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THYROID FUNCTION IN CHILDREN WITH MONOGENIC OBESITY

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ABSTRACT

OBJECTIVE: An association between obesity and thyroid function has mostly been investigated in adult subjects. Fewer studies have been performed to investigate the effect of excessive obesity on the thyroidal axis in children and have led to conflicting observations. In this study we have evaluated the thyroid function in children with monogenic obesityby measuring serum thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4). In addition we have determined serum leptin concentrations in the study subjects. **MATERIALS AND METHODS**: The present study was carried out in 27 children with severe early onset obesity (BMI SDS>3.5) due to known loss-of-function mutations in the leptin (*LEP),* leptin receptor *(LEPR)* or melanocortin 4 receptor (*MC4R)* gene. In addition 13 age-matched normal-weight subjects negative for these mutations served as the control group. Serum TSH, T_3, T_4, and leptin were analyzed by using commercially available kits. **RESULTS:** Remarkably, the circulating TSH, T_3 and T_4 were found to be within the normal range in all the 3 groups of children with severe obesity and the values of these hormones were not significantly different from those of the control group. As expected, leptin levels were non-detectable in children with

LEP mutations, and were raised over the reference values in subjects with LEPR or MC4R deficiency. **CONCLUSION:** The present study fails to establish an association of thyroid function with excessive adiposity in children due to monogenic causality. More importantly, complete leptin deficiency or a disruption of the leptin downstream signaling appears to have little effect on TSH or thyroid hormone secretions. However, there remains the possibility that any adverse effects of adiposity on pituitary-thyroid axis as reported in some studies, may arise due to other obesity associated metabolic dysfunctions becoming apparent at a later stage of life.

Keywords: Monogenic obesity; Thyrotropin; Thyroid hormones; Leptin

INTRODUCTION

Obesity presents a rapidly growing threat to human health that is now virtually felt by every country in the world. According to global statistics of World Health Organization (WHO), in 2016, almost 39% and 13% of adults were overweight and obese, respectively. Obesity initially considered as an adult problem, is now increasingly affecting children and adolescents. By the year 2016 the number of overweight children had grown to 41 million around the world [1] and according to Global Burden of Disease (GBD) study collaborators it is expected to rise to 70 million by 2025[2]. Obesity is generally accompanied by a number of co-morbidities such as type 2 diabetes, hypertension, cardiovascular disease, sleep apnea, some types of cancer amongst others [3]. Thus obesity has emerged as a major disease and a cause of one of the highest mortality risks. Obesity has also been associated with a number of neuroendocrine dysfunctions that are mainly the result of changes in the hypothalamic pituitary axis. Thyroid function has often been described as altered in adult and young subjects with obesity. Hyperthyrotropinemia or sub-clinical hypothyroidism has frequently been reported in adult subjects with obesity [4]. In some investigations, high adiposity and BMI have been positively associated with thyroid stimulating hormone (TSH) and triiodothyronine (T3) and with a mild decrease in free thyroxine levels (FT4)[5,6]. However, a number of other studies fail to show a remarkable relationship between adiposity and thyroid function [7, 8, 9].

The adipocyte derived hormone, leptin, has been implicated in influencing the thyroidal axis. A relationship between adiposity and thyroid function is expected since both leptin and thyroid hormones play a

central role in energy metabolism, energy expenditure and appetite. Furthermore, leptin has also been implicated in regulation of thyrotropin releasing hormone (TRH) expression through stimulation of paraventricular nucleus (PVN) neurons by up-regulating pro-TRH gene [10]. TSH has also been shown to affect an increase in adipocytes through its receptors on fat cells. Monogenic obesity though rare has provided useful insights into the molecular and physiological mechanisms regulating energy homeostasis, body weight and food-intake, and has led to the elucidation of the central leptin-driven melanocortin signalling. This form of obesity, therefore, provides an exceptionally useful human model to study the role of specific genetic deficiencies that may lead to extreme adiposity and its pathogenesis including neuroendocrine alterations. Systematic investigations to examine the impact of monogenic obesity on thyroid function are sparse and sporadic. The present study was, therefore, carried out to assess a possible association between severe obesity and the thyroidal status in children with monogenic obesity due to loss-of-function mutations in leptin (*LEP*), leptin receptor (*LEPR*) or melanocortin 4 receptor (*MC4R*) genes.

