

A Study of Serum Leptin Levels in Obese And Nonobese Adolescent Children of 11 – 18 Years

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I. Introduction

Childhood obesity has reached epidemic levels in developed as well as in developing countries and is one of the most serious public health challenges of the 21st century. The highest prevalence rates of childhood obesity have been observed in developed countries; however its prevalence is increasing in developing countries as well.¹ Obesity has emerged as one of the global health problems with 200 million school-aged children world-wide categorized as being overweight/obese, of which 40-50 million are obese.² Most of the earlier studies done in children and adolescents in India have reported prevalence based on international cut – off points³⁻¹¹, with a meta-analysis estimating the prevalence of overweight as 12.6% and obesity as 3.4%¹². Another multi-centric study reported an overall prevalence of overweight/obesity as 18.2%¹⁰. The factors attributing to increasing childhood obesity are increased intake of high-calorie foods that are low in vitamins, minerals and micronutrients coupled with decreased physical activity¹³. Some racial and ethnic minority populations, especially African American, Hispanic, and American Indian groups, are at particular risk for the development of overweight and obesity¹⁴. The increase in obesity prevalence in children is particularly alarming because obesity-related diseases rarely seen in children in the past, including obesity-associated sleep apnea¹⁵, non-alcoholic fatty liver disease¹⁶ with resultant cirrhosis¹⁷, and type 2 diabetes^{18,19} are increasingly diagnosed in pediatric patients.

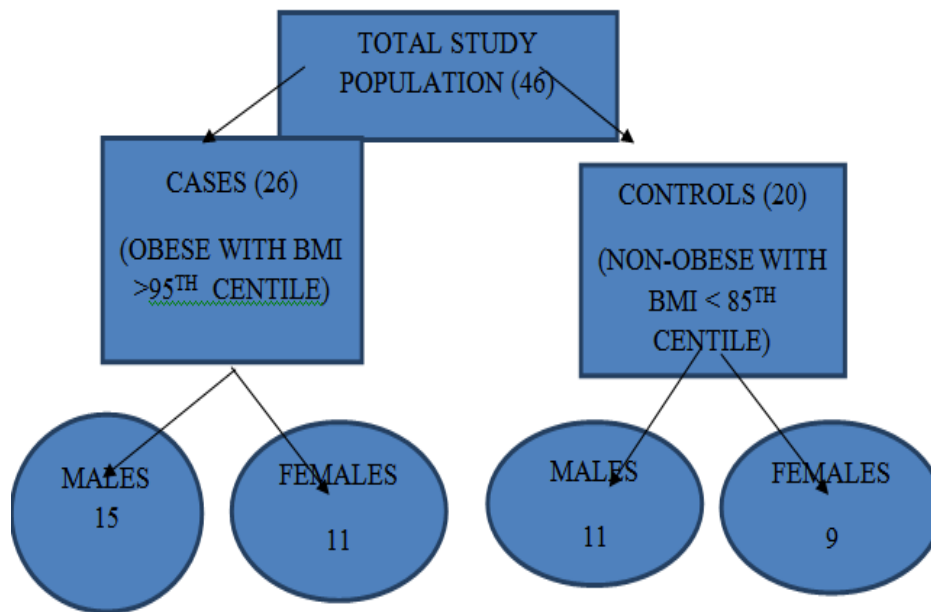
Obesity is a genetic disease, because all available data suggest that 60% to 80% of the observed variance in human body weight can be accounted for by inherited factors²⁰. More than 300 genetic loci that are potentially involved in human body weight regulation have been identified through analyses in humans, rodents, and *Caenorhabditis elegans*^{21,22}. One of the major advances in obesity science has been the elucidation of the leptin signalling pathway. Inactivating mutations affecting these genes may account for as much as 3% or 4% of severe, early onset obesity. Leptin is a member of cytokine family. It is a single chain 167 amino acid anti-obesity hormone secreted predominantly from white adipose tissue in an ultra-radian and circadian fashion. Leptin is considered to play an important role in appetite control, fat metabolism, and regulation of body weight. The dual effects of leptin result in a decrease in appetite and increase in energy expenditure, ultimately leading to an increase in fat metabolism²³. Leptin levels correlate with body mass index and percentage body fat and are elevated with increasing adiposity in both men and women. The relationship between serum leptin levels and several measures of adiposity demonstrates that leptin levels are elevated in obese patients²⁴.

Recent studies have suggested that elevated serum leptin concentrations among childhood population could be a marker for future BMI and metabolic disorders²⁵ and these children may be at high risk for adult obesity²⁶. Very few studies have been conducted in Indian children to study the serum leptin levels. In view of this we intend to study the levels of leptin in obese adolescent children and compare it with normal children of similar age. This study will add evidence for Indian studies related to Leptin and Obesity and Obesity indices. It also adds value for further studies exploring Leptin as treatment target for childhood and adult obesity.

II. Results

- The present study was undertaken to determine the correlation of serum leptin levels with obesity and BMI.
- A total of 46 adolescent children were included in the study.
- Out of 46 children, 26 were obese children with BMI > 95th centile who represented cases and 20 were normal weight children with BMI < 85th centile who were controls.
- Children with BMI between 85th to 95th centile being overweight were excluded from the study.
- Out of 26 obese children, 15 were males and 11 were females.
- Out 20 normal weight children 11 were males and 9 were females.

Figure 5: Distribution of study population

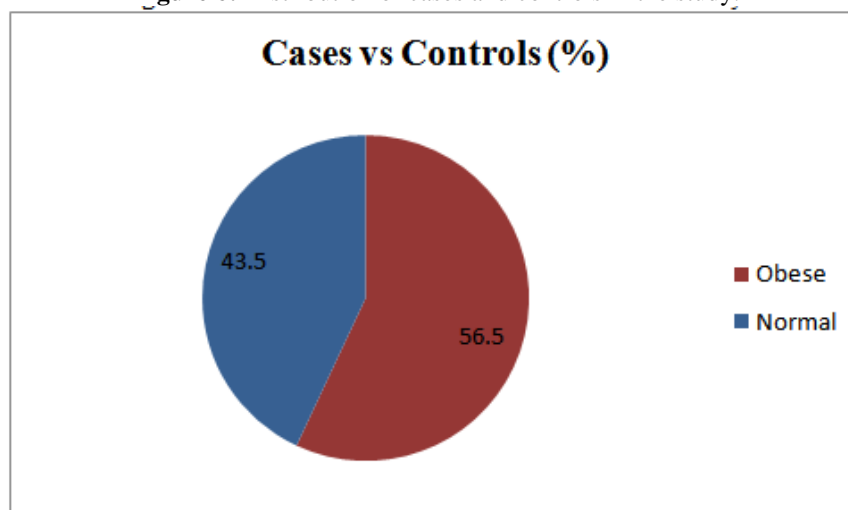


Distribution of cases and controls:

Table 2: Distribution of cases and controls in the study:

Cases	Controls
n=26 (56.5%)	n=20 (43.5%)

Figure 6: Distribution of cases and controls in the study.



- Total sample (n) was 46
- 56.5% (26/46) were cases (Obese)
- 43.5% (20/46) were controls (Normal weight).

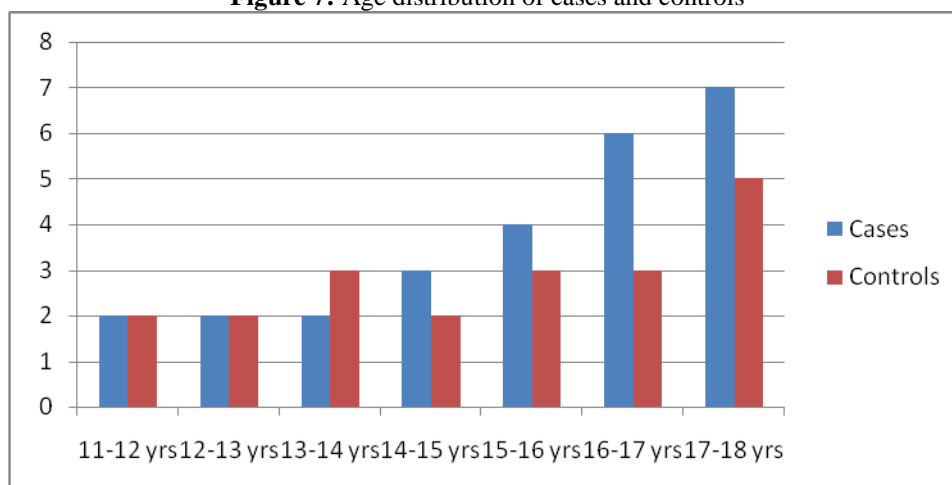
Distribution of age:

Table 3: Age distribution of cases and controls

Age in years	Cases		Controls	
	F	%	F	%
11-12 yrs	2	8.0	2	10.0
12-13 yrs	2	8.0	2	10.0
13-14 yrs	2	8.0	3	15.0
14-15 yrs	3	11	2	10.0

15-16 yrs	4	15	3	15.0
16-17 yrs	6	23	3	15.0
17-18 yrs	7	30	5	25.0
Total	26	100	20	100

Figure 7: Age distribution of cases and controls



- Number of obese children were more in 15-18 yrs age group compared to <15 yrs age.
- 7 cases (30%) out of 26 were of 17-18yrs of age, followed by 6 cases (23%) in 16-17 yrs of age and 4 cases (15%) in 15-16 yrs of age.
- In the controls, children of 15-18 yrs age group were 11(55%). Those <15 yrs were 9(45%).

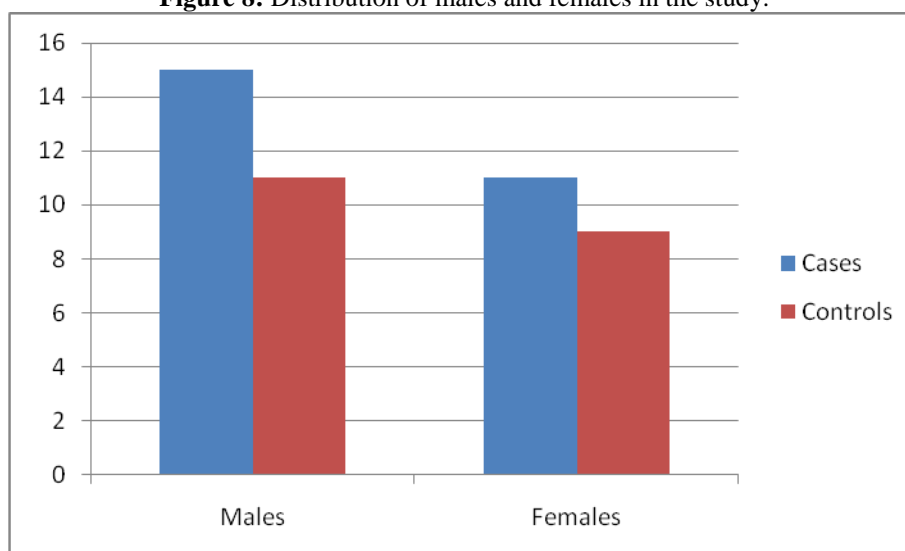
Gender distribution:

Table 4: Distribution of males and females in the study:

Gender	Cases	Controls
Males	15 (58%)	11 (55%)
Females	11 (42%)	9 (45%)
Total	26 (100%)	20 (100%)

P = 0.855

Figure 8: Distribution of males and females in the study:



- 58% of children were males, while 42% were females among cases.
- 55% of children were females, while 45% were females among controls.
- There is no significant difference in distribution of sex between obese and normal weight groups (p=0.855, Pearson Chi-square test).

