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REVIEW ARTICLE

RECENT ADVANCES IN HUMAN BROWN FAT PHYSIOLOGY

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Abstract : Of the two variants of adipose tissue, white fat is traditionally known as a lipid rich tissue which undergoes pathological expansion in obese conditions. To counter the excess accumulation of white fat in states of energy imbalance, the second and unique type of brown fat plays a key role by burning extra energy into heat through a special metabolic pathway. In addition brown fat also plays a vital role in thermoregulation in animals and newborn humans and infants. Recent progress in research areas of these two types of fat tissue has provided compelling evidence to show that they secrete a large number of chemicals that play an important role in body weight control that involves several mechanisms. Brown fat was considered absent in the adult humans until recently. But new techniques have provided ample support for its active existence. Based on the very recent data it has been suggested that brown fat can be a target organ in the treatment of obesity which can lead to exciting and informative outcomes in the future.

Key words : obesity, brown fat, white fat, energy balance, thermoregulation

INTRODUCTION

White fat was once considered as a lipid storage organ whereas the brown fat as important in the thermoregulation of smaller mammals and human neonates. Research has provided much evidence to show that white fat is now regarded as an endocrine organ secreting a large number of chemicals that play a vital role in food intake regulation and body weight control. The brown type on the other hand is the key to produce extra heat under special conditions of low environmental temperatures and following food intake thereby maintaining body temperature and energy balance. In the face of re-emergence of brown fat with a suggested role in obesity treatment it is intended to update the available knowledge and the recent progress made in understanding the role of this tissue.

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Origin, types, differences and functions of adipose tissue

Adipose tissue arises from the mesoderm along with skeletal muscle, cartilage and bone. The precise lineage though unknown, the mesenchyme produces common progenitors that develop into white adipose tissue (WAT) and brown adipose tissue (BAT) (1). This finding will have great bearing in understanding the functional importance of these two variants of fat.

The WAT is also referred to as white fat (WF) or yellow fat. It contributes to about 20-25% of body weight in normal humans and has important functions. The classical role of WAT as a storage of energy in the form of triglycerides is well known. The subcutaneous white fat provides insulation for heat conservation in body temperature regulation. The visceral fat acts as a protective cushion for the internal organs surrounding the fat (2). Of late, progress in adipose tissue research has established WAT as an 'endocrine organ' as it secretes a number of hormone or hormone like substances called 'adipokines' (Table I). Some of them, particularly leptin play a vital role in the pathophysiology of obesity, diabetes mellitus, hypertension and atherosclerosis (3).

The second variant of the fat tissue, the unique BAT also known as brown fat (BF) is a form of lipid containing connective tissue. It is found in almost all mammalian species and is distributed diffusely as small depots. Two very important physiological roles ascribed to BAT are i) 'thermoregulatory thermogenesis' to produce heat for defending body temperature in cold exposure, (cold induced thermogenesis – CIT or nonshivering thermogenesis – NST), during postnatal periods in newborns, during arousal from hibernation in animals and ii) 'metaboloregulatory thermogenesis' that burns excess energy to produce heat (diet induced thermogenesis – DIT) thus affecting energy balance to regulate body weight. Many experiments in animals on brown fat and its thermogenic response to overfeeding has strengthened the view that BAT could also be of importance in energy balance, body weight regulation and in the etiology of obesity in humans (4–7). Muralidhara and Desautels (8, 9) have shown that in diet-induced obese mice brown fat thermogenic function was reduced during fasting which recovered following nutritional rehabilitation was independent of the animal's prior energy reserves. The same authors (10, 11) have also shown that ethanol consumption in mice did not involve BAT functional alterations, though produced energy deposits as extra fat. Desautels and his coworkers (12) have reported an important and indirect role for histamine produced by the mast cells of BAT in its thermogenic response. Muralidhara and Shetty (13–16) have shown thermoregulatory insufficiency in the form of reduced CIT/NST, reduced BMR and low body temperatures in young malnourished Wistar rats that are directly related to brown fat activity levels.

Recent studies have shown a relationship between BAT activity and physiological status where men have less BF than women and older people have less BF than younger people. They also have reported that people with high blood sugar have less BF than people with normal blood sugar and obese people have lesser BAT than lean people indicating an inverse relationship between

body mass index (BMI) and BAT activity level in maintaining the lean phenotype (17, 18). These observations reveal some functional significance of BAT in relation to body fat content as heavier people are provided with better insulation in the form of white fat as they are deficient in metabolic thermoregulatory function due to lesser BAT and vice versa in the leaner group. Furthermore, BAT also secrete chemical substances in smaller quantities classified as autocrine, paracrine and endocrine secretions (Table I) of which some are also produced by WAT e.g. Resistin, Angiotensin (5).

