Fibroblast growth factor-21 is expressed in neonatal and pheochromocytoma-induced adult human brown adipose tissue

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Abstract

Objective. In rodents, brown (BAT) and white (WAT) adipose tissues are targets and expression sites for fibroblast growth factor-21 (FGF21). In contrast, human WAT expresses negligible levels of FGF21. We examined FGF21 expression in human BAT samples, including the induced BAT found in adult patients with pheochromocytoma, and interscapular and visceral BAT from newborns.

Methods. The expression of FGF21 and uncoupling protein-1 (UCP1, a brown adipocyte marker), was determined by quantitative real-time-PCR and immunoblotting. The transcript levels of marker genes for developmentally-programmed BAT (zinc-finger-protein of the cerebellum-1, ZIC1) and inducible-BAT (cluster of differentiation-137, CD137) were also determined.

Results. FGF21 and UCP1 are significantly expressed in visceral adipose tissue from pheochromocytoma patients, but not in visceral fat from healthy individuals. In neonates, FGF21 and UCP1 are both expressed in visceral and interscapular fat, and their expression levels show a significant positive correlation. Marker gene expression profiles suggest that inducible BAT is present in visceral fat from pheochromocytoma patients and neonates, whereas developmentally-programmed BAT is present in neonatal interscapular fat.

Conclusions. Human BAT, but not WAT, expresses FGF21. The expression of FGF21 is especially high in inducible, also called beige/brite, neonatal BAT, but it is also found in the interscapular, developmentally-programmed, BAT of neonates.

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Abbreviations: BAT, brown adipose tissue; CD137, cluster of differentiation 137; FGF21, fibroblast growth factor-21; UCP1, uncoupling protein-1; WAT, white adipose tissue; ZIC1, zinc finger protein of the cerebellum-1.

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1. **Introduction**

Fibroblast growth factor-21 (FGF21) is a hormonal factor that has systemic effects in promoting glucose uptake and oxidation [1]. The liver is the main site of FGF21 expression and production in rodents, but FGF21 is also expressed in white adipose tissue (WAT), where it may play an autocrine role [2], and in brown adipose tissue (BAT). In rodents, FGF21 targets BAT, where it induces mitochondrial uncoupling protein-1 (UCP1) gene expression, and favors glucose oxidation and energy expenditure [3]. Moreover, FGF21 promotes the “browning” of WAT; i.e. the appearance of brown adipocytes (also called “beige” or “brite” cells) in WAT depots [4]. Under thermogenic activation, FGF21 is highly expressed in BAT, which has been proposed to be source of FGF21 in this situation [5].

The liver is considered the main site of FGF21 expression in humans, and blood FGF21 levels and liver FGF21 expression have been correlated in some hepatic pathologies [6]. Muscle is also a potential source of FGF21 in patients with mitochondrial pathologies [7]. Unlike rodents, there is little or no expression of FGF21 in human WAT [6,8].

The presence of BAT has been traditionally recognized in human neonates, wherein the UCP1 content increases at the final trimester of gestation [9]. However, active BAT has also recently been found in adult humans [10]. BAT activity is reduced in obese individuals, and this has suggested, like in rodents, that BAT-mediated thermogenic energy expenditure may protect against obesity [10]. Some authors have recently claimed that BAT in adult humans arises largely via the inducible “browning” of WAT [11,12]. In fact, the capacity of adult humans to develop brown adipocytes has long been recognized, as brown adipocytes arise in WAT from pheochromocytoma patients due to the tumor-mediated release of catecholamines, which are known BAT-inducing agents [13,14]. However, recent data also claim for the presence of “classical” BAT in children and adult humans [15,16].

Considering the role of FGF21 in the thermogenic activation of BAT, the potential of BAT as a source of FGF21 in rodents, and the absence of FGF21 expression in human WAT, we herein investigated the expression of FGF21 in human BAT. We employed a unique collection of neonatal human BAT samples, as well as BAT samples from adult pheochromocytoma patients.

