A long journey to effective obesity treatments: is there light at the end of the tunnel?

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Abstract

As the obesity epidemic continues, more Americans are getting fatter, having more weight-related problems such as cardiovascular disease, and are experiencing new metabolic dysfunctions. For over 50 years, the adipose tissue (AT), commonly referred to as fat, has been of interest to academic and clinical scientists, public health officials and individuals interested in body composition and image including much of the average public, athletes, parents, etc. On one hand, efforts to alter body shape, weight and body fat percentage still include bizarre and scientifically unfounded methods. On the other hand, significant new scientific strides have been made in understanding the growth, function and regulation of anatomical and systemic AT. Markers of transition/conversion of precursor cells that mature to form lipid assimilating adipocytes have been identified. Molecular 'master' regulators such as peroxisome proliferator-activated receptor gamma and CCAAT-enhancer-binding proteins were uncovered and regulatory mechanisms behind variables of adiposity defined and refined. Interventions including pharmaceutical compounds, surgical, psychosocial interventions have also been tested. Has all of the preceding research helped alleviate the adverse physiologies of overweight and/or obese people? Does research to date point to new modalities that should be the focus of efforts to rid the world of obesity-related problems in the 21st century? This review provides a general overview of scientific efforts to date and a provocative view of the future for adiposity.

Keywords: obesity, adipocyte, differentiation, dedifferentiation, lipid metabolism, adipogenesis

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Introduction

The obesity pandemic is growing at an alarming rate with 2009 estimates of US$60 billion per year of medical costs in the USA alone.[1] Obesity has both personal and economic costs.[2] Obesity increases the risk of diabetes, heart disease, fatty liver and certain types of cancer[3,4] and the inter-relationships of these co-morbidities, referred to as metabolic syndrome, point to the complexity of this issue.[5] Obese adults and children appear to have lower quality of life ratings due to health related issues indicating psychosocial impact as well. Thus, the human and economic costs of obesity and its co-morbidities have, by necessity, resulted in vast research efforts in lipid and energy metabolism with additional heavy research emphasis on insulin function. Despite the impressive increase in understanding of various aspects of lipid metabolism, definitive treatments to combat obesity and its co-morbidities have not emerged. Remediation efforts for long-term, safe and sustained weight loss have not been very efficacious,

independent of the type of intervention. The most scientifically valid strategy to avoid obesity is currently prevention; the 'don't get fat' concept. But this has done little to address the crisis faced by millions who already have excess body fat and body weight.

Physiological processes to promote energy balance have been targeted to expedite the process of fat/weight loss in the fight against obesity through various weight loss protocols and medications. While not covered in this review, surgical, medical, physical activity and psychosocial interventions also have an extensive range of applications, but few prove to be successful in maintaining healthy weight and fat composition long term.[6] Therefore, the purpose of this review is to first highlight the various pharmacotherapy intervention strategies to remediate obesity,[7] including major lipid metabolism-related processes that may be regulated (include food intake [usually centrally acting anorectics or severe caloric restriction], fat digestion and absorption inhibitors, and direct regulation of adipogenesis, lipogenesis and lipolysis). Then some innovative treatment strategies based on bioactive edibles and more recent developments in adipose tissue (AT)/fat cells biology are discussed.

Current treatments for obesity

Historically, antiobesity medications have been plagued with either ineffectiveness or significant side-effects that warrant discontinuing the treatment.[8,9] For example, excessive thyroid hormone or amphetamines were previously used to treat obesity. Heart disease was a significant side effect of the drug combination fenfluramine and phentermine resulting in withdrawal of the medication.[10]

Physiological processes involved in lipid deposition and break down are necessary for energy balance and energy availability in humans and animals. Excess energy intake eventually causes excessive storage of lipids and dysregulation of lipid metabolism and development of obesity.[11] While excess energy intake is the starting point of obesity, obesity appears to result from a complex relationship between energy and fat intake, blood glucose, insulin and metabolic memory of fat synthesis.[12] Classical pharmacological approaches for obesity treatments include drugs that inhibit food intake through the central nervous system and compounds that block the absorption of lipids in the gastrointestinal tract. In some cases during drug discovery for centrally acting antidepressants and other psychosomatic compounds, workers noted that such compounds also affected food intake. This led to further work with such compound as food-intake regulators.[13,14]

Pharmacological approaches to prevent excess fat deposition would be an obesity prevention strategy rather than a treatment and have been conducted in farm animals. Extensive work in regulating lipid metabolism was done with immature, growing farm animals in an effort to decrease fat deposition.[15] These studies have provided much needed insight into AT growth regulation, and have resulted in successful strategies to maximize lean tissue growth while minimizing adipose accretion in growing animals. Currently used strategies include dietary manipulation, genetic selection, and environmental optimization. In addition, this research led to the development of pharmaceutical compounds, including growth hormone (GH) and beta-adreno-receptor agonists (BAA), for the promotion of lean tissue growth while limiting adiposity. Epinephrine was implicated early in activating lipolysis. This endogenous catecholamine/neurotransmitter, a BAA, binds to membrane G protein-coupled adrenergic receptors (GPCRs) and activates hormone-sensitive lipase (HSL) and perilipin via cAMP action.[12,15,16] Perilipin is bound to lipid droplets; HSL is translocated to lipid droplets followed by adipose triacyl-glycerol lipase (ATGL). The combination of these enzymes results in the break-down of triacylglycerides and the release of non-esterified fatty acids into the blood stream.[16] Isoproterenol, clenbuterol, and ractopamine are synthetic/exogenous BAA and demonstrate strong lipolytic effects when administered to animals.[17,18] However, these BAA compounds also attenuate *de novo* fatty acid synthesis via transcriptional regulation of fatty acid synthase.[19] In combination, these pharmacological effects of GPCR-ligands/beta-agonists lower fat deposition in growing animals and stimulate net protein deposition in skeletal muscle.[15,20] Even in growing animals, these BAA lose effectiveness within 3–4 weeks likely through beta adrenergic receptor down-regulation upon chronic stimulation.[19] While BAA may be useful for short term regulation of lipid deposition in growing animals, the use of highly potent BAA for any long-term human applications to remediate obesity would appear to be too risky and is not approved by the US Food and Drug Administration (FDA).

Similar to BAA, administration of GH to growing, finishing pigs resulted in increased lipolysis, attenuation of *de novo* fatty acid biosynthesis and increased lean gain. Surya *et al.*[21] utilized various modes of GH administration in a short-term study to evaluate rates of lipolysis and hepatic glucose production in obese subjects. They found that only pulsatile GH administration regimen would augment rates of lipolysis. In addition, GH had no effects on hepatic glucose production, but pulsatile and continuous GH infusions both impaired insulin sensitivity. These results indicate that human responses to exogenous GH administration mirror those reported in animal studies, but do not lead to any immediate long-term treatment paradigms.

Effective long-acting drugs have been eagerly searched out and developed, often with disappointing results. Lack of efficacy or unacceptable side-effects have derailed many of these drugs in translational work/clinical trials.[22] The potential of developing effective and safe centrally acting antiobesity drugs has received wide attention and major discovery and development research investments by pharmaceutical companies. To date, obesity medications are not cures and should only be considered treatments. Furthermore, information to date suggests permanent consequences to AT growth even after 'treatment' which implies we may be limited to prevention and treatments without the possibility of a cure. Hence issues of long-term efficacy, safety and minimal deleterious side-effects after

prolonged use have been primary factors in the translation of discovery research to clinical practice.

