BRIEF REPORT

Functional Brown Adipose Tissue in Healthy Adults

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SUMMARY

Using positron-emission tomography (PET), we found that cold-induced glucose uptake was increased by a factor of 15 in paracervical and supraclavicular adipose tissue in five healthy subjects. We obtained biopsy specimens of this tissue from the first three consecutive subjects and documented messenger RNA (mRNA) and protein levels of the brown-adipocyte marker, uncoupling protein 1 (UCP1). Together with morphologic assessment, which showed numerous multilocular, intracellular lipid droplets, and with the results of biochemical analysis, these findings document the presence of substantial amounts of metabolically active brown adipose tissue in healthy adult humans.

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Active brown adipose tissue helps maintain normal body temperature in newborn infants. It is believed that this tissue regresses with increasing age and is completely lost by the time a person reaches adulthood.[1] However, the capacity to produce brown adipose tissue in adulthood has been shown in patients with catecholamine-secreting tumors such as pheochromocytomas and paragangliomas, in whom distinct brown-adipose-tissue depots develop.[2,3] When scanning with a combination of PET and computed tomography (CT) — with the glucose analogue ^{18}F-fluorodeoxyglucose (^{18}F-FDG) as a tracer — is used in the diagnosis of neoplasms and their metastases, the results can be confounded by a high glucose uptake in the supraclavicular tissue; this increased glucose uptake has been thought to represent the presence of brown adipose tissue.[4] This view has been supported by the localization of the ^{18}F-FDG in adipose tissue on CT images[5] and its sensitivity to propranolol[6] and to environmental temperature before PET scanning[7]; furthermore, this phenomenon occurs more often during the cold winter months than in the summertime.[8] However, to our knowledge and as has been noted in a recent review,[9] there are no direct data that clearly show that tissue from these areas of cold-induced ^{18}F-FDG uptake in healthy adult subjects indeed has histologic features of brown adipose tissue and expresses mRNA and proteins that distinguish it from white adipose tissue. This is an important point, since such data would be necessary to identify bona fide brown adipose tissue in healthy adults and to indicate that such tissue is part of normal human physiology after infancy.

METHODS

SUBJECTS

We studied a group of five healthy volunteers, all of whom provided written informed consent. The study protocol was reviewed and approved by the ethics committee of the Hospital District of Southwest Finland. The study was conducted according to the principles of the Declaration of Helsinki. Subjects were recruited through advertisements in the local newspaper. All potential subjects were screened for metabolic status, and only those with normal glucose tolerance and normal cardiovascular status (as assessed on the basis of electrocardiograms and measured blood pressure) were included. Subjects had to be 20 to 50 years of age.

STUDY DESIGN

Each of the five subjects underwent two PET–CT (with ^{18}F-FDG) studies, one of which was performed during cold exposure and the other during warm conditions. Before being positioned in the scanner for the cold-exposure scan, the subject, while wearing light clothing, spent 2 hours in a room that had an ambient temperature of 17 to 19°C. While the PET–CT study was being performed, one of the subject's feet was placed intermittently in ice water (5 to 9°C; 5 minutes in the water alternating with 5 minutes out). The scan that was obtained in warm conditions was performed on a separate day, with the use of the same scanning protocol as that used for the scan with cold exposure, except that there was no cold exposure before the procedure and no ice-water immersion of a foot during the procedure. Both scans were obtained after the subject had fasted overnight and while the subject was in the supine position. Three of the volunteers provided written informed consent for a biopsy of fat tissue to be performed; the biopsy was performed while the subject was under local anesthesia, and specimens of both brown and white adipose tissue were obtained (for further information, see the Supplementary Appendix, available with the full text of this article at NEJM.org). Measurements from the images of activated brown adipose tissue, as observed on the cold-exposure scans, were used as a guide for the site of the biopsy.