2. **Methods**

Samples of visceral (perirenal, omental) adipose tissue were obtained during surgical removal of the pheochromocytoma tumors from Caucasian adult patients (Azienda Ospedaliero-Universitaria, Ancona, Italy, 6 patients; Hospital del Mar, Barcelona, Spain, 3 patients; clinical details provided in Supplemental Table 1). Visceral (omental) WAT samples from 10 Caucasian healthy control individuals were obtained during cholecystectomies. Mean age (51.3 ± 12.2 year in pheochromocytoma patients versus 57.5 ± 5.0 year in healthy controls, P = 0.17) and body mass index (24.2 ± 5.4 in pheochromocytoma patients versus 24.9 ± 2.1 in healthy controls, P = 0.75) were not significantly different in patient’s population relative to controls. Samples were frozen in liquid nitrogen until use for RNA and protein preparation. When possible, tissue sections were fixed and further processed for light and electron (JEM-1010, Japan) microscopy.

Samples of adipose tissue from the interscapular and visceral (perirenal/retrorenal) areas were obtained from human newborns (mostly premature neonates) who died during 1995–2006 in the Czech Republic. Autopsies were performed 2–3 h after the death. Some of these patients were previously reported in studies on developmentally-regulated gene expression in other tissues [17,18]. Clinical details are provided in Supplemental Table 2.

After RNA isolation, 1 μg RNA was retro-transcribed and gene transcript levels were quantified by quantitative RT-PCR, using TaqMan reagents as described by the supplier (Applied Biosystems) along with specific TaqMan probes (Supplemental Table 3). Expression levels of gene transcripts were considered negligible when, under the above standard RT-PCR conditions, cycle threshold was ≥ 40. Data of specific mRNA abundance were expressed relative to 18S rRNA. The protein levels of UCP1 and FGF21 were assessed using 35 μg protein/lane, standard immunoblotting procedures, and antibodies against FGF21 (sc-16842, Santa Cruz, USA) and UCP1 (a specific anti-serum kindly provided by E.Rial, CSIC, Madrid).

Where appropriate, statistical analyses were performed using the Mann-Whitney test or Kruskal-Wallis test with pairwise post-hoc analysis (Tukey adjustment). Spearman’s coefficients of correlation were determined. This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and it was approved by the committees of medical ethics at all of the collaborating institutions. Informed consent was obtained from all neonate parents and adult patients.

3. **Results**

3.1. **FGF21 is expressed in BAT of adult human pheochromocytoma patients**

We analyzed visceral adipose tissues from the perirenal and omental regions of pheochromocytoma patients. These samples, particularly those from the perirenal region, contained adipocytes with multilocular lipid droplets and enhanced mitochondrial abundance, which are characteristic of the brown adipocyte morphology (Fig. 1A). All samples from patients expressed detectable levels of UCP1 mRNA (median 9.3 × 10^{-6}, interquartile range (IR): 3.3 × 10^{-5}) the marker of the brown adipocyte cell identity relative to white adipocytes. In contrast, visceral adipose tissues from healthy adult controls showed low UCP1 (median 6.4 × 10^{-6}, IR: 1.2 × 10^{-07}, P = 0.0002 relative to pheochromocytoma patients), indicative of a main WAT phenotype. FGF21 mRNA was expressed at substantial levels in adipose samples from the pheochromocytoma patients (median 3.3 x10^{-06}, IR: 3.2 × 10^{-06}), whereas it was not detected in omental WAT from healthy controls (Fig. 1B, left). Subcutaneous abdominal or dorsal fat from healthy individuals did not show
**A**

Patient 7

Patient 9

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**B**

**FGF21 mRNA**

**UCP1 mRNA**

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**C**

**UCP1/FGF21**

$P = 0.017$

$r = 0.7883$
substantial expression of the FGF21 and UCP1 mRNAs. UCP1 and FGF21 protein levels were consistent with the above described transcript levels (Fig. 1B, right). Spearman’s coefficient of correlation analysis of FGF21 mRNA and UCP1 mRNA levels indicated a positive, significant, correlation (P = 0.017, r = 0.7883) (Fig. 1C). These findings indicated that FGF21 gene expression is directly associated with brown adipocyte abundance and activity in the adipose tissues of adult patients.