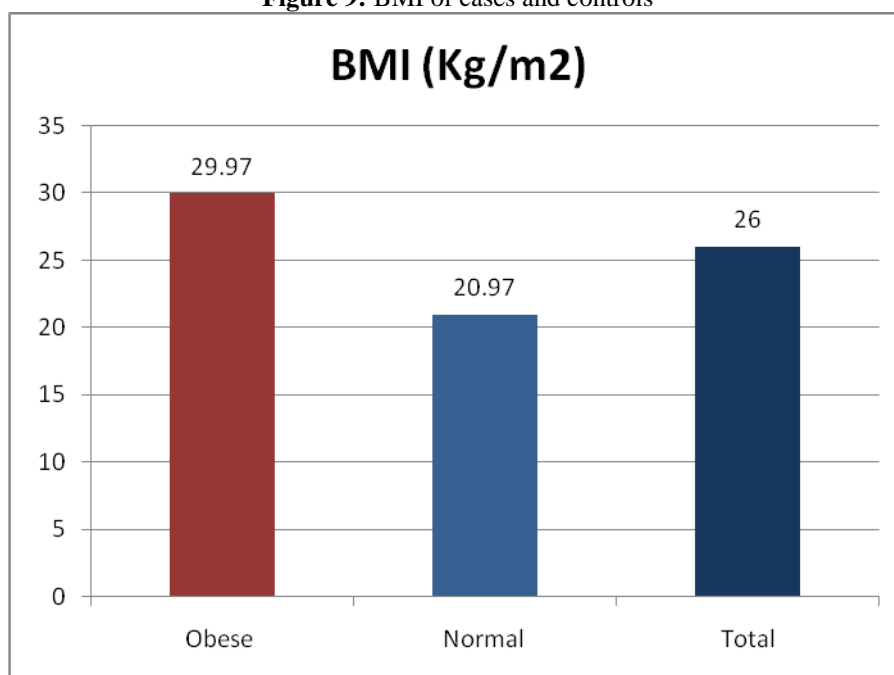
BMI distribution of cases and controls:

Table 5: BMI (kg/m²) of cases and controls

Age group	Cases n=26	Controls n=20
11 – 12 yrs	28.94	21.13
12 – 13 yrs	41.5	18.79
13 – 14 yrs	28.58	20.06
14 – 15 yrs	31.07	19.66
15 – 16 yrs	30.04	22.42
16 – 17 yrs	30.43	20.14
17 – 18 yrs	30.61	22.31
Mean ± SD	29.97 ±3.88	20.97 ± 2

P = <0.001

Figure 9: BMI of cases and controls



- Among cases 12 – 13 yrs age group had the highest BMI of 41.5kg/m² .
- BMI increased with increasing age in other age groups from 11 yrs to 18 yrs.
- The mean BMI of the total sample was 26.17 ± 5.515 Kg/m², with a minimum of 17 Kg/m²and a maximum of 38 Kg/m².
- The mean BMI of cases was 29.97 ± 3.88 kg/m² while the mean BMI of controls was 20.97 ± 2 Kg/m²
- The difference between the groups was significantly different (p<0.001, t-test for Equality of Means).

Blood Investigations

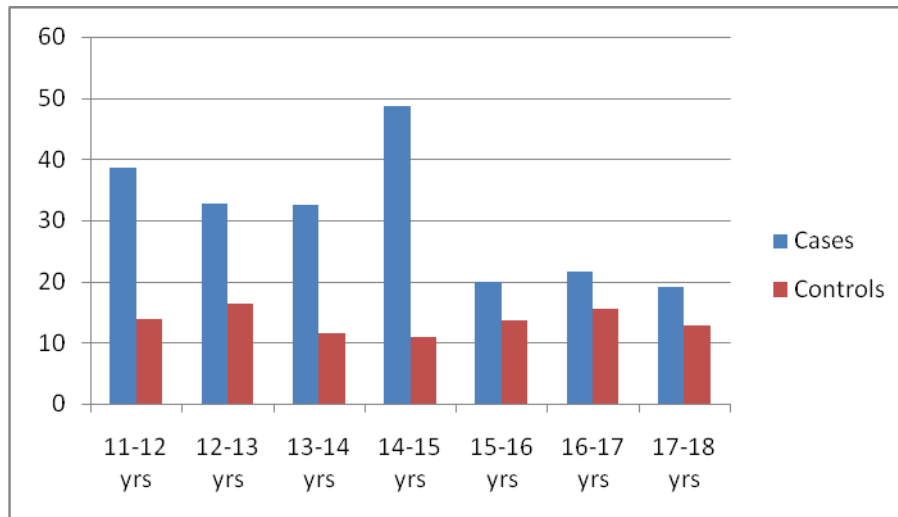
Serum Leptin Levles With Different Variables In The Study Population

1. AGE:

Table 6: Leptin in different age groups in cases and controls

Age (years)	Cases n=26	Controls n=20	Mean
11-12 yrs	38.5	13.85	26
12-13 yrs	32.73	16.4	24.5
13-14 yrs	32.5	11.47	21.9
14-15 yrs	48.66	10.9	29.75
15-16 yrs	19.95	13.56	16.75
16-17 yrs	21.68	15.43	18.55
17-18 yrs	19.11	12.73	15.92

Figure 10: Leptin in different age groups in cases and controls



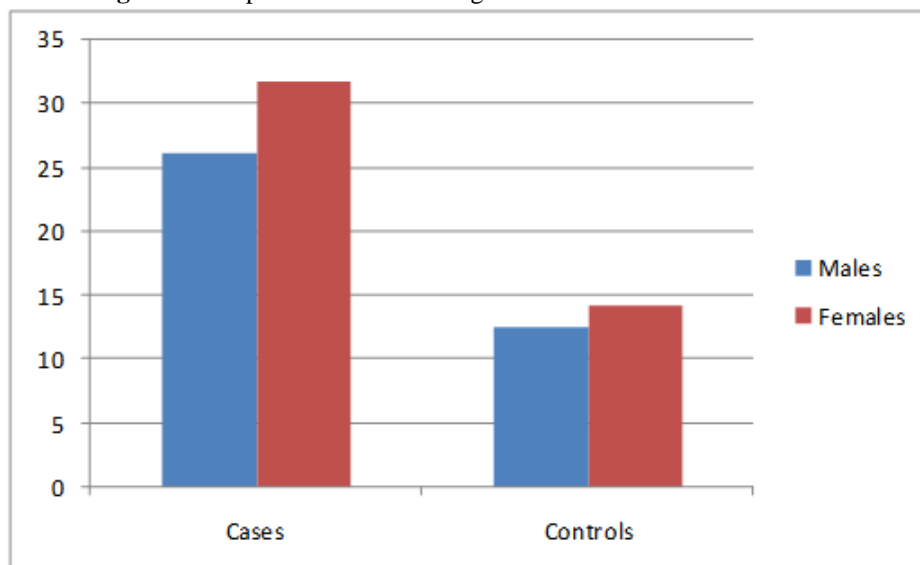
- Mean Leptin levels showed a decline with increasing age.
- Mean leptin values were highest in 14-15 yrs i.e. 29.75 pg/ml followed by 11-12 yrs i.e. 26 pg/ml and lowest in 17-18 yrs i.e. 15.92 pg/ml.
- The mean serum Leptin level of the total sample was 21.96 ± 13.350 pg/ml, with a minimum of 7.8 pg/ml and a maximum of 60 pg/ml.

Gender

Table 7: Leptin Levels According To Gender In Cases And Controls

Cases		Controls	
Males (n=15)	Females (n=11)	Males (n=11)	Females (n=9)
26.19	31.73	12.58	14.24
P = 0.50			

Figure 11: Leptin Levels According To Gender In Cases And Controls



- Serum leptin levels in cases among males were 26.19pg/ml while in females were 31.73 pg/ml.
- Serum leptin levels in controls among males were 12.58pg/ml while in females were 14.24pg/ml.
- Mean serum leptin levels in males were 19.38pg/ml while in females they were 22.98 pg/ml.
- Females had higher leptin levels than males in both cases and controls.

BMI

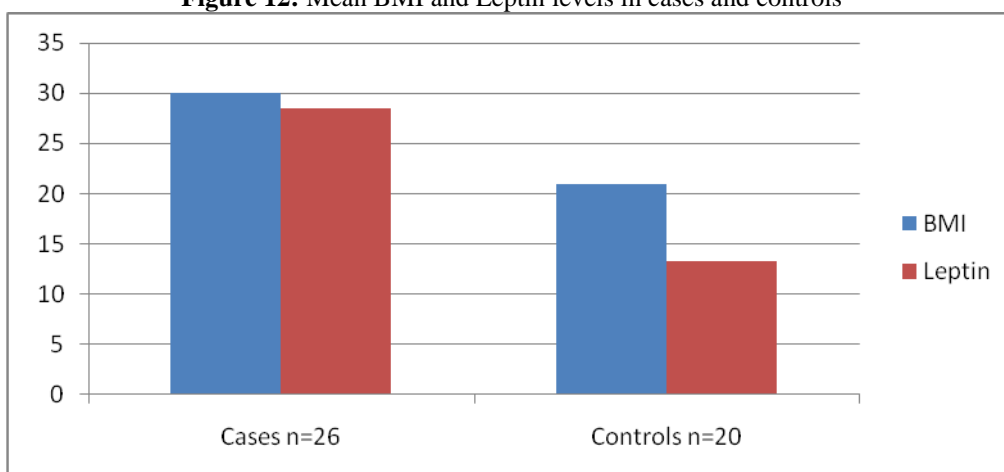
Table: 8 BMI and Leptin levels in cases and controls

Age(yrs)	Cases n=26		Controls n=20	
	BMI	Leptin	BMI	Leptin
11-12 yrs	28.94	38.5	21.13	13.85
12-13 yrs	41.5	32.73	18.79	16.4
13-14 yrs	28.58	32.5	20.06	11.47
14-15 yrs	31.07	48.66	19.66	10.9
15-16 yrs	30.04	19.95	22.42	13.56
16-17 yrs	30.43	21.68	20.14	15.43
17-18 yrs	30.61	19.11	22.31	12.73
Mean ± SD	29.97 ± 3.88	28.54 ± 14.38	20.97 ± 2	13.33 ± 3.63

P = 0.024

Table 9: Mean leptin values in cases and controls

Figure 12: Mean BMI and Leptin levels in cases and controls

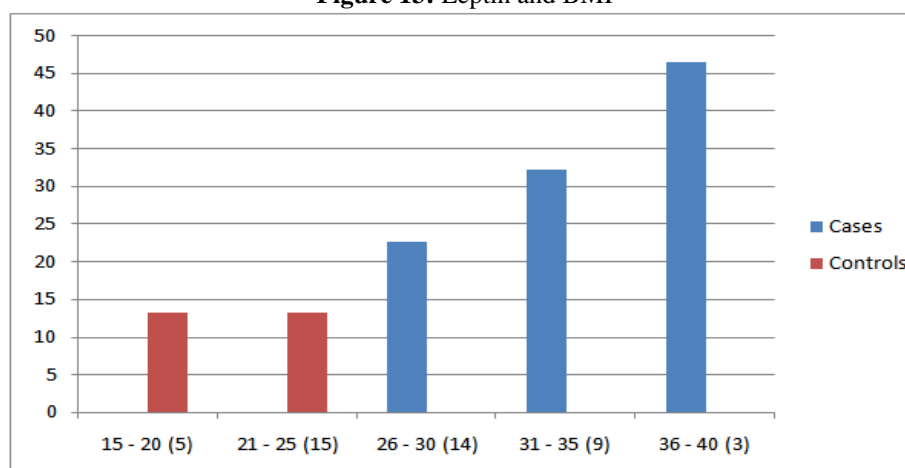


- The mean BMI in cases was 29.97±3.88 kg/m², while the mean BMI in controls was 20.97±2 kg/m².
- Cases had higher leptin levels of 29.97±14.38pg/ml, than controls of 13.33±3.63 pg/ml.
- There was statistically significant difference between the groups p = 0.024. (p<0.001, t-test for equality of means).