The drug orlistat, which inhibits fat absorption, has been for several years the only remaining drug approved by the FDA for long-term use in the treatment of obesity (for newer compounds, see below). Orlistat provides modest reduction of weight gain but side-effects including blood pressure lowering and negative implications of undigested fat in the gastrointestinal tract mean this medication is not appropriate for many individuals. The effect of this drug specifically on the gut microbiome has not been extensively evaluated which may have significant implications as new research suggests that the gut microbiome differs (shift from bacteroidetes to firmicutes) between mice fed on high- or low-fat diets.[23] Curiously, preventing fat absorption to decrease energy uptake as antiobesity pharmacotherapy may result in similar, but yet unexplored effects on gastointestional bacteria akin to high-fat diets *per se*. Thus, with the apparent limited application of orlistat the urgent need for new therapeutic approaches to treat obesity remains.[24]

Initial excitement for the potential use of leptin as a cure for obesity was tempered by the lack of effectiveness in much of the obese population. Once the role of leptin in food intake was established,[25] extensive work has been done with adipokines such as leptin and adiponectin and the brain leptin receptors. These studies have clearly outlined the role of such key adipokines in energy metabolism and food intake;[26] however, translation of such knowledge to clinical strategies has not been easy. For example, simple administration of leptin has not been fruitful in overcoming energy balance dysregulation.[22] Bray[27] and Heal *et al.*[22] have reviewed the efficacy and the safety of available antiobesity drugs and newly emerging drug candidates including neuropeptide Y (NPY), Y2, Y4 agonists, monoamine reuptake inhibitors, melanin concentrating hormone1 receptor antagonist, MC4 agonists, amylin-leptin agonists, ghrelin agonists, 5-HT 2C agonists, 5-HT6 antagonists, combination therapies and others. As such, these are not reviewed in this paper.

Initial data in rodents suggested that a combination of leptin and amylin (also known as islet amyloid polypeptide) together induced substantial weight loss by acting on appetite and energy expenditure.[28–30] The aim now is to evaluate the safety and long-term efficacy of this combined therapy because both leptin and amylin have been shown to affect the cardiovascular system. Indeed, a combined treatment of rats with leptin and amylin reduced blood pressure, an effect that is more specific to leptin.[31]

In summary, the modes of action of candidate drugs reviewed here are not completely elucidated and issues of efficacy and the development of long-term side-effects are relatively common.[22,27] Unfortunately, many more candidate drugs fail clinical trials and are removed from potential use than those that remain for development.[22,27]

Potential new treatment strategies

While the classic target of reducing net energy availability have not provided successful treatments of obesity, better molecular understanding of AT now provides us with alternative targets. The AT research field was markedly altered since the discovery of leptin and other adipokines, qualifying AT as an endocrine organ. Since then, a number of new factors and mechanisms have been identified, providing different pharmaceutical targets.

AT-derived adipokines and cytokines

The endocrine secretions of AT include numerous proteins. Some of these, including leptin and adiponectin affect food intake and energy homeostasis in multiple tissues while others, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, induce recruitment of macrophages into AT by largely unknown mechanisms. As such, mechanisms involved in maintenance of normal energy homeostasis are linked to obesity, inflammation, insulin resistance and the metabolic syndrome. While many additional details about the cross communication/crosstalk among adipocyte-released adipokines, chemokines and target tissues are emerging, we have not exploited these findings for the development of effective therapeutic agents against obesity and associated morbidities. Moreover, once established, obesity cannot really be cured but only treated. Importantly, the roles of many identified compounds have not been elucidated and may provide the essential key needed for successful obesity treatment.

Targeting the adipocyte

Successfully targeting the adipocyte to prevent adipose accumulation requires understanding of AT development and expansion. It is well known that AT growth during obesity occurs through both adipocyte hypertrophy and hyperplasia,[32,33] which provides opportunities to limit AT expansion at the cellular level. In addition, increasing adipocyte apoptosis re-programming of adipose progenitors to become brown-like adipocytes capable of oxidizing fatty acids provide intriguing possibilities.

Although the transcriptional cascade controlling adipogenesis has been well characterized over the past two decades and the mechanisms by which key adipogenesis regulators such as peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer binding protein α (CEBPa) act are now being fully elucidated,[34,35] only a little is known about adipocyte hyperplasia during normal AT growth and in diet-induced obesity. A new area of research on AT expansion is now emerging with the characterization of adipocyte progenitor cells. The factors that control the recruitment and the proliferation of these cells in response to excess nutrients are not known. Indeed, in the last four years, many studies have identified a stem cell population within white AT that could differentiate to mature adipocytes both *in vitro* and *in vivo*.[36,37] These cells were shown to proliferate within white AT depots in response to excess nutrients,[38] but the factors or the cells that impact their expansion are unknown. It is also unknown if the characteristics of human adipose progenitors are the same as in mice and if their response to excess nutrients is the same. Understanding the mechanisms

that regulate adipose progenitor cell proliferation/recruitment should provide additional targets that may be useful in the prevention, rather than the treatment, of obesity.

Other cell types within AT

Historically, AT was considered homogenous, but is known to consist of several different cell types – all that contribute to the respective transcriptome and proteome of the tissue. Mature adipocytes, stromal vascular cells (fibroblasts, preadipocytes and non-differentiated mesenchymal stem cells), endothelial cells, macrophages, and other undefined cells are present in varying percentages and in a depot-specific manner.[39] Adipokines, cytokines, and chemokines are produced and released by these different cells for communication and interaction among these cells and with other tissues.[40] Recently, the agouti signaling protein, a secreted protein linked to obesity,[41] was detected in mature adipocytes as well as in non-lipid containing cells within connective tissue.[42] Preadipocytes were shown to play a pivotal role in the development of insulin resistance via increasing cytokine/chemokine expression.[40] To better understand the role of adipokines in AT and in obesity, both the secreting cell and the target cell needs to be identified. Of late, numerous studies have indicated mature adipocytes could dedifferentiate spontaneously, *in vitro,* and the subsequent progeny cells (fibroblast-like) had the ability to re-differentiate into lipid-assimilating fat cells.[43–47] These studies indicate that mature adipocytes are not the terminally differentiated cell type once thought and they might be one of the precursor cells committed to repeated adipogenesis. Yet, the dedifferentiation phenomenon of mature adipocytes is still being explored *in vivo* and the regulation of the cellularity of mature adipocytes has not yet been examined. It remains to be shown whether it is possible to induce mature adipocytes to lose their lipid and prevent their progeny cells to re-assimilate lipids. Moreover, as recent evidence suggests, progeny cells may serve as a source of stem cells for a variety of applications.[48,49]

Targeting skeletal muscle: AT and muscle interaction

Special attention has also been given to developing progenitor cells within and between muscles. Studies have shown a positive correlation between the amounts of AT interspersed around and between skeletal muscle (determined with computed tomography) and insulin resistance.[50] In contrast, no similar relationship was found between subcutaneous AT and insulin sensitivity in the same individuals. The cellular composition of skeletal muscle is dominated by myocytes, which are heterogeneous in metabolic and contractile characteristics. Interactions between different cell types in the tissue – especially between adipocytes and myocytes – involve numerous factors with autocrine/paracrine and endocrine action. It is well established that adipocytes act as secretory cells[51] and myocytes produce and release a variety of bioactive compounds induced by contraction.[52] Adipokines and myokines facilitate bidirectional cross-talk between AT and skeletal muscle but also with other organs and tissues.[53] Trayhurn *et al.* noted that several proteins identified as adipokines are also components of the myokinome.[53] Therefore, they established the term 'adipo-myokine' for these proteins. Examples are IL-6, IL-8 and monocyte chemoattractant protein-1 (MCP-1), all having different functions depending on the physiological situation (exercise, lack of physical activity, inflammation, etc.). Processes such as lipolysis and insulin sensitivity are controlled by myokines and adipokines. Several adipokines and myokines are suggested to have reciprocal effects[54] and are involved in the recruitment of muscle stem cells into the adipogenic lineage and *vice versa*.[55]

Molecular mechanisms involved in the conversion of muscle stem cells into the adipogenic lineage include, among others, PPARs as well as muscle-derived factors like myostatin.[54] On the other hand, the activation of a myokine, called irisin, by the transcription factor peroxisome proliferator-activated receptor gamma coactivator-1α[56] can stimulate of white AT to metabolically active AT designated 'beige' AT.[57] Beige adipocytes express low basal levels of mitochondrial uncoupling protein (UCP-1) but express high levels of UCP-1 and exhibit high respiration rates following cyclic AMP stimulation.[57] Furthermore, the differentiation of beige adipocytes protects prolactin receptor-deficient mice from high-fat-induced obesity.[58] Irisin induces changes in AT s by inducing beige adipocytes which have thermogenic function, making irisin a promising target for the development of therapeutic strategies against obesity. Further study of factors that regulate beige adipocyte differentiation *per se* is a promising area of research that may impact the study of obesity.

