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Pathogenesis of type 2 diabetes and cardiovascular disease in South Asians: effects of dietary interventions on metabolism and cardiovascular function

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Brown adipose tissue: the body's own weapon against obesity?

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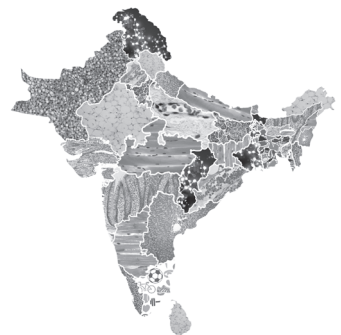
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*Translation of 'Bruin vet: een lichaamseigen middel in de strijd tegen obesitas?'
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ABSTRACT

Brown adipose tissue (BAT) dissipates energy stored in triglycerides as heat via the uncoupling protein UCP-1. It has recently been discovered that BAT is present and active in adults. BAT is situated predominantly around the aorta and in the supraclavicular area. BAT volume and activity are lower in individuals who are obese, suggesting that BAT significantly contributes to energy expenditure. Several pathological conditions that lead to activation of BAT, such as hyperthyroidism and pheochromocytoma, result in increased energy expenditure and in weight loss. Various ways in which BAT can be manipulated to increase expenditure of energy have been identified, e.g. exposure to cold, the use of so-called uncoupling agents or the administration of the hormone irisin. The activation of BAT could potentially be used to induce weight loss.

INTRODUCTION

Adipose tissue can be subdivided into 'white adipose tissue' and 'brown adipose tissue' (BAT). Until recently, it was thought that BAT disappears in adult life. However, modern techniques as ^{18}F -FDG-PET-CT-scans have proven otherwise. Interestingly, it has been discovered not long ago that BAT can be manipulated to increase energy expenditure.

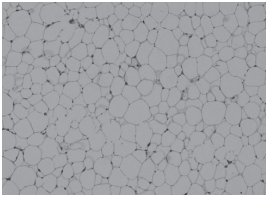
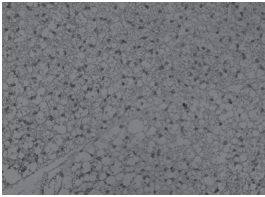
The function of white adipose tissue is to store triglycerides and to produce a large number of factors, the so called adipo(cyto)kines.¹ In contrast, BAT continuously burns triglycerides and glucose, thereby releasing energy as heat. This process is called thermogenesis. In neonates, thermogenesis contributes importantly to the maintenance of body temperature.

In this article we will give an overview of the anatomy, physiology and function of BAT and describe how BAT can be manipulated in order to increase energy expenditure and possibly induce weight loss.

Anatomy and origin of brown adipose tissue

BAT differs strongly from white adipose tissue in both volume and structure (**Table 1**). The total amount of BAT in an adult human is estimated to be 50-100 grams, while the amount of white adipose tissue is roughly 20% of total body weight. A white adipocyte consists of a big vacuole filled with triglycerides, surrounded by a thin rim of cytoplasm. The cytoplasm contains the nucleus and cell organelles, including a few mitochondria

Table 1 Differences between white and brown adipose tissue.

Characteristic	White adipose tissue	Brown adipose tissue
Microscopic image		
Amount in human body	12 – 35 kg	circa 50 – 100 g
Morphology	large cells filled with triglycerides few mitochondria	cells filled with small lipid droplets large number of mitochondria
Location	present throughout the whole body	in brown fat pads, predominantly in the subscapular area (neonates), and in the supra-clavicular region and along the aorta (adults); scattered as groups of cells in white adipose tissue and skeletal muscle
Function	storage of fat	conversion of triglycerides and glucose into heat

that provide for the formation of ATP, the main energy supplier of cells. In contrast, a brown adipocyte contains several small lipid droplets that are surrounded by a large number of mitochondria. The iron-containing proteins that are part of the respiratory chain inside the mitochondria give BAT its brownish colour.

