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Sexual dimorphism in age-related changes in UCP2 and leptin gene expression in subcutaneous adipose tissue in humans

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Abstract

The influence of age and gender on uncoupling protein 2 (UCP2) expression and its relationship with leptin expression in subcutaneous adipose tissue has been studied in humans. Samples of subcutaneous adipose tissue were obtained from 41 adult subjects (20 women and 21 men), with an age range of 28 to 84 years, and body mass index (BMI) of 19 to 36 Kgm^{-2}. UCP2 and leptin mRNA expression was determined by northern blot. In women, both leptin and UCP2 expression in the subcutaneous adipose tissue increased significantly with age ($r = 0.490$ $p < 0.05$ and $r = 0.475$ $p < 0.05$, respectively). In men, in contrast, a negative correlation was found between leptin expression and age ($r = -0.678$ $p < 0.001$), while no significant correlation was apparent between UCP2 expression and age ($r = -0.077$). In addition, there was a positive correlation between UCP2 and leptin expression in women ($r = 0.656$ $p < 0.01$). These data show important gender dependent differences in the age-related changes in leptin and UCP2 expression in subcutaneous adipose tissue in humans.

Keywords: UCP2; Leptin; Ageing; Adipose tissue

1. Introduction

The brown adipose tissue (BAT) uncoupling protein UCP1 (reviewed in [1]) has represented until recently the molecular basis for the only physiological mechanism known to mediate facultative thermogenesis. However, adult humans have little BAT and, in contrast to what is generally accepted for rodents, the importance of the UCP1 system in the regulation of body weight in humans is not clear (reviewed in [2]). Recently, novel members of the family of uncoupling proteins, termed UCP2 [3] and UCP3 [4,5], have been described in both rodents and humans. UCP2 is widely expressed in many tissues, including brown and white adipose tissue (WAT) [3], whereas UCP3 expression is restricted to muscle and BAT [4,5].

The physiological function of UCP2 is not well understood as yet. Evidence in rodents has suggested a role for this protein in energy balance: the levels of UCP2 expression in different mice strains correlate negatively with susceptibility to diet-induced obesity [3], and its expression is upregulated by feeding mice with a high-fat diet [3] or rats with a cafeteria diet [6]. In addition, mice lacking UCP1 are not obese, but have an upregulation of UCP2, which has been considered to compensate the lack of the UCP1-based thermogenesis [7]. However, more recent data obtained from mice lacking UCP2 [8] have revealed a main role for UCP2 in immunity and in the limitation of reactive oxygen species production.

Leptin, an anorexigenic hormone, which is produced mainly by the adipose tissue [9] and to a lesser extent by the stomach mucosa [10,11] plays an important role in the central regulation of energy balance by modulation of satiety signals, mainly in the hypothalamus [12–14], and by sympathetic nervous system mediated energy expenditure [15]. Leptin production reflects adipose tissue mass. Several studies have reported an increased expression of leptin in obese humans, both at mRNA levels and protein levels [16–21], probably related to a phenomenon of resistance to endogenous leptin.

It has been hypothesized that leptin may exert its weight-

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Table 1
Characteristics of subjects

Parameters	Women	Men
Number	20	21
Age (years)	59.2 ± 3.0 (31–79)	57.4 ± 3.7 (28–84)
BMI (Kgm^{-2})	27.1 ± 1.0 (19–36)	27.5 ± 0.5 (24–33)
Leptin mRNA levels (Au)	177 ± 21	149 ± 15
UCP2 mRNA levels (Au)	127 ± 18	183 ± 27

Values are means ± SEM. The ranges of age and BMI are also indicated within brackets. Leptin and UCP2 mRNA expression was measured by northern blot in subcutaneous adipose tissue and expressed relative to 18S rRNA in arbitrary units (Au).

reducing action not only through a hypothalamic neuroendocrine pathway, but also through an autocrine/paracrine action [22–24], by increasing fatty acid oxidation in the adipose tissue itself. Whether UCP2 is involved in such a direct action of leptin in human adipose tissue has not been previously considered.

In the present paper we have considered the well known implication of leptin in energy metabolism, and its possible direct relationship with UCP2 expression in adipose tissue, as an effector of a part of energy wasting; thus we thought it interesting to study whether age- and gender-related changes in leptin expression by adipocytes are linked to changes in the expression of UCP2 by these cells.