Table 10: Serum leptin with BMI

BMI (kg/m ²)	Leptin (pg/ml)	
	Cases (26)	Controls (20)
15 – 20 (n=5)	0	13.92
21 – 25 (n=15)	0	13.13
26 – 30 (n=14)	22.61	0
31 – 35 (n=9)	32.1	0
36 – 40 (n=3)	46.33	0

Figure 13: Leptin and BMI



- As the BMI increased serum leptin levels also increased.
- Children with BMI between 36 – 40 kg² had the highest mean leptin levels of 46.33 pg/ml.

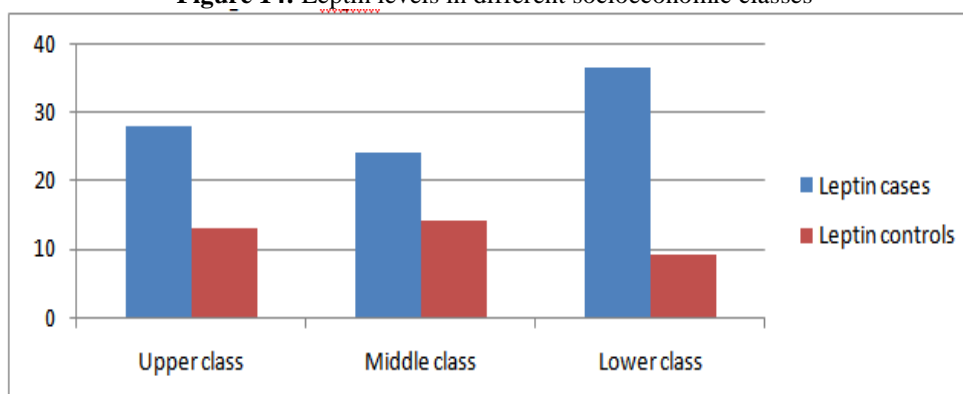
III. Risk Factors:

1. Socioeconomic class:

Table 11: Leptin levels in cases and controls according to different socioeconomic classes

Socioeconomic class	Cases		Controls	
	N	Leptin (pg/ml)	N	Leptin (pg/ml)
Upper class n=23 (50%)	13 (50%)	28	11 (55%)	13
Middle class n=19 (41%)	11 (42%)	24	8(40%)	14
Lower class n=4 (9%)	2 (8%)	36.5	1 (5%)	9
P = 0.9				

Figure 14: Leptin levels in different socioeconomic classes



- In cases the leptin levels in upper class were 28 pg/ml, middle class were 24 pg/ml, and lower class were 36.5 pg/ml.
- In controls the leptin levels of upper class were 13 pg/ml, of middle class were 14 pg/ml, and lower class were 9 pg/ml.
- There was no significant difference between the groups in terms of Leptin levels according to socioeconomic classes.

Cases	Controls	P value
28.54 ± 14.38	13.33 ± 3.63	<0.001

2. Life style:

Table 12 : Leptin (Pg/Ml) With Lifestyle In Cases And Controls

Lifestyle	Cases	Controls	P value
Exercise (mean duration)	50 min	75 min	0.001
Screen time (mean duration)	3 hrs	2.8 hrs	0.90
Junk food (% mean consumption)	58%	40%	0.01
Leptin (pg/ml)	28.50	13.45	0.001

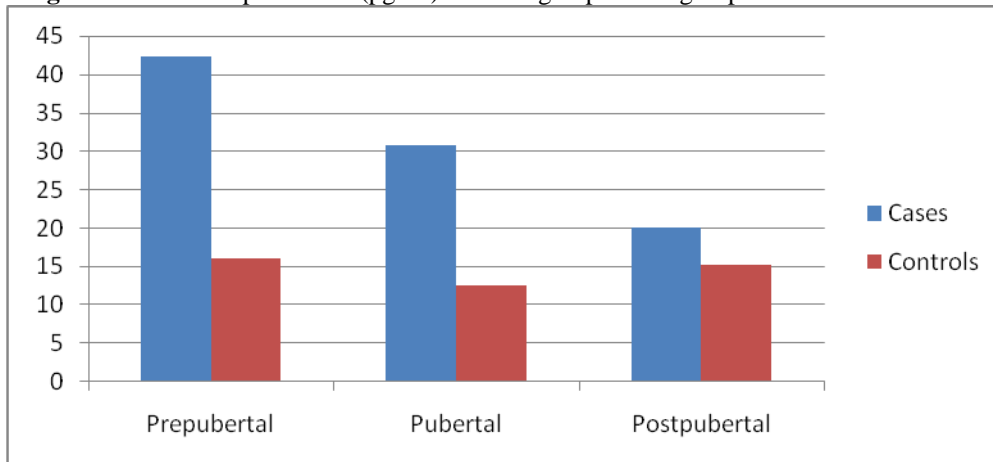
- Controls had a significantly higher mean duration of exercise (p = 0.001) and lower junk food consumption (p = 0.01) than cases.
- Screen time did not vary in cases and controls.

IV. Pubertal Stages

Table 13: Leptin levels (pg/ml) according to pubertal groups in cases and controls:

Pubertal group	Cases n=26			Controls n=20		
	males (15)	females (11)	mean	males (11)	females (9)	mean
Pre pubertal n = 10 (22%)	32.5	52	42.25	13.55	18.6	16.07
Pubertal n = 14 (30%)	28.94	32.48	30.71	12.18	12.8	12.49
Postpubertal n = 22 (48%)	16.85	23.23	20.04	13.8	16.5	15.15

Figure 15: Mean Leptin levels (pg/ml) according to pubertal groups in cases and controls:



- Leptin levels appeared to decrease with advancing pubertal stages from prepubertal to postpubertal with highest in females of prepubertal age group in both genders.
- This trend was more pronounced in cases than controls.

V. Correlations Between Different Variables
Correlation among cases and controls:

Table 14: Correlations between cases and controls

		Age	Height	Weight	BMI	Sr. Leptin	Tanner Stage
Sr. Leptin	Pearson Correlation	-.193	-.378**	.357*	.641**	1	-.208
	Sig. (2-tailed)	.199	.009	.015	.000		.165
	N	46	46	46	46	46	46

** . Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

- There is positive correlation found between the BMI and LEPTIN ($r = .641$) .
- As the BMI increased, LEPTIN levels also increased and this association is found statistically highly significant ($p < .001$)

Correlation between cases

Table 15: Correlation Between Cases:

		Age	Height	Weight	BMI	Sr. Leptin	Tanner Stage
Sr. Leptin	Pearson Correlation	-.415*	-.622**	-.113	.442*	1	-.506**
	Sig. (2-tailed)	.035	.001	.582	.024		.008
	N	26	26	26	26	26	26

*. Correlation is significant at the 0.05 level (2-tailed).
 **. Correlation is significant at the 0.01 level (2-tailed).
 There was a positive correlation between BMI and Sr. Leptin ($r = 0.442$) and it was statistically significant with a $p = 0.024$.

Correlation between controls:

Table 16: Correlation between controls

		Age	Height	Weight	BMI	Sr. Leptin	Tanner Stage
Sr. Leptin	Pearson Correlation	-.064	-.041	-.123	-.154	1	-.039
	Sig. (2-tailed)	.787	.864	.605	.517		.869
	N	20	20	20	20	20	20

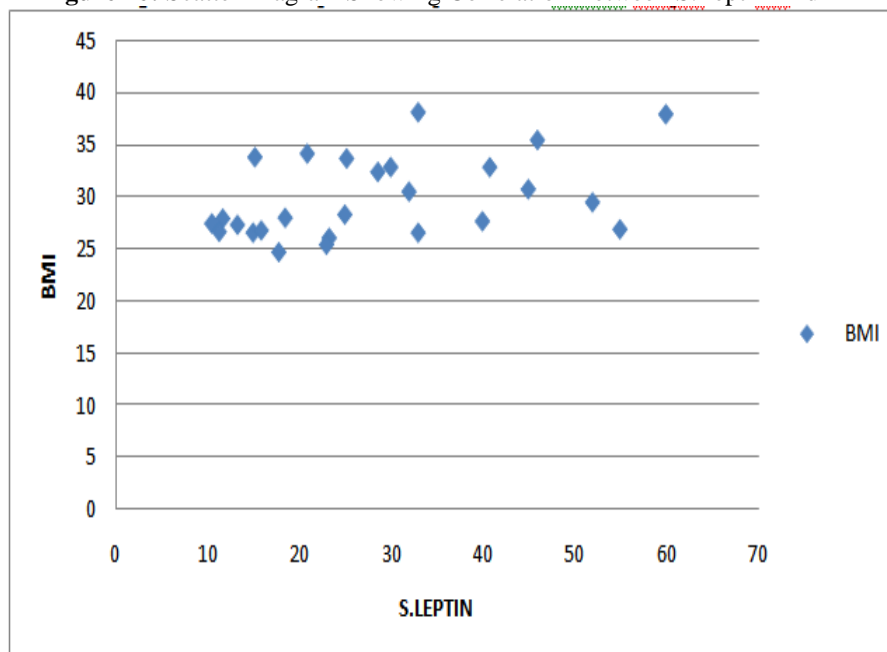
** . Correlation is significant at the 0.01 level (2-tailed).
 * . Correlation is significant at the 0.05 level (2-tailed).