Brown fat cells are present in two forms. On the one hand, they form brown fat pads, which are located in the subscapular area in neonates and along the aorta and in the supraclavicular region in adults.² In addition, individual brown fat cells lie scattered in other tissues, such as white adipose tissue and muscle, where they form a pool of 'peripheral brown adipocytes'. These cells are also called 'beige adipocytes', since their phenotype lies between a white and brown fat cell.

The two types of brown fat cells have different origins (**Figure 1**). Brown adipocytes present in the brown fat pads originate from a Myf5 (myogenic factor 5)-positive precursor

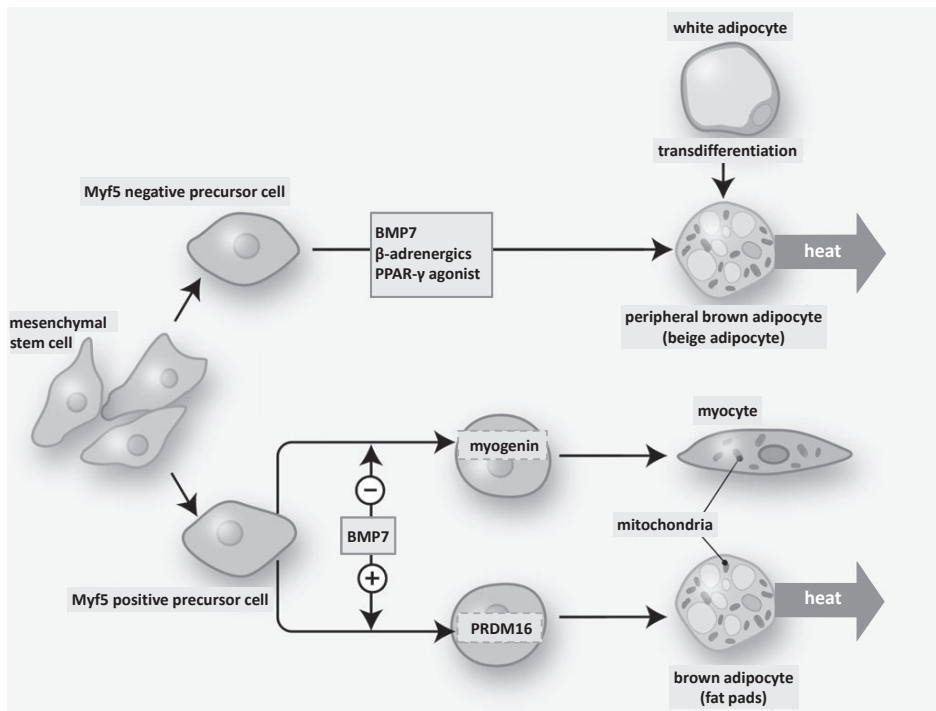


Figure 1. Differentiation of mesenchymal stem cells into brown adipocytes. Myf5-positive precursor cells can differentiate into skeletal muscle cells or brown fat cells, depending on the presence of BMP7. The skeletal muscle cells express myogenin, the brown fat cells PRDM16. This type of brown fat cell is present in fat pads. Myf5-negative precursor cells differentiate into peripheral brown fat cells ('beige fat cells') under influence of several stimuli, such as BMP7, β-adrenergics and PPAR-γ agonists. Peripheral brown fat cells can also originate from transdifferentiation of a white fat cell. (Myf5 = myogenic factor 5; BMP7 = 'bone morphogenetic protein-7'; PRDM16 = 'PR domain containing 16'; PPAR-γ = peroxisome proliferator-activated receptor-γ) (adaptation of a previously published figure).³

sor cell; this precursor cell can differentiate into both a brown fat cell and a skeletal muscle cell, depending on the presence of stimuli such as Bone Morphogenetic Protein 7 (BMP7). The peripheral brown fat cell – or beige fat cell – originates from a Myf5-negative precursor cell, as well as from transdifferentiation of a white fat cell into a brown fat cell.^{3,4} Several stimuli (such as BMP7, β -adrenergics and peroxisome proliferator-activated receptor- γ (PPAR- γ)-agonists) can activate the differentiation of Myf5-negative precursor cells into peripheral brown fat cells.³ These stimuli are therefore considered interesting therapeutic targets.