Though WAT and BAT have distinct physiological roles and differ in their distribution and histological features they are closely interrelated (Table II).

Location of human BAT

BAT contributes to about 5% of total body weight in infants but much less in adults (10). It is located between the scapulas, at the nape of the neck, along the great vessels in the thorax and abdomen and also scattered in other areas in the body (19-21). Some of these studies had confirmed that BAT persists into adult life. Though human adults

TABLE I: Secretions of the adipose tissue.

WAT secretions	*BAT secretions*		
	Autocrine	*Paracrine*	*Endocrine*
Adinopectin, Resistin, Apelin, Angiotensin, Visfatin, Vaspin, Tissue Necrotic Factor-a (TNF-α), Leptin	Basement membrane proteins e.g. Collagen IV, Laminin, Fibronectin, Adipsin Basic fibroblast growth factor, Insulin like growth factor I, Prostaglandins E2, F-alpha, Adenosine	Nerve growth factor, Vascualr endothelial growth factor, Nitric oxide, Angiotensinogen	IL-l,IL-6, Leptin, Adinopectin, Resistin, T3

TABLE II: Major differences between WAT and BAT. (From various sources).

Feature	*White fat*	*Brown fat*
Presence	Throughout life	Early in life, also in adults
Location	Subcutaneous, Viscera	Specific locations (See Table III)
Colour	White or yellow	Varies from dark red to tan. Due to rich blood supply and dense mitochondria that contains high levels of cytochrome.
Formation	Formed during the third trimester of intrauterine life.	Formed during the third trimester of foetal life.
Histology	Cell size 25-200 micron, single fat globule (unilocular), few mitochondria, peripheral flattened nucleus giving a 'signet-ring' appearance	Cell size 50-60 micron, many fat globule (multilocular, important for heat production), dense mitochondria, central round central nucleus
Innervation and vasculature	Sympathetic nerve supply more to vessels than the cells. Lesser vasculature to the tissue	Rich sympathetic innervations to both cells and blood vessels. Rich blood supply to the tissue
Biochemistry	Absence of UCP1, More ATP production, No regulatory heat production	Presence of UCP1, High cytochrome content, Less ATP Production, Heat production
Function	Storage of energy, Insulation, Cushion	Consumes energy to produce heat via CIT, NST, DIT

were assumed to have less or no functional BF with advancing age due to the loss of mitochondria, ^{18}F-flurodeoxyglucose positron emission tomography (^{18}F FDGPET), radioactive iodine uptake studies have shown that a majority of the adult subjects possess active and functional BAT in the upper chest and other parts of the body, the largest depot being in the neck region and supraclavicular area (17, 18, 20, 22-25). The other areas include paravertebral, mediastinal, para-aortic, suprarenal, perirenal, apex of the heart and so on. But the most interesting finding from these studies was that in the adult humans BF was not present in the interscapular area (IBAT) as supported from PET-CT scans. This report confirms the findings of an earlier study by Astrup (26).

Thermogenesis in the BAT

BAT acts like a 'furnace' or 'burner for the heater'. In other words, the function of BAT is to burn energy (from food) to heat by a unique metabolic pathway. The mitochondria in a eukaryotic cell utilize fuels to produce energy in the form of adenosine triphosphate (ATP). In tissues other than BAT, oxygen consumption by the mitochondria is closely coupled to ATP synthesis. The beta oxidation of fatty acids and the citric acid cycle lead to formation of 2 energy-rich electron donors, reduced nicotinamide adenine nucleotide (NADH) and a NADH phosphate (NADPH) in the mitochondrial matrix. In this process oxygen is reduced to water and protons (H^+) are transported from the mitochondrial matrix across the inner mitochondrial membrane. Because the inner mitochondrial membrane is generally impermeable to charged molecules, a proton gradient is generated across this membrane. Protons can enter the mitochondrial matrix only by the ATP-synthase complex. In this enzyme complex, energy derived from the flow of protons entering the mitochondrial matrix drives the phopsphorylation of adenosine diphosphate to ATP. This mechanism ensures the oxygen consumption is tightly coupled to ATP synthesis. The inner membrane of the mitochondria in BAT cells however, expresses a 32-kDa 'uncoupling protein,' (UCP) called UCP1 which allows protons to enter the mitochondrial matrix without ATP's is being synthesized. This means that ATP synthesis is uncoupled from oxygen consumption and that energy released from the oxidation of NADH and NADPH is completely converted to heat through this alternative proton conductance route (proton run back) or 'short-circuit' conductance (27).

