Author’s response to reviews

Title: Leptin, insulin and thyroid hormones in a cohort of Egyptian obese Down syndrome children: a comparative study

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Author's response to reviews: see over
Response to Reviewer’s comments
Reviewer: Kate Steinbeck
How pubertal development was determined? It was assessed by direct observation according to Tanner & Whitehouse staging (1976).

Major compulsory revisions:
1- Title has been changed into "Leptin, insulin and thyroid hormones in a cohort of Egyptian Down syndrome children: a comparative study"
2- The aim of the work is changed into: The current study was designed to clarify differences in some obesity-related hormones in a group of prepubertal Down syndrome children.
3- DISCUSSION: a) we did not mean or conclude that" Since leptin is not generally considered to have a major role in the pathogenesis of obesity, except in rare genetic deficiency or resistance states", but we concluded" increased leptin secretion, a marker of leptin resistance in obese children irrespective the cause of obesity (genetic or simple)", so we presumed the absence of genetic basis (extra-copy of 21 chromosome) for more severe leptin resistance in DS.
b) It was previously reported that BMI was the best predictor of insulin sensitivity in normal pubertal boys (Guercio et al., 2002) also, high BMI levels have consistently been found to be associated with cardiovascular disease risk factors such as insulin resistance, dyslipidemia, and increased blood pressure (Freedman & Sherry 2009). Other analyses indicate that BMI levels among children are as strongly correlated with various cardiovascular disease risk factors (lipid and insulin levels, blood pressure), as is the sum of the subscapular and triceps skin fold thicknesses (Freedman et al., 2009). In addition Flegal et al., (2010) concluded that current BMI cutoffs can identify a high prevalence of high adiposity in children with high BMI-for-age and a low prevalence of high adiposity in children with normal BMI-for-age but less than one-half of children with intermediate BMIs-for-age (85th to, 95th percentile) have high adiposity. A BMI for age at ≥95th CDC percentile has moderately high (70%–80%) sensitivity and positive predictive value, along with high specificity (95%), for identifying children with excess body fatness (Freedman & Sherry 2009). It correlates well with DEXA, with a true positive rate of 0.7 – 0.8 and a false positive rate of 0.1 or less (Lobstein et al., 2004). It is simple and more easily obtained than skin fold thickness measurement. The presence of standardized BMI curves and specified cut-off points can offer an opportunity to monitor the degree of obesity from childhood through adolescence and to define overweight and obesity in childhood (Husain 2003). However no internationally accepted cut points for waist circumference for either the classification of
obesity or presence of increased metabolic risk for children as in adult (Steinbeck 2004; Krebs et al., 2007), furthermore, no data on population with DS can be found on this regard (Gonzalez-Aguero et al., 2011). Although **Skin fold thicknesses** have often been considered an attractive, noninvasive tool for estimating the amount of subcutaneous fat (Freedman & Sherry 2009) previous results, suggest that skin fold measurements may only slightly improve (8%) the estimation of body fatness among children who are obese (BMI ≥95th CDC percentile) (Freedman et al., 2007). Furthermore it may help determine if an overweight child may have excess body fatness (Himes & Dietz 1994; Freedman & Sherry, 2009) and this group of children was not included in our study.

Also, there is no agreement about cutoff points for the percentage of body fat that constitutes obesity in children (WHO 1995; Freedman & Sherry 2009; Flegal et al., 2010). Body fat itself is not necessarily a precise measure of health risk. As stated by the recent AMA expert committee (Barlow 2007), “High levels of body fat are associated with increasing health risks. However, no single body fat value, whether measured as fat mass or as percentage of body weight, clearly distinguishes health from disease or risk of disease. Even if the body fat amount could be measured easily, other factors, such as fat distribution, genetics, and fitness, contribute to the health assessment.”

c- Elevated CK value in DS was previously reported (Hoorn et al., 2005) in a case report of an 18-year-old female with DS who presented with a hyperglycaemic coma that was complicated by hyponatraemia and rhabdomyolysis with marked elevation (46,390 U/L) in CK. Another relevant explanation for mildly elevated CK values in DS groups apart from hypothyroid related myopathy are infectious myopathy secondary to viral infection (kumar et al 2011), strenuous physical activity, racial variation (Gledhill et al., 1988).

d- Limitations were added.

**Minor revisions:**
All were considered especially revision of median and range values of FT.
Reviewer: **Alejandro Gonzalez-Aguero.**

**Major compulsory revisions:**

**Subjects & methods:**
1. Anthropometric data were collected as part of an ongoing research program ascertaining standing height to the nearest 0.1 cm in bare feet using portable Harpender stadiometer and weight to the nearest 0.1 kg using digital weight scale. The measurements were repeated twice and the average was recorded.

1. Statistical section: Kruskal Wallis was used for comparison between four groups while, Mann Whitney was used for comparison between each of the two groups.
2. We performed Spearman's correlation not Pearson's, it was a typing mistake.

**Results:**
1. Results are revised and modified and the last paragraph was deleted.
2. Reference range means those reported in the literature for children of the same age.
3. The last paragraph was deleted.

**DISCUSSION:** Were modified, we added the main findings, subheadings, limitations of our study. We tried to clarify the mysterious points.