Physiology of brown adipose tissue

In mitochondria ATP synthesis takes place. This process starts with the conversion of fatty acids and glucose into acetyl coenzyme A, that participates in the citric acid cycle in the mitochondrion. The cyclic acid cycle generates energy-rich compounds, such as

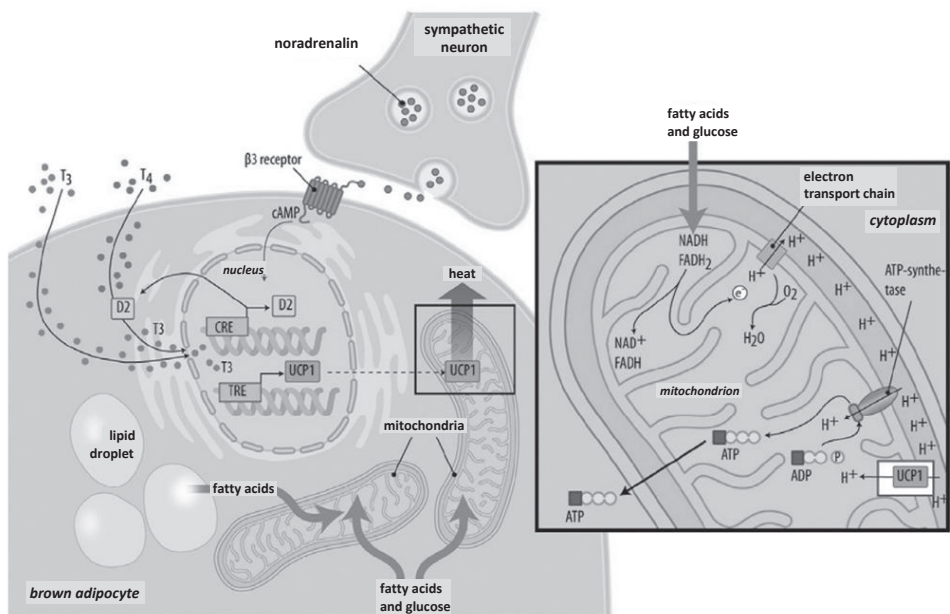


Figure 2. Activation of brown adipose tissue. In resting condition, the energy-rich compounds NADH and FADH₂ donate their energy in the form of electrons to the electron transport chain, thereby creating a gradient of H⁺-ions across the inner mitochondrial membrane (see inset right). The energy stored in this gradient is used by the enzyme ATP synthetase to convert ADP into ATP. However, in BAT the protein UCP-1 uncouples the H⁺-gradient of ATP-synthetase. This happens when a sympathetic nerve cell activates the β_3 -receptor of brown adipocytes by secretion of noradrenalin (left drawing), leading to the formation of cAMP and eventually to the production of UCP-1. Transport of H⁺ across the inner membrane via UCP-1 results in the production of heat instead of ATP. The active thyroid hormone T₃ also leads to production of UCP-1 (cAMP = cyclisch AMP; UCP-1 = ‘uncoupling protein 1’; T₃ = tri-iodothyronine; T₄ = thyroxine; D2 = type 2-deiodinase) (derived from a figure published elsewhere).²⁶

NADH and FADH₂, which donate their electrons during oxidative phosphorylation to the electron transport chain, thereby creating a gradient of H⁺-ions across the inner mitochondrial membrane (**Figure 2**). The energy stored in this gradient is used by the enzyme ATP synthetase to convert ADP into ATP.

The brown adipocyte is able to burn triglycerides and glucose via the process of 'uncoupling', in which energy is released as heat instead of ATP, resulting in increased energy expenditure in the cell. A detailed description of the physiology of a brown fat cell is shown in **Figure 2**.