PET STUDY

The tracer ^{18}F-FDG was synthesized in accordance with a standard operating procedure of the Turku PET Centre, with the use of a modified version of the method of Hamacher et al.[10] Technical details can be found in the Supplementary Appendix. At the beginning of the day on which the PET study was to be performed, a catheter was inserted in the subject's antecubital vein for a bolus injection of ^{18}F-FDG. Another catheter was inserted in the antecubital vein of the contralateral arm and was used to obtain samples of venous blood during the scanning.

BIOPSY PROCEDURE

The biopsy was performed while the subject was under local anesthesia (lidocaine supplemented with epinephrine). Guided by the high-resolution PET–CT images, a plastic surgeon collected open-biopsy specimens from areas that corresponded to the cold-induced areas of uptake in the first three subjects (hereafter referred to as Subjects 1, 2, and 3). Immediately after removal, the tissue sample was divided into two pieces: one was fixed in formalin for histologic examination, and the other was snap-frozen in liquid nitrogen. The frozen tissue was used for the preparation of mRNA and complementary DNA (cDNA) for use in real-time quantitative polymerase-chain-reaction (PCR) analysis; protein was also extracted from the frozen tissue (see the Supplementary Appendix). During the same surgical procedure, and through the same incision, an adjacent specimen of subcutaneous fat consisting of white adipose tissue (which served as control tissue) was obtained from all three subjects and was prepared in the same way as the specimens described above.

MICROSCOPICAL STUDIES

We performed immunohistochemical studies using an anti-UCP1 primary antibody (as described in the Supplementary Appendix), together with a horseradish peroxidase–conjugated secondary antibody. We also performed confocal microscopy (as described in the Supplementary Appendix).

RESULTS

As compared with the scans obtained in warm conditions, scans obtained with cold exposure showed enhanced ^{18}F-FDG uptake in all five subjects, most prominently in the supraclavicular area (Fig. 1A, 1B, and 1C). In response to cold exposure, glucose uptake in the supraclavicular area (as calculated with the use of graphical analysis)