2. Materials and methods

2.1. Chemicals

The RNA isolation reagent (Tripure), Hybond nylon membranes, and most reagents for northern blotting (digoxigenin-labeled probes, Dig-Easy Hyb, blocking reagent, anti-digoxigenin antibodies and CDP-Star) were from Boehringer Mannheim (Barcelona, Spain). Other reagents were purchased from Sigma (Madrid, Spain), and routine chemicals used were from Merck (Darmstadt, Germany) and Panreac (Barcelona, Spain).

2.2. Subjects

Abdominal subcutaneous adipose tissue biopsies were obtained from patients undergoing surgical procedures such as repair of inguinal hernia, endoscopic cholecystectomy, appendicitis or abdominal eventration. All patients were fasted at least 8 h preoperatively and all underwent general anesthesia. The study included 20 women (age 59.2 ± 3.0 years; BMI 27.1 ± 1.0 kgm^{-2}) and 21 men (age 57.4 ± 3.7 years; BMI 27.5 ± 0.5 kgm^{-2}). Characteristics of the study groups are shown in Table 1. None of the patients had diabetes or severe systemic illness, and none were on medications known to influence adipose mass metabolism. The experimental procedure was approved by the Ethical Committee of the Institution and all patients involved gave their informed consent. Samples were rapidly frozen in liquid nitrogen and stored at −70°C until RNA analysis.

2.3. Northern blot analysis

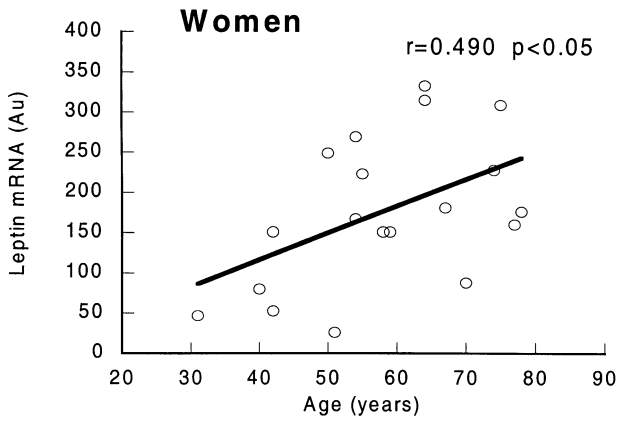
Tissue samples were homogenized in Tripure reagent and total RNA extracted following the instructions provided by Boehringer Mannheim. 15 μg of total RNA, denatured with formamide/formaldehyde, were fractionated by agarose gel electrophoresis as described in [6]. The RNA was then transferred onto a Hybond nylon membrane in 20 × SSC (saline sodium citrate buffer: 1 × SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0) by capillary blotting for 16 h, and fixed with UV light [6].

The mRNA for UCP2 was detected by a chemiluminiscence-based procedure, utilizing a 30-mer antisense oligonucleotide probe (5′-GGCAGAGTTCATGTATCTCGT CTTGACCAC-3′) [6] which was synthesized commercially (Boehringer Mannheim), labeled at both ends with a single digoxigenin ligand. Pre-hybridization was at 42°C for 15 min in DIG-Easy Hyb. Hybridization was at 42°C overnight in DIG-Easy Hyb containing the oligonucleotide probe (34 ng/ml). Hybridized membranes were submitted to 2 x 15 min washes at room temperature with 2 × SSC/0.1% SDS (sodium dodecyl sulfate), followed by 2 × 15 min washes at 48°C with 0.1 × SSC/0.1% SDS. After blocking, the membranes were incubated first with an anti-digoxigenin-alkaline phosphatase conjugate and then with the chemiluminescent substrate CDP-Star, essentially as in the protocols provided by Boehringer Mannheim. Finally, membranes were exposed to Hyperfilm ECL (Amersham, Buckinghamshire, UK); bands in films were analyzed by scanner photodensitometry and quantified using the BioImage program (Millipore, Bedford, MA, USA). Blots were sequentially stripped by exposure to boiling 0.1% SDS and re-probed for leptin mRNA, utilizing a 33-mer antisense oligonucleotide probe (5′-GGATAAGGTCAGGATGGG GTGGAGCCCAGG-3′) at the same concentration as for UCP2 and using the same chemiluminiscence-based procedure. Finally, blots were stripped and re-probed for 18S rRNA, to check the loading and transfer of RNA during blotting. For 18S rRNA detection, the 31-mer digoxigenin-labeled antisense oligonucleotide 5′-CGCCTGCTGCCT TCCTTGGATGTGGTAGCCG-3′ at a concentration of 70 pg/ml was used [25].

