EXPERIMENTAL STUDY

Comparison of leptin gene expression in different adipose tissues in children and adults

E Schoof, A Stuppy, F Harig¹, R Carbon², T Horbach³, W Stöhr⁴, W Rascher and J Dötsch

Departments of Pediatrics, ¹Cardiac Surgery, ²Pediatric Surgery, ³General Surgery and ⁴Medical Informatics, Biometry and Epidemiology, University of Erlangen-Nuremberg, Loschgestrasse 15, 91054 Erlangen, Germany

(Correspondence should be addressed to Jörg Dötsch, Klinik für Kinder und Jugendliche, Friedrich-Alexander-Universität Erlangen-Nürnberg, Loschgestrasse 15, 91054 Erlangen, Germany; Email: JoergWDoetsch@yahoo.com)

Abstract

Objective: Adipose tissue displays depot-specific metabolic properties and a predominant gene expression of leptin in subcutaneous tissue. The aim of the study was to evaluate leptin mRNA expression in various adipose tissues and to relate it to plasma leptin concentrations. Furthermore, developmental changes in leptin gene expression from childhood to adulthood were examined.

Design and methods: Thoracic subcutaneous and intrathoracic adipose tissue specimens were obtained in 22 adults (51–81 years) and 23 children (0.1–17 years) undergoing cardiac surgery, and abdominal subcutaneous, omental and mesenteric fat specimens were collected from 21 adults (38–79 years) and 22 children (0.2–17 years) before abdominal surgery. Preoperative plasma leptin concentrations were measured by RIA. Leptin mRNA expression was quantified by TaqMan real-time PCR.

Results: In adults, there was no difference between leptin gene expression in subcutaneous and intrathoracic fat, whereas in children leptin mRNA expression was significantly higher in subcutaneous adipose tissue. In omental fat, leptin mRNA levels were significantly lower compared with subcutaneous and mesenteric sites in both children and adults. Adults revealed a significantly higher leptin gene expression in subcutaneous, omental and mesenteric adipose tissues than children. Subcutaneous and omental leptin gene expression are independent factors for plasma leptin concentrations in children and adults.

Conclusion: Leptin is differentially expressed at different adipose tissue sites, a situation which is even more pronounced in children. There is a developmental increase in leptin mRNA expression in adipose tissue during childhood, reaching maximal capacity in adulthood.

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Introduction

Leptin is a peptide hormone controlling appetite and increasing energy expenditure (1, 2). It is mainly produced by differentiated adipocytes (3). Regional differences in leptin secretion (4) and gene expression in adipocytes from subcutaneous compared with omental origin are well established. Human omental adipocytes are known to express less leptin mRNA than subcutaneous adipocytes (5–10). In addition, these two adipocyte subtypes can be distinguished by several other biochemical properties such as an increased lipolytic responsiveness of omental adipocytes to catecholamines (11) and less responsiveness to the anti-lipolytic actions of insulin (12). All these different mechanisms in metabolic regulation may account for the fact that the type of body fat distribution is crucial for predicting metabolic outcomes in obese patients. The visceral quantity of fat, not the total body fat mass *per se*, is a strong predictor for metabolic adverse consequences

of obesity such as diabetes, hyperlipidemia and cardiovascular disease (13).

In children, there are only sparse data on leptin gene expression. An increase in leptin gene expression in subcutaneous adipose tissue with age has been described in obese children (14). In rats, it is known that leptin mRNA expression also increases with age, following characteristic ontogenetic patterns in different adipose tissue depots (15).

To see whether intrathoracic fat also shows different leptin gene expression compared with thoracic subcutaneous adipose tissue and to gain more information about regional differences as well as the influence of age on leptin mRNA expression, we measured the gene expression of leptin in subcutaneous, intrathoracic, omental and mesenteric fat in children and adults undergoing elective surgery. A secondary goal was to evaluate the impact of the different adipose tissues on leptin plasma concentrations in children and adults.