Every brown adipocyte is innervated by a sympathetic nerve. The most important stimulus of the sympathetic nervous system is cold; the sympathetic nerve is activated by the temperature centre in the brain, which receives input from sensory nerve fibres in the skin. Upon stimulation, the sympathetic nerve locally releases noradrenalin, which binds to β3-adrenergic receptors on the brown adipocyte. Activation of these receptors results in a cascade of intracellular reactions.

Uncoupling protein (UCP) First, the expression of the gene encoding for the uncoupling protein UCP-1 is induced via elevation of intracellular cyclic AMP. UCP-1 is unique for BAT. UCP-1 proteins are then incorporated into the inner membrane of mitochondria, forming pores. However, in the presence of UCP-1 the proton gradient is disturbed and the accumulated energy dissipates as heat rather than being converted into ATP. The greater the density of mitochondria (or the amount of UCP-1) in BAT, the more glucose and triglycerides will be burned and released as heat when BAT is activated.

Increased influx of substrate At the same time, sympathetic activation of BAT results in an increased amount and activity of the enzyme lipoprotein lipase (LPL), which cleaves fatty acids from triglycerides for uptake by BAT, and of GLUT-1, the glucose transporter that increases the uptake of glucose by BAT.⁵ In this way sympathetic activation of BAT increases not only thermogenesis, but also the influx of the necessary substrate.

Thyroid hormone Remarkably, thyroid hormone is also involved in the activation of brown fat. After uptake into the brown fat cell and translocation to the nucleus, T3, the active thyroid hormone, binds to thyroid hormone responsive elements located on the promoter of the UCP-1 gene.⁶ This leads to increased transcription of UCP-1 and consequently to increased conversion of triglycerides and glucose into heat.⁵ Furthermore, T3 is able to stabilize the UCP-1-mRNA, thereby reducing its degradation in the cell.⁵ During cold-induction, the activity of the enzyme type-2-deiodinase (D2) is increased in BAT, leading to locally increased amounts of T3. This is an additional and necessary mechanism to stimulate thermogenesis by BAT.

Presence of brown adipose tissue in adult humans

The primary function of BAT is the production of heat – nonshivering thermogenesis – to prevent a decrease in body temperature. In neonates this is particularly important,

since they have a relatively large body surface area and little capacity to shiver due to underdevelopment of their muscles.⁵

Although BAT remains present in large quantities in rodents and other mammals, in humans the amount of BAT declines fast after infancy. Until recently, it was even assumed that in adults BAT is almost completely absent; indeed, it is hardly necessary as in adults skeletal muscle contributes primarily to heat production. However, ¹⁸F-FDG-PET-CT scans performed in wintertime in adults in the context of visualization of malignancy showed increased glucose uptake in locations corresponding to BAT. Like malignant cells, brown fat cells take up glucose at an increased rate due to their high metabolism. Biopsies from these areas indeed showed a very high expression of UCP-1, the unique marker for BAT.

Further research by Van Marken Lichtenbelt and others with ¹⁸F-FDG-PET-CT scans showed that – following cold-induction – BAT is present in almost 100% of young adults (**Figure 3**).² However, recent genotyping of human brown fat biopsies showed that this brown fat resembles more the beige fat found in white fat depots in mice rather than the classical brown fat.⁷ Whether beige fat in humans has the same physiological properties as the classical brown fat in mice remains to be determined in the coming years.