Correlation of BMI and Leptin:

Table 17: Correlation between Serum Leptin and BMI

parameter	Correlation co-efficient (r)	p - value
S. Leptin - BMI	0.442	.024 * S

Figure 16: Scatter Diagram Showing Correlation Between S.Leptin And Bmi



r = 0.442 p = 0.024 *s

T- test

Table 18: Group Statistics

	GROUP	N	Mean	Std. Deviation	P value
Age	CASES	26	15.12	2.142	.477
	CONTROLS	20	14.65	2.231	
Height	CASES	26	158.77	9.035	.866
	CONTROLS	20	159.25	10.083	
Weight	CASES	26	75.50	11.190	.000
	CONTROLS	20	53.45	8.900	
BMI	CASES	26	30.08	3.877	.000
	CONTROLS	20	21.10	2.075	
Sr. Leptin	CASES	26	28.50	14.440	.000
	CONTROLS	20	13.45	3.605	
Tanner Stage	CASES	26	3.42	1.332	.237
	CONTROLS	20	2.95	1.317	

Note * : p value .000 means p < 0.001 *s Highly significant

VI. Discussion

Childhood obesity is a growing epidemic. With recent marked increase in childhood obesity prevalence, questions regarding the role of obesity-related factors such as leptin in children have increased in importance⁴⁵. However, information concerning childhood obesity and its relationship with adipokine levels remains limited. It is important to identify the obese children with abnormal leptin levels in order to prevent obesity-related diseases. Although the number of relevant publications show a great rise in recent years, most of these studies are about adult subjects and lower attention has been paid to childhood obesity and the role of leptin. During puberty, children experience weight gain rather than maintaining body weight . Hence the present study was done to identify adolescent children with high leptin levels as it predicts future risk of adult obesity and a state of leptin resistance.

• Distribution of cases and controls:

The total sample was 46 with 26 (56.5%) cases and 20 (43.5%) controls. This distribution is similar to a study by A Falorni et al¹¹⁰.

Table 19: Comparison of distribution of cases and controls with other studies

Study	Cases	Controls
Present study	56.5%	43.5%
A Falorni et al	56%	44%

• **Age distribution of cases and controls:**

Adolescent children from 11-18 yrs were selected for the study and categorised as cases and controls according to inclusion and exclusion criteria. The age distribution of the study population is similar to a study by EW Demerath¹¹¹ et al. The number of obese children in both studies were more in 15-18 yrs age group.

Table 20: Comparison of age distribution with other studies:

Age group	Present study	EW Demerath
11-12 yrs	9%	10%
12-13 yrs	9%	9%
13-14 yrs	11%	8%
14-15 yrs	10%	9%
15-16 yrs	15%	4%
16-17yrs	19%	8%
17-18 yrs	27%	20%

• **Gender distribution in the study:**

There were a total of 56.5% males comprising of 15 cases and 11 controls, while females were 43.5%, comprising of 11 cases and 9 controls.

This distribution was similar to a study by G Srinivasa Nageswara Rao¹¹² et al.

Table 21: Comparison of gender distribution with other studies

Gender	Present study	G Srinivasa Nageswara Rao
Males	56.5%	59%
Females	43.5%	41%

• **BMI distribution in the study:**

Mean BMI of cases was around 29.97 kg/m² and that of controls was 20.97kg/m². There was significant difference in the BMI of cases and controls p = 0.001

Mean BMI in cases and controls was similar to other studies by G Srinivasa Nageswara Rao¹¹² et al, Sandra G. Hassink et al⁵² and A Falorni et al¹¹⁰.

Table 22: Comparison of BMI distribution with other studies.

Study	Mean BMI (kg/m ²)	
	Cases	Controls
Present study	29.97±3.88	20.97±2
Srinivasa Nageswara Rao et al	28.77±3.06	17.97±2.57
Sandra G. Hassink et al	34.4	18.9
A Falorni et al	25.62	17.48

BMI tended to increase from 11 years to 18 years in both cases and controls. This was similar to studies by Garcia Mayor et al²⁹.

Serum leptin:

• **Serum leptin levels with age:**

The mean leptin levels tended to decrease with increasing age . This was more pronounced in males. Leptin levels decline with age due to alterations in adiposity and BMI. This trend was similarly observed in a study by Isidori AM et al¹¹³.

However highest mean leptin levels were seen among 14-15 yrs children i.e. 29.75 pg/ml. This can be explained by the fact that leptin levels transiently increase during puberty which is a state of physiological leptin resistance. Lowest values of leptin were seen in 17-18 yrs. This is the time when there is decreased fat mass and increase in muscle mass.

• **Serum leptin levels with gender:**

Leptin levels were higher in females than in males, both in cases (31.73 pg/ml vs 26.19 pg/ml) and controls (14.24 pg/ml vs 12.58 pg/ml). This finding was similar to earlier studies by Schoppen et al and Srinivasa Nageswara Rao et al. This variation in leptin levels in males and females can be explained by many factors. Blum et al¹¹⁴ proposed that lower leptin levels in boys may be partly explained by the suppressive effects of androgen.

Mc Conway et al¹¹⁵ proposed that females have higher total serum leptin levels, but lower leptin binding protein levels, than males, indicating higher free leptin levels.

Table 23: Comparison of serum leptin with gender with other studies

Study	Males		Females	
	Cases	Controls	Cases	Controls
Present study	26.19	12.58	31.73	14.24
Srinivasa Nageswara Rao et al	29.25	7.72	51.30	8.31
Camelia et al	15.84	4.03	28.02	5.16
Schoppen et al	20.04	3.1	36.4	13

Sexual dimorphism can also be explained by evidence that leptin secretion from adipose tissue is 2 to 3 fold higher for females than males. Nagy T et al¹¹⁶ suggested that higher circulating concentrations of leptin in females were due to their proportionately greater volume of subcutaneous tissue compared to males. Other studies have found that circulating concentrations of leptin, corrected for body composition, are significantly decreased in males compared to females at later stages of puberty.

• **Serum leptin levels with BMI:**

Leptin concentration in our study is significantly higher in obese children (28.54+/-14.38) than non-obese (13.33+/-3.63) children, with a p value of <0.001, which is similar to previous studies by Ehsaan Bahrami et al¹⁰⁸ and Doaa Mohammed Youssef¹¹⁷ et al.

Table 24: Comparison of BMI and leptin with other studies:

Study	Cases		Controls	
	BMI	Leptin	BMI	Leptin
Present study	29.97	28.54	20.97	13.33
Ehsan Bahrami et al	26.23	27.95	18.02	13.14
Doaa Mohammed et al	25,1	43.8	18.6	12.1

It was observed that despite good correlation of BMI and leptin, there was significant variability in leptin levels in subjects with similar BMI. This may be related to difference in body composition and fat distribution. Maffie et al⁴⁵ described significant heterogeneity in leptin concentrations in subjects with similar BMI. There may also be differential sensitivity of individuals to leptin.

• **Serum leptin levels with socioeconomic status:**

There was no significant difference in the distribution of socioeconomic status in the study population. There was no alteration in leptin levels with socioeconomic status in the present study similar to earlier studies by Abby F et al²⁶.

• **Serum leptin with lifestyle:**

There was significant difference in cases and controls in terms of mean duration of exercise, with controls having higher duration of exercise. Junk food which is high in calories and nutritionally inadequate, addictive, consisting of fast food and fizzy drinks was consumed more in cases than controls. The more it is consumed the more difficult it would be to opt for healthy foods. These life style habits like being sedentary and consuming junk food which is more in cases might lead to obesity in these individuals accounting to high BMI and also high leptin levels. A study did by Ali M et al¹¹⁸ in 2013 in female students in Kuwait revealed that most of the subjects had high leptin levels according to grade of obesity due to the high levels of junk foods consumed. Jane E Reseland, et al¹¹⁹ proposed that long term changes in lifestyle consisting of decreased intake of dietary fat and increased physical activity reduced leptin concentrations in humans beyond the reduction expected as a result of changes in fat mass. There was no significant difference in terms of screen time and leptin levels in cases and controls.

• **Serum leptin levels with puberty:**

The children in the study were categorised into prepubertal (Tanner I), pubertal (Tanner II, III, IV) and postpubertal (Tanner V) similar to the study by EW Demerath et al. Prepubertal children of both sexes had high leptin levels. In a preliminary report by Blum et al there was a transient increase in leptin concentrations just before the onset of puberty. Leptin also serves as a metabolic gate for puberty to progress. It was observed that there is a decline in leptin levels with advancing age in both males and females. Similar finding in observed in earlier studies by Hassink SG et al, Considine RV et al, Ellis KJ et al¹²⁰. It is hypothesised that prepubertal children manifest a central insensitivity to leptin or relative leptin resistance in the service of their dynamic energy needs. As adolescence approaches the end of puberty and adipose stores stabilise, and leptin sensitivity returns. This is part of normal growth and development. Horlick et al¹²¹, reported that there was a marked suppression of circulating leptin concentrations corrected for body composition in males at Tanner stages IV and

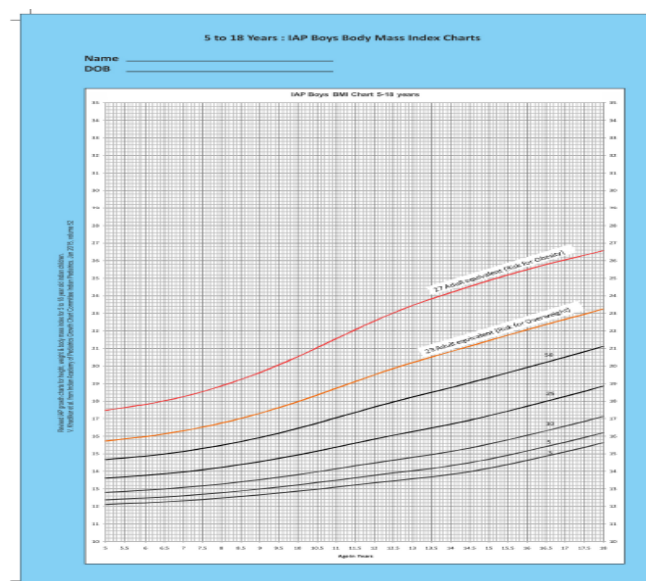
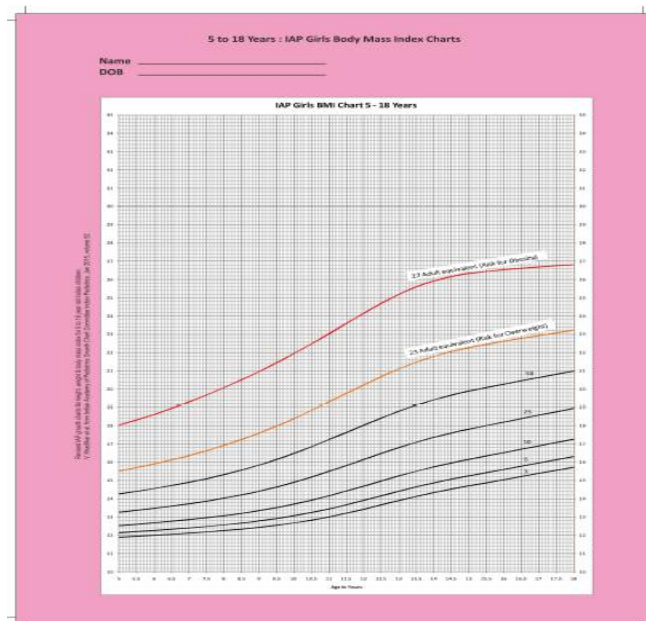
V and a smaller increase in circulating concentrations of leptin corrected for body composition in females at these later Tanner stages. They also noted a significant negative correlation of the ratio of leptin to fat mass and circulating testosterone concentration in males ($r=-0.46$, $P=0.0004$), but no significant correlation between leptin/fat mass and estradiol in females. They concluded that circulating androgens were the major cause the sexual dimorphism in circulating leptin concentrations.