Materials and methods

Patients

Two samples of adipose tissue from subcutaneous and intrathoracic fat were obtained immediately after the beginning and before the end of the operation from patients undergoing cardiac surgery, and from subcutaneous, omental and mesenteric fat from patients with elective abdominal surgery. The patients included 22 adults and 23 children with cardiac surgery and 21 adults and 22 children with abdominal surgery. Patients' characteristics are listed in Table 1. None of the patients was taking any steroidal medication or insulin. Body height and weight were measured one day before surgery. Body mass index (BMI) was calculated as kg/m^2 . Blood samples were drawn after the onset of anesthesia and were kept frozen at -18°C until analysis. The study was approved by the local ethics committee. Informed written consent was obtained on the day before surgery from all patients or parents as appropriate.

RNA extraction and reverse transcription

Tissue samples were kept frozen at -80°C until RNA extraction. Total RNA was extracted from the tissues using guanidine-thiocyanate acid phenol (TRIzol, WAK Chemie, Medical GmbH, Bad Homburg, Germany). The method was modified and optimized for extraction of RNA from adipose tissue. As opposed to the manufacturer's protocol, we used 1 ml TRIzol/100 mg adipose tissue and added 400 μl CHCl_3 .

The RNA concentration was determined spectrophotometrically. One microgram RNA was reversely transcribed in a volume of 20 μl at 39°C for 60 min (all chemicals were obtained from Boehringer Mannheim, Germany).

TaqMan real-time PCR

The method for the measurement of gene expression in various tissues has been described before (16, 17). This approach is based upon the 5' exonuclease activity of the Taq polymerase. Briefly, within the amplicon defined by a gene-specific oligonucleotide primer pair an oligonucleotide probe labeled with two fluorescent dyes is designed. As long as the probe is intact, the emission of a reporter dye (i.e. 6-carboxy-fluorescein, FAM) at the 5'-end is quenched by the second fluorescence dye (6-carboxy-tetramethyl-rhodamine, TAMRA) at the 3'-end. During the extension phase of the PCR, the Taq polymerase cleaves the probe releasing the reporter dye. An automated photometric detector combined with special software (ABI Prism 7700 Sequence Detection System, Perkin-Elmer Applied Biosystems, Foster City, CA, USA) monitors the increasing reporter dye emission. The algorithm normalizes the signal to an internal reference (ΔRn) and calculates the threshold cycle number (C_T), when the ΔRn reaches 10 times the standard deviation of the baseline. The C_T values of the probes are interpolated to an external reference curve constructed by plotting the relative or absolute amounts of a serial dilution of a known template vs the corresponding C_T values.

Table 1 Characteristics of adults ($n = 22$) and children ($n = 23$) undergoing cardiac surgery and of adults ($n = 21$) and children ($n = 22$) undergoing abdominal surgery.

	Adults	Children
Cardiac surgery		
Age (years)	68.7 ± 1.7 (range 51–81)	4.1 ± 1.0 (range 0.1–17)
Gender (male/female)	15/7	13/10
BMI (kg/m^2)	27.4 ± 0.8 (range 16.2–33.9)	15.9 ± 0.7 (range 12.8–27.2)
Medication	Beta blockers, $n = 12$ Other antihypertensive drugs, $n = 20$ Hemodilutants, $n = 19$ Glycerol nitrate, $n = 10$ Molsidomin, $n = 9$ Digitalis, $n = 3$ Diuretics, $n = 3$ HMG-CoA reductase inhibitors, $n = 12$	Antihypertensive drugs, $n = 1$ (no beta blockers) Haemodilutants, $n = 4$ Digitalis, $n = 10$ Diuretics, $n = 4$
Abdominal surgery		
Age (years)	59.5 ± 2.3 (range 38–79)	7.3 ± 1.4 (range 0.2–17)
Gender (male/female)	13/8	14/8
BMI (kg/m^2)	24.4 ± 0.79 (range 18.6–33.2)	17 ± 0.8 (range 11.6–25.6)
Medication	Beta blockers, $n = 1$ Other antihypertensive drugs, $n = 20$ Glycerol nitrate, $n = 3$ Digitalis, $n = 1$ HMG-CoA reductase inhibitors, $n = 2$	Antihypertensive drugs, $n = 1$ (no beta blockers) Immunosuppressants, $n = 2$ Antiepileptic drugs, $n = 1$

HMG-CoA, hydroxymethylglutaryl coenzyme A.