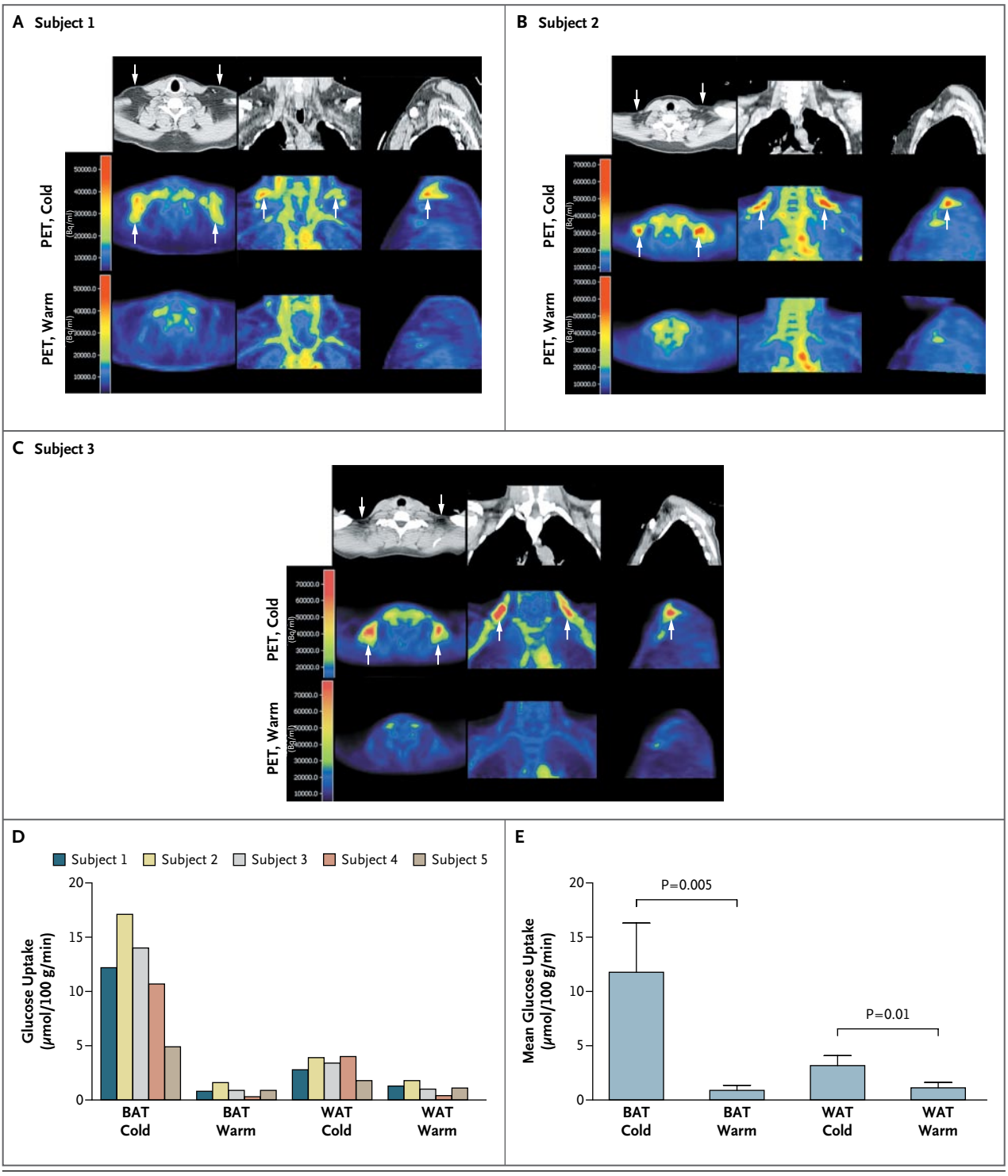

Figure 1 (facing page). Computed Tomographic (CT) and Positron-Emission Tomographic (PET) Images from the Neck and Upper Thoracic Region, Obtained during Cold and Warm Conditions.

Panels A, B, and C show images of the neck and upper thoracic region from Subjects 1, 2, and 3, respectively. The top row in each panel shows individual CT images, the middle row shows PET images, with the glucose analogue ^{18}F-fluorodeoxyglucose (^{18}F-FDG) as a tracer, during cold conditions, and the bottom row shows PET images with ^{18}F-FDG during warm conditions. The image on the left side of each row represents a transaxial slice, the image in the middle a coronal slice, and the image on the right side a sagittal slice from the region of activated brown adipose tissue. Cold-induced glucose uptake in supraclavicular tissue is marked by arrows. The color index to the left of the PET images shows the level of ^{18}F-FDG uptake, with red indicating the highest level. Glucose uptake, calculated with the use of graphical analysis of PET data, in each of the five study subjects is shown in Panel D. Glucose uptake rates in brown adipose tissue (BAT) were assessed in the supraclavicular region, and glucose uptake rates in white adipose tissue (WAT) were assessed in the subcutaneous region corresponding to the site of the biopsies. Panel E shows a comparison of mean glucose uptake in all five subjects, calculated with the use of a paired Student's t-test. T bars indicate standard deviations.

was higher than the uptake in adjacent white adipose (Fig. 1D), with the mean uptake in the supraclavicular area increased by a factor of approximately 15 ($P=0.005$), as compared with an increase by a factor of 4 in the white adipose tissue ($P=0.01$) (Fig. 1E).