Duplicates of RNA isolation and northern blot analysis were performed for all samples.

2.4. Statistics

The data were analyzed by simple regression analysis and the Pearson's correlation coefficient was used to estimate relationships between variables. $P < 0.05$ was the threshold of significance.



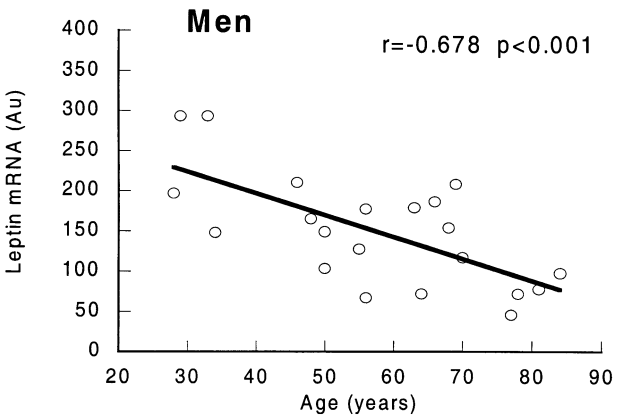
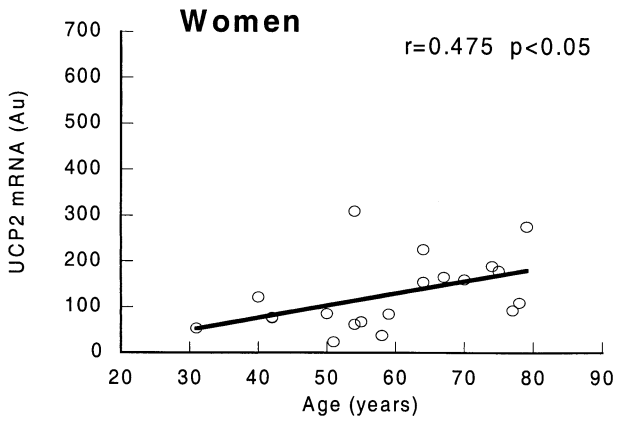


Fig. 1. Relationship between age and leptin mRNA expression in female and male subjects. Leptin mRNA expression was measured by northern blot in subcutaneous adipose tissue in 41 subjects (20 women and 21 men) and expressed relative to 18S rRNA (arbitrary units). Linear regression analysis of age and the relative leptin mRNA levels for individual female and male subjects are shown. The p- and r-values of linear regression analysis for both men and women are indicated. $p < 0.05$ was the threshold of significance.



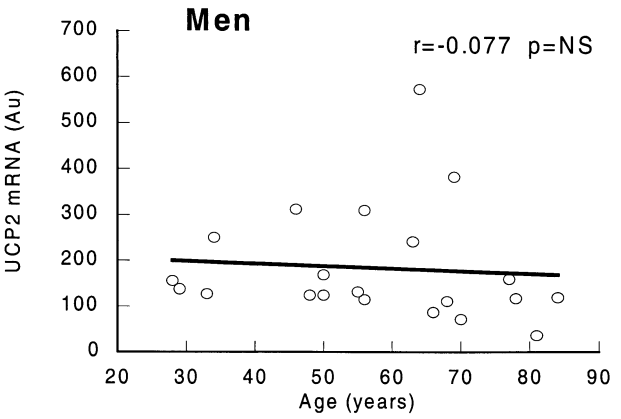


Fig. 2. Relationship between age and UCP2 mRNA expression in female and male subjects. UCP2 mRNA expression was measured by northern blot in subcutaneous adipose tissue in 41 subjects (20 women and 21 men) and expressed relative to 18S rRNA (arbitrary units). Linear regression analysis of age and the relative UCP2 mRNA levels for individual female and male subjects are shown *p*- and *r*- values for correlations are also shown. $p < 0.05$ was the threshold of significance. NS = non significant.