Commercial reagents (TaqMan PCR Reagent Kit, Perkin-Elmer) and conditions were employed according to the manufacturer's protocol. cDNA (2.5 µl) (reverse transcription mixture) and oligonucleotides with a final concentration of 300 nmol/l of primers and 200 nmol/l of TaqMan hybridization probe were added to 25 µl reaction mix. The oligonucleotides of each target of interest were designed by the Primer Express software (Perkin-Elmer) using uniform selection parameters that allowed the application of the same cycle conditions confirmed by primer optimization. All of the primers and probes were purchased from Perkin-Elmer Applied Biosystems. The thermocycler parameters were 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. A serial dilution of known copy numbers of a PCR product served as reference providing a relative quantification of the unknown samples.

Leptin gene expression was related to the housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β -actin.

The following primers and TaqMan probes were used. GAPDH: forward 5'-CCCATGTTTCGTCATGGGTGT-3', reverse 5'-TGGTCATGAGTCCCTCCACGATA-3', TaqMan probe 5' (FAM)-CTGCACCACCAACTGCTTAGCACCC-(TAMRA)3'; β -actin: forward 5'-GCGAGAAGATGACCCAGGATC-3', reverse 5'-CCAGTGGTACGGCCAGAGG-3', TaqMan probe 5'(FAM)-CCAGCCATGTACGTTGCTATC CAGGC-(TAMRA)3'; leptin: forward 5'-GTGCGGATTCT TGTGGCTTT-3', reverse 5'-GGAATGAAGTCCAAACCG GTG-3', TaqMan probe 5'(FAM)-CAATGACATTTTACA CACGTCAGTCTCCTCCAA-(TAMRA)3'.

Leptin measurement

Leptin was measured by a specific radioimmunoassay (18). For the measurement of plasma samples, the standard concentrations ranged from 15.6 to 0.06 µg/l. Samples were diluted 1:2 with assay buffer. Tracer activity was ~15 000 c.p.m. per tube, the final dilution of the specific antibody was 1:50 000. One hundred microliters of each tracer, appropriately diluted sample and antibody were mixed and incubated for 24 h at room temperature. Bound and non-bound fractions were separated by goat-anti-rabbit IgG (DSL, Sinheim, Germany) in 4% polyethyleneglycol (PEG) at a final dilution of 1:150. After 2 h at room temperature the tubes were centrifuged (3000 g × 15 min, 4 °C) and the radioactivity of the pellets was counted in a gamma counter. Fifty percent binding occurred at 2 µg/l. The sensitivity of undiluted samples was 0.03 µg/l. Inter- and intra-assay coefficients of variation were 8.5% and 3.8% respectively.

Statistical analysis

All values are expressed as means ± S.E.M. After testing for Gaussian distribution, parametric data of individual

groups were compared using Student's *t*-test, and non-parametric data were compared using Mann–Whitney-test. Wilcoxon signed rank test was used for comparison of related interval data. To analyze influence factors on plasma leptin concentrations, simple and multiple regressions were performed after log-transformation of plasma leptin levels. A *P*-value of ≤ 0.05 was considered significant.

Results

Thoracic tissue

In adult cardiosurgical patients there was no difference between leptin/GAPDH mRNA expression in subcutaneous and intrathoracic tissue (2.17 ± 0.32 vs 1.65 ± 0.26 relative units (RU), *P* = 0.22; leptin/ β -actin: 0.56 ± 0.07 vs 0.42 ± 0.05 RU, *P* = 0.11) (Fig. 1A). However, in children with congenital heart disease, we found a significantly higher leptin gene expression in subcutaneous compared with intrathoracic fat (0.89 ± 0.31 vs 0.15 ± 0.06 RU, *P* = 0.002) (Fig. 1B). This could be confirmed when relating leptin mRNA values to β -actin (2.55 ± 0.82 vs 0.51 ± 0.28 , *P* = 0.001).