MATERIALS AND METHODS

Study Subjects: This cross-sectional study is based on 40 children (22 males: 18 females), between the age of 0.5 to 8 years. Of these, 27 subjects with early onset severe obesity and a BMI SDS for age >3.5, were previously identified with homozygous loss-of-function mutations in *LEP* (n=12), *LEPR* (n=10) and *MC4R* (n=5) genes. Thirteen age-matched children with normal body weight and negative for mutations in the 3 obesity associated genes served as the control group. All study subjects were recruited from the Children's Hospital, Lahore and Fatima Memorial Hospital, Lahore, and were genetically screened at the Imperial College London, Department of Genomic and Common Disease for pathogenic mutations in *LEP*, *LEPR* and *MC4R* gene. The study was approved by the institutional ethical committees and written informed consent from parents/guardians was obtained in each case.

Study design: Following physical examination, medical history and anthropomorphic measurements in all children were recorded. The BMI SDS for age was calculated using the WHO software Anthro (version 3.2.2). Three to 4 ml of blood sample was drawn from the cubital vein and centrifuged at 4000 rpm for 10 min

for serum separation. The serum samples were aliquoted and stored at -80^{o} C until used for analysis.

Serum TSH, T3 and T4 levels were measured by an electrochemiluminescence immunoassay (ECLIA) with a Cobas e411 analyzer, Roche, Rotkreuz, Switzerland. Serum levels of leptin were measured by an enzyme linked immunosorbent assay (ELISA) using a commercially available kit (Labor Diagnostika Nord GmbH, Nordhorn, Germany).

Statistical analysis: All the statistical analyses were performed by using Statistical Package for Social Sciences (SSPS Version 20; Chicago, IL, USA). The significance of difference between groups was analyzed by Scheffe test and a *P* value of <0.05 was considered statistically significant.

RESULTS

The physical and biochemical characteristics of subjects enrolled in the study are summarized in Table 1. The mean age of LEP and LEPR deficient subjects was 1.2±0.3 and 1.8±0.4 years, respectively whereas children with *MC4R* mutation presented with obesity were at a somewhat later stage with a mean age of 6.5±0.5 years. The BMI SDS for age values were not statistically different among all the three groups of subjects with monogenic obesity and ranged from 7.6 to 9.3. The mean BMI SDS in the control group was 0.2±0.2 which was significantly different from those of the three mutant groups.

Remarkably, no statistically significant differences ($p<0.05$) were found in serum TSH and of thyroid hormones levels (T3 and T4) among all three groups of subjects with monogenic obesity from that of the control subjects (Table 1 and Figure 1). Further analysis of serum levels of TSH and thyroid hormones also did not show any marked deviation from reference values at individual basis. However, the median and third and fourth quartile values of TSH levels in children with leptin deficiency tended to be higher than control values (Figure 1). The serum leptin concentrations in LEP deficient children were ≤0.1 or non-detectable whereas levels in subjects with *LEPR* and *MC4R* mutations were 50.5±5.4 and 30.3±8.7 ng//ml, respectively and were significantly higher than the control values (3.7 ± 0.4 ng/ml) (Table 1; Figure 1). Serum leptin levels were markedly raised in LEPR deficiency compared to MC4R deficiency.