- **Correlation of serum leptin with BMI in the study:**
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Table 25: Comparison of Correlation of BMI and leptin with other studies

Study	r value (correlation)
Present study	0.442
Henedina Antunes et al	0.136
Sandra G Hassink et al	0.88
Schoppen et al	0.643
Sudisha Dubey et al	0.60

BMI is strongly correlated with serum leptin which is suggested by previous studies by Henedina Antunes et al²⁷, Sandra G Hassink et al⁵², Garcia Mayor RV²⁹, Schoppen et al³², Sudisha Dubey et al¹²², Camelia Alkhzouz et al²⁸. These findings suggest that BMI is the main determinant for the variations of leptin. In addition, it also suggests that resistance to the effects of leptin may start in early childhood.



List Of Figures

- Figure 1: Structure of leptin molecule
- Figure 2: Leptin actions on various systems
- Figure 3: Leptin's action in the brain during states of energy excess and energy deficiency
- Figure 4: Leptin signalling
- Figure 5: Distribution of study population
- Figure 6: Distribution of cases and controls in the study
- Figure 7: Age distribution of cases and controls
- Figure 8: Distribution of males and females in the study
- Figure 9: BMI of cases and controls
- Figure 10: Leptin in different age groups in cases and controls
- Figure 11: Leptin levels according to gender in cases and controls
- Figure 12: Mean BMI and Leptin levels in cases and controls
- Figure 13: Leptin and BMI
- Figure 14: Leptin levels in different socioeconomic classes
- Figure 15: Mean leptin levels according to pubertal groups in cases and controls
- Figure 16: Scatter diagram showing correlation between BMI and leptin

VII. Recommendations

1. Leptin and its role in obesity, both cause and effect, is still a subject under intensive research since its discovery.
2. Leptin resistance as evidenced by high leptin levels seen early in childhood should warn us Physicians, about future risks of obesity such as insulin resistance, cardiovascular morbidity, obstructive sleep apnea etc these children might face.
3. Hence early identification of these children should be done and as leptin resistance is reversible with exercise and changes in dietary habits, parents and children should be educated.
4. Diet induced obesity causing leptin resistance as evidenced by recent studies should be countered by prevention of diets rich in fructose, triglycerides, high calorie foods etc.
5. Strict exercise regime should be adopted early in the childhood and children should be encouraged both by parents and teachers to exercise regularly to prevent leptin resistance at the skeletal muscle level.
6. Community intervention programmes should be initiated to combat obesity.

VIII. Summary

1. **Name of Speciality:** PEDIATRICS
2. **Name of System of Body:** GENERAL
3. **Title of Thesis and year of submission of thesis:** "A STUDY OF SERUM LEPTIN LEVELS IN OBESE AND NONOBESE ADOLESCENT CHILDREN OF 11-18 YEARS". (Modified thesis)
4. **Name of candidate:** Dr. BANDARU VENKATA DEEPTI
5. **Name of supervisor:** Dr. YASHOWANTH RAO
6. **Name of hospital:** YASHODA SUPERSPECIALITY HOSPITAL
7. **Objectives of the study:**
 - To compare the serum leptin levels among obese and non-obese adolescent children of 11-18 yrs.
 - To find out whether there is any difference in serum leptin levels among obese and non-obese children and to see if there is any correlation between serum leptin levels and BMI.
8. **Materials and Methods:**
 - 8.1 Study area: School based study
 - 8.2 Reason for doing the study: Adolescence is the time of gaining weight. Leptin is a adipocytokine which is implicated as a biomarker in obesity. But very few studies have been done regarding the leptin levels in obese adolescent children, and more so in Indian population. This study was undertaken for this reason, as early identification of high leptin, indicating a state of leptin resistance in children may predict future risk of obesity related disorders and can help plan early intervention strategies.
 - 8.3 Study population: The study comprises of cases and controls. Children between the ages of 11-18 yrs were selected according to the inclusion and exclusion criteria of the study.

Cases consisted of 26 adolescent children of age group 11 – 18 years, of both sex, selected from the schools, junior colleges, Hyderabad, Telangana, with BMI > 95th percentile for age and gender after taking informed consent with the parents.

Controls consisted of 20 adolescent children of age group 11 – 18 years of both sex, selected from the schools, junior colleges, Hyderabad, Telangana, with BMI <85th percentile for age and gender after taking informed consent with the parents.

Children with known genetic and endocrine causes of obesity will be excluded.

8.4 Study design: A prospective observational comparative study

8.5 Sample size: A sample size of 40 (cases 20 and controls 20) is considered adequate depending on the mean values among obese and normal weight adolescents as per earlier studies¹⁰⁸, with a two sided confidence interval of 95%, power of 80% and an estimated dropout rate of 25%. 46 adolescent children of 11-18 years of both genders were studied. 26 were cases and 20 were controls.

8.6 Data collection technique and tools:

- **Subjects:**

Ethical committee approval was taken and all the subjects were included after explaining and taking Informed Consent from legal representative. Purposive sampling was conducted in 2 private schools and 3 junior colleges in the city of Hyderabad, Telangana. Obese and normal weight children of both genders, from 11-18 years were screened. Permission was obtained from the parents / guardians and the school Principal. Parents/ guardians and the School Principal were provided with a description of the study, and informed consent forms were sent to them. After getting consents from the parents and the School Principal, children willing to participate in the study were asked to give assent and the following data was collected.

- **Anthropometric measurements:**

Evaluation included detailed history including family history of obesity or endocrine diseases, lifestyle habits like hours spent in physical activity, screen time, dietary history with 24 hr dietary recall and consumption of junk food and socioeconomic status; physical examination, systolic and diastolic blood pressure, height, weight, body mass index, pubertal development according to Tanner staging, socioeconomic staging according to modified Kuppaswamy classification and blood test for serum leptin.

Height and weight of each subject was measured in a designated room to protect the subject's privacy. For all measurements, subjects were told to wear light clothing and be barefoot. Height was measured to the nearest 0.1 cm using a stadiometer, and body weight was measured to the nearest 0.1 kg using a scale.

Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m).

$$\text{BMI} = \text{weight} / \text{height}^2 \text{ (Kg/m}^2\text{)}$$

The reference data used to identify the cut-off points was taken from the Indian Academy of Pediatrics latest growth charts.

1. Underweight – less than 5th percentile
2. Healthy weight – 5th percentile to less than 85th percentile
3. Overweight – 85th to less than 95th percentile
4. Obese – equal to or greater than 95th percentile

- **Biochemical assessment:**

Blood samples were collected, around 3 milliliter of plain sample in a vacutainer, by a skilled and qualified technician by direct venipuncture around 8 am in the morning following an overnight fast (minimum 12 hr). After collection, the blood samples were centrifuged for 10 minutes and stored at -70 degrees centigrade. The materials used for collection were disposable, adequately labeled and of recognized quality. Leptin levels were measured using – The RayBio_ Human Leptin ELISA (Enzyme-Linked Immunosorbent Assay) kit according to the manufacturer's instructions and standard guidelines.

- **Data analysis:** Data was entered in Microsoft excel and analysis was done using SPSS version 20. Descriptive statistical analysis was done. Results on continuous measurements are presented as Mean & Standard Deviation. Results on categorical measurements are presented as Percentages. Significance is assessed at 5% level of significance. Student t test (independent, two tailed) was used to find out the significance of study parameters on a continuous scale between two groups. Pearson's correlation was used to find out the strength of linear relationship between study variables.

9. Salient findings:

- The present study was undertaken to determine the correlation of serum leptin levels with BMI.
- A total of 46 adolescent children were included in the study.
- Out of 46 children, 26 were obese children with BMI > 95th centile and 20 were normal weight children with BMI < 85th centile.
- Out of 26 obese children, 15 were males and 11 were females.
- Out 20 normal weight children 11 were males and 9 were females.
- Leptin concentration in our study is significantly higher in obese children (28.54+/-14.38) than non-obese (13.33+/-3.63) with a p value <0.001.
- BMI is strongly correlated with serum leptin

10. Conclusion:

- The present study found the mean serum leptin levels to be around 28.54+/-14.38 pg/ml in obese and 13.33+/-3.63 pg/ml in normal weight adolescent children.
- There was a strong positive correlation between serum leptin and BMI in adolescent children.
- The leptin levels were high in early stages of pubertal development, more so in obese adolescent children.
- There was a significant difference in leptin levels among cases and controls with physical activity and junk food consumption though no significance was found with screen time and socioeconomic status.
- High serum leptin levels associated with high BMI was a positive biomarker for obesity and indicates a state of leptin resistance in these obese children. This emphasises the need to start early intensive intervention by modification of lifestyle, and environment in order to prevent obesity associated complications.
- Randomized, placebo-controlled clinical trials are currently evaluating leptin as a potential treatment for weight loss maintenance, and the development of leptin sensitizers for common obesity is greatly anticipated in the near future.
- The present study concludes that serum leptin is a valuable indicator in predicting adult obesity and further studies in this direction are essential to help control this epidemic of childhood obesity in progressing further.
- Exploring the role of Leptin as therapeutic target may be a viable option for controlling the metabolic disorders like obesity in Indian patients.

11. Recommendations:

- Leptin and its role in obesity, both cause and effect, is still a subject under intensive research since its discovery.
- Leptin resistance as evidenced by high leptin levels seen early in childhood should warn us Physicians, about future risks of obesity such as insulin resistance, cardiovascular morbidity, obstructive sleep apnea etc these children might face.
- Hence early identification of these children should be done and as leptin resistance is reversible with exercise and changes in dietary habits, parents and children should be educated.
- Community intervention programmes should be initiated to combat obesity.

Limitations:

- The present study was conducted in a small sample, hence a larger sample size is required to study, the serum leptin levels indicative of the population.
- BMI determination represents a useful tool in overweight assessment, but does not offer accurate information about the body composition. For more accurate characterization of overweight, new methods are needed in order to permit the assessment of the percentage of body fat, muscle and bone, such as bio-impedance and MRI. This being associated with higher cost could not be possible in the present study.
- This study having small sample size limits us in accurately predicting the effect of leptin on pubertal development. Higher number of children have to be studied in order to see the variation of leptin levels with different stages of puberty.

Conclusion

1. The present study found the mean serum leptin levels to be around 28.54 ±14.38 pg/ml in obese and 13.33 ± 3.63 pg/ml in normal weight adolescent children.
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3. The leptin levels were high in males in early stages of pubertal development, more so in obese adolescent children.

4. There was a significant difference in leptin levels among cases and controls with physical activity and junk food consumption though no significance was found with screen time and socioeconomic status.
 5. High serum leptin levels associated with high BMI was a positive biomarker for obesity and indicates a state of leptin resistance in these obese children. This emphasises the need to start early intensive intervention by modification of lifestyle, and environment in order to prevent obesity associated complications.
 6. Randomized, placebo-controlled clinical trials are currently evaluating leptin as a potential treatment for weight loss maintenance, and the development of leptin sensitizers, for common obesity is greatly anticipated in the near future.
 7. The present study concludes that serum leptin is a valuable indicator in predicting adult obesity and further studies in this direction are essential to help control this epidemic of childhood obesity in progressing further.
- Exploring the role of leptin as therapeutic target may be a viable option for controlling the metabolic disorders like obesity in Indian patients.