Alternative approaches for a cure/obesity intervention

Long-term weight gain may be accounted for by a small extra increment of daily energy intake above daily requirements without any compensating exercise bouts.[27,59] This input/output model may not be the complete answer to obesity in its later clinical manifestations. It is apparent that, while challenging, simply targeting input and output with pharmaceutical interventions may be inadequate. Just as it took years of seemingly inconsequential calorie excess to accumulate the weight, losing the weight by seemingly insignificant caloric reduction should be considered as a long-term proposition as well. Despite extensive explorations into long-term energy balance mechanisms in humans and animals, we appear not to fully understand how to prevent this slight, persistent creep-up effect in food intake except via exceptional discipline. Recent work has shown that longterm, consistent increases in food intake apparently results in extensive metabolic re-modeling in both subcutaneous and visceral AT establishing a state of obese adipose, characterized by the development of blood vessel networks, increase in extracellular matrix, inflammation, cytokine secretion and cross talk with other tissues.[60,61] This would likely result in a more complex clinical state where loss and gain of fat depots is not simply a function of energy balance. One may speculate that the process of obesity development may include

counterbalancing between various central and peripheral tissues regulatory systems involved in energy balance.[60,61] Thus, it would appear that a much more successful approach to weight gain, obesity and associated morbidity issues requires either comprehensive approaches or prevention. A model for a pharmacological prevention approach may be the use of BAA in growing (farm) animals to counteract fat gain in the first place. In pigs, this is accomplished by lowering fat synthesis and repartitioning dietary energy to lean tissue deposition.[19,62] Preventive tactics such as local or statewide ordinances or legislation specifying serving sizes of fast food items and contents of food items are likely to meet stiff opposition because of privacy rights and constitutional protections against unwarranted interference by governments into private decisions. This approach is presently utilized in New York City, NY on some food/fast-food items; however, outcomes of such governmental actions remain unknown.

New role of edibles/diets in obesity prevention and remediation: bio-active compounds

The discovery of bio-active molecules in certain spices, nuts and other edible plant materials provided new opportunities for orally active antiobesity agents. Most of the bioactive compounds are able to inhibit adipogenesis and/or lipogenesis leading to reduced adiposity and inflammation as described below. For example, Park *et al.*[63] have recently reported that piperine, a component of black pepper, inhibits adipogenesis by antagonizing PPARγ activity in cultured 3T3-L1 cells. Commonly seen in pharmaceutical compounds, some bioactives appear to have greater efficacy *in vitro* than *in vivo*. Nonetheless, another pepper compound, capsiate, has shown small effects when consumed by people[64] and appears to act on very different pathways than those previously targeted. This compound appears to target the transient receptor potential vanilloid subtype 1 (TRPV1) receptors in the gastrointestinal tract but not those associated with taste perception found in the oral cavity. In addition, bioactive substances such as omega-3 polyunsaturated fatty acids or polyphenols have proven their efficacy on reducing adiposity and AT inflammation when used in mice fed a high-fat diet.[65–67] The exact mechanism by which these compounds reduce adiposity is not completely understood but their effect on inflammation and metabolic homeostasis is believed to be mediated (at least in part) via the antioxidant properties of these molecules. Central nutrient sensing mechanisms, such as intermediates in fatty acid biosynthesis, may be an additional potential approach of controlling obesity. For example, Lane and co-workers have reported studies on hypothalamic malonyl-CoA and carnitine palmitoyltransferase 1 in the putative treatment of obesity.[68] Unfortunately, previous drugs affecting food intake and absorption are generally ineffective long term regardless of whether the target is food intake, nutrient uptake or metabolic disruption. However, a few dietary supplement ingredients have been documented to have direct/different mechanisms of action that affect adipocyte differentiation, activation and lipolysis without resorting to stimulants. For example, rats overfed medium chain triglycerides for six weeks showed significantly decreased overall weight, fat pad weight and adipocyte size (but similar adipocyte number) compared with a cohort fed a similar amount of fat as long chain triglycerides.[69] In addition, composition of dietary macronutrient ingredients may affect adiposity through alternative processes such as increased thermogenesis and fatty acid oxidation – similar to the Atkins diet (high fat-pro-ketosis diet) to promote a fat reducing state.[70] Medium chain triglycerides (C_8 and C_{10}) have been used for years as an alternate (and rapid) energy source for parenteral and prenatal nutrition but have also been tested for weight loss.[69,71–81] Table 1 lists some recent ingredients and putative mechanisms with human clinical evidence.[69,71–91] In the absence of more novel approaches to remediate obesity than hitherto applied, the only options to avoiding obesity is disciplined eating or addressing the obesity issue with long-term, calorie-restricted weight loss programs and avoidance of the rebound effect.

Updates to antiobesity drugs with a link to diabetes

Weight loss is especially important but also more challenging for subjects with type 2 diabetes, than those without diabetes.[92] Current guidelines from the American Diabetes Association recommend that individuals with type 2 diabetes achieve modest weight loss (5–7%) to improve glycemic control and reduce cardiovascular risk.[93] Thus, weigh loss is especially important and can help patients with type 2 diabetes. While lifestyle interventions (caloric reduction and physical activity) can help achieve healthy weights, effective weight loss has been more consistently observed in individuals using antiobesity medications. Such drugs are typically prescribed to obese patients with a body mass index (BMI) $>30\,kg/m^2$ or to overweight patients with BMI $< 30\,kg/m^2$ with at least one co-morbid condition. Most antiobesity medications affect body weight via biological mechanisms that suppress appetite and a few of these drugs affect those mechanisms that regulate nutrient absorption. Major antiobesity drugs include pramlintide, AOD9604, oleoyl-estrone, trk-beta antagonists and melanin concentrating hormone and the newly approved drugs, phentermine/topiramate (Qsymia™, VIVUS, Inc., Mountain View, CA, USA) and lorcaserin (Belviq®, Arena Pharmaceuticals GmbH, Zofingen, Switzerland), all of which can reduce obesity and to some extent reduce some co-morbid conditions.

Until recently, very limited options for obesity treatment have been available. The FDA recently approved two new medications for weight loss. The first, Belviq (lorcaserin HCl) was approved in June 2012, and second, Qsymia™ (phentermine and topiramate), was approved in July 2012. These two drugs are the first to be approved in 13 years. Lorcaserin is a selective serotonin receptor agonist that works specifically on appetite signals in the brain. A recent double-blind placebo-controlled randomized trial

Table 1 Sampling of new dietary supplement ingredients for weight loss products with adipocyte mechanisms of action

Ingredient	Mechanism	Human study results	References
African mango seed extract (*Irvingia gabonensis*)	PPAR-γ, leptin and adiponectin gene expression changes	4–13 kg weight loss at 10 weeks 140% increase in adiponectin at 10 weeks	82,83,91
Veldt grape extract (*Cissus quadrangularis*)	Anti-inflammatory	4–8 kg weight loss at 8 weeks	83–85
Brown seaweed extract (Fucoxanthin) + Pomegranate seed oil	UCP, PPARγ gene expression changes; inhibition of G3PD activity in adipocytes	6 kg weight loss at 16 weeks	86,87
Green coffee extract (chlorogenic acid), decaffeinated	Decreased glucose absorption; inhibition of hepatic glucose-6-phosphatase	5 kg weight loss at 8–12 weeks	88–90
Medium chain triglycerides	Increased thermogenesis and fatty acid oxidation; decreased fat deposition	Various	69,71–81

PPARγ, peroxisome proliferator-activated receptor gamma

that lasted over one-year, named BLOOM-DM (Behavioral Modification and Lorcaserin for Obesity and Overweight Management in Diabetes Mellitus) evaluated effects of this drug in 604 obese and overweight participants with type 2 diabetes.[94] Lorcaserin use for up to one year in obese and overweight patients with type 2 diabetes was associated with statistically significant weight loss (5% versus 1.5% for placebos), and significant improvements in glycemic control. Previous clinical trials also demonstrated that lorcaserin decreased body weight in non-diabetic overweight and obese individuals.[95]

Qsymia™ is a newly approved medication for chronic weight management in adults who are obese, or overweight with at least one weight-related co-morbidity such as hypertension, type 2 diabetes mellitus or dyslipidemia. This drug is a combination of phentermine, a sympathomimetic amine approved by the FDA in 1959 and topiramate extended release, a fructose monosaccharide derivative, and an antiepileptic drug approved in 1996 with caloric reducing effects. In a recent randomized controlled trial, Qsymia™, in conjunction with lifestyle modification produced statistically significant weight loss, compared with placebo. Importantly, this combined therapy also led to better improvements in in co-morbidities compared with other antiobesity treatments.[96] The combination's efficacy of these two drugs exceeded the maximal response achieved with either individual agent alone at equivalent doses, likely because each is targeting a different mechanism(s) that impacts energy balance.

