

# A role of active brown adipose tissue in cancer cachexia?

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## Abstract

Until a few years ago, adult humans were not thought to have brown adipose tissue (BAT). Now, this is a rapidly evolving field of research with perspectives in metabolic syndromes such as obesity and new therapies targeting its bio-energetic pathways. White, brown and so-called *brite* adipose fat seem to be able to trans-differentiate into each other, emphasizing the dynamic nature of fat tissue for metabolism. Human and animal data in cancer cachexia to date provide some evidence for BAT activation, but its quantitative impact on energy expenditure and weight loss is controversial. Prospective clinical studies can address the potential role of BAT in cancer cachexia using <sup>18</sup>F-fluoro-deoxyglucose positron emission tomography-computed tomography scanning, with careful consideration of co-factors such as diet, exposure to the cold, physical activity and body mass index, that all seem to act on BAT recruitment and activity.

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## Introduction

### Cancer cachexia and hypermetabolism

Cancer cachexia is recognized as a distinct clinical disorder with a negative impact on quality of life, therapeutic success and prognosis. The recently redefined criteria for cachexia are weight loss of more than 5% over the previous six months or weight loss of more than 2% in individuals who already show a state of depletion according to decreased bodyweight (Body Mass Index or BMI < 20 kg/m<sup>2</sup>) or skeletal muscle index (sarcopenia).<sup>1</sup> Cancer cachexia frequency varies with the type of malignancy, occurring more frequently in patients with lung and upper gastrointestinal cancer and less often in patients with breast cancer or lower gastrointestinal cancer.<sup>2</sup>

Current evidence on the pathogenesis of the cachexia syndrome points to a central disturbance of the hypothalamic pathways controlling energy homeostasis, resulting in reduced energy intake and anorexia, increased energy expenditure, and loss of body tissue, *i.e.* muscle proteolysis and lipolysis.<sup>3</sup> Resting energy expenditure or metabolic rate (RMR) accounts for the major proportion of up to 70% of total energy expenditure in sedentary subjects.<sup>4</sup> Hypermetabolism has been found to be elevated in some but not all cancer patients,<sup>5</sup> for example, in approximately 50% of a mixed cancer population<sup>6,7</sup> and in up to 74% of primary lung cancer patients.<sup>8</sup> Cancer patients who lose weight have higher RMR than patients with a constant weight.<sup>6</sup>

Several pathophysiological mechanisms for an inappropriately elevated RMR in cancer cachexia have been proposed, *i.e.* abnormalities in the carbohydrate, fat or protein metabolism, systemic inflammation, hyperadrenergic activity and an increased liver mass.<sup>4,6,9-11</sup> In addition, it was suggested as early as 1981 that activation of brown adipose tissue (BAT) results in a hypermetabolic state and is partially responsible for weight loss in cancer patients<sup>12</sup> but this was in contrast to the widespread view that BAT is only present in newborns and that it disappeared in human adulthood. The human and animal studies discussed here were retrieved from a systematic literature search of relevant medical databases (Medline, Embase, Cochrane Library) using the key words *brown adipose tissue*, *cancer* and *cachexia* (Supplementary Figure S1).

### Brown adipose tissue activity

In 2002, Hany *et al.*<sup>13</sup> suggested the existence of functional BAT in adults with the introduction of combined positron emission tomography-computed tomography (PET-CT) scans, showing increased metabolism and glucose uptake with <sup>18</sup>F-fluoro-deoxyglucose (<sup>18</sup>F-FDG) in

areas that were unmistakably fat tissue on the basis of Hounsfield units on the CT.<sup>13</sup> Nedergaard *et al.*<sup>14</sup> discussed the perspectives presented by this observation to study BAT function in humans. So far, several independent studies<sup>15-18</sup> have confirmed the existence of functional BAT in adult humans, using cold-induced BAT activation, <sup>18</sup>F-FDG PET-CT scanning and biopsy verification.

The primary function of BAT is facultative thermogenesis, *i.e.* regulated heat production in response to a decrease in core body temperature with environmental cold exposure. For this, BAT cells are characterized by an abundance of mitochondria, in contrast to white adipose tissue (WAT). The thermogenic effect of BAT is due to the exclusive presence of uncoupling protein 1 (UCP1) which mediates proton leakage across the inner mitochondrial membrane, thus decreasing the level of coupling of respiration to ADP phosphorylation. In this way, heat is produced instead of energy stored as ATP (Figure 1). Environmental cold exposure causes BAT cells to increase their number of mitochondria and UCP1 synthesis through sympathetic adrenergic stimulation. Human studies have demonstrated that <sup>18</sup>FDG uptake in BAT is directly proportional to the expression of UCP1<sup>16,18,19</sup> and the cold-induced rise in energy expenditure.<sup>20</sup> Therefore, <sup>18</sup>FDG activity on PET-CT scan can be used as a surrogate marker as a non-invasive technique to measure BAT activity *in vivo* (Figure 2).

Another relevant recent discovery has been the identification of so-called *brite* (or *beige* or *systemic*) adipose cells which reside among the much larger area of WAT tissue in both subcutaneous and trunk (visceral) compartments. These were so named because they have the same origin (from a myogenic factor 5 (*myf5*)-negative mesodermal stem cell) as WAT cells, but different from the *myf5*-positive adipomyoblast that gives rise to myocytes and the *classical* brown adipocytes that occupy the deposits, as seen in Figure 2.<sup>21</sup> Although genotypically different, the thermogenic phenotype of brite and brown cells has been shown to be similar.<sup>22</sup> Importantly, differentiated brite and white fat cells have the capacity for reversible trans-differentiation into each other,<sup>23</sup> a process that is also closely related to the activity of the sympathetic system. This system provides a large metabolic reservoir for *recruitable* combustion (WAT→BAT conversion) or storage (BAT→WAT conversion) of energy and lipid tissue metabolites.