XIII. Recommendations

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IX. Limitations

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3. This study having small sample size limits us in accurately predicting the effect of leptin on pubertal development. Higher number of children have to be studied in order to see the variation of leptin levels with different stages of puberty.

X. Review Of Literature

Henedina Antunes, Cristina Santos and Susana Carvalho et al did a study to determine the relationship between serum leptin and clinical and biochemical features in overweight children and adolescents. This was published in British Journal of Nutrition in April 2009. Serum leptin was determined in 357 patients. The mean age was 9.5 (SD 3.2) years and mean BMI z-score was 1.72 (SD 1.34) (girls 1.71 (SD 1.61); boys 1.72 (SD 1.11)). They found that serum leptin levels were significantly related to sex (mean: girls 48.0 ng/ml, boys 34.4 ng/ml; $p = 0.009$). BMI z-score ($r = 0.25$; $p < 0.001$). They found that being female, and greater BMI, were significantly and independently associated with increased serum leptin²⁷.

Camelia Alkhzouz, Cecilia Lazea, Ioana Nascu, Alina Cotlet did a study to establish the correlation between the body mass index, percentage/amount of fat and the serum leptin level in overweight children published in 2015 in Jurnalul Pediatrului. They studied 126 children (study group: 86 overweight children and the control group: 40 normal weight children). Study methods included: anamnesis, clinical exam, anthropometric assessment (weight, height, BMI); body fat measurements by bio-impedance, lipid profile, glucose and serum leptin. They found that serum leptin correlated with BMI. They concluded that high level of leptin in obese children contributes to early development of metabolic syndrome²⁸.

Garcia-Mayor RV, Andrade MA, Rios M, Lage M, Dieguez C, Casanueva FF et al did a study to determine serum leptin in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage published in J Clin Endocrinol Metab. To understand whether leptin is the adipose

tissue signal that allows puberty, 789 normal children of both sexes, age 5-15 yr, were transversally studied. In an age-related analysis, leptin levels in girls rose from 5-15yr (from 4.3 +/- 0.4 to 8.5 +/- 0.9 micrograms/L) in parallel with body weight. Boys always had lower leptin levels than girls (3.3 +/- 0.3 micrograms/L at 5 yr), but they rose in parallel with weight until 10 yr (5.3 +/- 0.7 micrograms/L), when a striking decrease was observed until 15 yr (3.0 +/- 0.3 micrograms/L). They concluded that leptin appears to increase in both boys and girls before the appearance of other reproductive hormones related to puberty and leptin levels in boys are always lower than in girls, although they increase with age until the age 10 yr²⁹.

Robert V Considine, Ph.D., Madhur K Sinha, Ph.D., Mark L. Heiman, Ph.D., Aidas Kriauciunas, Ph.D., Thomas W. Stephens, Ph.D., Mark R. Nyce, et al did a study on serum immunoreactive-Leptin concentrations in normal weight and obese humans published in *Engl J Med*. 136 normal weight and 139 obese subjects (body mass index, >27.3 for men and >27.8 for women) were included and serum leptin concentrations measured. They found that mean (+/- SD) serum leptin concentrations were 31.3+/-24.1 ng/ml in obese subjects and 7.5+/-9.3 ng/ml in the normal weight subjects (p<0.001). They found a strong correlation between serum leptin concentrations and the percentage of body fat (r = 0.85, p <0.001). They concluded that serum leptin concentrations are correlated with the percentage of body fat, suggesting that most obese persons are insensitive to endogenous leptin production³⁰.

In 2005, Popruk S Tungtrongchitr R., Pongpaew P., Phourat B., Tungtrongchitr A., Tribunyatkul S., Paksanont S., Vudhivai N. and Schelp F., conducted a study in Thai children and found that Serum leptin, triglyceride and low density lipoprotein cholesterol/high density lipoprotein cholesterol ratios (LDL/HDL ratio) were significantly higher in the overweight and obese males and females³¹. In 2010 Schoppen S, Riesra P, Garcia-Anquita A, Lopez-Simon L, Cano B, de Oya I, et al. conducted a study on leptin and adiponectin levels in pubertal children: relationship with anthropometric variables and body composition. They studied 833 pubertal boys and girls. Leptin concentrations were significantly higher (p<0.0001) in obese or overweight children compared with normal weight children. Weight, Body mass index (BMI), waist circumference, hip circumference and waist to hip ratio were significantly correlated (p<0.01) with leptin concentrations in both genders. They concluded that, in pubertal children, leptin is related to weight, BMI, waist circumference, and hip circumference and correlates even more strongly with % fat mass³².

In 2011 Zhang M, Zhao X, Li M, Cheng H, Hou D, Wen Y, et al, did a study on abnormal adipokines associated with various types of obesity in Chinese children and adolescents. They studied 1788 boys and 1720 girls aged 6 to 18 years and assessed their body mass index, waist circumference, pubertal development, blood insulin, resistin, leptin, adiponectin, ASP and C3 levels. Serum resistin, leptin and adiponectin levels were higher in girls than those in boys (all p<0.001). With inclusion of body mass index and waist circumference in simultaneous regression analyses, both body size indices were independently and significantly correlated with insulin, leptin and adiponectin after age and gender adjustment. They concluded that obese children have a worse metabolic profile with high insulin, resistin, leptin, ASP and C3, and low adiponectin levels. The adipokine profile in mixed obesity is worse than that in peripheral or abdominal obesity. Identification of obese subjects with a malignant adipokine profile using a combination of body mass index and waist circumference is important for the prevention of obesity-related diseases³³.

In 2011 Friedman JM has demonstrated that there is a powerful biological basis for obesity. Majority of obese subjects are leptin resistant, which establishes that obesity is the result of hormone resistance. Leptin treatment results in weight loss in a subset of obese patients and can also synergize with other anti-obesity agents to reduce weight in the general population³⁴.

In 2011 Plonka M, Toton-Morys A, Adamski P, Suder A, Bielanski W, Dobrzanska MJ, et al, studied association of the physical activity with leptin blood serum level, body mass indices and obesity in schoolgirls. They studied 59 girls, aged 9-16 years (12.5+/-1.67) and divided into two groups: 1)PA: a physically active group of 29 girls and 2)PI: a group of 30 physically inactive girls. In all, physical activity was assessed using modified questionnaire concerning "activity for adolescents" and expressed in MET-h/week. Serum blood leptin concentrations in fasting girls were determined by RIA. Anthropometric parameters were measured. They found that the concentration of leptin was directly proportional to the degree of body fat and to the body composition³⁵.

In 2012 J.E. Park, H.J. Choi, I.K. Kim, H.J. Lee, J.H. Kang, J, Song, studied First-grade students, who entered elementary school at age 7 years in Gwacheon, a Korean city. These children were followed from 1st grade to 5th grade. Annual physical examinations from 2005 to 2009 were performed. In 2006, the levels of serum glucose, insulin, leptin and adiponectin and lipid profiles were examined. In 2008, the above parameters, except for adiponectin were measured again in 381 children (202 boys and 179 girls). In 2006, 10.2% of the children were overweight (BMI) ≥ 85th percentile), and after 2 years, an additional 3% became overweight. Compared with insulin and adiponectin, leptin was most highly associated with current and future BMI, and percent body fat. Boys in the highest tertile for initial leptin (T3) showed the highest prevalence of overweight

and metabolic risk scores among three leptin tertile groups. Girls showed the same trends as boys. High initial leptin levels could be predictive of greater future BMI and metabolic risk score ($p < 0.001$). They concluded that elevated serum leptin concentrations among the childhood population could be a marker for future BMI and metabolic disorders²⁵.

In 2007, Abby F. Fleisch, Neha Agarwal, Mary D. Roberts, Joan C. Han, Kelly R. Theim, Albert Vexler, et al studied children of 6–12 yrs at high risk for adult obesity because of early-onset childhood overweight and/or parental overweight from 1996–2004. Growth in body mass index (BMI) was studied in 197 children, and growth in total body fat mass was examined in 149 children over an average follow-up interval of 4.4 yr (range, 1–8 yr). At baseline, 43% of children studied were overweight (BMI \geq 95th percentile); during follow-up, an additional 14% became overweight. Independent of initial body composition, baseline leptin was a statistically significant positive predictor of increased BMI ($P = 0.0147$) and increased total body fat mass ($P < 0.007$). They concluded that high serum leptin, independent of body fat, may be an indicator of increased leptin resistance, which predisposes children at high risk for adult obesity to somewhat greater growth in weight and body fat during childhood²⁶.

A Cross-sectional study was conducted by David Jimenez-Pavon, Francisco B Ortega, Enrique G Artero, Idoia Labayen, German Vicente-Rodriguez, Inge Huybrechts et al published in 2012 in The Journal of Pediatrics were 902 (509 girls) adolescents aged 12.5-17.5 years were studied. Weight, height, and TBF (sum of 6 skinfold thickness) were measured, and fat free mass and body mass index were calculated. Physical activity was assessed by accelerometry. Physical fitness was assessed by the handgrip, standing long jump, 4 \times 10-m shuttle run, and 20-m shuttle run tests. Serum fasting leptin, insulin, and glucose concentrations were measured, and homeostasis model assessment was computed. Multiple linear regression models were used. Vigorous physical activity and fitness tests (all $P < .05$) were negatively associated with leptin, independently of several confounders including TBF and homeostasis model assessment. These associations remained significant after further controlling for each other (physical activity and fitness). They concluded that vigorous physical activity and fitness moderate the levels of leptin concentrations, regardless of relevant confounders including TBF. Intervention programs addressed to increase high intensity physical activity and fitness as well as to assess its impact on leptin concentration are required³⁶.

Aria M., Lacadie C, Seo D, Kubat J, Van Name MA, Giannini C et al conducted a study in twenty-five obese and fifteen lean adolescents published in 2014. They studied the children's behaviour and leptin levels with functional MRI during exposure to high calorie food, low calorie food and non-food visual stimuli 2hr after isocalorie meal consumption. They found that brain responses to high calorie food relative to non-food cues increased in obese versus lean adolescents in striatal-limbic regions, involved in motivation-reward and emotion processing. Higher endogenous leptin levels correlated with increased neural activation to high calorie food images in all subjects. They concluded that there is significant association between higher circulating leptin and hyper-responsiveness of brain motivation-reward regions to high calorie food images suggesting that dysfunctional leptin signalling may contribute to the risk of overconsumption of these foods, thus predisposing adolescents to the development of obesity³⁷.

XI. Physiology

Childhood obesity:

Obesity prevalence has been increasing among children and adolescents as it has in adults. The National Health and Nutrition Examination Survey³⁸, 2009-2010, found 32% of children, 2-19 yr old to be overweight or obese, and 17% in the obese range. Children's risk varies significantly by race/ethnicity. In 2009-2010, 24% of non-Hispanic Black, 21% of Hispanic, and $>20\%$ of American Indian/Alaskan Native children and adolescents were obese compared to 14% of white children.

