as TAG) and away from more harmful forms, such as ceramides, DAGs and LPCs. Redirecting lipids into safer storage forms has already been shown to be beneficial in muscle [44] and conversely preventing storage in the form of TAG by reducing DGAT2 levels in liver causes increased toxicity in terms of increased hepatic fibrosis in a model of NASH [55].

Increasing TAG formation in organs such as liver (seen as hepatosteatosis) can also be viewed as an allostatic mechanism. While accumulating TAG in organs is not desirable, the cellular systems that allow lipid droplet formation are in place in organs such as liver. Chronic activation of TAG storage appears to prevent the build up of more harmful lipid.

From an allostatic perspective, altering the types of lipid accumulated in non-adipose organs has some potential value as it should reduce the rate at which lipotoxic insults occur. An outstanding question is as to whether capacity for locking lipids up in less toxic forms such as TAG will eventually be exhausted. As it seems likely that the capacity for non-adipocyte lipid storage, even in safer forms such as TAG, must ultimately be limited it is perhaps not an ideal strategy to pursue in the long-term, but may provide a certain degree of buffer-capacity prior to implementing other therapeutic strategies.

18. Preventing glucolipotoxic effects on the beta cell

There is a large body of evidence that TG accumulation in beta cells leads to beta cell failure. Whether the TGs themselves are toxic, or if they are markers of the accumulation of other harmful lipid species such as ceramides is more debatable, but some evidence does point to TGs being directly toxic. Overexpression of DGAT1 in isolated islets (analogous to over expression of DGAT1 in muscle) dramatically reduced islet function while increasing islet TG levels [58]. Furthermore, blocking lipid uptake into islets via ablation of LPL has been shown to impair

glucose stimulated insulin release [59]. All of these processes suggest that fatty acid metabolism is essential for normal islet function, thus trying to blockade these processes may be counterproductive. Conversely, one study has demonstrated that specifically reducing ceramide production by using L-cycloserine reduced apoptosis and ceramide levels by 40–50% in the islets of Zucker *fa*/*fa* rats.

From an allostatic perspective there is likely to be little cost to the organism in preventing lipotoxic effects in islets. Unlike blocking lipid accumulation in liver, which results in repartitioning to other organs, blocking lipid accumulation in islets will not lead to a greatly increased lipid load on other organs. The question does remain, as with any strategy that looks to ameliorate the effects of lipotoxicity at such a late stage, as to whether blocking ceramide or other toxic lipid from accumulating will be an effective long-term strategy, or merely delay the disease progression until allostatic overload increases to a point where the treatment is no longer effective.

While altering lipid species is one approach to treat diabetes by targeting the beta cell, there are at least several others. The two major classes of antidiabetic drugs that target the beta cell aim to either increase beta cell mass or to promote elevated insulin secretion (function). Elevated beta cell function and beta cell hyperplasia are both allostatic mechanisms activated in response to the need for increased insulin production that occurs in insulin resistant states. In the case of established type 2 diabetes the insulin resistance occurs in multiple organs in a chronic manner, as opposed to physiological states of insulin resistance such as pregnancy which are relatively acute. The demand for increased insulin can be met by increasing beta cell function, which is the principal mode of action of the sulphonylurea class of antidiabetic drugs. From an allostatic perspective increasing beta cell function has some value, but also raises some concerns. Increasing insulin secretion reduces blood glucose, removing some of the pathogenic load on the beta cell. However, increasing insulin secretion alone may cause other toxic processes to occur. The unfolded protein response (UPR) is essential to beta cell function and is activated when the demand for insulin production increases physiologically (such as after a meal). However some evidence now shows that chronic activation of the UPR can cause beta cell failure and that sulphonylureas can actually be toxic to beta cells over a prolonged period of time due to this chronic activation of the UPR [60]. In humans sulphonylureas have been shown to have some efficacy in preventing IFG to diabetes transition, but these effects appear to be, as expected, relatively short term [70]. Other newer antidiabetic drugs that in part target the beta cell, such as the incretin analogues, may be more beneficial as they both stimulate beta cell hyperplasia as well as insulin secretion. Additionally drugs targeting the incretin system, such as Exenatide, have been shown to promote weight loss, reducing the allostatic load caused by demand on adipose tissue function. From an allostatic perspective incretin analogues are clearly a more attractive treatment when compared to sulphonylureas. However, it is doubtful if there is limitless capacity to expand beta cell mass and furthermore targeting beta cells will not alleviate the underlying allostatic load of failed adipose tissue function and lipotoxicity induced insulin resistance. From an allostatic perspective, treatments that exclusively target the beta cell are perhaps less suitable than those that try to treat the underlying causes that create the demand for insulin.