Several review publications over the last few years have addressed the developments in this growing field, hypothesizing on the possible importance of BAT function on substrate and energy homeostasis of the body. Many of these have focussed on obesity, with the prospect of therapeutic WAT→BAT conversion, thermogenic activity, weight loss and conversion of metabolic abnormalities.<sup>24-26</sup> Along these chain of events,

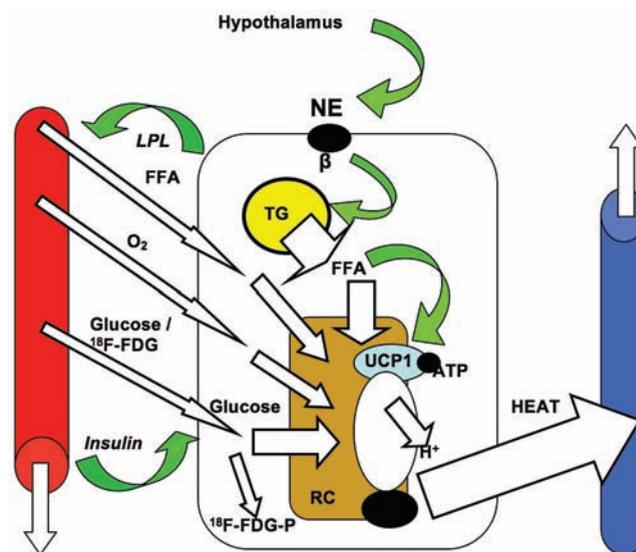


Figure 1. Schematic representation of the thermogenesis in brown adipose tissue (BAT) cells. Information such as body temperature and feeding status is integrated in the hypothalamus from where branches of the sympathetic nervous system innervate BAT through norepinephrine (NE) transmission and predominantly  $\beta_3$  beta-adrenergic agonism. The intracellular signal releases triglycerides from the lipid droplet (yellow) in the cytosol, as well as synthesis and release of lipoprotein lipase (LPL) and stimulation of glucose [or its equivalent <sup>18</sup>F-fluoro-deoxyglucose (<sup>18</sup>F-FDG)]. Released free fatty acid (FFA) activates uncoupling protein 1 (UCP1) by overcoming the chronic inhibition caused by cytosolic nucleotides such as ATP (or its experimental equivalent guanosine diphosphate). FFA, glucose and oxygen are taken up from the arterial vessels (red) and directed to the mitochondria (brown) for combustion. Insulin stimulates uptake of glucose (and <sup>18</sup>F-FDG) through the enhanced expression of glucose transporter GLUT-4 in the cell membrane, in addition to increasing LPL expression. <sup>18</sup>F-FDG is phosphorylated inside the cytosol and trapped from further metabolism. UCP1, by acting as an equivalent of an H<sup>+</sup> transporter, allows extrusion of H<sup>+</sup> and mitochondrial combustion of substrates in the respiratory chain, *uncoupled* from ATP production. Instead, heat is produced and dissipated to venous vessels (blue) for further transport to the body.



Figure 2. <sup>18</sup>F-fluoro-deoxyglucose positron emission tomography (PET) scans in normal volunteers, showing no brown adipose tissue (BAT) activity (left), multiple active deposits after cold exposure (16°C, center) and an example of BAT visualization on a routine PET scan in a patient with rectal cancer (right). Cervical, supraclavicular, axillary, mediastinal, paravertebral and perirenal deposits can be seen.

the possibility that cancer cachexia is partly mediated by BAT has also re-emerged. New research in this field is facilitated by the availability of PET-CT scans in cancer centers.

### Animal studies on cancer cachexia and brown adipose tissue function

Table 1 shows previous studies that examined the effects of subcutaneous tumor xenograft models on BAT activity in rodents.<sup>12,27-33</sup> In the early study of Brooks *et al.*,<sup>12</sup> which was later confirmed by Roe and co-workers,<sup>33</sup> resting oxygen consumption (VO<sub>2</sub>) as well as core body temperature showed significantly higher values in cachectic tumor-bearing mice as compared to pair-fed, non-tumor bearing controls, and these coincided with sympathetic activation of BAT, as judged by mitochondrial guanosine diphosphate (GDP) binding. These results seem to indicate the presence of a state of excessive energy expenditure and thermogenic activity of BAT in cancer cachexia, but they do not establish the corresponding degree of changes in BAT activity, energy expenditure and weight loss. On the other hand, in a study by Oudart,<sup>31</sup> significant differences were found between tumor-bearing animals and pair-fed controls with regard to BAT mitochondrial COX activity and GDP binding, but not with regard to UCP. Indeed, UCP1 protein levels in BAT tissue can be regarded as the most specific parameter of BAT thermogenic function.<sup>34</sup> These authors, therefore, suggested that BAT activity, as induced in tumor-bearing animals, is almost exclusively functional and that full fledged BAT tissue development or *recruitment* is unlikely in cancer cachexia. However, in this study there was no significant difference in final carcass mass between tumor-bearing animals and pair-fed control animals, so there seemed to be a lesser extent of cachexia. Other studies (Table 1) have focussed on the determination of BAT constituents but lack correlation to changes in body temperature and energy expenditure, while different parameters were used to measure BAT activity. For example, lipo-protein lipase (LPL) activity which is synthesized and released from the BAT cells in response to adrenergic stimulation to provide for combustible fatty acids is also strongly confounded by external factors such as insulin levels. These data demonstrate the importance of methodology to the results in these metabolic studies.