Abdominal tissue

Leptin gene expression in adult patients was significantly lower in omental compared with subcutaneous adipose tissue (GAPDH: 1.64 ± 0.30 vs 3.67 ± 0.61 RU, *P* = 0.005; β -actin: 1.64 ± 0.55 vs 4.18 ± 1.40 RU, *P* = 0.025). A comparison of omental and mesenteric fat revealed a significant difference when related to GAPDH (1.64 ± 0.30 vs 2.72 ± 0.39 RU, *P* = 0.035) and fell short of being significant when normalised to β -actin (1.64 ± 0.55 vs 2.99 ± 1.15 , *P* = 0.074). There was no significant difference in leptin gene expression between subcutaneous and mesenteric fat in adults (GAPDH: *P* = 0.196, β -actin: *P* = 0.521) (Fig. 1C).

In children, the same distribution of leptin mRNA expression in abdominal fat tissue could be observed as in adults. Omental fat revealed lower leptin mRNA values than subcutaneous (GAPDH: 0.05 ± 0.03 vs 0.74 ± 0.31 RU, *P* = 0.001; β -actin: 0.02 ± 0.01 vs 0.16 ± 0.05 RU, *P* = 0.001) or mesenteric (GAPDH: 0.05 ± 0.03 vs 0.30 ± 0.14 RU, *P* = 0.004; β -actin: 0.02 ± 0.01 vs 0.08 ± 0.03 RU, *P* = 0.01) fat. There was no significant difference between leptin gene expression in subcutaneous and mesenteric fat samples (GAPDH: *P* = 0.47; β -actin: *P* = 0.44) (Fig. 1D).

Adults revealed a significantly higher leptin gene expression than children in subcutaneous abdominal (GAPDH: *P* < 0.0001, β -actin: *P* < 0.0001), omental (GAPDH: *P* < 0.0001, β -actin: *P* < 0.0001), and mesenteric (GAPDH: *P* < 0.0001, β -actin: *P* = 0.006) adipose tissue (Fig. 2).

Figure 1 Leptin gene expression normalized to GAPDH in subcutaneous thoracic (sc th) and intrathoracic (ith) adipose tissue in (A) adult patients and (B) children undergoing cardiac surgery, and in subcutaneous abdominal (sc abd), omental (om), and mesenteric (mes) fat of (C) adult and (D) pediatric patients with abdominal surgery. * $P = 0.03$; ** $P < 0.005$; ns, not significant.

In none of our patient groups was there a significant difference in leptin mRNA expression between males and females.

A correlation between leptin mRNA expression and age could be found for children in subcutaneous thoracic (GAPDH: $r = 0.57$, $P = 0.005$, β -actin: $r = 0.59$, $P = 0.004$), intrathoracic (GAPDH: $r = 0.55$, $P = 0.007$, β -actin: $r = 0.57$, $P = 0.006$) and omental (GAPDH: $r = 0.45$, $P = 0.043$, β -actin: $r = 0.50$, $P = 0.019$), but not in subcutaneous abdominal (GAPDH: $r = 0.35$, $P = 0.109$, β -actin: $r = 0.50$, $P = 0.019$) and mesenteric (GAPDH: $r = 0.35$,

$P = 0.129$, β -actin: $r = 0.20$, $P = 0.386$) fat. Leptin gene expression revealed no relation to age in adult patients (subcutaneous thoracic, intrathoracic, subcutaneous abdominal, omental, and mesenteric fat, $P > 0.05$).

In patients with heart disease, a multiple regression analysis revealed that independent factors influencing plasma leptin levels are leptin gene expression in subcutaneous adipose tissue ($P = 0.015$), BMI ($P = 0.008$), gender (plasma leptin levels in women higher than in men, $P = 0.043$) and age (i.e. that in relation to low mRNA expression levels children have

Figure 2 Comparison of leptin/GAPDH gene expression in subcutaneous abdominal (subcutaneous abd.), omental, and mesenteric adipose tissue in children and adults. *** $P < 0.0001$.

relatively higher plasma leptin levels, $P = 0.047$), but not leptin gene expression in intrathoracic fat. In patients undergoing abdominal surgery, we found BMI ($P = 0.003$), age ($P < 0.0001$) and leptin gene expression in omental adipose tissue ($P = 0.027$) influenced plasma leptin levels independently. Subcutaneous abdominal and mesenteric fat did not correlate to plasma leptin.