The areas from which the open-biopsy specimens were obtained in Subjects 1, 2, and 3 are shown by arrows in Figures 1A, 1B, and 1C, respectively. Quantitative PCR analysis of mRNA expression levels for multiple genes is shown in Figure 2. Assessment of the tissue-biopsy specimens showed that expression of uncoupling protein 1 (*UCP1*), which is a marker gene for brown adipose tissue, was increased by a factor of more than 1000 as compared with expression in white adipose tissue. UCP1 allows protons to flow back over the mitochondrial inner membrane, generating heat instead of ATP — a process known as adaptive thermogenesis.[11] This process is believed to be important for maintaining normal body temperature in rodents, animals that hibernate, and human newborns.[12] The instrumental role of UCP1 in this process has been shown in studies of mice that have a targeted deletion of this gene; these mice have a severely blunted ability to maintain normal body temperature when they are acutely exposed to cold.[13]

Deiodinase, iodothyronine, type II (*DIO2*) mRNA was also significantly up-regulated in brown adipose tissue as compared with white adipose tissue in our three subjects (Fig. 2). This finding is of interest because it appears that *Dio2* is expressed by brown adipocytes in order to make triiodothyronine available to sustain the elevated metabolism of brown adipose tissue.[14,15] In addition, peroxisome-proliferator–activated receptor γ coactivator 1α (*PGC1α*) mRNA was significantly enhanced in brown adipose tissue (Fig. 2). This finding is not surprising, since cold induction of *UCP1* gene expression depends, to a large extent, on cold-induced activation of *PGC1α*.[16] Activation of *PGC1α* is crucial for *UCP1* induction, since deletion of *pgc1α* (in mice) dramatically reduces cyclic AMP–induced[17] and cold-induced[18] activation of *ucp1.* The master regulator of brown-adipose-tissue formation, PR domain containing 16 (*PRDM16*),[19] was also induced in all three subjects (Fig. 2). In mature brown adipose tissue from rodents, the β_3-adrenergic receptor (ADRB3) is the most important of the three subtypes of β-adrenergic receptors.[12] We found that there was a significant induction of this receptor subtype in brown adipose tissue as compared with white adipose tissue in the three subjects (Fig. 2). The data on mRNA expression presented here, which are based on samples from adipose-tissue depots identified by PET, display a gene-expression profile that is expected for brown adipose tissue. To determine whether tissue samples corresponding to areas of cold-induced ^{18}F-FDG uptake also expressed UCP1, the marker protein in brown adipose tissue, we performed a Western blot analysis, which showed the presence of UCP1 protein in all three subjects, whereas the control samples of white adipose tissue from these subjects did not express any detectable UCP1, as expected (Fig. 3A). We also investigated a mito-