The most recent studies have focussed on the effects of several tumor mediators on BAT activity (Table 2).<sup>35-41</sup> A recent systematic review found circulating levels of tumor necrosis factor-alpha (TNF $\alpha$ ) to be elevated, unchanged or undetectable in cancer patients with cachexia, and no association between systemic serum levels of TNF $\alpha$  and weight loss was found, with the exception of one study in advanced breast cancer.<sup>5</sup> Emphasizing the importance of a central nervous signalling pathway by TNF- $\alpha$ , Arruda *et al.*<sup>35</sup> demonstrated a moderate but significant body weight loss and BAT stimulation by intra-cerebro-ventricular administration of TNF- $\alpha$ . Importantly, BAT activity as well as coinciding body mass loss, body hyperthermia and inhibition of food intake could be attenuated by prior BAT denervation or administration of a  $\beta$ -3 antagonist (SR59230A), suggesting a causal link between BAT hyperactivity and cachexia hypermetabolism, as well as therapeutic activity of a  $\beta$ -3 adrenoceptor antagonist.

Another tumor mediator that has been implicated in cancer cachexia with regard to BAT activation has been zinc-alpha2-glycoprotein (ZAG), or lipid-mobilizing factor, which was isolated from the urine of cachectic cancer patients.<sup>4</sup> Its activity in subcutaneous fat biopsies was recently shown to inversely correlate with weight loss in patients with cancer cachexia.<sup>42</sup> In animal studies, ZAG purified from the murine adenocarcinoma MAC-16 induced profound cachexia by lipolysis, but not muscle breakdown.<sup>43</sup> Russell *et al.*<sup>38</sup> showed a dose-dependent increase in gross body weight loss and BAT UCP1 protein expression with increasing doses of i.p. administered ZAG. This was accompanied by an increase in BAT oxygen consumption indicating thermogenesis, although its effect on overall energy expenditure was not reported. Presumably, according to these authors, increased levels of plasma fatty acids as a result of ZAG induced lipolysis contributed to an increase in BAT UCP1 and thermogenesis. Interestingly, TNF- $\alpha$  but not ZAG has also been found to up-regulate other UCP subtypes, *i.e.* UCP2 that is found in low levels in many tissues, and UCP3 found in BAT and skeletal muscle.<sup>44,45</sup> One should, therefore, also consider the possibility of cytokine driven heat production in other tissues.

Up to now, not many studies have explored the functional response of BAT activity in response to cold in this context. Edstrom *et al.*<sup>46</sup> examined cold-induced thermogenesis in cachectic tumor-bearing

**Table 1. Rodent studies on cancer cachexia and brown adipose tissue activity, studying the effect of tumour implantation.**

Study	Cancer group	Control group	Cachexia induction (vs control group) *	BAT activation (parameter) *
Bing <sup>27</sup>	MAC-16 adeno-sarcoma from cachectic animals in NMRI mice, 18 days	Pair fed animals	++: P<0.01 (BW -20% vs -4%)	++: P<0.01 (BAT UCP1 mRNA)
Bing <sup>28</sup>	MAC-16 adeno-sarcoma from cachectic animals in CD-1 mice, 16 days	Sham operated animals	+: P=n.s. (BW -19% of controls)	++: P<0.001 (BAT ZAG mRNA and protein)
Oudart <sup>31</sup>	Yoshida sarcoma in Wistar rats, 10 days	Pair fed, sham operated animals	- P=n.s.d. (carcass mass 256.5 +/-4.5 g. vs 262.6 +/-5.1 g.)	++ P<0.01 (mitochondrial GDP binding)
Lopez-Soriano <sup>29</sup>	Yoshida AH-130 hepatoma cells in Wistar rats, 7 days	Pair fed, NaCl sham injected animals	++ P<0.01 (carcass mass 173.8 +/-4.2 g. vs 191.0 +/-3.0 g.)	- P=n.s.d. (BAT LPL / BAT mass in g)
Lopez-Soriano <sup>30</sup>	Yoshida AH-130 hepatoma cells in Wistar rats, 11 days	NaCl sham injected animals	++ P<0.001 (BW -30% vs +30%)	++ P<0.001 (BAT LPL)
Seelaender <sup>32</sup>	Walker 256 carcinosarcoma cells in Wistar rats, 14 days	NaCl sham injected animals	+ P=n.s. (BW -13% vs +32%)	+ P=n.s. (BAT LPL / BAT fat %)
Roe <sup>33</sup>	T-cell leukemia cells in Piebald Variegated rats, 17 days	Pair fed, NaCl sham injected animals	++ P<0.01 (BW -14% vs +18%)	+ P<0.05 (mitochondrial GDP binding)
Brooks <sup>12</sup>	Human hypernephroma in immunosuppressed CBACa mice, 13 days	Sham operated animals	- P=n.s.d. (BW -10% vs -5%)	+ P<0.05 (mitochondrial GDP binding)

BAT, brown adipose tissue; UCP1, uncoupling protein 1; BW, body weight; GDP, guanosine diphosphate; ZAG, zinc- $\alpha$ 2-glycoprotein; LPL, lipo-protein lipase; n.s., not specified; n.s.d., not significant.