Gene/hormone therapy

The fact that most pharmacological alternatives used to treat type 2 diabetes and obesity eventually fail suggests that a targeted approach may be more effective in achieving results that are more durable. Furthermore, the lifelong commitment of these drugs raises the risk for toxicity and resistance. Gene therapy may offer the possibility for a targeted approach without the requirement for a daily treatment. During the last few years several genes have been identified that are related with biochemical pathways that regulate glucose and lipid metabolism with potential for gene therapeutic strategies (including suppression of enhancement of targeted gene or protein expression) that may be used for the treatment of obesity and type 2 diabetes.

Leptin

Deregulation of the glucose–insulin axis is associated with a leptin-deficit and insufficient hypothalamic leptin signaling.[97,98] Transgene over-expression of leptin antagonizes leptin insufficiency thereby producing hyperleptinemia and suppressing, over several weeks, weight gain, adiposity and blood insulin levels in normal adult rats.[99,100] Furthermore, a single intramuscular injection of a recombinant adeno-associated virus (AAV) vector encoding mouse leptin (rAAV-leptin) in *ob/ob* mice (genetically deficient in leptin) lead to normalization of hyperglycemia and appetite and repressed obesity and blood insulin in obese mice, for up to the 6-month duration of the experiment.[101] In a recent study using *db/db* mice (a naturally diabetic mouse with a genetic defect in the receptor for the fat-lowering hormone leptin) neurons were transplanted into the hypothalamus partially restoring leptin responsiveness and ameliorating hyperglycemia and obesity.[102]

Adiponectin

Adiponectin is an adipocyte-secreted adipokine that regulates fatty acid oxidation and glucose uptake[103] via AMP-activated protein kinase (AMPK)-dependent pathway.[104] AMPK is an enzyme also involved in energy control, appetite regulation, adipocyte differentiation and myogenesis.[105] Short-term over-expression of AMPK in the liver by adenovirus-mediated transfer reduces blood glucose levels and results in a switch from glucose to fatty acid utilization to supply energy needs.[106] Studies showed that adiponectin decreases insulin resistance by decreasing triglyceride content in muscle and liver in obese mice and decreased adiponectin is closely related with insulin resistance.[107] Transgenic expression of human adiponectin decreased the excessive fat accumulation of subcutaneous and visceral fat in mice fed a high-calorie diet.[108] Adiponectin gene therapy might provide s new treatment for insulin resistance and obesity. However, while several examples exist that show the potential for gene based therapy, more studies are required to understand the gene and metabolic networks in which these targets are involved and the impact of using viral vectors before gene therapy can be applied to humans.

Glucose transporter 4

Several studies aimed at up-regulating the expression of glucose transporter 4 (GLUT-4) have yielded some

interesting possibilities. Over-expression of GLUT4 in skeletal muscle of GLUT4$^{+/-}$ mice normalized insulin sensitivity and glucose tolerance.[109] Furthermore, it increased glucose utilization, glycogen synthesis and decreased body weight.[110] Over-expression of GLUT4 in the AT of muscle-specific GLUT4-deficient mice circumvents glucose intolerance and diabetes.[111]

Adipose triglyceride lipase

ATGL is newly identified lipase which is expressed at high levels in white AT and muscle tissue. Age-associated changes in ATGL expression in white AT are depot and species dependent with an increase noted in rat brown fat tissue.[112,113] In pigs and young rats, caloric restriction/fasting resulted in increased ATGL expression in several ATs whereas fasting produced no change in ATGL expression in white ATs of older rats (7- and 13-month old).[112,113] Possibly, nutritional regulation of ATGL expression in white AT is impaired with age. The importance of ATGL was further demonstrated by silencing adipocyte ATGL gene expression which almost completely abolished basal and TNF-α-induced adipocyte lipolytic rate in 3T3-L1 adipocytes.[114] Furthermore, the lipolytic rates in cells with silenced HSL or CGI-58 (an ATGL coactivator) were restored by over-expression of ATGL indicating a predominant role for it.[114] The most exciting results demonstrated that, in mice, over-expression of ATGL in adipocytes increased lipolysis and reduced adipocyte lipid content and attenuated diet-induced obesity.[115] Collectively these studies indicate that ATGL is a most promising candidate gene for potentially reducing obesity.

Fat mass and obesity-associated protein

Fat mass and obesity-associated protein (FTO) is widely expressed in fetal and adult tissues and abundantly expressed in the brain.[116] FTO gene was first found to be strongly associated with obesity using a genome-wide search in 2007[116] and appears to be involved in energy homeostasis by the control of energy expenditure.[117] Lack of FTO in mice leads to postnatal growth retardation and a significant reduction in AT and lean body mass,[117] while over-expression of FTO leads to obesity in mice.[118] It would be worthwhile to determine whether effective therapies can be best achieved by suppressing, rather than enhancing gene expression. FTO is widely expressed in fetal and adult tissues and in the brain.[116]

Peroxisome proliferator-activated receptor γ

Suppression of PPARγ expression in white AT could possibly limit the extent of depot lipid storage by negating preadipocyte differentiation. Two feasible approaches may be the over-expression of delta-like 1 (DLK1)/Pref1 or the use of specific micro-RNA to suppress PPARγ expression.[119–122] Over-expression of DLK1, which blocks PPARγ expression, has been used in experimental protocols to establish a role for this transcription factor in adipogenesis of fat cell lines in culture. Most recently, miR-27a was identified as a negative regulator of adipocyte differentiation by suppressing PPARγ expression in 3T3-L1 cells.[120] It was also reported that levels of miR-27a in mature adipocytes of obese mice were much lower than those noted in lean mice.[120] While the long-term effects and exogenous administration regimens of appropriately targeted vectors to utilize these novel aspects of regulation of preadipocytes in animals/humans have not been developed, the specificity of targeting the master regulator for adipogenesis (PPARγ) may indeed have minimal side-effects and high efficacy.[120]

Angiopoietins

A novel potential agent for the treatment of obesity has arisen from the study of a family of proteins structurally similar to angiopoietins but identified and designated 'angiopoietin-like proteins' (ANGPTLs).[123] ANGPTL1–7 are encoded by seven genes and possess some structures characteristic of angiopoietins.[123] Several ANGPTLs regulate angiogenesis, but ANGPTL3, and -4 influence AT metabolism by directly inhibiting lipoprotein lipase activity independent of angiogenic effects.[124] ANGPTL4 is a signal protein secreted into the blood in many forms to modulate many physiological activities.[125] Consideration of ANGPTL4 as a potential agent to treat obesity and type 2 diabetes is based on numerous and varied studies. In particular, animal studies have provided great insight into ANGPTL4. Porcine ANGPTL4 was cloned and mapped to chromosome 2q21→q24 region and many other QTL and functional genes associated with back fat thickness are also located in close proximity on this chromosome.[126] Several studies indicate that ANGPTL4 is expressed to the greatest degree in AT.[126,127] Expression profiling analysis of porcine AT shows a significant influence of age on AT ANGPTL4 expression.[128] Furthermore, 80% caloric restriction (8 days) significantly up-regulated porcine AT ANGPTL4 expression.[129] And, treatment of pigs with melanocyte-stimulating hormone via intracerebroventricular canulation markedly up-regulated porcine AT ANGPTL4 expression within 24 hours.[130] Finally, in related studies a novel target gene induced by PPAR-γ ligands (i.e. PPAR-γ angiopoietin related [PGAR]) encoding a novel member of the angiopoietin family of secreted proteins was characterized and designated PGAR. The expression of PGAR is primarily restricted to ATs and placenta and is consistently elevated in genetic models of obesity.[131] Clearly, the evidence is considerable that ANGPTL4 represents a potential agent for treatment of obesity.