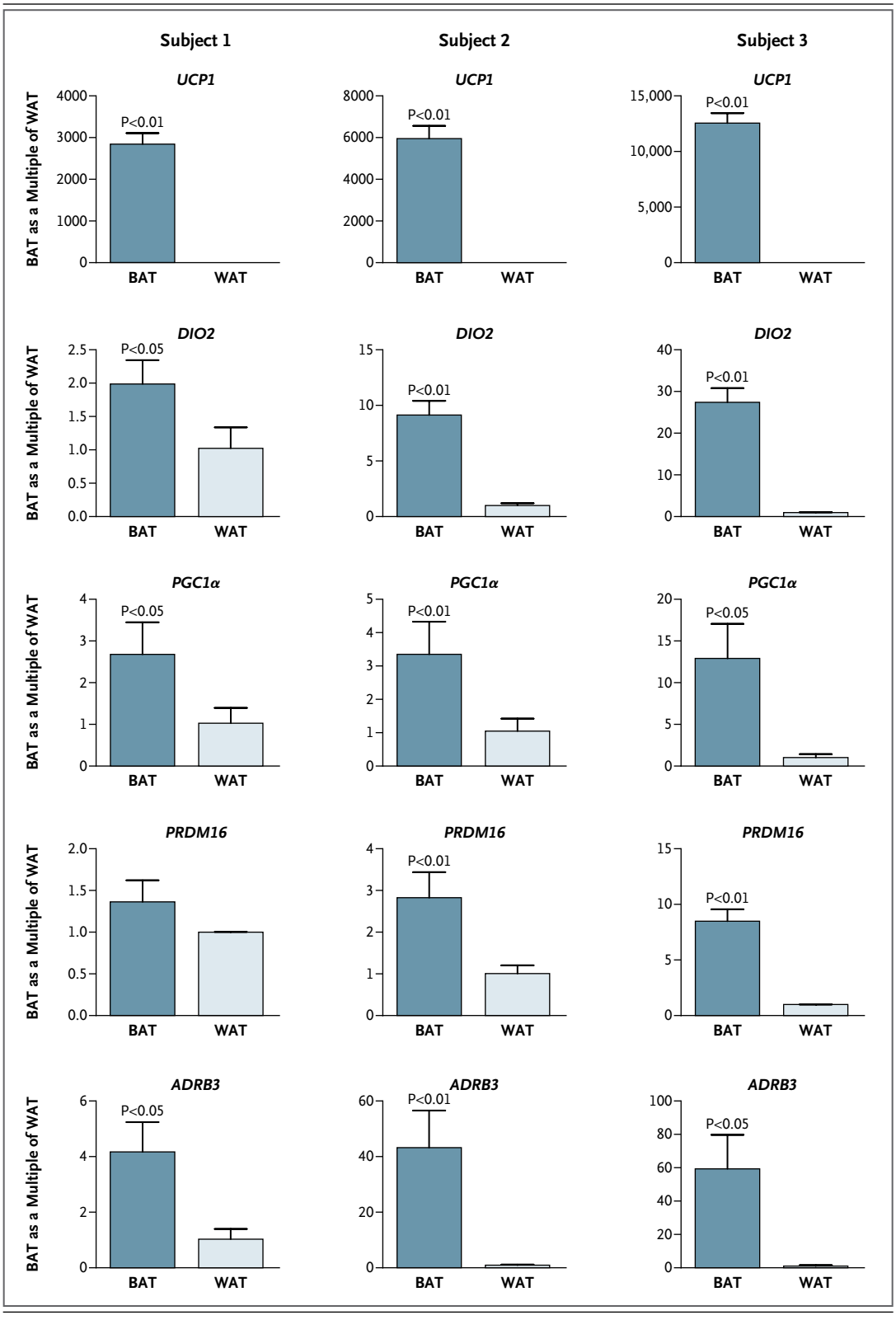

Figure 2 (facing page). Gene Expression in Brown and White Adipose Tissue.

The mean levels of expression of *UCP1, DIO2, PGC1α, PRDM16,* and *ADRB3,* based on the results of quantitative real-time PCR analysis, are shown for Subjects 1, 2, and 3. Expression levels were normalized to that of β-actin and are shown as the levels in brown adipose tissue (BAT) as a multiple of the levels in white adipose tissue (WAT). T bars indicate standard deviations.

chondrial marker, cytochrome *c*, and found it to be more abundant in the brown adipose tissue than in the white adipose tissue (Fig. 3B).

Histologic analysis of the biopsy samples from our three subjects clearly showed cells with multilocular lipid droplets in the supraclavicular depot of brown adipose tissue but not in adjacent white adipose tissue (Fig. 3C). The results of immunohistochemical staining for UCP1 strengthen the indirect connection between gene expression and histologic features, since the brown adipose tissue had substantial levels of UCP1, whereas the white adipose tissue did not (Fig. 3D). Laser confocal microscopy also showed that in brown adipose tissue, the UCP1 signal colocalized with the signal for a mitochondrial marker (cytochrome oxidase subunit I), resulting in an overlay that indicated nearly perfect colocalization (Fig. 3E; see also the Supplementary Appendix). There was no detectable UCP1 in white-adipose-tissue sections (Fig. 3E).