\*P values represent the strength of the difference in mean +/- standard deviation or standard error of mean between the intervention group (cancer, cachexia) and the methodologically best available control group (pair fed rather than freely fed, carcass weight rather than body weight) and BAT activity parameter (UCP1, functional test rather than BAT, fat or protein weight).

mice. Exposure of cold in tumor-bearing mice produced a significantly elevated BAT thermogenic response, although there was no significant difference in carcass weight between animals kept at a temperature of 5°C compared to those kept at 25°C. In this study, neither  $\beta$ -blockade nor BAT tissue extirpation was shown to impact on body composition. Therefore, these authors concluded that full-blown BAT proliferation and activation was unlikely in cancer cachexia. However, these experiments were not pair-fed controlled because food restriction at 5°C resulted in 100% mortality. Animals kept at 5°C had double the food intake of those kept at 25°C. Recently, Tsoli *et al.*,<sup>47</sup> found increased BAT activity in C26-induced cachectic mice, as judged by BAT UCP1 protein. This persisted when the animals were acclimatized to 28°C, despite a dramatically reduced food intake. According to these authors, this confirmed the existence of an inappropriate activation of BAT, representing a maladaptive response to nutrient deprivation in cancer cachexia. Furthermore, <sup>18</sup>F-FDG PET imaging showed similar kinetics in these cachectic C26 mice as compared to freely-fed, non-tumor bearing animals despite the fact that uptake of <sup>18</sup>F-FDG is normally reduced in fasting mice,<sup>48</sup> supporting the concept of an increase in metabolic BAT activity in cancer cachexia.

In summary, Tables 1 and 2 show that most animal studies indicate some degree of BAT activation in cancer cachexia. However, these data do not establish the quantitative impact on the amount of hypermetabolism that is relevant to the development of cachexia. The human and animal studies were retrieved from a systematic literature search of relevant medical databases (Medline, Embase, Cochrane Library) using the key words *brown adipose tissue*, *cancer* and *cachexia* (Supplementary Figure S1).

## Human studies on cancer and brown adipose tissue function

Hibernoma, a rare soft tissue benign tumor composed of brown fat cells, has been associated with massive weight loss as a primary symptom,<sup>49</sup> suggesting the potential of BAT to significantly contribute to body weight loss in cachexia in humans. Abundant BAT hyperactivity can also be seen on <sup>18</sup>F-FDG PET scanning in cases of pheochromocytoma as a result of chronic stimulation of the sympathetic nervous system by high levels of circulating catecholamines, such as epinephrine and norepinephrine.<sup>50</sup> In one case, after removing the pheochromocytoma, and thus the excess of norepinephrine, the intense uptake of <sup>18</sup>F-FDG in BAT was no longer seen,<sup>51</sup> presumably reflecting an involution of BAT. However, such extreme levels of circulating catecholamines in pheochromocytoma are not representative of other cancer types.

In retrospective studies of mixed cancer populations referred for routine <sup>18</sup>F-FDG PET scanning, single scan observations have shown a prevalence of 5-10% of BAT positivity in a general oncology patient group<sup>52</sup> (Figure 2) Rousseau analyzed serial PET-CT scans performed during neo-adjuvant breast cancer chemotherapy in 33 patients.<sup>53</sup> A total of 82% of patients showed BAT activity on at least one occasion. However, positive PET-CT scans were randomly distributed between patients and time points which, according to the authors, suggested an association of an increased chance of a sufficiently cold environment activating BAT with an increasing number of scans, rather than any correlation to cancer progression or response. Three retrospective studies<sup>18,54,55</sup> addressed a possible relationship of BAT <sup>18</sup>F-FDG activity

**Table 2. Rodent studies on cancer cachexia and brown adipose tissue activity, studying the effect of tumour mediators.**

Study	Cachexia group	Control group	Cachexia induction (vs control group)*	BAT activation (parameter)*
Coombes <sup>36</sup>	I.v. TNF- $\alpha$ in Wistar rats, 6 days	NaCl sham injected animals	++ P<0.01 (BW -10% of controls)	+ P<0.05 (mitochondrial GDP binding)
Lopez-Soriano <sup>37</sup>	Lewis lung carcinoma cells in rTNF- $\alpha$ 1 <sup>0</sup> Wistar rats, 11 days	Wild type and non-tumor bearing animals	++ P<0.01 (carcass weight loss up to -26% in wild type vs -18% in TNF- $\alpha$ 1 <sup>0</sup> )	+ P<0.05 vs non-tumour (BAT LPL activity)
Arruda <sup>35</sup>	ICV TNF- $\alpha$ in Wistar rats, 4 days	Pair fed, sham injected animals	+ P<0.05 (final BM 241.02 $\pm$ 5.70 g vs 270.06 $\pm$ 7.23 g)	+ P<0.05 (BAT UCP1 mRNA and protein)
Russell <sup>39</sup>	I.v. LMF from urine of <i>weight losing</i> pancreatic cancer patients, in NMRI mice, 2 days	PBS sham injected animals	++ P=n.s. (BW - 42%)	++ P<0.001 (BAT utilization of <sup>3</sup> H-2-deoxyglucose (2DG))
Bing <sup>40</sup>	I.v. LMF from the urine of <i>cachectic</i> cancer patients (> 10% body weight loss), in NMRI mice, 3 days	Weight matched, PBS sham injected animals	++ P<0.01 (BW -2.24 g vs +0.06 g.)	++ P<0.01 (BAT UCP1mRNA)
Russell <sup>38</sup>	I.p. ZAG in NMRI and MF1 mice, 4 days	PBS and heat-inactivated ZAG sham injected animals	++ P<0.001 (BW -3.8 $\pm$ 0.5 g till -1.26 $\pm$ 0.46 g vs -1.2 $\pm$ 0.3 g till -0.89 $\pm$ 0.5 g <i>dose-dependent</i> )	++ P<0.001 ( <i>dose-dependent</i> ) (BAT UCP1 protein)
Johnen <sup>41</sup>	I.p. MIC-1 in BALB/c nude mice after s.c. MIC-1 transfected 5-7 $\times$ 10 <sup>6</sup> DU145 prostate carcinoma cells, n.s.	PBS sham and empty plasmid vector injected animals	++ P<0.001 (BW -5.3 g vs -1.1 g)	-- P<0.001 (BAT weight)

BAT, brown adipose tissue; UCP1, uncoupling protein 1; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; ZAG, zinc- $\alpha$ 2-glycoprotein; BW, body weight; BM, body mass; 2-DG, 2-deoxyglucose; GDP, guanosine diphosphate; i.p., intraperitoneal; s.c., subcutaneous; i.v., intravenous; ICV, intra-cerebro-ventricular; LMF, lipid mobilizing factor; LPL, lipo-proteinase; MIC, macrophage inhibitory cytokine; n.s., not specified; PBS, phosphate buffered saline.