Obesity or increased adiposity is defined using the Body Mass Index which is an extent proxy for more direct measurement of body fat.

$$\text{BMI} = \text{Weight in Kg}/(\text{Height in Meters})^2$$

During childhood, levels of body fat change, beginning with high adiposity during infancy. Body fat levels decrease for approximately 5.5 years until the period called adiposity rebound, when body fat is typically at the lowest level. Adiposity then increases until early adulthood.

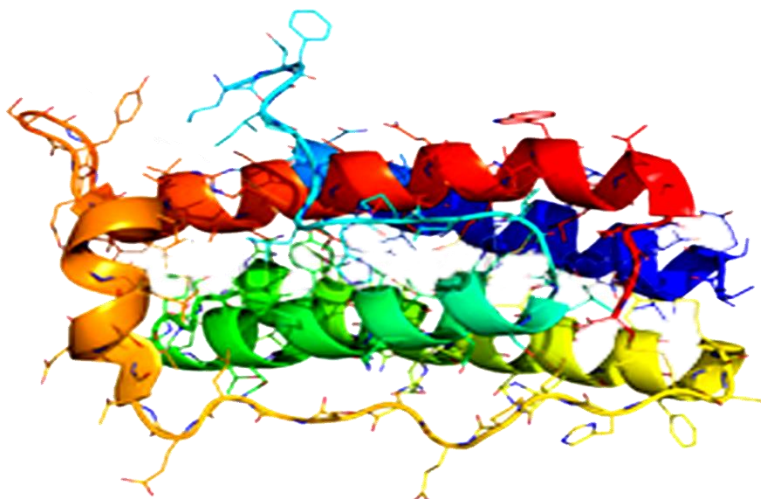
Obesity and overweight are defined using BMI percentiles; Children more than 2 yr old with BMI \geq 95th percentile meet the criterion for obesity, and those with BMI between 85th and 95th percentiles fall in the overweight range.

Leptin biology:

Several genes have been identified to disclose a physiological system that maintains body weight within a range of about twenty pounds³⁹. A key element of this system is leptin, the 16-kDa hormonal product of the obesity (ob) gene⁴⁰ located at chromosome 7 (7q31.1)⁴¹. Leptin is a prototypical adipokine consisting of 167-

amino acids with a four-helix bundle motif similar to that of a cytokine. Leptin is primarily secreted by adipocytes⁴² and is a classic member of more than 50 identified adipocytokines that participate in adipose tissue hormonal signalling⁴³. Other tissues such as the stomach, intestines, placenta and testes also secrete leptin⁴⁴. Plasma leptin is generally proportional to adipose mass⁴⁵. It regulates the expression of hypothalamic neuropeptides involved in energy metabolism by suppressing food intake and stimulating energy expenditure⁴⁶.

Figure 1: Structure of leptin molecule

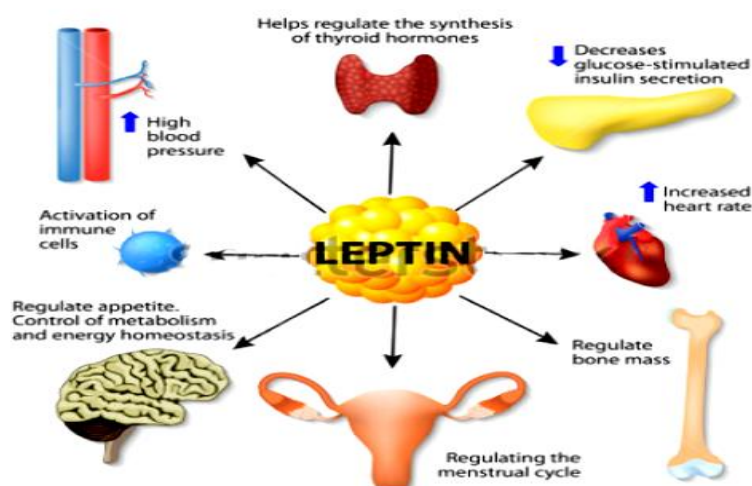


Leptin levels are pulsatile and follow a circadian rhythm, with highest levels between midnight and early morning and lowest levels in the early- to mid- afternoon. Specifically, the concentration of circulating leptin may be up to 75.6% higher during the night as compared to afternoon trough levels. The pulsatile characteristics of leptin secretion are similar in obese and lean individuals, except that, obese have higher pulse amplitudes⁴⁷.

The absence of leptin therefore leads to increased appetite and food intake that result in morbid obesity. Initially, it was thought that human obesity might occur as a result of lack of leptin (as in the ob/ob mouse). Only rare cases of severe early childhood obesity have been associated with leptin deficiency^{48,49}. It was found that the majority of humans did not lack leptin; in contrast they had circulating leptin concentrations that were highly correlated with adipose tissue mass⁵⁰. Correlations between measures of body fat and leptin concentration range from 0.7 to 0.9 in both adults and children^{45,51,52}. The failure to induce weight loss in these cases is thought to be the result of leptin resistance⁵³.

Leptin has effects on different systems of the body: central nervous system (inhibition of food intake, reduction of adipose mass, increased thermogenesis), glucose homeostasis, reproductive system (signalling onset of puberty, maintenance of reproductive function), autonomic nervous system, haematopoietic system, skeletal system, oncogenesis and transplantation (contributing to rejection of transplants)⁴⁴.

Figure 2: Leptin actions on various systems



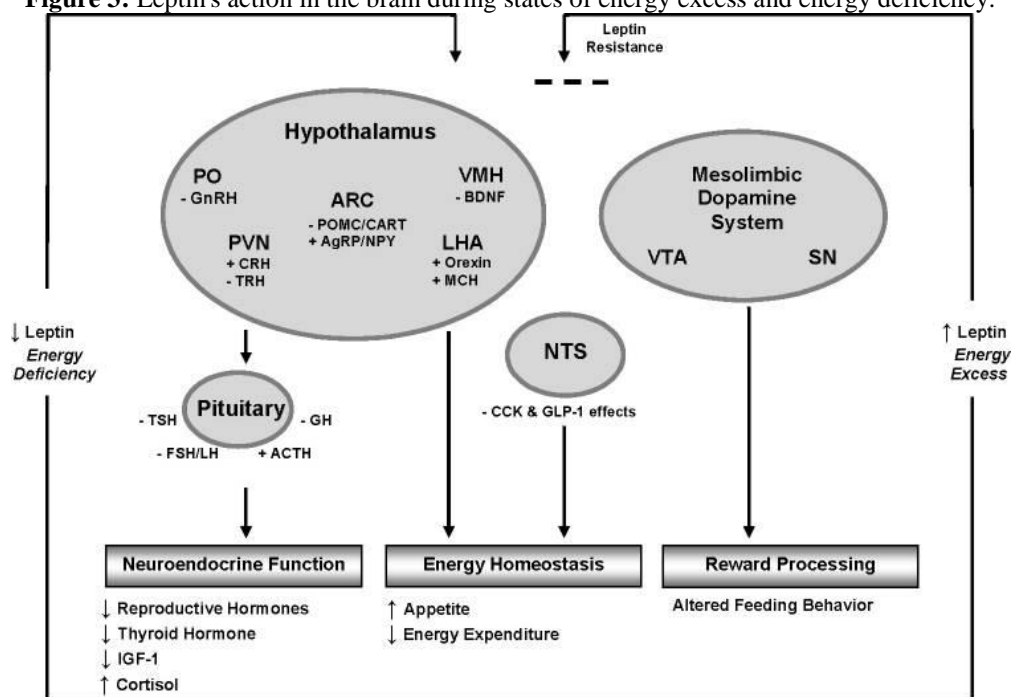
XII. Leptin signalling

Leptin And Leptin Receptor:

Leptin is primarily secreted by adipocytes and circulates at a level of 5 to 15 ng/ml in lean subjects⁵⁴. Its expression is increased by overfeeding, insulin, glucocorticoids, endotoxin, and cytokines and is decreased by fasting, testosterone, thyroid hormone, and exposure to cold temperature^{55,56}. Six isoforms of the obesity receptors Ob-R (a to f) have been identified, and they are closely related to the class I cytokine receptor family. These isoforms have homologous extracellular domains but distinct intracellular domains, which vary by length and sequence due to alternative mRNA splicing. The short isoforms *ObRa* and *ObRc* are thought to play important roles in transporting leptin across the blood–brain barrier (BBB). The long leptin receptor isoform *ObRb* is primarily responsible for leptin signalling. This functional leptin receptor *ObRb*, expressed in several organs, is strongly expressed throughout the central nervous system but particularly in the hypothalamus, where it regulates energy homeostasis and neuro-endocrine function. Most Ob-Rs in the CNS are located in the basomedial hypothalamus^{57,58}. In the neurons that synthesize proopiomelanocortin and express Ob-Rb, leptin stimulates the synthesis of proopiomelanocortin, which is processed to produce alpha-melanocyte-stimulating hormone and activate downstream melanocortin-3 and -4 receptors to decrease appetite⁵⁹. Leptin also inhibits the appetite-stimulating hormone neuropeptide Y in the arcuate nucleus, and causes inhibition of agouti-related peptide that restrain melanocortin-3 and -4 receptor signalling^{60,61}.

Leptin interacts with the mesolimbic dopamine system and modulates the hedonic drive to feed^{62,63}. Neurons in the ventral tegmental area (VTA) expressing LepRb respond directly to leptin, resulting in suppression of feeding. Moreover, leptin modulates the mesolimbic dopamine system indirectly through the lateral hypothalamus^{64,65}.

Figure 3: Leptin's action in the brain during states of energy excess and energy deficiency.

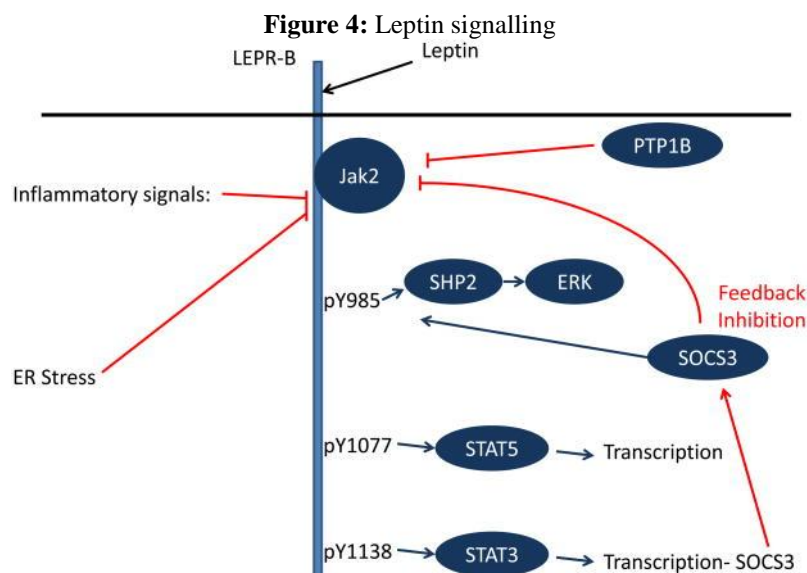


During states of leptin and energy excess, leptin's access to the hypothalamus and other brain areas is impaired and leptin's action is blunted. In states of leptin and energy deficiency, neuropeptides that are normally inhibited by leptin are elevated (+) and neuropeptides stimulated by leptin are suppressed (-). A change in the concentrations of these neuropeptides leads to alterations in neuroendocrine function and energy homeostasis. Alterations in leptin levels may also affect the hedonic aspects of feeding behavior.

