Serum Concentrations of Leptin in Heart, Liver and Kidney Transplant Recipients

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Abstract

Background: Leptin is a 16 kDa hormone synthesized by adipocytes and involved in body weight regulation.

Objectives: To determine serum leptin concentrations in heart, liver and kidney transplant recipients.

Methods: We investigated 57 patients: 18 male heart transplant recipients (age 25–69 years) at 1–66 months after transplantation, 6 female and 8 male liver transplant recipients (age 33–70) at 11–73 months after transplantation, and 10 female and 15 male kidney transplant recipients (age 20–61) at 3–138 months after transplantation. All recipients were receiving immunosuppressive therapy, including prednisone 0–20 mg/day, azathioprine 75–125 mg/day, cyclosporin 100–250 mg/day or tacrolimus 2–10 mg/day. The results were compared to those of 10 female and 10 male healthy controls. Morning serum concentrations of leptin were measured with a commercial radioimmunoassay (Linco Research Inc., USA), and serum insulin and cortisol levels were measured by radioimmunoassay.

Results: Patients (both men and women) after heart, liver and kidney transplantation exhibited significantly higher serum concentrations of leptin and leptin/body mass index ratios than controls. Serum leptin concentrations were significantly higher in women than in men and correlated very significantly with BMI in all cases. The multivariate stepwise analyses showed that among parameters including BMI, gender, age, time after transplantation, prednisone dose, hematocrit, serum concentrations of glucose, albumin, creatinine, cortisol and insulin, only BMI, gender, cortisol and insulin were significant independent determinants of serum leptin levels in these patients.

Conclusions: This is the first report showing that, in addition to body mass index and gender, basal cortisol and insulin levels affect the hyperleptinemia in transplant patients. The clinical relevance of hyperleptinemia in these patients will require further investigation.

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Leptin is a 16 kDa protein that is encoded by the ob gene [1], synthesized in adipocytes, and is thought to play an important role in regulation of food intake and energy expenditure in animal models [2,3] and in humans [4].

Glucocorticosteroids, the most often used immunosuppressive drugs following transplantation, may lead to hyperleptinemia since they increase leptin mRNA expression and protein production by adipocytes *in vitro* [5] and *in vivo* [6]. Given that leptin is cleared partly by the kidney [7,8], and that heart, liver and kidney transplantation is frequently followed by some impairment of renal function in the post-transplant period due to drug nephrotoxicity and/or transplant organ failure, serum leptin levels may also be affected in these patients. It was recently shown that hyperinsulinemia contributes to elevated serum leptin concentrations in patients with chronic renal failure [9] and in those undergoing hemo- or peritoneal dialysis [10]. Hyperinsulinemia is often associated with transplantation, suggesting that serum leptin concentrations may be increased in transplant patients. However, the recently published studies are contradictory. Two studies [10,11] found an increase in serum leptin concentrations in kidney transplant recipients, while another [12] showed normal levels. Kokot et al. [13] demonstrated a decrease in serum leptin concentrations in the early post-transplant period after kidney transplantation followed by an increase in later years. Surprisingly, a fall in serum leptin levels after successful orthotopic liver transplantation was reported in children [14]. To the best of our knowledge, no study of leptin levels in heart and adult liver transplant recipients has been performed.

The present study was designed to assess the effect of glucocorticosteroids, hyperinsulinemia and kidney function on serum leptin levels in heart, liver and kidney transplant recipients during the long-term post-transplant periods.

Patients and Methods

Patients

We investigated 57 patients: 18 male heart transplant (Tx-heart) recipients at 1–66 months after transplantation, age 25–69 years; 6 female and 8 male liver transplant (Tx-liver) recipients at 11–73 months after transplantation, age 33–70; and 10 female and 15 male kidney transplant (Tx-kidney) recipients at 3–138 months after transplantation, age 20–61. All recipients were receiving immunosuppressive therapy, including prednisone 0–20 mg/day, azathioprine 75–125 mg/day, cyclosporin 100–250 mg/day or tacrolimus 2–10 mg/day. Mean serum concentrations of cyclosporin A were 418.0 $\pm$ 27.6 ng/ml in Tx-heart patients (n=18), 138.7 $\pm$ 11.3 ng/ml in Tx-liver patients (n=8), and 143.2 $\pm$ 7.3 ng/ml in Tx-kidney patients (n=22). Mean serum levels of tacrolimus were 9.6 $\pm$ 0.6 ng/ml in Tx-liver patients (n=6) and 6.5 $\pm$ 0.9 ng/ml in Tx-kidney patients (n=2). These results were compared to those of 10 female and 10 male healthy controls [10]. The mean $\pm$ SEM values of age (47.0 $\pm$ 6.1