3. Results

UCP2 and leptin mRNA expression in subcutaneous adipose tissue of 41 adult subjects (20 women and 21 men) were determined by northern blot. See Table 1 for details on age and body mass index (BMI) of subjects.

Age-related changes in UCP2 and leptin gene expression in the subcutaneous adipose tissue were considerably different in both genders. In women, leptin expression increased significantly with age ($r = 0.490$ $p < 0.05$), while in men a strong negative correlation was noted between leptin expression and age ($r = -0.678$ $p < 0.001$) (Fig. 1). Concerning UCP2 expression (Fig. 2), a positive correlation ($r = 0.475$ $p < 0.05$) was seen between relative UCP2 mRNA levels and age in women. However, there was no statistically significant correlation in men ($r = -0.077$). Interestingly, a positive correlation was found between leptin and UCP2 mRNA levels in women ($r = 0.656$ $p < 0.01$) but not in men ($r = -0.076$) (Fig. 3).

It is remarkable that in our study no significant correlation was found between BMI and leptin expression in either gender (results not shown), although slightly better relationships with age were found when the relative levels of leptin mRNA were adjusted for BMI ($r = 0.511$ for women and $r = -0.691$ for men). The lack of correlation between leptin expression and BMI contrasts with previous published results [15–18], although this may in part reflect that in our study the subjects encompassed a limited range of BMI, while wide age ranges were selected.

No relationship was found between BMI and UCP2 expression in either gender (results not shown).

4. Discussion

The results of this study reveal a close relationship between leptin and UCP2 expression in subcutaneous adipose tissue in women, but not in men. Age- and gender- related differences in the expression of both genes are also shown.

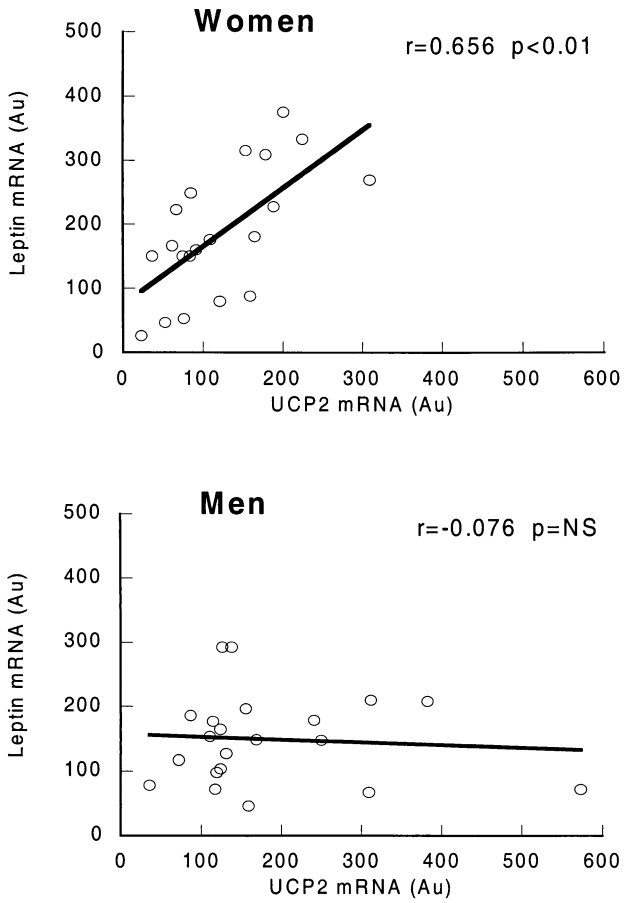


Fig. 3. Relationship between UCP2 and leptin mRNA expression in female and male subjects. See Fig. 1 and 2 for further details.

We have reported here on UCP2 and not on UCP3 expression, since we found no significant expression of UCP3 mRNA in subcutaneous adipose tissue. Previously published results also show no expression or only small amounts of UCP3 mRNA levels in WAT [4,5].