\* P values represent the strength of the difference in mean  $\pm$  standard deviation or standard error of mean between the intervention group (cancer, cachexia) and the methodologically best available control group (pair-fed rather than freely fed, carcass weight rather than body weight) and BAT activity parameter (UCP1, functional test rather than BAT, fat or protein weight).

with cancer (Table 3). However, none of these observed a significantly higher incidence of BAT activity in active and/or  $^{18}\text{F}$ -FDG PET-positive cancer patients. Lee *et al.*<sup>55</sup> found BAT activity in 50% of the scans in patients with evidence of active malignancy compared with 44% of scans in which there was no  $^{18}\text{F}$ -FDG PET-CT evidence of active disease. Other studies<sup>18,56</sup> have applied a univariate and multivariate analysis on patients with BAT activity and found outdoor temperature, age, sex, BMI and diabetes to be significant variables, but not active cancer<sup>56</sup> or indication for PET scanning (cancer/no cancer).<sup>18</sup> The study of van Marken Lichtenbelt *et al.*<sup>17</sup> showed a BAT prevalence of as high as 96% in healthy, young volunteers after a standardized protocol of cold exposure, suggesting that almost all adults possess active or at least potentially active BAT, depending on the degree of BAT stimulation at that moment. The prevalence of BAT activity in cancer patients may thus be considerably higher than that observed so far because these non-controlled studies do not indicate the degree of BAT stimulation represented by the scan at that moment. Routine preparation for  $^{18}\text{F}$ -FDG PET scanning in oncology is aimed at reducing BAT uptake by keeping patients warm and sober to avoid false positive findings for a tumor. The sharp discrepancy between the reported 5-10% in the general population and 96% in stimulated conditions has led some to conclude that quantitative and qualitative data from retrospective studies must not be considered as indications of the true prevalence of BAT and its physiological control.<sup>25</sup> Careful consideration of factors co-acting on BAT recruitment and activity, such as diet, cold exposure, physical activity, insulin levels and BMI makes this type of metabolic study challenging, especially for patients.

Virtanen and colleagues<sup>16</sup> estimated that, by calculating the metabolic rate of glucose uptake (rGU) from dynamic scanning and pharmacokinetic modelling, constant activation of the supraclavicular BAT deposit by moderate cold could burn energy levels equivalent to approximately 4.1 kg of adipose tissue in one year in healthy subjects. It is unclear how this can relate to the situation in cancer cachexia patients. This amount may be considered an overestimation when comparing the intensity of BAT activity after cold with that in the occasional cancer patient (Figure 2) and considering the fact that most of the patients are *thermoneutral*, *i.e.* an environmental temperature at which heat production is not stimulated, which is during most of the day. However, it may be an underestimation if one considers a much larger area of BAT tissue that is activated, not only the supraclavicular deposit, and if brite fat thermogenesis is to be taken into account. These issues need further investigation and require more refined quantitative methods for the  $^{18}\text{F}$ -FDG-PET-CT scanner to include dif-

fuse nests of brite fat that may falsely appear as areas of low activity as a result of the limited spatial resolution of the PET scanner.

Two previous studies examined fat tissue in cancer patients. Bianchi *et al.*<sup>57</sup> assessed BAT activity from GDP binding to mitochondria prepared from peroperative samples, and found this to be significantly elevated (by 3-fold) in children with various malignancies as compared to children undergoing exploratory renal surgery without cancer. In another study by Shellock *et al.*,<sup>58</sup> the presence of BAT, as assessed by histological light-microscopic criteria, was determined in peri-adrenal fat tissue acquired during autopsy. In a group of *cachectic* adult cancer patients BAT was found in 80% but in only 13% of patients who died from other illnesses without cancer or cachexia.

In summary, in humans there is no evidence linking BAT activity with cancer cachexia, although studies report a widely variable prevalence of BAT activity depending on the individual metabolic demand at the time of  $^{18}\text{F}$ -FDG-PET scanning.

### Brown adipose tissue energetics

Humans and rodents share an identical cell composition of the adipose organ, as well as functional BAT characteristics: dense different vascularization, sympathetic innervation with predominately  $\beta 3$  adrenoceptors, UCP1 as the characteristic mitochondrial membrane protein in brown adipocytes, dependency on ambient temperature.<sup>59</sup> In smaller mammals, such as rodents, cold activated BAT thermogenesis has a substantial impact on total energy expenditure. From mice studies, the maximum heat-producing capacity has been established to be 300 W/kg.<sup>34</sup> For the moment, no information is available on the contribution of BAT thermogenesis to whole body energy expenditure in humans, although a recent study using  $^{11}\text{C}$ -acetate PET-CT provides evidence of metabolic factors contributing to non-shivering thermogenesis.<sup>60</sup> In mammals, mass-specific energy expenditure (*i.e.* per unit tissue mass) is negatively related to body size. Taking such allometric relations into account, it can be calculated from the data from rodents that human activated BAT would spend 45 W/kg.<sup>61</sup> Rothwell and Stock estimated 40-50 g BAT in humans;<sup>62</sup> this would result in 3-5% of basal metabolic rate (BMR). Using  $^{18}\text{F}$ -FDG PET-CT scanning, Virtanen<sup>16</sup> estimated BAT volume to be slightly higher at 63 g, while assuming the contribution of glucose to overall BAT substrate use to be 10%.<sup>63</sup> This resulted in an estimated heat production of activated BAT of 55 W/kg, or approximately 4.5% of BMR. These calculations suggest that BAT activity can have a substantial impact on daily energy expenditure.