Table 1: Physical characteristics and hormone profile in children with mutations and controls

Characteristics	*LEP* deficient	*LEPR* deficient	*MC4R* deficient	Controls (Wild type)
N	12	10	5	13
M/F ratio	6/6	6/4	3/2	7/6
Age (Years)	1.2±0.3 [c, d]	1.8± 0.4 [c, d]	6.5±0.5 [a, b, d]	4.6±0.4 [a, b, c]
BMI SDS*	9.3±1.0[d]	7.6±1.0[d]	8.5±1.7[d]	0.3±0.3 [a, b, c]
TSH (uIU/ml)	2.5±0.4	1.8±0.3	2.7±0.6	2.2±0.2
T3 (ng/ml)	1.7±0.1	1.7±0.1	1.6±0.1	1.6±0.1
T4 (ug/dl)	8.2±0.6	7.2±0.6	8.9±0.8	9.3±0.5
Leptin (ng/ml)	0.1±0.0 [b, c]	50.5±5.4 [a, c, d]	30.3±8.7 [a, b, d]	3.7±0.4 [b, c]

*WHO Anthro, v 3.2.2; AnthroPlus, v 1.0.4; Values represent mean±SEM

Significant difference (Scheff's multiple t test; P <0.05):[a] *vsLEP*; [b] *vsLEPR*; [c] *vsMC4R*;[d]*vs*Controls

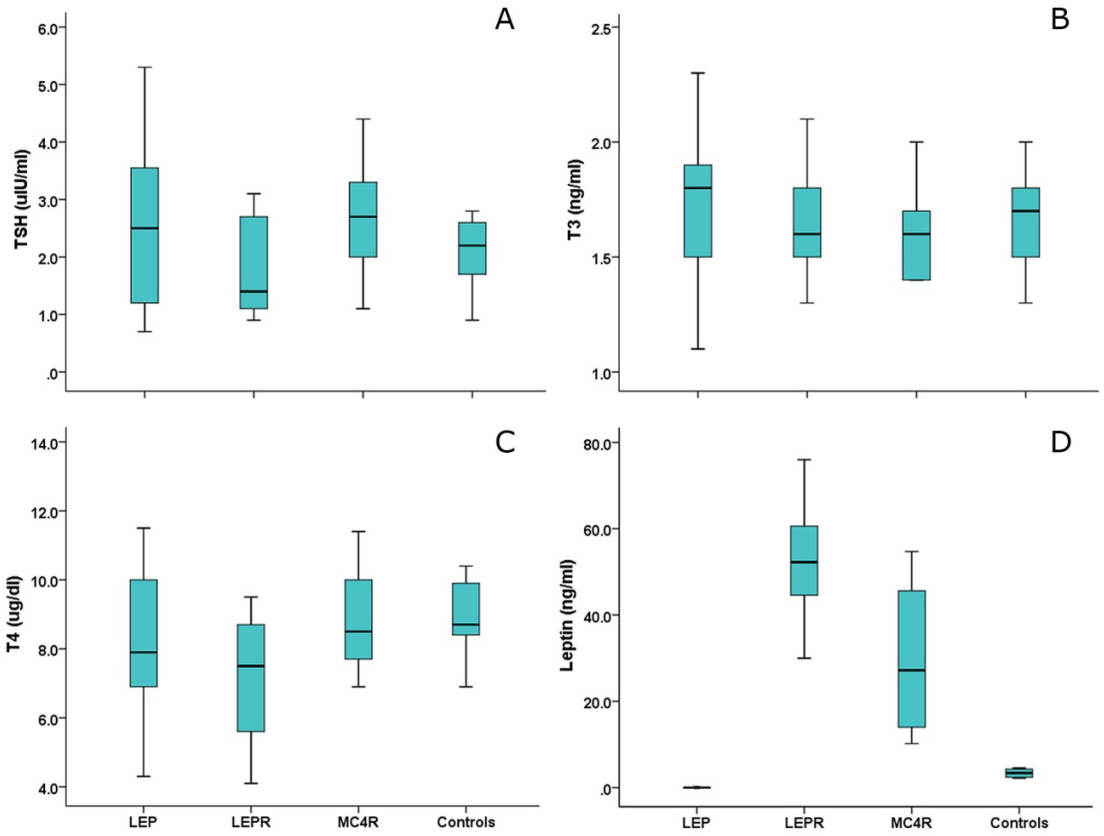

Fig 1 TSH (A), T3 (B), T4 (C) and Serum leptin (D) (mean±SEM) in mutants and controls