BMI = body mass index

Table 1. Clinical characteristics of transplant recipients (mean ± SEM)

	Gender (M/F)	Age (yr)	BMI (kg/m^2)	Hematocrit (%)	Creatinine (mg/dl)	*Ccr (ml/min)	Albumin (g/dl)	Glucose (mg/dl)	Cortisol (nmol/L)	Insulin (mU/L)	Prednisone (mg/kg/24 hr)	Time** (mo)
Heart	18/0	53.6±2.9	27.4±0.9	38.2±0.7	1.9±0.1	54.4±6.3	4.3±0.1	106.8±7.9	340.2±64.6	14.9±4.2	0.07±0.02	23.7±5.3
Liver	8/6	50.0±2.7	28.0±2.0	38.4±2.1	1.8±0.3	62.4±8.0	3.9±0.2	94.5±5.5	229.5±49.0	11.4±3.0	0.05±0.00	36.6±4.4
Kidney	15/10	46.0±2.6	26.8±1.0	40.8±1.3	1.5±0.1	58.3±3.7	4.2±0.0	82.7±1.4	107.6±15.0	8.8±1.4	0.13±0.00	59.4±8.2
Total	41/16	49.4±1.6	27.3±0.7	39.4±0.8	1.7±0.1	58.1±3.2	4.1±0.0	93.2±4.3	211.0±27.5	11.4±1.6	0.09±0.00	42.5±4.5

* Creatinine clearance in males: Ccr = (140-Age, yr) x (weight, kg)/(72 x serum creatinine, mg/dl) and in females: Ccr = 15% less than in males [16].
** Time after transplantation.

years) and body mass index (26.2 ± 1.5 kg/m^2) in control subjects were similar to those of studied patients. The clinical characteristics of the studied individuals are shown in Table 1.

Causes of end-stage organ disease

- In heart transplant recipients: dilated cardiomyopathy (n=7), ischemic cardiomyopathy (n=7), idiopathic cardiomyopathy (n=3), and glycogen storage disease (n=1).
- In liver transplant recipients: primary sclerosing cholangitis (n=2), hepatitis B cirrhosis (n=5), hepatitis C cirrhosis (n=3), fulminant hepatitis (n=1), cryptogenic cirrhosis (n=1), alcoholic cirrhosis (n=1), and hepatocellular carcinoma (n=1).
- In kidney transplant recipients: chronic glomerulonephritis (n=6), interstitial nephritis (n=4), nephrosclerosis (n=3) and polycystic kidney disease (n=3), and unknown (n=9).

Patients with biopsy-proven rejection of transplant organ (heart, liver and kidney) were not included in the study. All patients were clinically free from symptoms of heart or liver failure. Informed consent was obtained from all participants.

Methods

Venous blood was sampled at 8 a.m. after a 12 hour fast. Serum concentrations of glucose, urea and creatinine were analyzed by standard autoanalyzer techniques (Hitachi 747, Boehringer Mannheim Corp., Indianapolis, IN, USA). Serum albumin was determined in serum using the bromcresol green method. Serum insulin and cortisol concentrations were measured by radioimmunoassay. Serum leptin concentrations were measured using a commercial radioimmunoassay (Linco Research Inc., St Charles, MO, USA) with a sensitivity limit of 0.5 ng/ml and intra-assay coefficient of variation of 4.5%. Body mass index was calculated as weight (kilograms) divided by height (square meters). Renal function as creatinine clearance was calculated by the Cockcroft-Gault formula, i.e., males: Ccr = (140-Age) x (weight, kg)/(72 x serum creatinine, mg/dl) and females: Ccr = 15% less than in males.

Ccr = creatinine clearance

Statistical analysis

Correlation coefficients were calculated according to the Spearman Rho test. The Mann-Whitney test was used for analysis of leptin values**.** Stepwise multiple regression analysis was used to find significant independent predictors of leptin in the transplant patients. Results are expressed as mean ± SEM.