In addition, BAT may be differently activated depending upon the physiological stimulus. Orava *et al.*<sup>64</sup> showed that BAT is a highly

**Table 3. Human studies on cancer cachexia and brown adipose tissue activity, studying the effect of tumour presence.**

Study	Cancer group	Control group	Cachexia induction (vs control group)*	BAT activation (parameter)*
Cypess <sup>18</sup>	Adults; <i>lymphoma and other cancers</i> from mixed PET population (n=1972)	No-cancer	n.s.	- P=n.s.d. (BAT $^{18}\text{F}$ -FDG activity)
Quellet <sup>56</sup>	Adults; <i>active cancers</i> from mixed PET population (n=4842)	No-cancer	n.s.	- P=n.s.d. (BAT $^{18}\text{F}$ -FDG activity)
Lee <sup>55</sup>	Adults; active (PET positive) <i>malignancies</i> from mixed cancer population (n=2934)	Tumor PET negative	n.s.	- P=n.s.d. (BAT $^{18}\text{F}$ -FDG activity)
Bianchi <sup>57</sup>	Pediatric; mixed cancer types (n=8)	No-cancer (pyelo-plasty) (n=9)	+ P=n.s. ( <i>evidence of recent weight loss</i> )	+ P<0.05 (mitochondrial GDP binding in perirenal fat)
Shellock <sup>58</sup>	Adults; mixed cancer types (n=25); all cachexia; autopsy study	Age-matched, no-cancer, no-cachexia (n=15)	+ P=n.s. ( <i>cachexia according to pathologist</i> )	+ P=n.s. (microscopic BAT in peri-adrenal fat)

BAT, brown adipose tissue; PET, positron emission tomography;  $^{18}\text{F}$ -FDG,  $^{18}\text{F}$ -fluoro-deoxyglucose; GDP, guanosine diphosphate; n.s., not specified; n.s.d., not significant difference.

\*P values represent the strength of the difference in mean  $\pm$  standard deviation or standard error of mean between the intervention group (cancer, cachexia) and the methodologically best available control group (pair-fed rather than freely fed, carcass weight rather than body weight) and BAT activity parameter (uncoupling protein 1, functional test rather than BAT, fat or protein weight).

insulin-sensitive tissue. Insulin administration by an euglycemic, hyperinsulinemic clamp technique led to a 5-fold increase in BAT glucose uptake. This reinforces the view that BAT plays a role in human metabolism and may be of relevance in cancer cachexia in which many of the metabolic abnormalities of type II diabetes mellitus, including glucose intolerance, increased hepatic glucose production and recycling, and insulin resistance have been reported, even prior to the development of severe malnutrition.<sup>65</sup>

## Conclusions

Animal experiments provide some evidence for an association between BAT activation and cancer cachexia. Given the similarities in anatomy and BAT function between rodents and humans, such a relation may also exist in humans. The proliferation of <sup>18</sup>F-FDG PET-CT scanners in cancer centers supplies researchers with a new tool to study BAT and human energy balance. However, current retrospective observations in cancer patients do not support a clinically relevant association between BAT and cancer cachexia. Still, the large discrepancy between retrospective and prospective data shows that methodology is critical in these types of metabolic studies. Therefore, further prospective and well controlled studies are warranted to provide more solid evidence in this field. In animal studies, cancer cachexia models should resemble the human situation as close as possible in terms of end points, degree of anorexia, muscle and fat loss, tumor to body weight, metabolic abnormalities, hormonal changes and cytokine profile.<sup>66</sup> In human studies, <sup>18</sup>F-FDG PET-CT scanning seems the preferred examination mode to establish patterns of BAT activation and recruitment during pre-cachexia development as compared to age and gender matched normal adults. Direct correlation to measurements of energy expenditure, body temperature and weight loss is essential. Scans should be performed in stimulated circumstances such as at cold temperatures, in order to evaluate the full amount of active BAT present. Co-factors that act on BAT should be controlled or simultaneously measured: age, gender, temperature conditions, season, BMI, physical activity, prior diet, medication ( $\beta$ -adrenoceptor agents), cytokines (CRP, TNF- $\alpha$ , IL-6), hormonal profile (thyroid hormones, insulin), body composition changes (fat mass, lean mass), presence of metabolic pathologies such as obesity, diabetes. In animal and human studies, UCP1 protein is the preferred parameter in brown and white fat tissue biopsies to assess specific thermogenic activity. In intervention studies, <sup>18</sup>F-FDG PET-CT can be used to study the effects of a  $\beta$ 3-adrenoceptor antagonist on BAT activation and cachexia before, during and after initiation of treatment.