DISCUSSION

Both the PET–CT studies and the studies of tissue-biopsy specimens indicate that normal adult humans have brown adipose tissue. Brown adipose tissue expresses substantial amounts of UCP1 protein; it also contains more cytochrome *c* than white adipose tissue, as one would expect, since human brown adipose tissue is mitochondria-dense, in contrast to white adipose tissue, which has relatively few mitochondria.

On the basis of the data presented here and previous findings regarding the metabolism of brown adipose tissue, we speculate that activation of brown adipose tissue by cold exposure may be important in terms of energy expenditure in humans. For example, on the basis of data derived from the PET–CT scans in one of our subjects, the weight of the supraclavicular brown-adipose-tissue depot (both sides included) was 63 g. The rate of glucose uptake was 12.2 μmol per 100 g per minute, which is equivalent to 7.7 μmol for the entire depot. During a 24-hour activation period, 11 mmol of glucose would have been taken up by the brown-adipose-tissue depot. Since the substrate for activated brown adipose tissue consists predominantly of fatty acids, this finding may be important. In fact, during activation of brown adipose tissue, only approximately 10% of the total metabolism of brown adipose tissue is derived from glucose uptake.[20] Thus, if the brown adipose tissue in this example were fully activated, it would burn an amount of energy equivalent to approximately 4.1 kg of adipose tissue over the course of a year. We believe that this is a modest assumption, since the degree of activation of brown adipose tissue most likely is submaximal (we estimate it to be 50%). Furthermore, studies in rodent models indicate that the contribution of glucose to the metabolism of fully activated brown adipose tissue is an estimated 2% at maximal stimulation.[20] On the basis of this example, we speculate that in humans, activated brown adipose tissue has the potential to contribute substantially to energy expenditure.

In conclusion, our studies in healthy subjects show that cold-induced glucose uptake in supraclavicular adipose-tissue depots is increased by a factor of 15 and that this tissue expresses mRNA for markers of brown adipose tissue — namely *UCP1*, *DIO2*, *PGC1α*, *PRDM16*, and *ADRB3*. In addition, the tissue expresses substantial levels of UCP1 protein and cytochrome *c*, as assessed by Western blot analysis, and is characterized morphologically by multilocular, intracellular lipid droplets. Finally, the tissue shows mitochondrial localization of the UCP1 protein. In our opinion, these findings constitute direct identification of functional human brown adipose tissue. On the basis of these biochemical, molecular, and morphologic criteria, we believe that brown adipose tissue is present in healthy adults. We suggest that the presence of brown adipose tissue in normal adults is worthy of further study and speculate that this tissue might provide a pharmacologic target, given the current obesity pandemic.