Results

As shown in Figure 1, heart, liver and kidney transplant recipients exhibited significantly higher serum concentrations of leptin and leptin/BMI ratios than did control subjects [10]. These values were significantly higher in women than in men. No significant

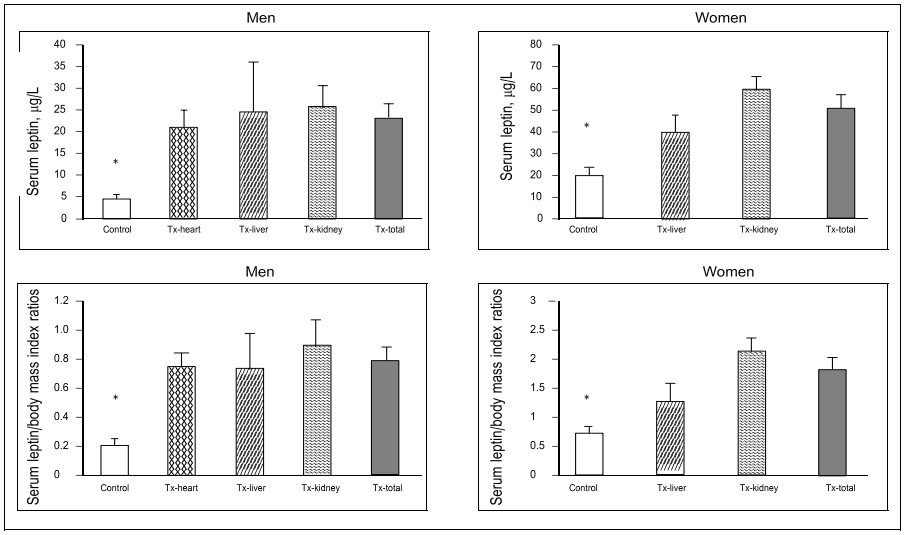

Figure 1. Serum concentrations of leptin and leptin to body mass index ratios in heart, liver and kidney transplant patients. The results, including control subjects [ref. 10], are expressed as mean ± SEM. The mean values of BMI in control subjects were similar to those of transplant recipients.

Leptin concentrations **(**µg/L): Men: control 4.8 ± 0.9 (n = 10), Tx-heart 21.7 ± 4.2 (n = 18), Tx-liver 25.0 ± 11.2 (n = 8), Tx-kidney 26.1 ± 5.9 (n = 15), Tx-total 24.0 ± 3.4 (n = 41). Women: control 20.8 ± 3.8 (n = 10), Tx-liver 40.1 ± 11.0 (n = 6), Tx-kidney 60.4 ± 8.0 (n = 10), Tx-total 52.8 ± 6.7 (n = 16).

Leptin to body mass index ratios**:** Men: control 0.2 ± 0.0 (n = 10), Tx-heart 0.7 ± 0.1 (n = 18), Tx-liver 0.7 ± 0.2 (n = 8), Tx-kidney 0.9 ± 0.1 (n = 15), Tx-total 0.8 ± 0.0 (n = 41). Women: control 0.8 ± 0.0 (n = 10), Tx-liver 1.3 ± 0.3 (n = 6), Tx-kidney 2.2 ± 0.2 (n = 10), Tx-total 1.9 ± 0.2 (n = 16). * $P < 0.05$ vs. Tx-heart, Tx-liver, Tx-kidney and Tx-total groups

differences in serum concentrations of leptin and leptin/BMI ratios were observed between the three groups of transplanted patients. All patients were arbitrarily divided into two subgroups according to basal insulin levels: insulin <10 mU/L and insulin >10 mU/L (hyperinsulinemia). Of all transplant recipients 45.6% (21 men and 5 women) demonstrated hyperinsulinemia. Men with hyperinsulinemia showed significantly higher serum leptin and leptin/BMI values than those with basal levels of insulin <10 mU/L. In women these differences did not reach statistical significance [Figure 2].

The influence of various parameters on the serum leptin concentration and leptin/BMI ratios was investigated using Spearman Rho test analysis [Table 2]. A strong positive correlation of leptin and leptin/BMI values with BMI and gender was documented. There were also a significant positive correlation of serum leptin concentrations and leptin/BMI ratios with serum concentrations of creatinine and creatinine clearance and an inverse correlation with basal cortisol levels, but not with the duration time after transplantation [Table 2]. It is obvious that the low basal cortisol levels in these patients reflect the glucocorticoid treatment that they presently receive or have received in the past.