## References

1. Fearon K, Strasser F, Anker SD, et al. Definition and classification of cancer cachexia: an international consensus. *Lancet Oncol* 2011;12:489-95.
2. Wys WDD. Weight loss and nutritional abnormalities in cancer patients: incidence, severity and significance. Calman kc Fk, editor. London: Saunders; 1986.
3. Laviano A, Inui A, Marks DL, et al. Neural control of the anorexia-cachexia syndrome. *Am J Physiol Endocrinol Metab* 2008;295: E1000-8.
4. Tisdale MJ. Mechanisms of cancer cachexia. *Physiol Rev* 2009;89:381-410.
5. Blum D, Omlin A, Baracos VE, et al. Cancer cachexia: A systematic literature review of items and domains associated with involuntary weight loss in cancer. *Crit Rev Oncol Hematol* 2011;80:114-44.
6. Cao DX, Wu GH, Zhang B, et al. Resting energy expenditure and body composition in patients with newly detected cancer. *Clin Nutr* 2010;29:72-7.
7. Bosaeus I, Daneryd P, Svanberg E, Lundholm K. Dietary intake and resting energy expenditure in relation to weight loss in unselected cancer patients. *Int J Cancer* 2001;93:380-3.
8. Staal-van den Brekel AJ, Schols AM, ten Velde GP, et al. Analysis of the energy balance in lung cancer patients. *Cancer Res* 1994;54: 6430-3.
9. Staal-van den Brekel AJ, Dentener MA, Schols AM, et al. Increased resting energy expenditure and weight loss are related to a systemic inflammatory response in lung cancer patients. *J Clin Oncol* 1995;13:2600-5.
10. Hyltander A, Drott C, Korner U, et al. Elevated energy expenditure in cancer patients with solid tumours. *Eur J Cancer* 1991;27:9-15.
11. Lieffers JR, Mourtzakis M, Hall KD, et al. A viscerally driven cachexia syndrome in patients with advanced colorectal cancer: contributions of organ and tumor mass to whole-body energy demands. *Am J Clin Nutr* 2009;89:1173-9.
12. Brooks SL, Neville AM, Rothwell NJ, et al. Sympathetic activation of brown-adipose-tissue thermogenesis in cachexia. *Biosci Rep* 1981;1:509-17.
13. Hany TF, Gharehpapagh E, Kamel EM, et al. Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. *Eur J Nucl Med Mol Imaging* 2002;29:1393-8.
14. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab* 2007;293:E444-52.
15. 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;58:1526-31.
16. Virtanen KA, Lidell ME, Orava J, et al. Functional brown adipose tissue in healthy adults. *N Engl J Med* 2009;360:1518-25.
17. van Marken Lichtenbelt WD, Vanhomerig JW, Smulders NM, et al. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 2009;360:1500-8.
18. Cypess AM, Lehman S, Williams G, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009; 360:1509-17.
19. Baba S, Engles JM, Huso DL, et al. Comparison of uptake of multiple clinical radiotracers into brown adipose tissue under cold-stimulated and nonstimulated conditions. *J Nucl Med* 2007;48:1715-23.
20. Yoneshiro T, Aita S, Matsushita M, et al. Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obesity (Silver Spring)* 2011;19:13-6.
21. 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 U S A* 2007; 104:4401-6.
22. Cinti S. Between brown and white: novel aspects of adipocyte differentiation. *Ann Med* 2011;43:104-15.
23. Cinti S. Reversible physiological transdifferentiation in the adipose organ. *Proc Nutr Soc* 2009;68:340-9.
24. Ravussin E, Galgani JE. The implication of brown adipose tissue for humans. *Annu Rev Nutr* 2011;31:33-47.
25. Nedergaard J, Bengtsson T, Cannon B. Three years with adult human brown adipose tissue. *Ann N Y Acad Sci* 2010;1212:E20-36.
26. Tews D, Wabitsch M. Renaissance of brown adipose tissue. *Horm Res Paediatr* 2011;75:231-9.
27. Bing C, Brown M, King P, et al. Increased gene expression of brown fat uncoupling protein (UCP)1 and skeletal muscle UCP2 and UCP3 in MAC16-induced cancer cachexia. *Cancer Res* 2000;60: 2405-10.

