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REVIEW

TARGET ADIPOSE TISSUE NERVES TO TREAT OBESITY: SPECIAL REFERENCE TO β3 ADRENOCEPTOR AGONISTS

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Abstract

Adipocytes exist as white, brown and beige phenotype. White adipocytes are major metabolic and secretory components of the white adipose tissue, whereas brown adipocytes are responsible for non-shivering thermogenesis. A subset of white adipocytes can be activated to thermogenic beige or brite (brown in white) adipocytes. This conversion is enhanced by the drug Mirabegron, a β3 adrenoceptor agonist, resulting in improved glucose, lipid and thermogenic homeostasis. These exciting initial results could be a forerunner for additional studies on the pharmacotherapy for human obesity and related cardiometabolic disorders.

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Introduction

Accumulation of white adipose tissue (WAT) and dysfunction of brown adipose tissue (BAT) are pivotally related to the pathogenesis of obesity and related cardiometabolic diseases. Accordingly, current studies in this field are focused on investigating the ability of different drugs to suppress WAT and/or activate BAT. Browning of WAT is one such. It is mediated through intermittent cold exposure, exercise, and β3 adrenoceptor (ADRβ3) agonists. Even though the latter can be used in the therapy of obesity and related disorders, they remain largely unexplored.

White adipose tissue was initially considered an inert repository of energy storage. It is now emerging to have endocrine and paracrine activities, secreting a variety of signalling proteins (adipokines) that regulate various aspects of metabolism, inflammation and other biological functions (1, 2). Brown adipose tissue, the main thermogenic tissue in humans, is a target for drugs acting on its nerve supply, as a potential treatment of obesity and type 2 diabetes mellitus (T2DM). At the cellular level both WAT and BAT comprise of adipocytes, pre-adipocytes, fibroblasts, macrophages and other immune-inflammatory

cells (for adipose mast cells, see Tunçel *et al* in this volume of *Adipobiology*). A variety of adipokines were identified such as leptin, adiponectin, visfatin, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), apelin, chemerin, and omentin; more than 500 are established (1, 2 and references therein).

The evolutionary pathways of energy metabolism originated whereby genes were retained that ensured periods of hunger were tolerated. During times of 'feasting' energy accumulates in adipose tissue to enable periods of involuntary fasting (3). Hunter-gatherer populations had slow transit of food in the gastrointestinal system until animal fat was available as a source of food. Over time, as meat became staple diet, muscle development was enhanced, which enhanced the ability to hunt for food.

Principally, adipose tissue serves as a repository of fats so that other body tissues are not exposed to excess fats resulting in lipotoxicity. Excess fat accumulation in non-adipose tissues results in disturbed fluidity of membranes, ultimately leading to insulin resistance *via* the release of lipidic mediators and oxygen metabolites (4). New adipocytes are produced in response to a positive energy balance, resulting in infiltration of immune cells, angiogenesis and hypertrophy of adipocytes that already exist.

Types of adipocytes

There are three classes of adipocytes in mammals: white, brown and brite (or beige). They have different regions of origin, morphology and genes related to thermogenesis. White adipocytes measure between 25 and 200 μm. They have a large lipid droplet with few mitochondria, consistent their role in storing triglycerides as energy. Generally, adipose tissue surrounding visceral organs results in adverse metabolic phenotype, in contrast to that deposited in subcutaneous tissues. Accumulated visceral fat results in metabolic syndrome *via* dysregulated secretion of adipokines.

Brown adipocytes which are rich in mitochondria, dissipate stored energy as heat. They do so by uncoupling protein 1 (UCP1) which is found in the inner mitochondrial membrane on its inner surface. UCP1 uncouples fuel oxidation from the synthesis of ATP (4). BAT is found in the perirenal region in the newborn. Adults were shown to have BAT in lower neck and in the supraclavicular areas. Brown adipocytes are served by a rich supply of β3 adrenergic nerves, respectively β3 adrenoceptors (ADRβ3). They regulated thermogenesis *via* the activation of UCP1. Animal studies have shown that BAT is responsible for cold adaptation and for adaptation to low carbohydrate ingestion in diets (4-6). There is an inverse correlation of body mass index with BAT, leading to the possibility of its role in insulin resistance (7). This led to targeting BAT as well as engineering BAT for leads into the management of obesity in humans (8). Recent studies have focussed on the genetic and epigenetic involvement of climate on the prevalence of human obesity (9). Genetic factors have a role in the development of obesity; differences in adaptive thermogenesis may account for variations in daily energy expenditure.