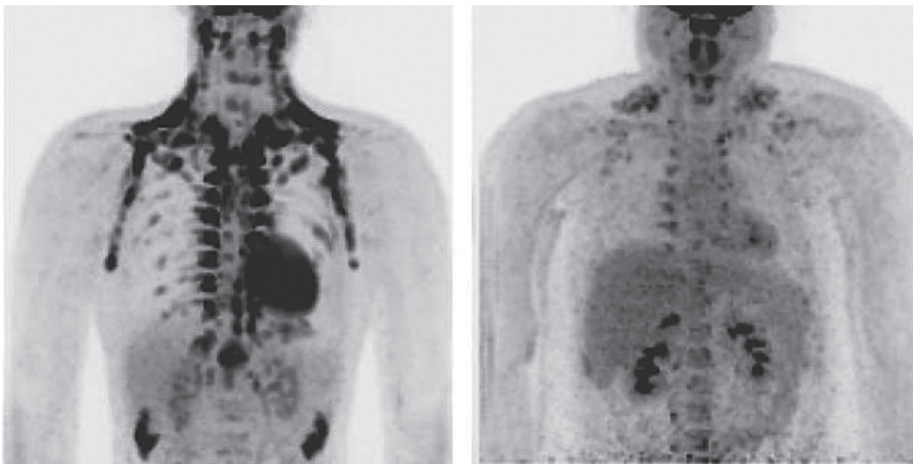


Figure 3. ¹⁸F-fluorodeoxyglucose (FDG)-PET-CT-uptake of brown adipose tissue (BAT) in adults. BAT can be visualized with a FDG-PET-CT-scan. To do this, a patient is exposed to cold (circa 16°C) during 2 hours to activate BAT. After 1 hour of cold-induction the radioactive tracer ¹⁸F-FDG is injected intravenously. ¹⁸F-FDG, a glucose analog, is taken up by organs that have a high glucose usage, especially the brain, heart and BAT. After 2 hours of cold-induction, and 1 hour after administration of the tracer, the uptake of ¹⁸F-FDG is measured in circa 30 minutes using a low-dose CT-scan, immediately followed by a PET-scan. The CT-scan is used for localization of the FDG uptake sites. The activity and volume of BAT can be quantified by auto-contouring the areas in which FDG-uptake has taken place with a set threshold. Remarkable in this figure is the increased presence of BAT in a lean subject (left) compared to an obese subject (right) (figure is derived from a previous publication).²

Involvement of brown adipose tissue in pathology

Hibernomas and pheochromocytomas

In adults, the presence of BAT was already noticed in two clinical conditions: hibernomas and pheochromocytomas. Hibernomas are rare, benign tumours, named for their resemblance to BAT in animals that go into hibernation. They are clearly visible on ^{18}F -FDG-PET-CT scans, indicating that the tumour is metabolically active.⁸ Pheochromocytomas, which are neuroendocrine tumours, secrete excessive amounts of noradrenalin, an important activator of BAT. Indeed, on ^{18}F -FDG-PET-CT scans in patients with this tumour, an increased volume and activity of BAT is seen. Moreover, after resection of the tumour FDG uptake decreases dramatically. The increased activity of BAT probably contributes significantly to the increased energy expenditure typical for this condition.⁹

Hyperthyroidism and hypothyroidism

In people with hyperthyroidism energy expenditure is increased, while this is decreased in hypothyroidism. In mice, thyroid hormone has been shown to be both directly – via the T3-receptor – and indirectly – via the sympathetic nervous system – involved in the activation of BAT.^{10;11} The weight loss and excessive transpiration in hyperthyroidism, and the weight gain and reduced cold tolerance in hypothyroidism can therefore be (partly) attributed to an increased, respectively decreased activity of BAT.⁵

Obesity and type 2 diabetes mellitus

An interesting finding is that in adults the amount of BAT is inversely related to BMI and percentage of body fat.² More specifically: the volume and activity of BAT are inversely correlated with parameters of central obesity, such as visceral fat volume on CT-scan and waist circumference.⁹ These findings suggest that obesity is associated with a low level of BAT activity. On the one hand, a reduced activity of BAT may predispose to obesity and obesity-related diseases such as type 2 diabetes mellitus by accumulation of triglycerides in the blood and subsequent storage in white adipose tissue, including ectopic fat depots such as skeletal muscle and the liver. Indeed, it has been shown in mice that excision of BAT results in hypertriglyceridemia and obesity.¹² Alternatively, insulation, due to the thick subcutaneous white fat layer in obese individuals, may be sufficient for the maintenance of body temperature, making active BAT redundant; low activity of BAT could then be the consequence of obesity.¹³

BAT burns both triglycerides and glucose. Therefore, BAT could also contribute to glucose homeostasis, particularly in resting conditions when glucose utilization by skeletal muscle is minimal. A low activity of BAT might, thus, not only predispose to type 2 diabetes via the aforementioned relation to obesity, but also via reduced glucose uptake at rest.¹⁴

Manipulating volume and activity of brown adipose tissue

The above-mentioned findings underscore that increasing the volume and activity of BAT is a promising target to increase total energy expenditure and consequently induce weight loss. Theoretically, BAT volume and activity can be increased in several ways. Generally, a distinction is made between methods that activate already present brown fat cells, and methods that stimulate the recruitment of new brown fat.