While several examples exist that show the potential for gene-based therapies, more studies are required to understand the gene and metabolic networks in which these targets are involved and the impact of using viral vectors before gene therapy can be applied to humans.

Metabolically healthy obese

While it is well established that obesity clearly increases the risk for type 2 diabetes and metabolic syndrome, it has been

reported that 20–30% of obese subjects remain at low risk for these disorders. These are referred to as 'metabolically healthy obese (MHO)' and typically exhibit insulin sensitivity comparable to healthy non-obese subjects. MHOs also have lower incidence of liver steatosis and heart disease compared with most metabolically unhealthy obese patients. Recent studies suggest that protection against development of hepatic steatosis, ectopic fat deposition, inflammation of visceral AT, and AT dysfunction contributes to healthy obesity.[132–134] Taken together these findings clearly indicate that obesity is a complex metabolic condition that cannot be simply attributed to increased fat mass. Moreover, detailed studies of AT cellularity and inflammation, as well as ectopic fat accumulation will further enhance our understanding of the link between obesity and metabolic disorders.

Summary

Despite our fairly detailed, and growing, understanding of adipocytes, to date we have not yet been able to design clinical treatments for obesity, insulin resistance and associated morbidities. Once established, obesity cannot be cured – only treated. In addition to weight loss protocols based on caloric restriction, various remediation strategies against obesity have also been studied, but are either ineffective or cause substantial side-effects. Novel avenues based on emerging understanding of body depot fat biology are being explored in the attempt to find hitherto unknown strategies which may be able to regulate the adverse symptoms of obesity or reverse obesity all together.

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REFERENCES

1 Friedman JM. Obesity: causes and control of excess body fat. *Nature* 2009;**459**:340–2
2 Fernyhough ME, Bucci LR, Hausman GJ, Antonio J, Vierck JL, Dodson MV. Gaining a solid grip on adipogenesis. *Tissue Cell* 2005;**37**:335–8
3 Kopelman PG. Obesity as a medical problem. *Nature* 2000;**404**:635–43
4 Haslam DW, James WP. Obesity. *Lancet* 2005;**366**:1197–209
5 Dodson MV, Hausman GJ. Metabolic syndromes: resolving a malady that involves numerous tissues, cells, regulators and regulatory pathways. *J Metab Syndr* 2011;**1**:e101
6 Lagerros YT, Rössner S. Managing obesity – from childhood and onwards. *Int J Pediatr Obes* 2011;**6**:74–8
7 Colon-Gonzalez F, Kim GW, Lin JE, Valentino MA, Waldman SA. Obesity pharmacotherapy: what is next? *Mol Aspects Med* 2013;**34**:71–83
8 Misra M. Obesity Pharmacotherapy: current perspectives and future directions. *Curr Cardiol Rev* 2012 [Epub ahead of print]
9 Lauer MS. Lemons for obesity. *Ann Intern Med* 2012;**157**:139–40
10 Griffen L, Anchors M. The 'phen-pro' diet drug combination is not associated with valvular heart disease. *Arch Intern Med* 1998;**158**:1278–9
11 Hardie DG. Organismal carbohydrate and lipid homeostasis. *Cold Spring Harb Perspect Biol* 2012;**4**:a006783
12 Miller RS, Becker KG, Prabhu V, Cooke DW. Adipocyte gene expression is altered in formerly obese mice and as a function of diet composition. *J Nutr* 2008;**138**:1033–8
13 Foltin RW. Effects of dietary and pharmacological manipulations on appetitive and consummatory aspects of feeding in non-human primates. *Appetite* 2005;**45**:110–20
14 Blundell JE. Serotonin manipulations and the structure of feeding behaviour. *Appetite* 1986;**7**:39–56
15 Hausman GJ, Dodson MV, Ajuwon K, Azain M, Barnes KM, Guan LL, Jiang Z, Poulos SP, Sainz RD, Smith S, Spurlock M, Novakofski J, Fernyhough ME, Bergen WG. Board invited review: The biology and regulation of preadipocytes and adipocytes in meat animals. *J Anim Sci* 2009;**87**:1218–46
16 Carmen GY, Victor SM. Signalling mechanisms regulating lipolysis. *Cellular signalling* 2006;**18**:401–8
17 Mersmann HJ. Regulation of porcine adipose tissue lipolytic activity by adrenergic agonists and antagonists. *Comp Biochem Physiol C* 1992;**102**:407–11
18 Mills SE. Biological basis of the ractopamine response. *J Anim Sci* 2002;**80**:E28–32
19 Halsey CH, Weber PS, Reiter SS, Stronach BN, Bartosh JL, Bergen WG. The effect of ractopamine hydrochloride on gene expression in adipose tissues of finishing pigs. *J Anim Sci* 2011;**89**:1011–9
20 Bergen WG, Johnson SE, Skjaerlund DM, Babiker AS, Ames NK, Merkel RA, Anderson DB. Muscle protein metabolism in finishing pigs fed ractopamine. *J Anim Sci* 1989;**67**:2255–62
21 Surya S, Horowitz JF, Goldenberg N, Sakharova A, Harber M, Cornford AS, Symons K, Barkan AL. The pattern of growth hormone delivery to peripheral tissues determines insulin-like growth factor-1 and lipolytic responses in obese subjects. *J Clin Endocrinol Metab* 2009;**94**:2828–34
22 Heal DJ, Gosden J, Smith SL. What is the prognosis for new centrally-acting anti-obesity drugs? *Neuropharmacology* 2012;**63**:132–46
23 Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen YY, Knight R, Ahima RS, Bushman F, Wu GD. High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology* 2009;**137**:1716–24
24 Visscher TL, Seidell JC. The public health impact of obesity. *Annu Rev Public Health* 2001;**22**:355–75
25 Friedman JM. Leptin at 14 y of age: an ongoing story. *Am J Clin Nutr* 2009;**89**:973S–9S
26 Clarke IJ. Models of 'obesity' in large animals and birds. *Front Horm Res* 2008;**36**:107–17
27 Bray GA. Medical therapy for obesity. *Mt Sinai J Med* 2010;**77**:407–17
28 Roth JD, Hughes H, Kendall E, Baron AD, Anderson CM. Antiobesity effects of the beta-cell hormone amylin in diet-induced obese rats: effects on food intake, body weight, composition, energy expenditure, and gene expression. *Endocrinology* 2006;**147**:5855–64
29 Roth JD, Roland BL, Cole RL, Trevaskis JL, Weyer C, Koda JE, Anderson CM, Parkes DG, Baron AD. Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. *Proc Natl Acad Sci USA* 2008;**105**:7257–62
30 Trevaskis JL, Coffey T, Cole R, Lei C, Wittmer C, Walsh B, Weyer C, Koda J, Baron AD, Parkes DG, Roth JD. Amylin-mediated restoration of leptin responsiveness in diet-induced obesity: magnitude and mechanisms. *Endocrinology* 2008;**149**:5679–87

31 Seth R, Knight WD, Overton JM. Combined amylin-leptin treatment lowers blood pressure and adiposity in lean and obese rats. *Int J Obes (Lond)* 2011;**35**:1183–92

32 Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Näslund E, Britton T, Concha H, Hassan M, Rydén M, Frisén J, Arner P. Dynamics of fat cell turnover in humans. *Nature* 2008;**453**:783–7

33 Hirsch J, Batchelor B. Adipose tissue cellularity in human obesity. *Clin Endocrinol Metab* 1976;**5**:299–311

34 Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. *Genes Dev* 2000;**14**:1293–307