While UCP1 could have evolved in eutherian mammals as early as the Cretaceous period, humans may have had a different trajectory. Hot and sub-Saharan conditions, where *Homo sapiens* originated must have adapted them to such environments (10). Over time, when humans were exposed to cold, genes responsible for non-shivering thermogenesis in BAT could have been selected. Evidence comes from a high frequency of variant ADRβ3 gene in nonhuman primates. Interestingly the energy wasting variant of ADRβ3 gene is seen only in humans.

Evidence for the selective advantage of cold-adaptive genes is not robust, a few reports suggest it could have operated. Intuits from Greenland harbour thermogenesis by BAT, showing a potential adaptive response. Lending support, populations who are not exposed to extreme cold, such as south Asians have reduced non-shivering thermogenesis and BAT volume. Both these influence energy expenditure and basal metabolism (11). However, genes along cannot explain the differences in obesity. In addition, even those living in the cold stay mainly in temperature-regulated environments.

Regulation of beige adipocyte plasticity

The third kind or adipocytes, beige or brite, is a special phenotype of brown adipocytes, which express UCP1 and thus is capable of thermogenesis. Browning of adipocytes occurs mainly by exposure to cold temperatures, in addition to stimulation by ADRβ3 agonists. Recent reports showed that beige adipocytes develop by stimulation of other proteins (12). Interest in this subgroup of adipocytes lies in their potential for being a target of treatment of metabolic diseases (13). Beige adipocytes may have roles other than thermogenesis *via* secretion of *batokines* (from BAT and -kines) that regulate glucose metabolism (14).

Formation of beige adipocytes (UGP^{1+}) can occur either by stimuli such as physical exercise (see Lakshmi and Sridhar in this volume of *Adipobiology*) or exposure to cold, or by white adipocyte trans-differentiation. Principal stimulus includes sympathetic nerves, besides others such as thyroxin, IL-6, irisin, BDNF and other molecules collectively termed "browning hormones" (15).

Activation of UCP1 protein, which is highly expressed in the

inner mitochondrial membrane results in dissipation of electrochemical gradient due to synthesis of fatty acid across mitochondrial membranes. Many other substrates transverse BAT including fatty acids, glutamate, glucose and nonesterified fatty acids (16).

Thermogenesis and adipocytes

Thermogenesis of fat occurs due to a dense network of transcription factors. These include peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α), peroxisome proliferator-activated receptor gamma (PPARγ), and PR domain containing 16 (PRDM16) (16). PPARs are well known mediators of fat storage in adipocytes (17), which are targeted by the thiazolidinedione group of drugs. Rosiglitazone, an agonist of PPAR receptor leads to increased mass of mitochondria, which results in enhanced oxygen consumption and palmitate oxidation (18). Attempts are being made to assess whether rosiglitazone, which had adverse consequences in the treatment of diabetes, can be re-purposed to enhance thermogenesis. Thermogenic programming is postulated to be upregulated by post-translational modifications of PPARγ during adipogenesis.

PGC1-α, by interacting with nuclear factors plays a role in biogenesis and oxidative metabolism in the mitochondria (19). Translation of animal studies to humans failed initially because it requires maximal exposure to cold is necessary for maximal BAT thermogenesis; as referred to earlier, modern humans generally stay in thermally controlled environments and are not exposed to the severe cold that is required for activation of BAT. In addition, body size and energy expenditure are inversely related, which makes humans less able to utilize BAT thermogenesis (20). Early efforts to manipulate thermogenesis in adipose tissue were futile: dinitrophenol and related compounds had serious side effects leading to death, because of their potential effects on non-BAT cells. While other physiological activators such as catecholamines and thyroxine had adverse consequences, selective ADRβ3 agonists also faced roadblocks. Oral bioavailability was poor, until Mirabegron, an oral ADRβ3 agonist was approved for use in overactive bladder.

Role of adrenergic system in adipocyte thermogenesis

Brown adipose tissue is well innervated by nerves from the sympathetic nervous system; fall in temperature or emotional stress can activate β3 adrenergic nerves selectively, or alternatively directly activate ADRβ3 (21). While UCP1 in the inner mitochondrial membrane leads to heat generation, norepinephrine from the sympathetic nerves binds to brown adipocyte ADRβ3, thus inducing lipolysis within these cells (22). UCP1-independent thermogenesis has also been recognised, being mediated by FGF21 and IL-6. Despite the existing evidence, one must consider the many other variables that can influence adipose tissue *via* ADRβ3 (23-25).