19. Other treatment modalities

One of the most commonly used antidiabetic drugs is metformin. While the mode of metformin action remains somewhat controversial it is now largely believed to act via activating AMPK. In humans the beneficial metabolic effects of metformin seem to be largely focussed on reducing hepatic glucose output [71]. In rodents, AMPK activation by compounds such as AICAR have shown beneficial effects in multiple organs including adipose tissue, muscle, liver and brown adipose tissue. One possible reason for the more limited spectrum of tissues affected by metformin comes from studies suggesting that cellular uptake of metformin is mediated by the Oct8 transporter [73]. Oct8 is expressed almost exclusively in liver and kidney. From an allostatic point of view metformin would not necessarily be expected to be particularly beneficial in the longer term, as it would only suppress glucose output without necessarily ameliorating issues such as failed adipose tissue storage. Perhaps surprisingly metformin has been shown to be effective over long periods [74]. It is worth noting that metformin does appear to cause a small but significant reduction in body weight, which may help to contribute to its prolonged efficacy. It is also worth noting that during a washout period in the Diabetes Prevention Program Outcomes Study that occurred between the first and second phases of this study, there was a spike in the incidence of diabetes on patients previously treated with metformin that did not occur in the lifestyle intervention group [75]. This result suggests that the removal of metformin's suppressive effects on blood glucose unmasked an underlying worsening of other systemic factors leading to insulin resistance.

Another class of drug with considerable therapeutic potential are Sirt1 inhibitors. Drugs such as resveratrol and srt1720 activate the histon deacetylase Sirt1. Sirt1 activators, in rodents at least, lead to a multifaceted improvement in metabolic profile, increasing energy expenditure, reducing weight, increasing muscle and brown adipose tissue fatty acid oxidative machinery and promoting insulin sensitivity [76–78]. From an allostatic perspective Sirt1 activators are very attractive candidates for treatment of diabetes given that they target the obesity to diabetes transition at multiple points and critically alleviate allostatic load.

20. Conclusions

Overall this article discusses how a state of positive energy balance can lead to insulin resistance via a principle failure in the body's ability to store excess lipid appropriately in adipose tissue. We attempt to rephrase these processes, which have been reviewed extensively elsewhere, in the context of allostasis. Thinking of the steps leading from the initiation of positive energy balance through to ultimate beta-cell failure and Type 2 diabetes in terms of a series of processes being activated to try to maintain glucose homeostasis is particularly valuable when considering rational treatment strategies.

In our opinion the therapeutic strategies should not only try to correct the manifestation of the disease such as hyperglycaemia or dyslipidaemia. Instead, we think there is an added benefit to considering the underlying causes of a disease. The load on the systems that regulate blood glucose lead to series of pathophysiological manifestations, some of which may be markers of the underlying allostatic load, such as dyslipidaemia, whereas others may represent allostatic mechanisms that are trying to protect the organism from the worst effects of the pathological state, such as TAG accumulation in liver. Therefore, there may be long-term benefit in modifying these allostatic mechanisms, even if the short-term benefits may not appear to be obviously related to the disease. Treating hyperglycaemia has obvious value in that it reduces malaise. However, from an allostatic point of view, if treatments that target hyperglycaemia do not also address the underlying cause of the disease state, these treatments are likely to only have short-term value. If underlying causes of diseases are not addressed eventually allostatic overload will exceed the capacity of treatments to ameliorate symptoms.

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