Leptin, a protein produced by adipose tissue, has been found to be important in the regulation of fat mass and body weight from studies in rodents [1]. Leptin is not only a factor involved in the induction of a hypothalamic state of satiety, but also has many peripheral actions, including modulation of the hypothalamic-pituitary-adrenal axis [2], promotion of angiogenesis [3], regulation of hematopoiesis [4], and control of the development of reproductive systems [5]. Several studies have already shown that circulating leptin concentrations are increased in obese humans, and positively associated with body fat and body mass index (BMI) [1, 6–9]. However, the mechanisms regulating leptin synthesis and secretion are still unknown. It has been proposed that increased leptinemia in obese humans is due to resistance to leptin [10] and increased gene expression in adipose tissue [11].

Leptin can regulate fat mass via hypothalamic control of food intake and thermogenesis [6, 12], and can also directly affect fat mass regulation through peripheral tissues, including adipocytes, without the involvement of the brain [13, 14]. Leptin can enhance glucose...
utilization and lipolysis in adipose tissue via activation of the Jak/STAT pathway [15]. The existence of leptin sensors in adipose tissue is further supported by the nerve reflex activation induced by leptin [16]. Thus, adipose tissue not only has the capacity to secrete leptin, but also serves as a site of action for leptin.

Four splice variants of the leptin receptor have been identified in humans: a long isoform, Ob-Rb, and three shorter isoforms, HuB219.1, HuB219.2, and HuB219.3 [4]. Leptin receptors have a wide distribution in the human body. It has been suggested that only the long isoform has full intracellular signaling capacity and is responsible for anorectic action in the hypothalamus and peripheral actions in other tissue [17]. However, the function of the shorter isoforms in humans and animals remains unknown.

It is well known that central obesity, especially intra-abdominal fat accumulation, is closely related to insulin resistance and its related disorders, including diabetes, hypertension, and dyslipidemia [18]. Although the expression of different isoforms of the leptin receptor in human omental adipose tissue has been studied [19], there has been no report regarding the relationship between serum leptin concentration, BMI, and expression of different leptin receptor isoforms. In this study, a quantitative polymerase chain reaction (PCR) technique was used to measure the relative amounts of different isoforms of leptin receptors in human omental tissue.

**Methods**

**Subjects, tissues, and blood specimens**

Omental adipose tissue was obtained from 57 non-diabetic women who underwent surgery for myoma uteri or ovarian cyst. None was receiving or had a prior history of hormone replacement therapy at the time the tissue samples were obtained. Fasting serum leptin concentration was measured using a radioimmunoassay (RIA) kit (Linco Research, Inc, St. Louis, MO, USA). The intra-assay coefficient of variation was 3.5% and the inter-assay coefficient of variation was 6.7%. Demographic data and blood leptin concentrations are listed in Table 1. This study was approved by the Ethics Committee of National Taiwan University Hospital.

**Quantitative PCR**

The omental adipose tissue samples each weighed about 0.5 g. RNA was extracted from the samples using Trizol reagent (Life Technologies, Inc., Montgomery County, MD, USA) according to the manufacturer’s recommendations. The primer and probe sequences of β-actin and four leptin receptor isoforms — Ob-Rb (long form), HuB219.1, HuB219.2, and HuB219.3 — were designed by computer software (Table 2). The obtained sequences were located across two exons in each of the mRNAs so that no genomic DNA was amplified. Quantitative PCR was performed using real-time Taqman™ technology and the products were analyzed using a Model 7700 Sequence Detector (Perkin-Elmer Co, Foster City, CA, USA). Cycle threshold (Ct) values corresponding to the cycle number at which the fluorescent emission monitored in real time reached a threshold of 10 standard deviations above the mean baseline emission from cycle 1 to 15 were measured [20]. The mean intra-assay coefficient of variation for Ct was less than 3%. Cycling parameters were 2 minutes at 50°C, 30 seconds at 60°C, and 5 minutes at 95°C, followed by 40 cycles of 15 seconds at 94°C, and 1 minute at 60°C.

**Statistical analyses**

BMI was calculated as weight in kg/(height in m)². Pearson correlation analysis was used to describe the relationships among age, BMI, leptin concentration, and HuB219.1 ΔCt, where the relative amount of each mRNA normalized to β-actin was calculated from 2⁻ΔΔCt. A smaller ΔCt indicated a greater abundance of mRNA. Multiple linear regression analysis was applied to describe the relationship between BMI and other variables, including age, menopausal status, leptin concentration, and HuB219.1 ΔCt. All statistical analyses were performed using SPSS 8.0 software (SPSS Inc, Chicago, IL, USA).

**Results**

Among the leptin receptor isoforms, Ob-Rb, HuB219.1, and HuB219.3 were expressed in human omental adi-
Table 2. Primer and probe sequences of β-actin and the different isoforms of leptin receptor

<table>
<thead>
<tr>
<th>Gene (accession No.)*</th>
<th>Forward primer† (5’–3’)</th>
<th>Reverse primer‡ (5’–3’)</th>
<th>Probe§ (FAM/TAMRA)</th>
<th>PCR product (bp)</th>
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expression in adipose tissue [25]. Boado et al demonstrated that the expression of Ob-Ra receptor (homologous to human HuB219.3) in the blood-brain barrier was upregulated by a high-fat diet [26]. More recently, Barr et al found that all isoforms of human leptin receptor were internalized via clathrin-mediated endocytosis and could be downregulated by leptin [27]. These observations suggest that a short receptor isoform might be functionally important in tissues other than the hypothalamus. Whether and how the short HuB219.1 receptor isoform signals relate to human obesity remain to be determined.

In conclusion, higher BMI is correlated with increased circulating leptin concentrations and reduced tissue expression of HuB219.1 receptor, the predominant leptin receptor isoform in human omental adipose tissue. The expression of tissue leptin receptor is independent of the circulating leptin concentration. Our findings indicate that the shorter leptin receptor isoforms in human omental adipose tissue might play an important role in body weight control. Further studies of the inter-relationship between leptin concentrations and multiple leptin receptor isoforms are needed to elucidate the exact mechanism of obesity.

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References