Our results show no significant correlation between UCP2 mRNA levels and BMI in either sex, in contrast with those of Millet et al [26] who reported a positive correlation between UCP2 mRNA levels in subcutaneous adipose tissue and BMI. Explanations for these discrepancies are unclear, even considering the different ranges of BMI in both studies. In our study, the range of BMI involved lean and overweight individuals, but not extremely obese individuals, in contrast to the above mentioned study [26] which included morbidly obese subjects. According to us, two more recent papers in this field [27,28] also including very obese subjects, did not find differences in UCP2 mRNA expression in subcutaneous adipose tissue between lean and obese subjects either. The lack of correlation between UCP2 expression and BMI, is contrary to what should be expected under the assumption that UCP2 is a regulatory effector of thermogenesis.

A number of studies have shown that leptin mRNA expression in adipose tissue [16–19] and plasma levels [19,20] are increased in obese subjects, and a significant relationship between BMI and plasma leptin has also been described [19–21]. However, heterogeneity in serum leptin concentration among individuals at each BMI has still been observed [18]. In our study, we found no significant correlation between BMI and leptin expression in subcutaneous adipose tissue; changes in leptin expression were mainly dependent on age and gender and only weakly associated with changes in the body weight. There is evidence from many studies that leptin production corresponds to the size of the body fat stores [16–21], but also depot- and sex-specific differences in leptin expression have been described [29].

A sexual dimorphism in age-related changes in leptin expression has been demonstrated. In female subjects, we have found a significant positive correlation between age and leptin mRNA levels in subcutaneous adipose tissue, while in men a significant negative relationship between these parameters was apparent. Previous studies [30] in elderly men aged 62 to 98 years, pointed out a positive association of serum leptin levels with age, and this was associated with the decrease in serum testosterone, an inhibitor of leptin production. In the same study, no significant correlation with age was found in elderly women [30]. Other studies, involving subjects with an average age of 29 years in the normal-weight group and 37 years in the obese group, have reported no association of age with serum leptin, independently of body fatness or the BMI [19].

It is difficult to reconcile apparently controversial data concerning age-related changes in serum leptin levels and those of leptin expression by the adipocytes, although a possible clue is to consider the changes in body composition with age. Body fatness increases with age up to about 60 years, and then remains stable or decreases during old age. In addition, distribution of adipose tissue becomes more centralized with age, particularly in women during and following menopause. Since in humans leptin mRNA is mainly expressed in subcutaneous adipocytes [29,31,32] and plasma leptin levels have been suggested to be attributed mainly to the extent of subcutaneous adiposity [33], age-related changes in body composition may account for the described decrease in serum leptin levels in elderly women [34], even when, according to our data, specific leptin expression in the subcutaneous adipose tissue is increased.

Concerning UCP2 expression in adipose tissue, sex differences were also found with age. While in men UCP2 expression did not seem to depend on the individual age, in women both parameters were positively correlated. The profile in women was similar to that of leptin, and interestingly a strong positive correlation was found between leptin and UCP2 mRNA levels in the subcutaneous adipose tissue. While the hypothalamus was the first described target organ for leptin, recent evidence has suggested that leptin may also exert its weight-reducing action by acting directly on target cells in other tissues [22–24,35]. In particular, leptin

has been shown to positively regulate UCP2 gene expression of adipocytes and pancreatic islets in rats by acting directly on these cells [22]. Thus, although several factors may be involved, leptin could be a likely candidate to underlie the age-related increase in UCP2 mRNA levels. However, this association is influenced by sex, as it occurs only in women. It is not clear how a relationship between leptin, a key factor in the control of energy disposal, and UCP2, a potential effector of part of energy wasting, can be so closely connected only in females. To our knowledge, there is no information on the effect of sexual hormones on UCP2 expression, and no differences have been found between men and women concerning UCP2 expression [26, 36]. In contrast, the influence of sex on plasma leptin levels has been well established, women having a higher plasma leptin concentration than men, regardless of body fatness [20,34]. Sexual hormones may contribute in part to determining this gender difference, since a suppressive effect of testosterone on leptin expression has been described [30, 37], whereas no clear effects of ovarian sex steroids have been found [38]. Taking into account the above considerations, the differences between the role of sexual hormones and age-related changes in hormones do not apparently help to explain the differences between genders in the interrelationship between leptin and UCP2 expression, and further investigation is needed for a final explanation.

In summary, our results show that, at least in individuals from normal weight to moderately obese, the expression of leptin and UCP2 in subcutaneous adipose tissue is mainly dependent on age and gender, while the degree of adiposity may be a less important contributor.

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