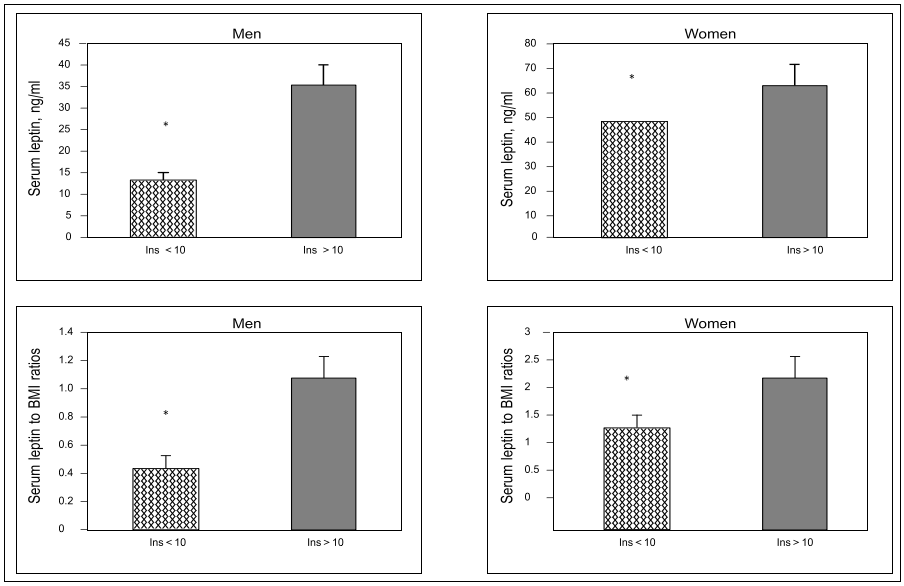

Figure 2. Serum concentrations of leptin and leptin to body mass index ratios in transplant patients with basal insulin levels more or less than 10 mU/L. The studied patients are divided by basal concentrations of insulin into two groups: < 10 mU/L (Ins < 10) and > 10 mU/L (Ins > 10). Results are expressed as mean ± SEM. ***Leptin concentrations*** **(**μg/L**):** Men: Ins < 10, 13.1 ± 2.2 (8 Tx-heart, 2 Tx-liver and 10 Tx-kidney); Ins > 10, 34.3 ± 5.5 (10 Tx-heart, 6 Tx-liver and 5 Tx-kidney). Women: Ins < 10, 48.0 ± 8.4 (4 Tx-liver and 7 Tx-kidney); Ins > 10, 63.3 ± 10.7 (2 Tx-liver and 3 Tx-kidney).
Leptin to body mass index ratios**:** Men: Ins < 10, 0.4 ± 0.0 (n = 20); Ins > 10, 1.0 ± 0.1 (n = 21). Women: Ins < 10, 1.7 ± 0.2 (n = 11); Ins > 10, 2.3 ± 0.3 (n = 5). * $P < 0.05$ vs. Ins > 10.

As expected, the strongest inverse correlation was observed between serum creatinine levels and calculated creatinine clearances ($r = –0.750$, $P<0.0001$) and between basal cortisol levels, as a marker of previous and current glucocorticoid treatment, and daily prednisone doses ($r = –0.519$, $P<0.0001$).

When stepwise multiple regression analysis was used, BMI as the independent variable had the highest correlation with leptin ($R^2 = 0.327$, the value of Mallow's Cp = 60.2; $P<0.0001$). The addition of gender into the respective equation increased the correlation coefficient ($R^2 = 0.611$) and reduced the value of Mallow's Cp (Cp = 14.3) with $P<0.0001$, followed by basal cortisol ($R^2 = 0.654$, Cp = 9.2; $P = 0.0135$) and basal insulin ($R^2 = 0.691$; Cp = 5.0; $P = 0.0160$) respectively. In contrast, the combination of this model with the other variables studied did not measurably improve the prediction of leptin.

Table 2. Spearman Rho coefficients (r) and P values between serum leptin concentrations, serum leptin to body mass index ratios (leptin/BMI) and other measured variables in 57 transplant recipients

	Leptin		Leptin/BMI	
	r	*P*	*r*	*P*
Body mass index	0.496	<0.0001	0.345	0.0086
Gender*	0.462	0.0003	0.524	<0.0001
Age	–0.117	NS	-0.147	NS
Time after transplantation	0.066	NS	0.019	NS
Prednisone doses**	–0.097	NS	0.003	NS
Hematocrit	0.209	NS	0.214	NS
Creatinine clearance ***	0.503	<0.0001	0.462	0.0003
Serum creatinine	–0.312	0.0179	–0.326	0.0133
Serum albumin	0.101	NS	0.185	NS
Serum glucose	–0.029	NS	–0.080	NS
Basal cortisol	–0.239	0.0755	–0.288	0.0311
Basal insulin	0.223	0.0955	0.187	NS