Recognition of the importance of BAT

Several techniques from light microscopy to more sophisticated positron emission tomography and computed tomography (PET-CT) scanning have been of great help in providing ample evidence for the existence of BAT in animals and humans. The other important techniques include excision studies, measurement of noradrenaline stimulated oxygen consumption, blood flow measurements, infrared thermography, immuno-histochemistry, enzyme histochemistry, radioimmunoassay, molecular biotechniques and so on.

In 1551 Konrad Gessner recognized BAT as 'Embryonal fat' in hibernating marmots. He explained it as 'neither fat, nor flesh – but something in between'. At different time points in the history of BAT over 450 years,

it was considered as a 'glandular organ' or a 'hibernating gland' or an 'endocrine gland' subserving different functions. BAT is found in almost all mammalian species and is located in various parts of the body depending on the species and the age of the animal (2, 5). Histologically detectable BAT in human infants and adults in a number of locations is well established (19, 21, 28) (Table III). In the first decade of life active BF seems to be widely distributed. With advancing age, BAT starts disappearing and seen only in deeply situated areas as late as 8th decade of life (19, 21). Interscapular BAT (IBAT) depots may not be quantitatively important in therrnogenesis in adult humans as there was no histological evidence for its presence (21, 26) where as all other internal sites contain at least isolated or islands of BAT (19-21). This view is supported by recent findings using newer techniques (17, 18, 22, 25).

The 'thermogenic' function of BAT and its 'consequences' on body temperature regulation and body weight control through energy balance mechanisms (5, 7) was clearly established only after a great deal of research carried out in the late 1960s in small laboratory animals (5, 7, 29, 30). The presence of UCP1 in BAT and the role of BAT in influencing the metabolic efficiency was identified later in 1970s (31, 32).

BAT deposits become more visible in cold exposed conditions (7, 17, 18, 33). Experiments in genetically obese rats and mice have shown reduced capacity for cold induced thermogenesis (34). The evidence for BAT heat production also comes from the observation that in animals reared in thermoneutral conditions the tissue subsequently involutes and heat producing capacity is lost whereas cold exposure and feeding may activate heat production and prevent the involution of BF (35). Blood flow and sympathetic nerve discharge in BAT increases after food intake so that heat production is increased (6, 29-31). Reduced BAT thermogenic capacity or BAT atrophy was associated with deposition of extra fat leading to obesity in animals (i.e. increased metabolic efficiency) while increased heat generation following cafeteria diet was associated with reduced metabolic efficiency (31, 36). BAT thermogenesis, both CIT/NST and DIT are controlled by the hypothalamus via sympathetic nervous system involving norepinephrine (NE) as the neurotransmitter and UCP1 plays a vital role in all these cases (33, 37, 38). NE controls the thermogenic process in the brown fat cells through beta-3 receptors by activating UCP1. The functional activity of BAT in any given physiological condition is determined by NE through the maximal capacity and the total thermogenic capacity. NE also promotes proliferation and differentiation of brown adipocytes and regulates the genetic expression of UCP1. NE plays an important role in preventing the death of brown fat cells (39). It also has been shown that within the white fat deposits in adult humans, islets of brown fat are found along with UCP1 and its level can be elevated by NE. Thus the life of brown fat cells is under adrenergic control to a large extent.

TABLE III : Locations of BAT in the humans. (Ref 10).

Interscapular area, Neck vessels, Neck muscles, Calvicles, Axillae, Intercostal vessels, Mammary vessels, Trachea, Oesophagus, Pericardium, Lung hilum, Para-aortic region, Pancreas, Splenic hilus, Kidneys, Suprarenals, Mesocolon, Greater omentum, Inguinal areas, Anterior abdominal wall.