3.2. FGF21 is expressed in BAT of human neonates

All samples of interscapular and visceral (perirenal and retrorenal) adipose tissue from human neonates expressed detectable levels of the UCP1 and FGF21 mRNAs. UCP1 mRNA expression levels were: median 3.1 × 10^{-05}, IR: 8.8 × 10^{-05} in interscapular fat; and median 1.9 × 10^{-04}, IR: 3.1 × 10^{-04} in visceral fat, P = 0.04 and P = 0.0009 versus control WAT, respectively. UCP1 mRNA levels were not significantly different in interscapular versus visceral comparison FGF21 mRNA levels were: median 6.9 × 10^{-08}, IR: 8.2 × 10^{-08} in interscapular fat, median 3.8 × 10^{-07}, IR: 7.1 × 10^{-07} in visceral fat, P = 0.036. The FGF21 mRNA levels showed positive, significant, correlation with gestational age at birth (Fig. 2A, left). The mRNA levels of UCP1 and FGF21 showed a positive and significant correlation (Fig. 2A, right), indicating that as much UCP1 gene is expressed (and, therefore, as much extent of brown adipose tissue maturation and differentiation occurs), as much the FGF21 gene is expressed in the neonatal samples. Separate analysis of FGF21 mRNA gene expression in samples from neonates with systemic inflammatory conditions (sepsis, necrotizing enterocolitis, N = 12, median 0.66 × 10^{-07} IR 1.25 × 10^{-07}) relative to neonates without those conditions (N = 7, median 0.37 × 10^{-07} IR 0.58 × 10^{-07}) revealed no significant changes in FGF21 gene expression (P = 0.083).

3.3. FGF21 gene expression and the molecular signature of fetal/neonatal BAT and pheochromocytoma-induced adult BAT

We compared gene expression levels of marker genes for distinct types of BAT in the interscapular and visceral (perirenal and retrorenal) fat samples from neonates of similar gestational age (N = 7, 28.0 ± 7.9 months, interscapular; N = 7, 32.1 ± 5.3 months, visceral). ZIC1, a marker gene expressed in developmentally-programmed brown adipocytes but not in beige/brite adipocytes [12,19], was highly expressed in interscapular neonatal BAT (median 9.8 × 10^{-06}, IR: 5.4 × 10^{-06}), but it was lower in visceral neonatal adipose tissue (median 6.1 × 10^{-07}, IR: 5.4 × 10^{-07}, P = 0.032 relative to interscapular adipose tissue), in visceral adipose tissue from adult pheochromocytoma patients (median 1.7 × 10^{-07}, IR: 3.8 × 10^{-07}, P = 0.04 relative to interscapular neonatal adipose tissue) and in adult control WAT (median 1.2 × 10^{-05}, IR: 8.0 × 10^{-11}, P = 0.03 relative to adult pheochromocytoma patients, P = 0.0002 relative to interscapular neonatal fat, and P = 0.04 relative to visceral neonatal adipose tissue) (Fig. 2). Conversely, the proposed beige/brite marker gene CD137 [11,12], was much more expressed in visceral (median 4.1 × 10^{-07}, IR: 6.6 × 10^{-07}) relative to interscapular adipose tissues from neonates of similar age (median 2.6 × 10^{-08}, IR: 2.5 × 10^{-08}, P = 0.0004 relative to neonatal visceral fat), and relative to WAT from healthy adults (median 1.2 x10^{-07}, IR: 9.3 x 10^{-08}, P = 0.04 relative to neonatal visceral adipose tissue), but its expression was not significantly different relative to adipose tissue from pheochromocytoma patients (median 1.6 × 10^{-07}, IR: 1.2 × 10^{-07}, P = 0.42 relative to visceral fat from neonates).

4. Discussion

We found that human adipose tissues containing brown adipocytes systematically express FGF21, which shows a positive association with UCP1 expression, an indicator of the amount and activity of brown adipocytes in fat tissue. Neonatal interscapular BAT shows the molecular signature of developmentally-programmed BAT (e.g. high expression of ZIC1); and fetal/neonatal visceral BAT more closely resembles fat from the inducible, beige/brown, lineage. This is consistent with recent reports on a beige/brite molecular signature in intra-abdominal adipose depots from human children and neonates [12] but preferential expression of marker genes of developmentally-programmed BAT (e.g. ZIC1) in interscapular fat from children [15]. Our data suggest that the two cell lineages known to result in thermogenic, UCP1-expressing cells, co-exist at distinct anatomical sites in the human fetal/neonatal period. In any case, FGF21 expression (and, therefore the capacity of synthesizing and releasing FGF21) occurs in both types of BAT-related fat. However, at least in neonates, FGF21 appears to be more intensely expressed in beige/brite BAT. Cell culture experiments showed that FGF21 was expressed in cells differentiated into developmentally-programmed brown adipocytes and in beige/brite adipocytes, which much higher expression seen in beige/brite cells [11,12]. Recently, Lee et al. [20] reported that
differentiation of adult human precursor cells from neck fat into a beige/brite phenotype leads to expression and release of FGF21. Overall, present results in neonates and pheochromocytoma patients confirm these observations.