28. Bing C, Bao Y, Jenkins J, et al. Zinc-alpha2-glycoprotein, a lipid mobilizing factor, is expressed in adipocytes and is up-regulated in mice with cancer cachexia. *Proc Natl Acad Sci USA* 2004;101:2500-5.
29. Lopez-Soriano J, Argiles JM, Lopez-Soriano FJ. Lipid metabolism in rats bearing the Yoshida AH-130 ascites hepatoma. *Mol Cell Biochem* 1996;165:17-23.
30. Lopez-Soriano J, Argiles JM, Lopez-Soriano FJ. Sequential changes in lipoprotein lipase activity and lipaemia induced by the Yoshida AH-130 ascites hepatoma in rats. *Cancer Lett* 1997;116:159-65.
31. Oudart H, Calgari C, Andriamampandry M, Le Maho Y, Malan A. Stimulation of brown adipose tissue activity in tumor-bearing rats. *Can J Physiol Pharmacol* 1995;73:1625-31.
32. Seelaender MC, Nascimento CM, Curi R, Williams JF. Studies on the lipid metabolism of Walker 256 tumour-bearing rats during the development of cancer cachexia. *Biochem Mol Biol Int* 1996;39:1037-47.
33. Roe S, Cooper AL, Morris ID, Rothwell NJ. Mechanisms of cachexia induced by T-cell leukemia in the rat. *Metabolism* 1996;45:645-51.
34. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004;84:277-359.
35. Arruda AP, Milanski M, Romanatto T, et al. Hypothalamic actions of tumor necrosis factor alpha provide the thermogenic core for the wastage syndrome in cachexia. *Endocrinology* 2010;151:683-94.
36. Coombes RC, Rothwell NJ, Shah P, Stock MJ. Changes in thermogenesis and brown fat activity in response to tumour necrosis factor in the rat. *Biosci Rep* 1987;7:791-9.
37. Lopez-Soriano J, Llovera M, Carbo N, et al. Lipid metabolism in tumour-bearing mice: studies with knockout mice for tumour necrosis factor receptor 1 protein. *Mol Cell Endocrinol* 1997;132:93-9.
38. Russell ST, Zimmerman TP, Domin BA, Tisdale MJ. Induction of lipolysis *in vitro* and loss of body fat *in vivo* by zinc-alpha2-glycoprotein. *Biochim Biophys Acta* 2004;1636:59-68.
39. Russell ST, Tisdale MJ. Effect of a tumour-derived lipid-mobilising factor on glucose and lipid metabolism *in vivo*. *Br J Cancer* 2002;87:580-4.
40. Bing C, Russell ST, Beckett EE, et al. Expression of uncoupling proteins-1, -2 and -3 mRNA is induced by an adenocarcinoma-derived lipid-mobilizing factor. *Br J Cancer* 2002;86:612-8.
41. Johnen H, Lin S, Kuffner T, et al. Tumor-induced anorexia and weight loss are mediated by the TGF-beta superfamily cytokine MIC-1. *Nat Med* 2007;13:1333-40.
42. Mracek T, Stephens NA, Gao D, et al. Enhanced ZAG production by subcutaneous adipose tissue is linked to weight loss in gastrointestinal cancer patients. *Br J Cancer* 2011;104:441-7.
43. Tisdale MJ. Zinc-alpha2-glycoprotein in cachexia and obesity. *Curr Opin Support Palliat Care* 2009;3:288-93.
44. Eckardt K, Schober A, Platzbecker B, et al. The adipokine zinc-alpha2-glycoprotein activates AMP kinase in human primary skeletal muscle cells. *Arch Physiol Biochem* 2011;117:88-93.
45. Masaki T, Yoshimatsu H, Chiba S, et al. Tumor necrosis factor-alpha regulates *in vivo* expression of the rat UCP family differentially. *Biochim Biophys Acta* 1999;1436:585-92.
46. Edstrom S, Kindblom LG, Lindmark L, Lundholm K. Metabolic and morphologic changes in brown adipose tissue from non-growing mice with an isogenic sarcoma. Evaluation with respect to development of cachexia. *Int J Cancer* 1986;37:753-60.
47. Tsoli M MM, Painter A, Berghofer P, Gregoire M-C, Katsifis A, Clarke S, Robertson, editor. Activation of BAT in cancer cachexia/anorexia syndrome: a maladaptive response to reduced food intake driven by tumour-derived IL-6. Pre-congress meeting on brown adipose tissue and human obesity; 2010 July, 10-11; Stockholm.
48. Fueger BJ, Czernin J, Hildebrandt I, et al. Impact of animal handling on the results of <sup>18</sup>F-FDG PET studies in mice. *J Nucl Med* 2006;47:999-1006.
49. Essadel A, Bensaid Alaoui S, Mssrouri R, et al. [Hibernoma: a rare case of massive weight loss]. *Ann Chir* 2002;127:215-7.
50. Wang Q, Zhang M, Ning G, et al. Brown adipose tissue in humans is activated by elevated plasma catecholamines levels and is inversely related to central obesity. *PloS One* 2011;6:e21006.
51. Yamaga LY, Thom AF, Wagner J, et al. The effect of catecholamines on the glucose uptake in brown adipose tissue demonstrated by (18)F-FDG PET/CT in a patient with adrenal pheochromocytoma. *Eur J Nucl Med Mol Imaging* 2008;35:446-7.
52. Cohade C. Altered biodistribution on FDG-PET with emphasis on brown fat and insulin effect. *Semin Nucl Med* 2010;40:283-93.
53. Rousseau C, Bourbouloux E, Champion L, et al. Brown fat in breast cancer patients: analysis of serial (18)F-FDG PET/CT scans. *Eur J Nucl Med Mol Imaging* 2006;33:785-91.
54. Ouellet V, Routhier-Labadie A, Bellemare W, et al. Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of <sup>18</sup>F-FDG-detected BAT in humans. *J Clin Endocrinol Metab* 2011;96:192-9.
55. Lee P, Greenfield JR, Ho KK, Fulham MJ. A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab* 2010;299:E601-6.
56. Ouellet V, Routhier-Labadie A, Bellemare W, et al. Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of <sup>18</sup>F-FDG-detected BAT in humans. *J Clin Endocrinol Metab* 2011;96:192-9.
57. Bianchi A, Bruce J, Cooper AL, et al. Increased brown adipose tissue activity in children with malignant disease. *Horm Metab Res* 1989;21:640-1.
58. Shellock FG, Riedinger MS, Fishbein MC. Brown adipose tissue in cancer patients: possible cause of cancer-induced cachexia. *J Cancer Res Clin Oncol* 1986;111:82-5.
59. Zingaretti MC, Crosta F, Vitali A, et al. The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J* 2009;23:3113-20.
60. Ouellet V, Labbe SM, Blondin DP, et al. Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. *J Clin Invest* 2012;122:545-52.
61. van Marken Lichtenbelt WD, Schrauwen P. Implications of nonshivering thermogenesis for energy balance regulation in humans. *Am J Physiol Regul Integr Comp Physiol* 2011;301:R285-96.
62. Rothwell NJ, Stock MJ. A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 1979;281:31-5.
63. Ma SW, Foster DO. Uptake of glucose and release of fatty acids and glycerol by rat brown adipose tissue *in vivo*. *Can J Physiol Pharmacol* 1986;64:609-14.
64. Orava J, Nuutila P, Lidell ME, et al. Different metabolic responses of human brown adipose tissue to activation by cold and insulin. *Cell Metab* 2011;14:272-9.
65. Tayek JA. A review of cancer cachexia and abnormal glucose metabolism in humans with cancer. *J Am Coll Nutr* 1992;11:445-56.
66. Bannani-Baiti N, Walsh D. Animal models of the cancer anorexia-cachexia syndrome. *Support Care Cancer* 2011;19:1451-63.