Adult human BAT

BAT studies in the adult humans are controversial. Several authors have shown the presence of BAT in adult humans (17-21, 24, 25). BAT deposits in humans are located primarily in the supraclavicular and neck regions. It is also present in paravertebral, mediastinal, paraaortic and suprarenal areas. But IBAT deposits are not seen in adult humans unlike in the newborn infants or in rodents which form the main BAT deposits in them. Although the evidence for the presence of BAT in adult humans was presented by Lean (21) in late 80's it was largely ignored. However, Lean seems to be right as Nedergaard and his co-workers (20) in their recent review have concluded with evidences that a substantial fraction of adult humans possess active BAT which can be of metabolic importance in normal physiological conditions.

Adult men exposed to <10°C cold have responded with increased energy expenditure with no change in the basal metabolic rate (BMR). Shivering may also contribute to energy expenditure in addition to BAT metabolic heat. But Neilsen (40) has shown that shivering was not the source of heat produced during cold exposure in the adult men. NE injections as well as cold exposure increases heat production in adult humans (41, 42). Sims with his co-worker (43) has shown increased energy expenditure in individuals overfed a high fat diet for several months. On the other hand, a few authors have reported reduced regulatory thermogenesis in obese diabetic subjects (44). Jung et al (45) and others have shown that obese subjects have a decreased response to NE injections. These findings led to the speculation that BAT may be involved in the development of obesity (46). Lean and his co-workers (47) have found a positive correlation of the results of the experimental animals with altered thermogenic capacity in cold exposure with Cushing's disease, diabetes and phaeochromocytoma. More convincing evidence for the functional capability of BAT comes from NE secreting tumors phaeochromocytoma. This condition is associated with abundant brown fat in the perirenal fat and significantly increased UCP1 levels (47, 48). Other reports published also have shown a good correlation between reduced heat production, increased BAT lipid contents and clinical characteristics of hypothyroidism (49).

With ageing or with body size increasing brown fat function relatively reduces. This is because of the higher ratio of heat production and smaller surface area in all adult animals including humans. It also is due to the reduced requirement of heat production as clothing and indoor life can protect the humans from cold exposures. But given a necessity or chance to increase the thermogenic function of the BAT as in severe cold conditions one can expect the BAT in humans to revive their function.

BAT in human neonates

Newborns have relatively large deposits of brown fat and UCP1 (21). The wide distribution of BAT in early years of life may thus be related to the immaturity of the heat regulating mechanisms in the new born children. It is well known that the new born child needs to adapt to low environmental temperatures in the initial hours after birth by increasing metabolic heat generation. Heat generation is proportional to body mass. Premature babies have smaller body size and hence produce less heat. Furthermore, they are not able to generate heat by shivering

due to lack of muscle mass and unable to move away from cold stress. They do not have much BAT to assist metabolic heat production as it is laid down only in the 3rd trimester of intrauterine life and do not have much insulation as white fat is also laid down during the last trimester of pregnancy. The control on vascular function (vasodilation and vasoconstriction) is inadequate due to incomplete development of nervous system. BAT seems to be the major means of metabolic heat production until the 2nd year of life in the new born and therefore premature babies can be helped only to prevent heat loss and not heat generation. The consequences of hypothermia in such children are many which include neonatal cold injury, high metabolic rate and increased oxygen consumption leading to hypoglycemia, jaundice and kernicterus, increased susceptibility to infection and poor weight gain. Most of these complications are related to the dysfunctions of immature organ systems in the premature/ malnourished babies.

Following Bruck's earlier report (50) much work has been done on human neonates to show that BAT is largely responsible for heat production in cold exposure. Newborn infants have been shown to also increase oxygen consumption without shivering or involvement of other muscular activity. Normally 100-300% increase in metabolism is seen in normal neonates at 25°C cold exposure (21, 50). Hull (51) has suggested that approximately 30 g of BAT in the human infant is sufficient to account for total heat producing capacity in response to cold or NE injections which is equivalent to double or triple the normal metabolic rate (200-300%) or increase the core temperature by 5-6°/h. Aherne & Hull (52) have shown that 394 infants who died before the age of 4 weeks and the premature babies were at risk of thermoregulatory deficiency due to the lack of well developed BAT and WAT. Heim et al (53) have also reported that histological changes in BAT in infants exposed to different temperatures ranging from 22–35°C.

Studies on malnourished children of 4-16 months age have shown that they had lower basal metabolic rate, slow pulse rate, low body temperature and low RQ. Metabolic response of malnourished babies to cold exposure at 25°C was insufficient to produce increase in total oxygen consumption and also failure to raise the body temperature. Furthermore, IBAT atrophy was also seen in these children (28). Similar thermoregulatory deficiencies have been reported in children with low birth weights (54). These reports reveal very clearly the importance of brown fat in the new born malnourished babies.