35 Wu Z, Rosen ED, Brun R, Hauser S, Adelmant G, Troy AE, McKeon C, Darlington GJ, Spiegelman BM. Cross-regulation of C/EBP alpha and PPAR gamma controls the transcriptional pathway of adipogenesis and insulin sensitivity. *Mol Cell* 1999;**3**:151–8

36 Rodeheffer MS, Birsoy K, Friedman JM. Identification of white adipocyte progenitor cells in vivo. *Cell* 2008;**135**:240–9

37 Tang W, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, Tallquist MD, Graff JM. White fat progenitor cells reside in the adipose vasculature. *Science* 2008;**322**:583–6

38 Lee YH, Petkova AP, Mottillo EP, Granneman JG. In vivo identification of bipotential adipocyte progenitors recruited by beta3-adrenoceptor activation and high-fat feeding. *Cell Metab* 2012;**15**:480–91

39 Hauner H. Secretory factors from human adipose tissue and their functional role. *Proc Nutr Soc* 2005;**64**:163–9

40 Chung S, Lapoint K, Martinez K, Kennedy A, Boysen Sandberg M, McIntosh MK. Preadipocytes mediate lipopolysaccharide-induced inflammation and insulin resistance in primary cultures of newly differentiated human adipocytes. *Endocrinology* 2006;**147**:5340–51

41 Yen TT, Gill AM, Frigeri LG, Barsh GS, Wolff GL. Obesity, diabetes, and neoplasia in yellow A(vy)/- mice: ectopic expression of the agouti gene. *FASEB J* 1994;**8**:479–88

42 Albrecht E, Komolka K, Kuzinski J, Maak S. Agouti revisited: transcript quantification of the ASIP gene in bovine tissues related to protein expression and localization. *PLoS One* 2012;**7**:e35282

43 Tholpady SS, Aojanepong C, Llull R, Jeong JH, Mason AC, Futrell JW, Ogle RC, Katz AJ. The cellular plasticity of human adipocytes. *Ann Plast Surg* 2005;**54**:651–6

44 Fernyhough ME, Hausman GJ, Dodson MV. Progeny from dedifferentiated bovine adipocytes display protracted adipogenesis. *Cells Tissues Organs* 2008;**188**:359–72

45 Nobusue H, Endo T, Kano K. Establishment of a preadipocyte cell line derived from mature adipocytes of GFP transgenic mice and formation of adipose tissue. *Cell Tissue Res* 2008;**332**:435–46

46 Yagi K, Kondo D, Okazaki Y, Kano K. A novel preadipocyte cell line established from mouse adult mature adipocytes. *Biochem Biophys Res Commun* 2004;**321**:967–74

47 Chen J, Dodson MV, Jiang Z. Cellular and molecular comparison of redifferentiation of intramuscular- and visceral-adipocyte derived progeny cells. *Int J Biol Sci* 2010;**6**:80–8

48 Fernyhough ME, Hausman GJ, Guan LL, Okine E, Moore SS, Dodson MV. Mature adipocytes may be a source of stem cells for tissue engineering. *Biochem Biophys Res Commun* 2008;**368**:455–7

49 Obinata D, Matsumoto T, Ikado Y, Sakuma T, Kano K, Fukuda N, Yamaguchi K, Mugishima H, Takahashi S. Transplantation of mature adipocyte-derived dedifferentiated fat (DFAT) cells improves urethral sphincter contractility in a rat model. *Int J Urol* 2011;**18**:827–34

50 Goodpaster BH, Thaete FL, Kelley DE. Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. *Am J Clin Nutr* 2000;**71**:885–92

51 Kim S, Moustaid-Moussa N. Secretory, endocrine and autocrine/paracrine function of the adipocyte. *J Nutr* 2000;**130**:3110S–5S

52 Febbraio MA, Pedersen BK. Contraction-induced myokine production and release: is skeletal muscle an endocrine organ? *Exerc Sport Sci Rev* 2005;**33**:114–9

53 Trayhurn P, Drevon CA, Eckel J. Secreted proteins from adipose tissue and skeletal muscle – adipokines, myokines and adipose/muscle cross-talk. *Arch Physiol Biochem* 2011;**117**:47–56

54 Vettor R, Milan G, Franzin C, Sanna M, De Coppi P, Rizzuto R, Federspil G. The origin of intermuscular adipose tissue and its pathophysiological implications. *Am J Physiol Endocrinol Metab* 2009;**297**:E987–98

55 Aguiari P, Leo S, Zavan B, Vindigni V, Rimessi A, Bianchi K, Franzin C, Cortivo R, Rossato M, Vettor R, Abatangelo G, Pozzan T, Pinton P, Rizzuto R. High glucose induces adipogenic differentiation of muscle-derived stem cells. *Proc Natl Acad Sci USA* 2008;**105**:1226–31

56 Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Boström EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Højlund K, Gygi SP, Spiegelman BM. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012;**481**:463–8

57 Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerbäck S, Schrauwen P, Spiegelman BM. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 2012;**150**:366–76

58 Auffret J, Viengchareun S, Carré N, Denis RG, Magnan C, Marie PY, Muscat A, Fève B, Lombès M, Binart N. Beige differentiation of adipose depots in mice lacking prolactin receptor protects against high-fat-diet-induced obesity. *FASEB J* 2012;**26**:3728–37

59 Kushner RF. Pharmacologic management of obesity. *Pediatr Blood Cancer* 2012;**58**:140–3

60 Catalán V, Gómez-Ambrosi J, Rodríguez A, Ramírez B, Rotellar F, Valentí V, Silva C, Gil MJ, Salvador J, Frühbeck G. Increased Tenascin C and toll-like receptor 4 levels in visceral adipose tissue as a link between inflammation and extracellular matrix remodeling in obesity. *J Clin Endocrinol Metab* 2012;**97**:E1880–9

61 Alligier M, Meugnier E, Debard C, Lambert-Porcheron S, Chanseaume E, Sothier M, Loizon E, Hssain AA, Brozek J, Scoazec JY, Morio B, Vidal H, Laville M. Subcutaneous adipose tissue remodeling during the initial phase of weight gain induced by overfeeding in humans. *J Clin Endocrinol Metab* 2012;**97**:E183–92

62 Mersmann HJ. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. *J Anim Sci* 1998;**76**:160–72

63 Park UH, Jeong HS, Jo EY, Park T, Yoon SK, Kim EJ, Jeong JC, Um SJ. Piperine, a component of black pepper, inhibits adipogenesis by antagonizing PPARgamma activity in 3T3-L1 cells. *J Agric Food Chem* 2012;**60**:3853–60

64 Ludy MJ, Moore GE, Mattes RD. The effects of capsaicin and capsiate on energy balance: critical review and meta-analyses of studies in humans. *Chem Senses* 2012;**37**:103–21

65 Lin J, Della-Fera MA, Baile CA. Green tea polyphenol epigallocatechin gallate inhibits adipogenesis and induces apoptosis in 3T3-L1 adipocytes. *Obes Res* 2005;**13**:982–90

66 Prior RL, Wu X, Gu L, Hager T, Hager A, Wilkes S, Howard L. Purified berry anthocyanins but not whole berries normalize lipid parameters in mice fed an obesogenic high fat diet. *Mol Nutr Food Res* 2009;**53**:1406–18

67 Kalupahana NS, Claycombe K, Newman SJ, Stewart T, Siriwardhana N, Matthan N, Lichtenstein AH, Moustaid-Moussa N. Eicosapentaenoic acid prevents and reverses insulin resistance in high-fat diet-induced obese mice via modulation of adipose tissue inflammation. *J Nutr* 2010;**140**:1915–22

68 Wolfgang MJ, Lane MD. Hypothalamic malonyl-coenzyme A and the control of energy balance. *Mol Endocrinol* 2008;**22**:2012–20

69 Baba N, Bracco EF, Hashim SA. Enhanced thermogenesis and diminished deposition of fat in response to overfeeding with diet containing medium chain triglyceride. *Am J Clin Nutr* 1982;**35**:678–82