Thermogenesis can be approached in ways other than cold exposure and use of ADRβ3 agonists. A number of others can be addressed employed including phosphodiesterase inhibitors, melatonin, β-aminobutyric acid, irisin, BMP-7, FGF21, follistatin, IL-6, etc (26-28).

β3 adrenoceptor agonist: before Mirabegron

Despite the encouraging results in animals, activators of ADRβ3 have not had success in humans. The limitations ranged from poor effectiveness, the need for parenteral administration, or toxicity, the last when used for other indications (29). In addition, rodents have a proportionately higher surface area to volume. Humans have little BAT which makes drug stimulation of little clinical significance.

Mirabegron: effects on adipose tissue browning

Mirabegron, a selective orally active ADRβ3 agonist, is approved in the treatment of overactive bladder (30). It is given 25-50 mg/day and is primarily excreted in faeces and urine after being metabolised by CYP3A4 (31, 32). A preliminary study assessed the effect of WAT from subcutaneous tissue to beige following administration of Mirabegron to the application of ice in lean and obese subjects (33). Biopsies were taken to assess UCP1 and transmembrane protein 26 (THEM26) expression, which were increased in the WAT of the cold-treated leg as well as the other leg not exposed to ice. To confirm the functional consequences of UCP1 expression, mitochondria from lean subjects were studied after unilateral exposure to cold. It was found that cold led to increased UCP1 and TMEM26 staining.

There have been two recent human studies on the outcomes of chronic Mirabegron on human glucose homeostasis, insulin sensitivity, HDL cholesterol and BAT. A higher than recommended dose of the drug (100 mg/day for four weeks) was administered to 14 healthy young women, to induce greater stimulation of BAT. The drug led to increased BAT activation, particularly among those who had little BAT at baseline (34). Increased activation of BAT occurred without any changes in body weight, adipose mass or any alterations in self-reported dietary intake.

Three patterns of metabolite responses were observed with Mirabegron: no effect in most, acute increase in β-hydroxybuturate and non-esterified fatty acids on day 28 or changes which could be responsible for improved metabolic milieu:

increased HDL cholesterol, ApoE, apolipoprotein A1, and adiponectin (34). This study demonstrated that chronic Mirabegron administrated induced changed in BAT similar to exposure to cold. The postulated mechanisms included adiponectin acting through skeletal and liver, increased incretin or a direct effect on pancreatic β cells (35).

Another publication in the same issue studied the effect of Mirabegron on glucose metabolism in insulin resistant obese subjects. They were administered 50 mg/day for 12 weeks. The drug improved glucose tolerance and reduced glycosylated haemoglobin (36). There were no alterations of resting energy expenditure, body composition or plasma lipids. No adverse effects were reported. Improved glucose tolerance resulted from simultaneous increase in pancreatic β cell function and improved insulin sensitivity. Mirabegron led to increased expression of beige adipose tissue markers UCP1, TMEM26 and cell death inducing DNA fragmentation factor subunit alpha (DFFA)-like effector in white adipocytes. Gene expression studies on adipose tissue showed that Mirabegron treatment resulted in altered mRNA of fatty acid-binding protein, FGF 21, and retinol-binding protein 4. All these genes involved in adipocyte function are related to inflammatory response (37).

Summing up and conclusion

Given both animal and clinical studies, the metabolic benefit of Mirabegron is being unravelled. Published evidence showed that the drug positively affects circulating adipokines, glucose metabolism, and liposomes. Together with lifestyle changes, the drug appears to be the most promising ADRβ3 agonist currently available for the potential treatment of obesity and related cardiometabolic diseases.

In conclusion, Mirabegron administration improved the homeostasis of glucose among obese insulin-resistant subjects by enhancing insulin sensitivity and pancreatic β cell function. Still to be known are the sustainability of its beneficial effects. An accompanying editorial concluded that "the potential therapeutic utility of ADR β3 for cardiometabolic disorders is down, but it's not yet out" (37). There are yet questions to be answered along with their potential role as a supplement to the synergy of other pathways for metabolic and mental health (38-44).

Conflict of interest statement

The authors declare that no conflicts of interest exists.

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