The other clinical condition that supports the function of BAT thermogenic function is sudden cot-death which is a problem amongst the small infants. Lean & Jennings (55) has reported two cases of cot deaths with no other pathology other than high core temperature of > 40°C. Hibernoma – a rare benign tumor of BAT – is another condition that shows the presence of functional BAT in the humans characterized by increased BMR and weight loss (56, 57).

Recent advances in human BAT

Considering the total mass of BAT in adult humans, its physiological importance in whole body energy homeostasis was overlooked or considered negligible until now. But recent studies suggest that BAT activity could have a significant impact on

daily energy expenditure by dissipating energy as heat and can thus counteract weight gain. Therefore, therapeutic interventions or activation of BAT may be an effective approach for limiting obesity. Molecular biotechnology has greatly assisted in greater understanding of the factors and their mechanisms in the regulation of BAT functions. During the last few years research work related to transcriptional control of BAT development, differentiation and functions has kindled more hopes in that direction. The prime regulator of BAT formation and function PRDM16 (a zinc-finger protein selectively expressed in BAT) can simultaneously induce BAT gene expression while suppressing WAT gene expression. It is suggested that PRDM16 and other associated co-regulators – PPARy coactivator-lcc (PGGC -1α) and C-terminal binding protein (CtBPl/2) which controls the switch from WAT to BAT are potential targets for the development of obesity related therapeutics (58-60). Recent report on bone morphogenetic protein 7 (BMP 7), a bone growth messenger protein seems to be another important regulator of BAT (61). This protein is reported to play an important role in BAT cell differentiation, induction of PRDM16 and UCP gene expression. This new findings may suggest BAT as one of the possible effectors of pharmacological protection for human excessive adipose tissue deposition, diabetes mellitus, hypertension and arteriosclerosis and also to overcome the thermoregulatory insufficiencies. Although new findings are stimulating and looks rational, the possible counter effects of increased appetite, increased heat generation due to heightened BAT activity of such approaches must be kept in mind.

Conclusion

The cumulative information from both animal and human experiments has enhanced the knowledge of BF functions and its control to a great extent. There have always been questions raised on the relevance of the experimental results of animals and its application to humans. Some argue that there is no reason to believe that the experimental results of rodents on BF functions are not applicable to humans just because they are large mammals. However, it must be remembered that though BAT thermogenesis may contribute to 1-2% of energy balance and thereby to body weight regulation, a rough estimate of a defect of this order of thermogenic capacity could lead to weight gain at about 1-2 kg/year in humans. But it is very clear that adult humans have functional BAT that can be a new target for antiobesity and antidiabetes therapies focusing on increasing energy expenditure. Future studies may refine methodologies to measure BAT mass and activity and expand our knowledge of critical-control points in BAT regulation. Focus on future research may also help achieve long-lasting weight loss and an improved metabolic profile by testing pharmacological agents that increase BAT thermogenesis (62).

REFERENCES

1. Timmons JA, Wennmalm K, Larsson O et al. Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proc Natl Acad Sci* 2007; 104: 4401–4406.
2. Albright AL, Stern JS. (1998) Adipose tissue. In:

Fahey T.D. (ed.) Encyclopedia of sports medicine and science, Internet society for sport science, http://sportsci.org.