Activation of existing brown adipose tissue

BAT is strongly innervated by the sympathetic nervous system (**Figure 2**). This offers potential targets for intervention. Furthermore, the uncoupling phenomenon is a possible target.

Cold The most important activator of BAT via the sympathetic nervous system is cold. Several studies have shown a relation between the volume and activity of BAT and the outdoor temperature, with the highest activity during the coldest month of the year.¹⁵ Therefore, the simplest method to activate BAT seems to be cold induction, for instance via creating a colder living or working environment. Whether this will actually affect BAT activity, and thus may induce weight loss, remains to be investigated.

Sympathomimetics In addition to indirect activation of β_3 -adrenergic receptors by cold, sympathomimetics could also be used. In mouse models these seemed to be very successful.¹⁶ Unfortunately, the expected weight-reducing effect failed to occur in humans. In addition, side effects – sometimes life threatening – were experienced.^{17,18} So far, these agents are therefore not used for clinical purposes.

Uncouplers Increasing the uncoupling of ATP synthesis towards heat in BAT or other tissues, such as white adipose tissue or skeletal muscle (ectopic expression), might be an effective method to increase energy expenditure. Already in the thirties of the last century, the chemical uncoupler 2,4-dinitrophenol (DNP) was successfully used as an ingredient in diet pills. However, chemical uncouplers influence oxidative processes in all tissues, and not specifically in BAT. Due to serious side effects, like hyperthermia, this agent was therefore withdrawn from the market in 1938.¹⁹ Current research now focuses on increasing (ectopic) expression of naturally occurring uncouplers, such as UCP-1.^{20,21}

Recruitment of new brown adipose tissue

New BAT could be recruited by stimulating the differentiation of precursor cells of white adipocytes into brown adipocytes.

PPAR- γ agonists One of the key regulators in the differentiation of adipocytes is PPAR- γ . Animal studies have shown that PPAR- γ -agonists can recruit precursor cells of BAT and, in addition, can “brown” white adipocytes that thereby obtain UCP-1.²² PPAR- γ -agonists are already used in the treatment of type 2 diabetes: the thiazolidinediones (TZDs). Research has shown that the improvement in insulin sensitivity with TZDs is

partly due to an accelerated clearance of glucose in BAT.²³ Remarkably, though, use of TZDs leads to weight gain – partly due to fluid retention – and a different fat distribution, making their use as a weight loss agent less likely. Moreover, prescription of these agents is limited due to side effects such as heart failure and osteoporotic fractures.

BMP7 Another important regulator in the differentiation of BAT is BMP7. Recent studies in mice have shown that BMP7 is an effective agent to increase the amount of BAT – both in fat pads as peripherally – leading to an increase in energy expenditure and weight loss (Boon *et al.*, unpublished data).^{3,24}

Irisin Irisin is a recently discovered hormone that, in both mice and humans, is secreted by skeletal muscle during exercise. A recent study in mice demonstrated that exogenous administration of irisin induced 'browning' of subcutaneous white adipose tissue. This resulted in an increase in energy expenditure, a decrease in weight and an improvement in glucose tolerance.²⁵

CONCLUSION

BAT burns triglycerides and glucose towards heat via the uncoupling protein UCP-1, and thus has a significant share in total energy expenditure. The recent observation of active BAT in adult humans might therefore offer new possibilities in the fight against obesity. Currently, various studies focus on activating BAT as a treatment strategy against obesity. In mouse models we seem to be close to success; the coming years will tell us whether BAT may be a novel therapeutic target organ in humans to combat obesity and related disorders such as type 2 diabetes.

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