DISCUSSION

Obesity and thyroid dysfunction are among the most common health problems prevalent worldwide and frequently coexist in the same subject. Thyroid hormones regulate energy metabolism and may, therefore, play a significant role in control of food-intake and appetite [09]. Also, thyroid deficiency has been associated with increased fat accumulation due to a deficit in energy expenditure [10]. These considerations have prompted studies attempting to establish a link between obesity and abnormal thyroid function. Elevated TSH levels have frequently been reported in adults with obesity in the absence of autoimmune thyroiditis. Moderately elevated levels of TSH above the normal range in association with a normal free thyroxine (FT4) concentration, referred to as subclinical hypothyroidism, is frequently observed in adults with obesity who have no evidence of any other thyroidal disease such as autoimmune thyroiditis [4, 13]. However, the results from other studies present inconsistent results and fail to find an association between adiposity and TSH or thyroid hormones [10].

There have been fewer studies to assess thyroidal function in obese children. In the present study we did not find an association between extreme adiposity and serum concentrations of TSH, T3 or T4 in children between the age of 0.5 to 8 years. In a more recent study, Krause and colleagues have examined an association of BMI with thyroid function in a relatively large sample of children, 5-18 years old, with excessive obesity. Their findings indicated a positive correlation of TSH was with BMI and fat mass whereas FT4 had a negative correlation to adiposity. The authors suggest that leptin concentrations may partially explain obesity's effects on thyroid function most likely through its effects on TSH secretion [11]. Also a possible role of leptin in conversion of T4 to T3 has been indicated [13].

Earlier *in vivo* and *in vitro* studies in animal models provided evidence of a direct effect of adipocyte derived leptin on up-regulation of pro-TRH expression through stimulation of neurons of PVN [14]. Also food deprivation in laboratory mice has been shown to lead to a down-regulation of TRH expression and a decrease in T4 and T3 concentrations [15] whereas exogenously administered leptin restores the normal levels of thyroid hormones [16]. It has also been suggested that leptin may have a direct stimulatory effect on thyroid gland and on the release of thyroid hormones [17]. On the other hand, the relationship of obesity and the role of leptin in modulating thyroidal

function in the humans have remained controversial and conflicting results have been presented by different investigators [18]. In the present investigation we did not find any significant changes in thyroid function as a result of complete leptin deficiency. Our results corroborate some of the previous findings in congenitally leptin deficient children [19, 20]. In another study on leptin deficient subjects, leptin replacement resulted in a moderate increase in thyroid hormones but not in TSH levels [21]. Similar results were obtained in some other studies where leptin administration to hypoleptinemic subjects prompted an increase in T3 and T4 levels with no change in TSH secretion [17]. Together these results suggest that whereas leptin may have a marginal or permissive effect on thyroid hormone synthesis, it does not play a major and definitive role in regulation of the thyroidal axis. Also in this study disruption of the downstream leptin signaling due to MC4R deficiency, did not bring about a discernible change in TSH or thyroid hormone levels. Our observations are consistent with a previous study that reports normal TSH level in severely obese *MC4R* mutated subject [21].

In summary, we have failed to find a significant association between extreme adiposity due to leptin or MC4R deficient states, and the thyroid function, in children between the age of 0.5-8 years. The incongruent results obtained in some of the previous studies suggesting an association of obesity with thyroid function may be a secondary outcome of other neuroendocrine or metabolic changes accompanying obesity, and/or possibly due to differences in age, ethnicity and environmental conditions. Whether leptin has a permissive effect on thyroidal axis in normal or leptin deficient subjects, has yet to be ascertained.

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