Discussion

Leptin gene expression shows regional differences and changes from childhood to adulthood. When analyzing leptin gene expression in different adipose tissues, we found two- to tenfold higher mRNA levels in adults than in children. The well known increase in plasma leptin concentrations from childhood to adulthood (19) might, therefore, be caused not only by the age-dependent increasing fat mass but also by a higher capacity of adult fat cells to produce leptin.

In children, we found increasing leptin mRNA levels with age at some sites of adipose tissue (subcutaneous thoracic, intrathoracic, and omental fat). There is one previous study showing an increase in leptin gene expression with age in the subcutaneous adipose tissue of obese children (14). Our results indicate that there is a differentially regulated development of leptin mRNA expression at different adipose sites during childhood.

In a multiple regression analysis, BMI, gender and leptin gene expression in subcutaneous and omental fat could be shown to independently influence plasma leptin levels. The relation between BMI and plasma leptin levels has been recognised for some time (1). However, studies on the impact of leptin mRNA expression in different adipose tissues are quite inconsistent (4, 5, 6, 9, 10, 20). In particular, data on the impact of leptin gene expression in children have not been shown previously.

Our study showed that leptin mRNA expression is lower in omental than in abdominal subcutaneous or mesenteric adipose tissue. These data are in line with previous observations that found the same difference in adults (5–10). An identical finding of reduced omental leptin mRNA expression has now been demonstrated in children.

Different metabolic features between subcutaneous and omental adipocytes have been described. Omental adipocytes reveal less sensitivity to the suppressive effect of insulin on lipolysis (12). As insulin promotes leptin mRNA expression, this mechanism might be an explanation of reduced leptin synthesis in omental fat. Furthermore, omental adipocytes are more sensitive to the lipolytic effect of catecholamines (11). On the other hand, catecholamines induce elevated intracellular cAMP levels that can lead to diminished leptin mRNA production (21). It may be suggested, therefore,

that increased lipolysis parallels reduced leptin mRNA expression. Another finding that omental adipocytes have more glucocorticoid receptors (22) and display higher glucocorticoid sensitivity (21) than subcutaneous adipocytes cannot explain the reduction in leptin gene expression, as glucocorticoids produce an increase in serum leptin levels (23) and increased leptin production *in vitro* (24). It has also been discussed whether the regional difference in leptin gene expression might be due partly to a difference in mean fat cell volume (8). In lean mice, depot-specific differences in leptin gene expression are mainly related to the volumes of the constituent adipocytes (25).

We are able to demonstrate for the first time that in children adipose tissue from intrathoracic origin expresses less leptin mRNA than thoracic subcutaneous fat. In adults, there was no significant difference in leptin gene expression from subcutaneous compared with intrathoracic sites. This difference in children might resemble different developmental changes of leptin mRNA expression found at several adipose sites in rats (15). Further studies have to be performed examining the metabolic properties of intrathoracic fat to find out whether there are similar differences as between subcutaneous and omental adipose tissue.

The question arises whether regional, not completely specified, regulatory mechanisms account for these different findings. As mentioned above, in rats as well, characteristic patterns in the 'maturation' of the leptin system can be found at every adipose tissue depot (15).

In our study, there was no difference in leptin mRNA expression in men and women in bivariate comparison. However, in the multiple regression analysis, gender could be outlined as an independent factor influencing plasma leptin levels. It is well known that women reveal higher plasma leptin levels than men (26). This may be partly due to testosterone-induced suppression of leptin production, but may also be explained by the different distribution of fat depots in men and women. In contrast to men, in whom most of the adipose tissue is located intra-abdominally, in women fat distribution favours subcutaneous sites. We have shown that subcutaneous fat has a higher capacity to produce leptin than omental fat and may, therefore, contribute to the higher plasma leptin levels in females. In children as well, subcutaneous fat seems to have the highest impact on plasma leptin concentrations.

In summary, we have shown that leptin mRNA is expressed at a lower level in children. In children and adults, regional fat depot specific mechanisms seem to lead to a different influence of leptin gene expression on plasma leptin levels.

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