There is a current debate in the literature on whether human fat derived from the supraclavicular region should be classified as brown, or as the white fat-derived less potent, brite/beige. This commentary addresses whether the existing classification defined in mice is sufficient to describe the types of thermogenic adipocytes in humans. We recently published a contradictory mRNA expression signature of human supraclavicular fat defined by an upregulation of the brite marker TBX1 along with the classical brown markers ZIC1 and LHX8, as well as genes indicating brown fat activity including UCP1, PGC-1α, and PRDM16; and, finally, a downregulation of the white/brite markers HOXC8 and HOXC9. Subcutaneous fat was used as reference material. Another recent study presents a higher expression of ZIC1 and a lower expression of TBX1 in interscapular compared with supraclavicular fat. Here, however, there was no difference in UCP1, PGC-1α, PRDM16, suggesting both depots had equal brown fat potency. Taken together, supraclavicular brown fat derived from adult humans seems to represent a type of brown fat with distinct features from both subcutaneous white/brite and interscapular brown fat. Therefore, the classification of adipocyte subtypes defined in mice may need reconsideration when applying to humans.

Classification of Human and Murine Fat

Following on from initial reports demonstrating that metabolically active brown adipose tissue (BAT) exists in adult humans, several studies have recently confirmed and provided additional molecular evidence to support this biological finding. Importantly, some of the anatomical regions which in earlier studies have been reported as metabolically active in adult humans have now been studied in more detail, including the neck and the supraclavicular region. There is some debate in the literature on whether human supraclavicular BAT should be classified as brown or whether it rather resembles the brown-in-white (brite, also termed beige) adipocytes. Brite/beige fat was identified within murine white fat depots and represents a type of white adipocytes that in response to cold stimuli (in vivo) or PPARγ protein activation (in vitro) adapt brown-like characteristics, i.e., increased uncoupling of mitochondria. Importantly, the levels of UCP1 in brite fat, even when activated, do not reach the levels of classical brown fat, suggesting a higher uncoupling capacity and anti-obesity potential of brown fat compared with brite/beige. In mice, the inguinal, cardiac, and retroperitoneal white fat depots have been re-classified into brite, while visceral depots including the mesenteric and the epididymal white fat depots, seem to represent a true white phenotype where thermogenesis is not induced despite chronic cold exposure. Whether this is due to white and brite fat having specific precursor cells or if it is rather a matter of differences in location and exposure of adrenergic stimuli in vivo is not completely clear. Indeed, following immortalization of inguinal adipocytes using the 3T3 approach, a subset of cultures were able to induce UCP1 in response to isoprenaline while other cultures remained unresponsive, suggesting that brite/beige precursors exist side by side with...
side with white precursors within the subcutaneous depot. However, it cannot be excluded that the cloning process in itself results in loss of responsiveness in some of the cultures, and that in fact the brite program could be induced in all white fat cells. Indeed, following chronic exposure to a PPARγ agonist, it was possible to induce a brite/beige phenotype in murine primary cultures isolated from the visceral epididymal depot, indicating that the differences in response to cold between the visceral and subcutaneous depot might be due to differences in innervation rather than in composition of different precursor cells. This idea is supported by the phenomena observed in pheochromocytoma patients where catecholamine secreting tumors have been shown to induce white-to-brown transdifferentiation of omental adipocytes. Nevertheless, it is clear that, in both mice and humans, distinct functional differences between fat depots exist. Therefore, in order to study the roles of different fat types and the impact on full body metabolism, there is a need for a clear classification.

In contrast to what has been suggested in the literature, we recently demonstrated that supraclavicular fat from adult humans demonstrate molecular features of classical brown fat. We collected BAT from the supraclavicular region in 21 patients undergoing surgery for suspected cancer in the neck area. Only one of the patients had cachexia while none had hyperthyroidism or pheochromocytoma, thus these known cancer-associated activators of BAT were not confounders of our material, although any unknown effects of cancer cannot be excluded. We assessed the gene expression of established markers for brown, brite/beige, and white adipocytes. These markers have been defined from global analyses in mice and their validity in humans could therefore be questioned. However, several recent human studies, have found that some of the murine fat markers can distinguish between different fat types also in humans, whereas a global analysis on human material is still warranted. It is important to note that many of these markers separate the fat types in a relative, rather than absolute manner, making the classification of the reference material equally important. To minimize sample variation, it is ideal to collect both target and reference material from the same subjects. We were unfortunately unable to obtain this so instead we utilized subcutaneous fat from the abdominal region of age, gender, and BMI-matched subjects (n = 10) as reference material. Here, it is important to note that human abdominal subcutaneous fat is composed of two different layers with different molecular characteristics. The superficial subcutaneous fat is located between the skin and a fascia, whereas the deep subcutaneous fat is located beneath this fascia. We obtained all our reference material from the superficial layer, which have been described to express more metabolically associated genes compared with the deep subcutaneous fat. As subcutaneous fat depots in mice have been classified as brite, this is potentially also the case in humans. We obtained all our reference material from the superficial layer, which have been described to express more metabolically associated genes compared with the deep subcutaneous fat.