3. Fonseca-Alaniz MH, Takada J, Alonso-vale MI et al. Adipose tissue as an endocrine organ: from theory to practice. *J Pediatr (Rio J)* 2007; 83: suppl 5. S192–S203.
4. Smalley RL, Dryer RL. Brown fat: thermogenic effect during arousal from hibernation in the bat. *Science* 1963; 21; 140: 1333–1334.
5. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004; 84: 277–359.
6. Leibel RL. Molecular physiology of weight regulation in mice and humans. *Int J Obes* 2008; 32: Suppl 7: S98–S108.
7. Smith RE, Horwitz BA. Brown fat and thermogenesis. *Physiol Rev* 1969; 49: 330–425.
8. Muralidhara DV, Desautels M. Changes in brown adipose tissue composition during fasting and refeeding of diet-induced obese mice. *Am J Physiol* 1994; 266: R1907–R1915.
9. Muralidhara DV, Desautels M. Differing response of body weight gain to high fat diet treatment in the mouse. *Indian J Physiol Pharmacol* 1998; 42: 113–118.
10. Muralidhara DV, Desautels M. Effects of ethanol consumption on brown adipose tissue thermogenic capacity in mice. *Physiology and Behavior* 1996; 60: 645–652.
11. Muralidhara DV, Desautels M. Alcohol dehydrogenase activity in mouse brown adipose tissue. *Indian J Physiol Pharmacol* 1996; 40: 167–170.
12. Desautels M, Wollin A, Halvorson I, Muralidhara D V, Thornhill J. Role of mast cell histamine in brown adipose tissue thermogenic response to VMH stimulation. *Am J Physiol* 1994; 266: R831–R837.
13. Muralidhara DV, Shetty PS. Effects of preweaning nutritional deprivation on basal metabolism and thermoregulatory therniogenesis in the rat. *Brit J Nutr* 1986; 56: 615–623.
14. Muralidhara DV, PS Shetty. Basal metabolic rate, nonshivering thermogenesis and cold tolerance in rat during undernutrition and subsequent nutritional rehabilitation. Indian *J Exp Biol* 1990; 10: 972–976.
15. Muralidhara DV, Shetty PS. Sucrose feeding stimulates basal metabolism and nonshivering thermogenesis in undernourished rats. *Indian J Med Res [B]* 1990; 92: 447–451.
16. Muralidhara DV, Shetty PS. Metabolic responses to episodes of partial dietary restriction in undernourished Wistar rats. *Indian J Med Res* 1994; 100: 190–195.
17. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM et al. Cold-activated brown adipose tissue in healthy man. *N Engl J Med* 2009; 360: 1500–1508.
18. Cypess AM, Lehman S, William SG et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009; 360: 1509–1517.
19. Heaton JM. The distribution of brown adipose tissue in the human. *J Anat* 1972; 112: 35–39.
20. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab* 2007; 293: E444–E452.
21. Lean MEJ. Brown adipose tissue in humans. *Proc Nutr Soc* 1989; 48: 243–256.
22. Okuyama C, Sakane N, Yoshida T et al. $(123)^{1}$– or $(125)^{1}$ - metaiodibenzylguanididne visualization of brown adipose tissue. *J Nucl Med* 2002; 43: 1234–1240.
23. Cunningham S, Leslie P, Hopwood D et al. The characterization and energetic potential of brown adipose tissue in man. *Clin Sci* 1985; 69: 343–348.
24. Saito M, Okamatsu-Ogura Y, Matsushita M, et al. High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* 2009; 58: 1526–1531.
25. Virtanen KA, Liddel ME, Orava J et al. Functional brown adipose tissue in healthy adults. *N Engl J Med* 2009; 360: 1518–1525.
26. Astrup A, Bulow J, Christensen NJ et al. Ephedrine-induced thermogenesis in man: no role for interscapular brown adipose tissue. *Clin Sci* 1984; 66: 179–186.
27. Wolfgang A, Weber MD. Brown adipose tissue and nuclear medicine imaging. *J Nucl Med* 2004; 45: 1101–1103.
28. Brooke OG, M Harris, Salvosa CB. The response of malnourished babies to cold. *J Physiol* 1973; 233: 75–91.
29. Foster DO, Frydman ML. Nonshivering thermogenesis in the rat. II. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorigenesis induced by noradrenaline. *Can J Physiol Pharmacol* 1978; 56: 110–122.
30. Andrews JF. Comparative physiology of brown adipose tissue. *Proc Nutr Soc* 1989; 48: 237–241.
31. Rothwell NJ, MJ Stock. A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 1979; 281: 31–35.

32. Heaton GM, Wagenvoord RJ, Kemp JA, Nicholls DG. Brown-adipose-tissue mitochondria: photoaffmity labeling of the regulatory site of energy dissipation. *Eur J Biochem* 1978; 82: 515–521.

33. Klingenspor M. Cold induced recruitment of brown adipocyte thermogenesis. *Experimental Physiology* 2003; 88: 141–148.

34. Trayhurn P, Thurlby PL, James WP. A defective response to cold in the obese (ob/ob) mouse and the obese Zucker (fa/fa) rat [proceedings]. *Proc Nutr Soc* 1976; 35: 133A.