70 Johnstone AM, Horgan GW, Murison SD, Bremner DM, Lobley GE. Effects of a high-protein ketogenic diet on hunger, appetite, and weight loss in obese men feeding ad libitum. *Am J Clin Nutr* 2008;**87**:44–55

71 Baba N, Bracco EF, Hashim SA. Role of brown adipose tissue in thermogenesis induced by overfeeding a diet containing medium chain triglyceride. *Lipids* 1987;**22**:442–4

72 Clegg ME. Medium-chain triglycerides are advantageous in promoting weight loss although not beneficial to exercise performance. *Int J Food Sci Nutr* 2010;**61**:653–79

73 Kasai M, Nosaka N, Maki H, Negishi S, Aoyama T, Nakamura M, Suzuki Y, Tsuji H, Uto H, Okazaki M, Kondo K. Effect of dietary medium- and long-chain triacylglycerols (MLCT) on accumulation of body fat in healthy humans. *Asia Pac J Clin Nutr* 2003;**12**:151–60

74 Matsuo T, Matsuo M, Kasai M, Takeuchi H. Effects of a liquid diet supplement containing structured medium- and long-chain

triacylglycerols on bodyfat accumulation in healthy young subjects. *Asia Pac J Clin Nutr* 2001;**10**:46–50
75 St-Onge MP. Dietary fats, teas, dairy, and nuts: potential functional foods for weight control? *Am J Clin Nutr* 2005;**81**:7–15
76 St-Onge MP, Bosarge A. Weight-loss diet that includes consumption of medium-chain triacylglycerol oil leads to a greater rate of weight and fat mass loss than does olive oil. *Am J Clin Nutr* 2008;**87**:621–6
77 St-Onge MP, Bosarge A, Goree LL, Darnell B. Medium chain triglyceride oil consumption as part of a weight loss diet does not lead to an adverse metabolic profile when compared to olive oil. *J Am Coll Nutr* 2008;**27**:547–52
78 St-Onge MP, Bourque C, Jones PJ, Ross R, Parsons WE. Medium- versus long-chain triglycerides for 27 days increases fat oxidation and energy expenditure without resulting in changes in body composition in overweight women. *Int J Obes Relat Metab Disord* 2003;**27**:95–102
79 St-Onge MP, Jones PJ. Greater rise in fat oxidation with medium-chain triglyceride consumption relative to long-chain triglyceride is associated with lower initial body weight and greater loss of subcutaneous adipose tissue. *Int J Obes Relat Metab Disord* 2003;**27**:1565–71
80 St-Onge MP, Ross R, Parsons WD, Jones PJ. Medium-chain triglycerides increase energy expenditure and decrease adiposity in overweight men. *Obesity Res* 2003;**11**:395–402
81 Tsuji H, Kasai M, Takeuchi H, Nakamura M, Okazaki M, Kondo K. Dietary medium-chain triacylglycerols suppress accumulation of body fat in a double-blind, controlled trial in healthy men and women. *J Nutr* 2001;**131**:2853–9
82 Ngondi JL, Oben JE, Minka SR. The effect of *Irvingia gabonensis* seeds on body weight and blood lipids of obese subjects in Cameroon. *Lipids Health Dis* 2005;**4**:12
83 Oben JE, Ngondi JL, Momo CN, Agbor GA, Sobgui CS. The use of a *Cissus quadrangularis*/*Irvingia gabonensis* combination in the management of weight loss: a double-blind placebo-controlled study. *Lipids Health Dis* 2008;**7**:12
84 Oben J, Kuate D, Agbor G, Momo C, Talla X. The use of a *Cissus quadrangularis* formulation in the management of weight loss and metabolic syndrome. *Lipids Health Dis* 2006;**5**:24
85 Oben JE, Enyegue DM, Fomekong GI, Soukontoua YB, Agbor GA. The effect of *Cissus quadrangularis* (CQR-300) and a Cissus formulation (CORE) on obesity and obesity-induced oxidative stress. *Lipids Health Dis* 2007;**6**:4
86 Miyashita K, Hosokawa M. Antiobesity effect of allenic carotenoid, fucoxanthin. In: Mine Y, Miyashita K, Shahidi F, eds. *Nutrigenomics and Proteomics in Health and Disease Food Factors and Gene Interactions*. Iowa: Wiley-Blackwell, 2009
87 Abidov M, Ramazanov Z, Seifulla R, Grachev S. The effects of Xanthigen in the weight management of obese premenopausal women with non-alcoholic fatty liver disease and normal liver fat. *Diabetes Obes Metab* 2010;**12**:72–81
88 Dellalibera O, Lemaire B, Lafay S. Svetol®, green coffee extract, induces weight loss and increases the lean to fat mass ratio in volunteers with overweight problem. *Phytotherapie* 2006;**4**:1–4
89 Nardon K, Lemaire B, Lafay S. Green coffee extract Svetol® can manage weight: a review. *Agrofood Industry Hi-Tech* 2007;**18**:37–9
90 Thom E. The effect of chlorogenic acid enriched coffee on glucose absorption in healthy volunteers and its effect on body mass when used long-term in overweight and obese people. *J Int Med Res* 2007;**35**:900–8
91 Ngondi JL, Etoundi BC, Nyangono CB, Mbofung CM, Oben JE. IGOB131, a novel seed extract of the West African plant *Irvingia gabonensis*, significantly reduces body weight and improves metabolic parameters in overweight humans in a randomized double-blind placebo controlled investigation. *Lipids Health Dis* 2009;**8**:7
92 Pi-Sunyer FX. Weight loss in type 2 diabetic patients. *Diabetes Care* 2005;**28**:1526–7
93 American Diabetes Association. Standards of medical care in diabetes – 2012. *Diabetes Care* 2012;**35**:S11–63
94 O'Neil PM, Smith SR, Weissman NJ, Fidler MC, Sanchez M, Zhang J, Raether B, Anderson CM, Shanahan WR. Randomized Placebo-Controlled Clinical Trial of Lorcaserin for Weight Loss in Type 2 Diabetes Mellitus: the BLOOM-DM Study. *Obesity (Silver Spring)* 2012;**20**:1426–36
95 Smith SR, Weissman NJ, Anderson CM, Sanchez M, Chuang E, Stubbe S, Bays H, Shanahan WR; Behavioral Modification and Lorcaserin for Overweight and Obesity Management (BLOOM) Study Group. Multicenter, placebo-controlled trial of lorcaserin for weight management. *N Engl J Med* 2010;**363**:245–56
96 Allison DB, Gadde KM, Garvey WT, Peterson CA, Schwiers ML, Najarian T, Tam PY, Troupin B, Day WW. Controlled-release phentermine/topiramate in severely obese adults: a randomized controlled trial (EQUIP). *Obesity (Silver Spring)* 2012;**20**:330–42
97 Cohen P, Zhao C, Cai X, Montez JM, Rohani SC, Feinstein P, Mombaerts P, Friedman JM. Selective deletion of leptin receptor in neurons leads to obesity. *J Clin Invest* 2001;**108**:1113–21
98 Prieur X, Tung YC, Griffin JL, Farooqi IS, O'Rahilly S, Coll AP. Leptin regulates peripheral lipid metabolism primarily through central effects on food intake. *Endocrinology* 2008;**149**:5432–9
99 Chen G, Koyama K, Yuan X, Lee Y, Zhou YT, O'Doherty R, Newgard CB, Unger RH. Disappearance of body fat in normal rats induced by adenovirus-mediated leptin gene therapy. *Proc Natl Acad Sci U S A* 1996;**93**:14795–9
100 Muzzin P, Cusin I, Charnay Y, Rohner-Jeanrenaud F. Single intracerebroventricular bolus injection of a recombinant adenovirus expressing leptin results in reduction of food intake and body weight in both lean and obese Zucker fa/fa rats. *Regul Pept* 2000;**92**:57–64
101 Murphy JE, Zhou S, Giese K, Williams LT, Escobedo JA, Dwarki VJ. Long-term correction of obesity and diabetes in genetically obese mice by a single intramuscular injection of recombinant adeno-associated virus encoding mouse leptin. *Proc Natl Acad Sci USA* 1997;**94**:13921–6
102 Czupryn A, Zhou YD, Chen X, McNay D, Anderson MP, Flier JS, Macklis JD. Transplanted hypothalamic neurons restore leptin signaling and ameliorate obesity in db/db mice. *Science* 2011;**334**:1133–7
103 Diez JJ, Iglesias P. The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur J Endocrinol* 2003;**148**:293–300
104 Satoh H, Nguyen MT, Trujillo M, Imamura T, Usui I, Scherer PE, Olefsky JM. Adenovirus-mediated adiponectin expression augments skeletal muscle insulin sensitivity in male Wistar rats. *Diabetes* 2005;**54**:1304–13
105 Rojas J, Arraiz N, Aguirre M, Velasco M, Bermudez V. AMPK as target for intervention in childhood and adolescent obesity. *J Obes* 2011;**2011**:252817
106 Foretz M, Ancellin N, Andreelli F, Saintillan Y, Grondin P, Kahn A, Thorens B, Vaulont S, Viollet B. Short-term overexpression of a constitutively active form of AMP-activated protein kinase in the liver leads to mild hypoglycemia and fatty liver. *Diabetes* 2005;**54**:1331–9
107 Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001;**7**:941–6
108 Otabe S, Yuan X, Fukutani T, Wada N, Hashinaga T, Nakayama H, Hirota N, Kojima M, Yamada K. Overexpression of human adiponectin in transgenic mice results in suppression of fat accumulation and prevention of premature death by high-calorie diet. *Am J Physiol Endocrinol Metab* 2007;**293**:E210–8
109 Tsao TS, Stenbit AE, Factor SM, Chen W, Rossetti L, Charron MJ. Prevention of insulin resistance and diabetes in mice heterozygous for GLUT4 ablation by transgenic complementation of GLUT4 in skeletal muscle. *Diabetes* 1999;**48**:775–82
110 Tsao TS, Li J, Chang KS, Stenbit AE, Galuska D, Anderson JE, Zierath JR, McCarter RJ, Charron MJ. Metabolic adaptations in skeletal muscle overexpressing GLUT4: effects on muscle and physical activity. *FASEB J* 2001;**15**:958–69
111 Carvalho E, Kotani K, Peroni OD, Kahn BB. Adipose-specific overexpression of GLUT4 reverses insulin resistance and diabetes in mice lacking GLUT4 selectively in muscle. *Am J Physiol Endocrinol Metab* 2005;**289**:E551–61
112 Deiuliis JA, Shin J, Bae D, Azain MJ, Barb R, Lee K. Developmental, hormonal, and nutritional regulation of porcine adipose triglyceride lipase (ATGL). *Lipids* 2008;**43**:215–25
113 Caimari A, Oliver P, Palou A. Impairment of nutritional regulation of adipose triglyceride lipase expression with age. Int J Obes (Lond) 2008;**32**:1193–200