### Table 1. Transcriptional markers of adipose depots in humans and mice discussed in this commentary

| Supraclavicular adult human tissue | Interscapular infant human tissue | Deep neck adult human tissue | Subcutaneous adult human tissue | Epididymal (WAT) murine cell cultures | Inguinal (B/B) murine tissue | References |
|-----------------------------------|----------------------------------|-----------------------------|--------------------------------|-------------------------------------|---------------------------|------------|
| UCP1 PGC-1α PRDM16 ZIC1 LHX8 TBX1 | UCP1 PRDM16 PGC-1α ZIC1 TBX1 | Hoxc8 Hoxc9                 |                                |                                     |                           |            |
| TBX1                              |                                  | UCP1 ZIC1 LHX8              |                                |                                     |                           |            |
|                                   |                                  | Hoxc8 Hoxc9                 |                                |                                     |                           | 7          |
|                                   |                                  | Hoxc9                       |                                |                                     |                           | 8          |

WAT, white adipose tissue; B/B, brite/beige.

Human Supraclavicular Fat: More Brown than Brite?

In contrast to what has been suggested in the literature, we recently demonstrated that supraclavicular fat from adult humans demonstrate molecular features of classical brown fat. We collected BAT from the supraclavicular region in 21 patients undergoing surgery for suspected cancer in the neck area. Only one of the patients had cachexia while none had hyperthyroidism or pheochromocytoma, thus these known cancer-associated activators of BAT were not confounders of our material, although any unknown effects of cancer cannot be excluded. We assessed the gene expression of established markers for brown, brite/beige, and white adipocytes. These markers have been defined from global analyses in mice and their validity in humans could therefore be questioned. However, several recent human studies, have found that some of the murine fat markers can distinguish between different fat types also in humans, whereas a global analysis on human material is still warranted. It is important to note that many of these markers separate the fat types in a relative, rather than absolute manner, making the classification of the reference material equally important. To minimize sample variation, it is ideal to collect both target and reference material from the same subjects. We were unfortunately unable to obtain this so instead we utilized subcutaneous fat from the abdominal region of age, gender, and BMI-matched subjects (n = 10) as reference material. Here, it is important to note that human abdominal subcutaneous fat is composed of two different layers with different molecular characteristics. The superficial subcutaneous fat is located between the skin and a fascia, whereas the deep subcutaneous fat is located beneath this fascia. We obtained all our reference material from the superficial layer, which have been described to express more metabolically associated genes compared with the deep subcutaneous fat. As subcutaneous fat depots in mice have been classified as brite, this is potentially also the case in humans. We observed a distinct expression signature in the supraclavicular fat samples with a higher expression of UCP1, LHX8, ZIC1, PGC-1α, PRDM16, and TBX1 and a lower expression of the white/brite/beige markers Hoxc8 and Hoxc9, compared with the subcutaneous fat reference material. Importantly, this expression signature was conserved in preadipocytes that were isolated from the same subjects and regions that the tissue biopsies were obtained from.
and differentiated in vitro into mature fat-droplet containing adipocytes. Based on our markers we could conclude that, consistent with previous positron emission tomography/CT (PET/CT)-scan data, the supraclavicular fat depots had a pronounced brown fat expression signature in relation to the subcutaneous depots. Interestingly, as mentioned above, subcutaneous fat depot has previously been classified as brite in mice. Thus, although it is currently not clear whether this classification translates to humans we cannot exclude the possibility that we compared brown fat to brite/beige fat rather than to white fat.

**Human Brown Fat Identity: Novel Nuances of Brown**

While brown fat activity in adult humans was demonstrated by several groups in 2009, it was recently suggested that human brown fat from the supraclavicular region consisted of brite/beige fat. This idea originated from the relative gene expression of some brite/beige markers defined in murine adipocytes, among others including TBX1, which were expressed in supraclavicular fat samples, $n = 24$, and subcutaneous fat samples, $n = 10$ as well as in cultured cells (supraclavicular adipocytes, $n = 6$, and subcutaneous adipocytes, $n = 6$), supporting the idea of a brite/beige fat phenotype in the human supraclavicular region. Intriguingly, however, several markers for classical brown fat were more highly expressed in human supraclavicular fat than in subcutaneous fat. Indeed, we were able to confirm this finding in our larger sample set of tissue (supraclavicular fat samples, $n = 24$, and subcutaneous fat samples, $n = 10$) as well as in cultured cells (supraclavicular adipocytes, $n = 6$, and subcutaneous adipocytes, $n = 6$). Supporting the idea of a brite/beige phenotype in the human supraclavicular region, this separation occurred even despite any overlaps between the cell cultures if supraclavicular would have been brite/beige. However, we saw a clear separation without any overlaps between the cell cultures from the two depots ($n = 6$ in each group). This separation occurred even despite chronic resiglitazone treatment (200 nM) previously demonstrated to induce a brite/beige phenotype in white fat cells.

Taken together, supraclavicular brown fat derived from adult humans seems to represent a type of brown fat with distinct features from both the more brite/beige/white subcutaneous fat and from the interscapular brown fat found in infants. Whether the supraclavicular brown fat also has a distinct function in human energy homeostasis remains to be investigated. To further understand this, it would be useful to analyze biopsies from a large cohort of subjects including lean and obese. Nevertheless, as present in adult humans, with clear molecular features of brown fat, supraclavicular fat holds great potential in future development of anti-obesity and anti-diabetes strategies.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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