Abbreviations: ACTH, adrenocorticotrophic hormone; AgRP, agouti-related peptide; ARC, arcuate nucleus; BDNF, brain-derived neurotrophic factor; CART, cocaine- and amphetamine- regulated transcript; CCK, cholecystokinin; CRH, corticotropin-releasing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; GLP-1, glucagon-like peptide 1; GnRH, gonadotropin-releasing hormone; IGF-1, insulin-like growth factor 1; LH, luteinizing hormone; LHA, lateral hypothalamic area; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; NST, nucleus of the solitary tract; PO, preoptic area; POMC, proopiomelanocortin; PVN, paraventricular nucleus; SN, substantia nigra; TRH, thyrotropin-releasing

hormone; **TSH**, thyrotropin-stimulating hormone; **VMH**, ventromedial hypothalamus; **VTA**, ventral tegmental area.

Ob-Rs have been shown to activate Janus-activated kinase (JAK), signal transducers and activators of transcription (STAT), insulin receptor substrate, and the mitogen-activated protein kinase (MAPK) pathways. The best-characterised pathway in leptin signalling is the JAK/STAT pathway⁶⁶.



Leptin decreases food intake while increasing energy expenditure by binding to, and activating the long form of its receptor (LEPR-B) in the brain. LEPR-B is a type 1 cytokine receptor, that upon leptin binding to its extracellular domain, undergoes a conformational change to activate its associated Jak2 tyrosine kinase⁶⁵. Activated Jak2 promotes the tyrosine phosphorylation of a number of intracellular residues on LEPR-B (as well as on Jak2 itself), and each tyrosine phosphorylation site recruits a specific set of downstream molecules to promote specific intracellular signals. LEPR-B contains three distinct tyrosine phosphorylation sites: Tyr₉₈₅, Tyr₁₀₇₇, and Tyr₁₁₃₈⁶⁶. Tyr₁₁₃₈ recruits signal transducer and activator of transcription-3 (STAT3), a latent transcription factor, which subsequently becomes tyrosine phosphorylated (pSTAT3) by Jak2, enabling its nuclear translocation and promoting its transcriptional effects. The detection of pSTAT3 is used as an important bioassay of LEPR-B signalling *in vivo*⁶⁷. Similarly, Tyr₁₀₇₇ recruits and mediates the phosphorylation and activation of a related transcription factor, STAT5^{66,68}. Tyr₉₈₅ recruits the tyrosine phosphatase PTPN11, and also binds the suppressor of cytokine signalling-3 (SOCS3; an inhibitor of LepRb/Jak2 signalling)^{69,70}.

Table 1: Leptin Signaling

Signaling Pathway	Primary Site of Action	Known Mechanisms of Action	Clinical Results
JAK-STAT3	Hypothalamus	Stimulates transcription of POMC and suppresses transcription of NPY .	Regulates appetite and, thus, body weight . May also contribute to neuroendocrine function as neural-specific STAT3 deletion results in decreased linear growth and infertility .
P13K	Hypothalamus	Stimulates POMC neurons. Inhibits FOXO1, an inhibitor of POMC transcription, to increase POMC expression .	Regulates appetite and body weight . May contribute to leptin resistance in obesity, given the overlapping pathway with insulin. May mediate the stimulation of sympathetic outflow.
MAPK	Hypothalamus, liver, pancreas, adipose tissue, and myocytes	Stimulates POMC neurons and inhibits AgRP/NPY neurons .	Regulates appetite and body weight . Increases sympathetic activity to brown adipose tissue . Increases fatty acid oxidation in peripheral tissues . Promotes cardiomyocyte hypertrophy.
AMPK	Hypothalamus, muscle	Stimulates ACC activity in the hypothalamus to regulate food intake and weight .	Regulates appetite and weight . Stimulates fatty-acid oxidation in muscle and may sensitize muscle to insulin.

		Inhibits ACC activity in muscle.	
mTOR	Hypothalamus	Induces phosphorylation of S6K1 to regulate protein synthesis.	Regulates appetite and weight.

Abbreviations: ACC, acetyl coenzyme A carboxylase; AMPK, 5'adenosine monophosphate-activated protein kinase; ATP, adenosine triphosphate; FOXO1, forkhead box O1; JAK-STAT3, janus kinase-signal transducers and activator of transcription 3; K⁺, potassium; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; P13K, phosphatidylinositol 3-kinase; S6K1, S6 Kinase 1.

In addition to regulating food intake, leptin increases energy expenditure through sympathetic nerve activity. Leptin has a neurotrophic effect on hypothalamic neurons implicated in feeding and energy homeostasis, as well as cortical and hippocampal neurons^{71,72}. In addition to its role in early neuronal development, leptin modulates synaptic plasticity in adults. Brain imaging studies have also revealed structural and functional deficits reversible by leptin treatment in humans with congenital leptin deficiency^{73,74}.

Leptin and metabolism:

Total leptin deficiency results in insulin resistance, diabetes, steatosis and other features of metabolic syndrome. In morbidly obese individuals with congenital leptin deficiency, leptin replacement dramatically decreases insulin resistance, steatosis, dyslipidemia and glucose levels^{75,76}. Similarly, central or peripheral leptin administration decreases insulin resistance, steatosis and glucose in generalized lipoatrophy^{77,78,79}.

- **Glucose metabolism:**

It has been proposed that leptin replacement may serve as an adjunct therapy to insulin in patients with type 1 diabetes, by improving glucose and lipid metabolism^{80,81}. Studies suggest that leptin affects peripheral insulin sensitivity via CNS mechanisms independent of its effects on food intake and weight⁸². Leptin alters glucose homeostasis through insulin. Leptin inhibits insulin gene expression and glucose-stimulated insulin secretion, and these actions adapt glucose levels to body fat stores. In turn, insulin stimulates both leptin synthesis and secretion, thus establishing an adipose-islet axis.

- **Fat metabolism:**

Leptin regulates lipid metabolism independently of food intake. Central leptin administration inhibits *de novo* lipogenesis and stimulates lipolysis in adipose tissue and liver via activation of the sympathetic nervous system^{83,84}. Leptin stimulates fatty acid oxidation by up-regulating peroxisome proliferator-activated receptor γ -coactivator-1 α , and decreasing triglyceride stores within white adipocytes and liver. Leptin also stimulates fatty acid oxidation by activating AMPK in skeletal muscle, and preventing the accumulation of lipid metabolites associated with lipotoxicity. Disruption of LepR in peripheral organs has no significant impact on metabolism, suggesting that leptin acts mainly in the brain to influence glucose and lipid metabolism⁸⁵.

Leptin and exercise:

Human skeletal muscle expresses low levels of LepRb. Leptin is thought to directly increase glucose uptake and fatty acid oxidation in skeletal muscle. Leptin receptors and leptin signaling in skeletal muscle particularly in the leg muscles are reduced in obese subjects, suggesting a potential mechanism of leptin resistance in obesity.

Leptin and immune function:

leptin has important roles in modulating innate and adaptive immunity⁸⁶. Leptin stimulates neutrophil chemotaxis and promotes macrophage phagocytosis, as well as production of pro-inflammatory cytokines such as interleukin (IL)-6, IL-12, tumor necrosis factor (TNF)- α . Recently, it has also been shown that leptin acts as a negative signal for the proliferation of regulatory T cells, while stimulating T helper 1 cells^{86,87}. Thus, leptin may contribute to the protection from infections and the development of autoimmunity.

Mechanisms of cellular leptin resistance:

SOCS3, PTP1B (Protein Tyrosine Phosphatase 1B), hypothalamic ER (endoplasmic reticulum) stress, and inflammation are the molecular and cellular mediators that directly attenuate LEPR-B signalling in states of obesity, and represent mediators of cellular leptin resistance⁸⁸⁻⁹³. Alterations in LEPR-B to STAT3 signalling also lead to leptin resistance^{94,95,96,97}.

Central leptin resistance can also occur from the dependency of leptin on saturable transport across the vascular barrier and, across the choroid plexus to reach the arcuate nucleus⁹⁸. The activity of this blood-brain barrier decrease in diet-induced obese (DIO) rodents⁹⁹, resulting in failure of circulating leptin to reach its

targets in brain. Increasing evidence suggests that leptin signalling is preferentially reduced in the arcuate nucleus of the hypothalamus¹⁰⁰.

Proopiomelanocortin neurons (POMC) in the arcuate nucleus of the hypothalamus (ARC) project to downstream targets where they release POMC-derived peptides, including alpha-MSH, that activate CNS melanocortin receptors to reduce food intake and increase energy expenditure^{101,102}. Disruption of melanocortin action by physical lesions of the ARC, by pharmacological means, or by various genetic alterations at the level of the melanocortin peptide or its receptors, causes obesity and proportionate hyperleptinemia.

In Diet Induce Obesity (DIO), increased food intake and associated adiposity promotes cellular leptin resistance and this prevents LEPR-B signalling from reaching the level that it would otherwise attain in response to the increased ambient leptin, thereby further facilitating the weight gain associated with the consumption of high-calorie diet.

A sustained increase in the strength of anorexic signals will favour the maintenance of a reduced level of body fat stores, as would interfere with orexigenic signals and mediators of cellular leptin resistance. Hence intervention at multiple independent points is likely to produce synergistic effects. We have to better understand the mechanisms governing food intake and to modify them therapeutically, including the neural pathways that modulate food palatability and reward.

There is significant association between higher circulating leptin and hyperresponsiveness of brain motivation-reward regions (striatal-limbic regions) to high calorie food images as evidenced by functional MRI suggesting that dysfunctional leptin signaling may contribute to the risk of overconsumption of these foods, thus further predisposing adolescents to the development of obesity and T2D³⁸.

Clinical applications of leptin.

- **Leptin treatment in states of leptin deficiency:**

Congenital leptin deficiency - Leptin reduces body fat and reverses neuroendocrine and metabolic abnormalities¹⁰³.

Congenital and acquired lipoatrophy – Leptin improves insulin sensitivity, dyslipidemia and hepatic steatosis^{104,105}.

- **Leptin in leptin resistance (obesity, Type 2 DM):**

Combination therapy of leptin and leptin sensitizers – Amylin has been suggested to overcome leptin resistance. Clinical studies have shown that Pramlintide, amylin analog can be combined with leptin to reduce weight.

- **Leptin in Alzheimers:**

Future potential areas of leptin therapy include neurodegenerative disorders such as Alzheimer's disease. A growing body of evidence suggests that leptin has a positive influence on neurogenesis, axon growth, synaptogenesis, and neuroprotection¹⁰⁶. Prospective studies have shown that high leptin levels, especially among non-obese individuals, are associated with a lower risk of dementia and Alzheimer's disease, implying a possible therapeutic role of leptin¹⁰⁷. Furthermore, leptin may be targeted for diagnostic or therapeutic uses in the regulation of immunity and bone health. Much remains to be learned about mechanisms underlying cellular leptin resistance and the relative importance of various mediators of cellular leptin resistance.

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