114 Yang X, Zhang X, Heckmann BL, Lu X, Liu J. Relative contribution of adipose triglyceride lipase and hormone-sensitive lipase to tumor necrosis factor-alpha (TNF-alpha)-induced lipolysis in adipocytes. *J Biol Chem* 2011;**286**:40477–85

115 Ahmadian M, Duncan RE, Varady KA, Frasson D, Hellerstein MK, Birkenfeld AL, Samuel VT, Shulman GI, Wang Y, Kang C, Sul HS. Adipose overexpression of desnutrin promotes fatty acid use and attenuates diet-induced obesity. *Diabetes* 2009;**58**:855–66

116 Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 2007;**316**:889–94

117 Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Bruning JC, Rüther U. Inactivation of the Fto gene protects from obesity. *Nature* 2009;**458**:894–8

118 Church C, Moir L, McMurray F, Girard C, Banks GT, Teboul L, Wells S, Brüning JC, Nolan PM, Ashcroft FM, Cox RD. Overexpression of Fto leads to increased food intake and results in obesity. *Nature Genet* 2010;**42**:1086–92

119 Sul HS. Minireview: Pref-1: role in adipogenesis and mesenchymal cell fate. *Mol Endocrinol* 2009;**23**:1717–25

120 Kim SY, Kim AY, Lee HW, Son YH, Lee GY, Lee JW, Lee YS, Kim JB. miR-27a is a negative regulator of adipocyte differentiation via suppressing PPARgamma expression. *Biochem Biophys Res Commun* 2010;**392**:323–8

121 Mitterberger MC, Lechner S, Mattesich M, Kaiser A, Probst D, Wenger N, Pierer G, Zwerschke W. DLK1(PREF1) is a negative regulator of adipogenesis in CD105(+)/CD90(+)/CD34(+)/CD31(−)/FABP4(−) adipose-derived stromal cells from subcutaneous abdominal fat pats of adult women. *Stem Cell Res* 2012;**9**:35–48

122 Romao JM, Jin W, Dodson MV, Hausman GJ, Moore SS, Guan le L. MicroRNA regulation in mammalian adipogenesis. *Exp Biol Med (Maywood)* 2011;**236**:997–1004

123 Kadomatsu T, Tabata M, Oike Y. Angiopoietin-like proteins: emerging targets for treatment of obesity and related metabolic diseases. *FEBS J* 2011;**278**:559–64

124 Sukonina V, Lookene A, Olivecrona T, Olivecrona G. Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. *Proc Natl Acad Sci USA* 2006;**103**:17450–5

125 Grootaert C, Van de Wiele T, Verstraete W, Bracke M, Vanhoecke B. Angiopoietin-like protein 4: health effects, modulating agents and structure-function relationships. *Expert Rev Proteomics* 2012;**9**:181–99

126 Feng SQ, Chen XD, Xia T, Gan L, Qiu H, Dai MH, Zhou L, Peng Y, Yang ZQ. Cloning, chromosome mapping and expression characteristics of porcine ANGPTL3 and -4. *Cytogenet Genome Res* 2006;**114**:44–9

127 Köster A, Chao YB, Mosior M, Ford A, Gonzalez-DeWhitt PA, Hale JE, Li D, Qiu Y, Fraser CC, Yang DD, Heuer JG, Jaskunas SR, Eacho P. Transgenic angiopoietin-like (angptl)4 overexpression and targeted disruption of angptl4 and angptl3: regulation of triglyceride metabolism. *Endocrinology* 2005;**146**:4943–50

128 Li M, Zhu L, Li X, Shuai S, Teng X, Xiao H, Li Q, Chen L, Guo Y, Wang J. Expression profiling analysis for genes related to meat quality and carcass traits during postnatal development of backfat in two pig breeds. *Sci China C Life Sci* 2008;**51**:718–33

129 Lkhagvadorj S, Qu L, Cai W, Couture OP, Barb CR, Hausman GJ, Nettleton D, Anderson LL, Dekkers JC, Tuggle CK. Microarray gene expression profiles of fasting induced changes in liver and adipose tissues of pigs expressing the melanocortin-4 receptor D298N variant. *Physiol Genomics* 2009;**38**:98–111

130 Barb CR, Hausman GJ, Rekaya R, Lents CA, Lkhagvadorj S, Qu L, Cai W, Couture OP, Anderson LL, Dekkers JC, Tuggle CK. Gene expression in hypothalamus, liver and adipose tissues and food intake response to melanocortin-4 receptor (MC4R) agonist in pigs expressing MC4R mutations. *Physiol Genomics* 2010;**41**:254–68

131 Yoon JC, Chickering TW, Rosen ED, Dussault B, Qin Y, Soukas A, Friedman JM, Holmes WE, Spiegelman BM. Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation. *Mol Cell Biol* 2000;**20**:5343–9

132 Blüher M. Are there still healthy obese patients? *Curr Opin Endocrinol Diabetes Obes* 2012;**19**:341–6

133 Bradshaw PT, Monda KL, Stevens J. Metabolic syndrome in healthy obese, overweight, and normal weight individuals: the atherosclerosis risk in communities study. *Obesity (Silver Spring)* 2012 [doi:10.1038/oby.2012.173]

134 Hamer M, Stamatakis E. Metabolically healthy obesity and risk of all-cause and cardiovascular disease mortality. *J Clin Endocrinol Metab* 2012;**97**:2482–8