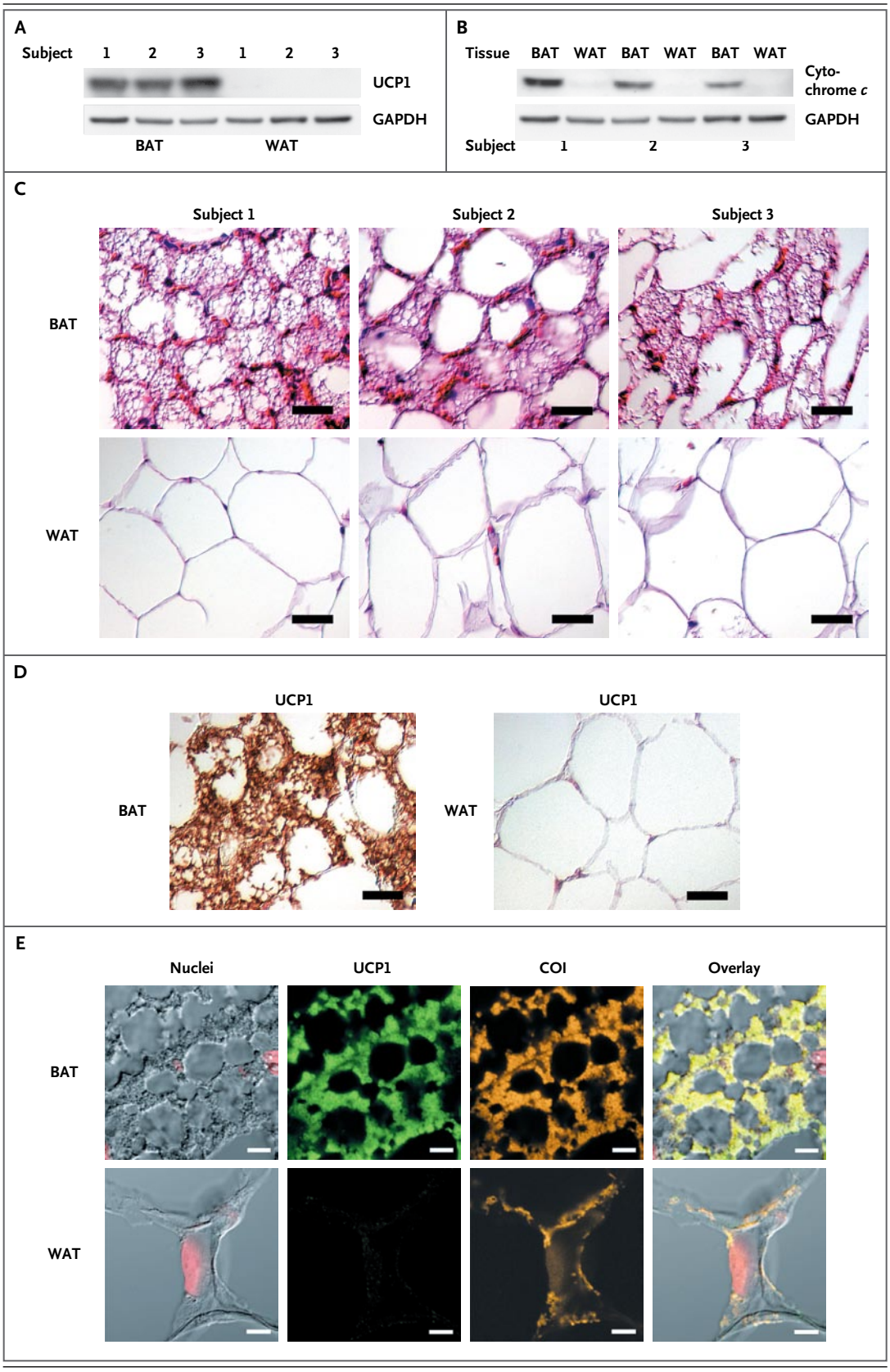

Figure 3 (facing page). Western Blot, Histologic, and Immunofluorescence Analyses of Brown and White Adipose Tissue.
Western blots show levels of UCP1 (Panel A) and cytochrome *c* (Panel B) in brown adipose tissue (BAT) and white adipose tissue (WAT) from Subjects 1, 2, and 3. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a protein loading control. Sections of brown adipose tissue and white adipose tissue from Subjects 1, 2, and 3 are shown in Panel C (hematoxylin and eosin). Multilocular, intracellular lipid droplets are present in brown adipose tissue but not in white adipose tissue. Immunohistochemical staining of brown adipose tissue and white adipose tissue with a UCP1-specific antiserum (Panel D) shows that brown adipose tissue is positive for UCP1, whereas no staining is seen in white adipose tissue. Immunofluorescence staining of brown adipose tissue and white adipose tissue (Panel E) shows colocalization of UCP1 (green) and a mitochondrial marker, cytochrome oxidase subunit I (COI, orange), in brown adipose tissue. No UCP1 could be detected in mitochondria of white adipose tissue. Nuclei were stained with TO-PRO-3 (red). Scale bars represent 30 μm in Panels C and D and 5 μm in Panel E.

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No potential conflict of interest relevant to this article was reported.

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