35. Benito M. Contribution of brown fat to the neonatal thermogenesis. *Biol Neonate* 1985; 48: 245–249.

36. Trayhurn P, Thurlby PL, James WP. Thermogenic defect in pre-obese (ob/ob) mice. *Nature* 1977; 266: 60–62.

37. Morrison SF, Nakimura K, Madden CJ. Central control of thermogenesis in mammals. *Experimental Physiology* 2008; 93: 773–797.

38. Sell H, Deshaies Y, Richard D. The brown adipocyte: update on its metabolic role. *Int J Biochem Cell Boil* 2004; 36: 2098–2104.

39. Lindquist JM, Rehnmark S. Ambient regulation of apoptosis in brown adipose tissue: ERK 1/2 promotes norepinephrine dependent survival. *J Biol Chem* 1998; 273: 30147–30156.

40. Neilsen B. Metabolic reactions to changes in core and skin temperature in man Acta Physiologica Scandinavica 1975; 97: 129–138.

41. Kurpad AV, Khan K, Calder AG, Elia M. Muscle and whole body metabolism after norepinehrine. *Am J Physiol Endocinol Meatb* 1994; 266: E877–E884.

42. Lesna I, Vybiiral S, Jansky L, Zeman V. Human nonshivering thermogenesis. *J Thermal Biol* 1999; 24: 63–69.

43. Sims EAH, Horton ES. Endocrine and metabolic adaptation to obesity and starvation. Am J Clin Nutr 1968; 21: 1455–1470.

44. Golay A, Schutz Y, Meyer HU *et al.:* Glucose-induced thermogenesis in nondiabetic and diabetic obese subjects. *Diabetes* 1982; 31: 1023–1028.

45. Jung, RT, Shetty, PS, James WPT et al. Reduced thermogenesis in obesity. *Nature* 1979; 279, 322–323.

46. James WPT, Trayhurn P. Thermogenesis and obesity. *Br Med Bull* 1981; 37: 43–48.

47. Lean MEJ, James WPT, Jennings G et al. Brown adipose tissue in patients with pheochromocytoma. *Int J Obesity* 1986; 10: 219–227.

48. Melicow MM. One hundred cases of pheochromocytoma (107 tumors) at the Columbia-Presbyterian medical center, 1926-1976: a clinicopathological analysis. Cancer 1977; 40: 1987–2004.

49. Shattock SG. On Normal Tumour-like Formations of Fat in Man and the Lower Animals. *Proc R Soc Med* 1909; 2 (Pathol Sect): 207–270.

50. Bruck K. Temperature regulation in the newborn infant. *Biologia Neonat* 1961; 3: 65.

51. Hull D. Brown adipose tissue and the newborn infant's response to cold. In: Philipp EE, Barnes J, Newton M, *eds.* Scientific foundation of obstetrics and gynaecology. London: William Heinemann, 1977: 545–550.

52. Aherne W, Hull D. Brown adipose tissue and heat production in the newborn infant. *J Pathol Bacter* 1966; 91: 223–234.

53. Heim T, Kellermayer M, Dani M. Acta Paed Acad Sci Hungaricae 1968; 9: 109–120.

54. Hey EN, Katz G. Temporary loss of a metabolic response to cold stress in infants of low birth weight. *Archs Dis Childh* 1969; 44: 323–330.

55. Lean MEJ, Jennings G. Brown adipose tissue activity in pyrexial cases of cot death, *din. Pathol* 1989; 42: 1153–1156.

56. Allegra SR, Gmuer C, O'Leary GP. Endocrine activity in a large hibernoma. *Hum Pathol* 1983; 14: 1044–1052.

57. Tsuchiya T, Osanai T, Ishikawa A et al. Hibernomas show intense accumulation of FDG positron emission tomography. *J Comput Assist Tomogr* 2006; 30: 333–336.

58. Kajimura S, Seale P, Tomaru T et al. Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. *Genes & Dev* 2008; 22: 1397–1409.

59. Seale P, Bjork B, Yang W, et al. PDRM16 controls a brown fat/skeletal muscle switch. *Nature* 2008; 454: 961–967.

60. Wijers SL, Saris WH, van Marken Lichtenbelt WD. Recent advances in adaptive thermogenesis: potential implications for the treatment of obesity. *Obes Rev* 2009; 10: 218–226.

61. Tseng YH, Kokkotou E, Schulz TJ et al. New role for bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 2008; 454: 1000–1004.

62. Cypess AM, Kahn C R. Brown fat as a therapy for obesity and diabetes. Current Opinion in Endocrinology, *Diabetes & Obesity* 2010; 17: 143–149.