The present study has obvious limitations, mostly derived from the small sample size due to ethical considerations and to the relatively infrequency of pheochromocytoma among human populations. For neonates, the autopsy-derived samples, derived time span for sampling post-mortem individuals plus the diversity in morbidity and mortality causes are limitations whose influence on gene expression data and their interpretation cannot be currently evaluated. We couldn’t exclude totally that pre-existing inflammatory conditions –a reported stimuli of FGF21 levels [21,22]- in a sub-set of neonate population may have some influence in the extent of FGF21 gene expression in some samples. Moreover, the use of cholecystectomy surgical procedures in adult controls to obtain intra-abdominal adipose tissue is also a potential bias, difficult to address due to the obvious ethical constraints for obtaining intra-abdominal adipose samples from healthy individuals. In contrast with the above weaknesses, the current study took advantage of the analysis of a unique collection of human neonatal BAT samples, as well as BAT from several adult pheochromocytoma patients. Thus, even taking into account the caution in the interpretations arising from the limitations mentioned above, present data reinforce the concept that BAT

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**Fig. 2** – FGF21 expression in BAT from human neonates. A, Linear correlation of FGF21 mRNA expression in adipose tissue from neonatal samples in relation to gestational age at birth (left), and linear correlation of FGF21 mRNA and UCP1 mRNA in these samples (right). Spearman’s coefficient of correlation is shown. B, Transcript levels of the UCP1, FGF21, ZIC1 and CD137 genes in neonatal adipose tissue from interiscapular or visceral (perirenal/retrorenal) sites, compared with the levels in healthy adult visceral adipose tissue (control, C) and adipose tissue from adult pheochromocytoma patients. Results are shown as box-and-whisker plot, the line within the box marks the median, and the upper boundary of the box indicates the interquartile range. Error bars above and below the box denoted the 100th and 0th percentiles, respectively. Kruskal-Wallis test and pairwise post-hoc analysis (Tukey adjustment) was used for between-group comparisons. Significant differences with respect to controls are denoted by * (P < 0.05), ** (P < 0.01) and *** (P < 0.001), shown only when controls values were detectable (see Methods). Significant differences between groups are marked denoting the P value.
may be a relevant site of FGF21 release in humans. Given the strong capacity of FGF21 to promote BAT activity and the recruitment of inducible brown adipocytes, we speculate that FGF21 could play autocrine (and, perhaps endocrine) roles in these processes both during development and in the adult response to environmental stimuli. Considering that the identification of novel molecular actors capable of influencing BAT activity are of potential interest in promoting energy expenditure, the current evidence of preferential expression of FGF21 in BAT warrants further research on this regulatory factor in relation to foreseeable strategies to treat or prevent obesity by promoting energy expenditure.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.metabol.2013.11.014.

Author’s contributions

Clinical data and biopsy samples from pheochromocytoma patients were obtained by A.F., J.P. and A.G; and those from neonates by P.F. and P.K. Isolation and subsequent assays with RNA were done by A.F., E.H. and J.M.G-E. Immunoblot assays were done by R.C, and microscopy analysis was done by M.G. RNA were done by A.F., E.H. and J.M.G-E. Immunoblot assays with patients were obtained by A.F., J.P. and A.G; and those from neonates by P.F. and P.K. Isolation and subsequent assays with RNA were done by A.F., E.H. and J.M.G-E. Immunoblot assays were done by R.C, and microscopy analysis was done by M.G. Data were analyzed by S.C., J.K. and F.V. The paper was written by F.V.

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Conflict of interest

All authors declare no conflict of interest.

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