* Dummy variables were used for genders (men = 1, women = 2)
** Prednisone doses (mg/kg body weight/24 hr)
*** Creatinine clearance = (140–Age, yr) x (Weight, kg)/(72 x Serum creatinine, mg/dl); in females it was 15% less than in males [16].
NS = not significant; $P>0.1$

Discussion

In this study we show that serum leptin concentrations as well as serum leptin levels adjusted for BMI (leptin/BMI ratios) were significantly higher in heart, liver and kidney transplant patients than in normal subjects [10]. These findings concur with the results of Howard et al. [11], Kokot et al. [15] and our previous report [10] showing markedly elevated serum concentrations of leptin in kidney transplant recipients. On the other hand, these findings conflict with those of Landt and colleagues [12] and Kokot et al. [13], who found a significant decline of leptin levels in adults in the early post-transplant period after successful kidney transplantation. Roberts and co-workers [14] also found a reduction in serum leptin

in children after orthotopic liver transplantation despite significant increases in body fat mass. A possible explanation for these discrepant results [12–14] may be the fact that most of our patients were investigated during the long-term period after transplantation and we did not compare the changes in serum leptin concentrations in the same patients before and after transplantation. Hyperleptinemia in heart and liver transplant patients has not been previously reported.

Poor appetite and malnutrition is commonly seen in advanced disease of the heart, liver and kidney. Appetite is suppressed when renal function is decreased [16]. Most patients with severe heart and liver disease, in addition to patients with end-stage kidney disease, have some degree of renal failure. Renal failure itself contributes to hyperleptinemia [10]. Increased leptin concentrations, which are negatively correlated to food intake [17], may also inhibit appetite. Successful transplantation, whether of heart, liver or kidney, improves renal function and improves nutritional status and appetite. However, the serum leptin levels are still increased and surprisingly show a significant positive correlation with creatinine clearance and significant negative correlation with serum concentrations of creatinine. This apparent discrepancy is due to the fact that serum creatinine is a marker of both body weight (a parameter of nutrition) and renal function. In patients with mild renal failure, in whom the elevation in serum creatinine is too small to significantly affect serum leptin concentrations, body weight may be a stronger factor affecting leptin levels. Hyperleptinemia accompanying the improvement in renal function and associated with the normalized nutritional status in these patients may be attributed in part to an elevation in body fat mass [18], a stimulating effect of glucocorticosteroids [5,6] and of hyperinsulinemia [9,10,19]. Indeed, results of the stepwise multiple regression analysis show that BMI, a relative marker of obesity and percent of body fat mass, was the best predictor of serum leptin concentrations in transplant recipients together with gender. In addition, basal cortisol levels – as a relative marker of glucocorticosteroid treatment, and basal insulin levels – as a relative evidence of insulin resistance, were also independent predictors of hyperleptinemia. This is the first report demonstrating that basal cortisol and insulin levels affect the hyperleptinemia in transplant patients. We suggest that leptin overproduction rather than shortage of leptin degradation by kidney [7,8] could account for hyperleptinemia in these patients. The underlying cause of leptin overproduction is unknown, but is likely the result of the balance between leptin secretion by adipocytes and leptin sensitivity to its receptors [20].

In line with the results reported by us previously [10], women in this study had markedly higher leptin values than did men in all the groups assessed, suggesting a specific gender-related effect [21].

Leptin enhances the human T cell response (proliferation and interleukin-2 production) primarily by binding to its receptor (ObRb) on CD4+ helper T cells [22]. The mechanism of this response may be related to the stimulatory effect of leptin on calcineurin (protein phosphatase 2B) activity caused by up-regulation of calcineurin gene expression [23]. Calcineurin is a critical enzyme for T lymphocyte activation [24] and is inhibited in a dose-dependent manner by the immunosuppressive drugs cyclosporin A and tacrolimus [25]. The clinical relevance of the possible interaction between hyperleptinemia and degree of cyclosporin A and tacrolimus-induced inhibition of the T cell calcineurin activity in these patients is unknown.

It is theoretically possible that increased serum leptin levels found in patients after heart, liver or kidney transplantation may decrease the immunosuppressive effect of cyclosporin A and FK-506 in inhibiting the phosphatase activity of calcineurin. This mechanism could be involved in the initiation of chronic rejection of the transplanted organ; this hypothesis requires experimental confirmation.

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