**Minor essential revisions:**
All were considered including:

1. Trisomy 21 is the most common cause of DS, this is why we mentioned it in the background.
4. The sentence was referenced (Cernovich et al., 2008).
11. Leptin resistance index is one of the laboratory parameters performed in our study so it is a part of subjects & methods.

**Discretionary revisions:**
References were updated.
Reviewer: Véronique A. Bricout.

General comments:
2-Methods: laboratory methods were prescribed in subjects and methods, anthropometric measurements were added and methods for statistical analysis were revised and corrected.
7-Flore et al., (2008) reported mean values for HOMA-IR (1.09±0.16) in a group of non obese (BMI:23.3 ± 0.6) young adults (mean age22 ±1 years) while our study reported median and range for HOMA-IR in prepubertal obese and non obese children DS – trisomy 21 evidenced by cytogenic study. However we refer to this valuable work in the discussion of our assumption of liability to IR in obese DS children.

Major compulsory revisions:

Background:
I- Our background was brief but relevant to the studied parameters however, we added hints about incapacity to do physical exercise as well as dietary habits among DS children to fulfill the potential risk factors for obesity. Regarding chromosomal abnormalities we choose trisomy 21 DS children evidenced by cytogenic studying and we exclude translocation and mosaicism.
2- Alteration of catecholamine secretion in these endocrine disorders in DS children and their effect on glucose metabolism. A negative feedback loop exist between leptin and catecholamines as a part of leptin regulatory mechanism (Ren, 2004), while in obese individuals there is hyper-leptienemia and ‘selective leptin resistance’ that posits, persistence of certain actions of leptin (sympathoexcitatory actions) and resistance to others (metabolic actions) (Martin etal., 2008), the activation of sympathetic nervous system adrenergic effects on the heart, blood vessels and kidney promotes the development of hypertension. All these factors contribute highly to rise cardiovascular risk factors (Bricout et al., 2008), this necessitates a longitudinal study to follow up those patients from childhood to adulthood period. So our preliminary study cannot fulfill all these frontier ideas about hormonal profile including catecholamines and their effects on glucose metabolism, leptin receptors in DS children.

Subjects and methods:
One of our study limitations is that we relied solely on BMI as an indicator of body fatness however the underlying assumptions for using BMI to
assess adiposity is that height is correlated with the body fatness of children (Freedman et al., 2004) and the higher BMIs of taller children correctly identify their increased fatness. Furthermore, the correlation between childhood BMI and body fatness is close to the maximum that is possible for any power index (Mei et al., 2002; Freedman & Sherry, 2009). The utility of BMI in measuring adiposity was assessed by Flegal et al., (2010) who concluded that "Current BMI cutoffs can identify prevalence of adiposity in children with high and normal BMI-for-age but, less than one-half of children with intermediate BMIs-for-age (85th to, 95th percentile) have high adiposity". Freedman & Sherry (2009) determined the sensitivity, positive predictive value (70%–80%), and specificity (95%) of BMI for age at ≥95th CD Percentile, for identifying children with excess body fatness. It also correlates well with DEXA, with a true positive rate of 0.7 – 0.8 and a false positive rate of 0.1 or less (Lobstein et al., 2004). Furthermore, other analyses indicated that BMI levels among children are as strongly correlated with various cardiovascular disease risk factors (lipid and insulin levels, blood pressure), as is the sum of the subscapular and triceps skin fold thicknesses (Freedman et al., 2009). It is also simple and more easily obtained than skin fold thickness measurement. The presence of standardized BMI curves and specified cut-off points can offer an opportunity to monitor the degree of obesity from childhood through adolescence and to define overweight and obesity in childhood (Al Husain, 2003).

Although Skin fold-thicknesses have often been considered an attractive, noninvasive tool for estimating the amount of subcutaneous fat (Freedman & Sherry, 2009) previous results, suggest that skin fold measurements may only slightly improve (8%) the estimation of body fatness among children who are obese (BMI ≥95th CDC percentile) (Freedman et al., 2007). Furthermore it may help determine if an overweight child may have excess body fatness (Himes & Dietz, 1994; Freedman & Sherry, 2009) fortunately this group of children was not included in our study. Due to racial variation and poor inter- and intra-observer reliability skin fold thickness equations should be validated in each population and it is difficult to standardize (Freedman & Sherry, 2009).

While for waist circumference, no internationally accepted cut points for either the classification of obesity or presence of increased metabolic risk in children as in adult (Steinbeck, 2004), furthermore, no data on population with DS can be found on this regard (Gonzalez-Aguero et al., 2011). Also, there is no agreement about cutoff points for the percentage of body fat that constitutes obesity in children (WHO, 1995; Freedman & Sherry, 2009; Flegal et al., 2010). Body fat itself is not necessarily a precise measure of health risk.
As stated by the recent AMA expert committee (Barlow 2007), “High levels of body fat are associated with increasing health risks. However, no single body fat value, whether measured as fat mass or as percentage of body weight, clearly distinguishes health from disease or risk of disease. Even if the body fat amount could be measured easily, other factors, such as fat distribution, genetics, and fitness, contribute to the health assessment.”

Finally although bioelectric impedance analysis (BIA) is non invasive and has a high repeatability it is not a true direct method since the measurement is obtained through statistical equations which is equipment and population specific (NIH 1994).

Minor revisions:
1-We added some information on life-style and diet in subjects &methods. The DS children did not practice particular type of sport however; they were enrolled in a physical therapy program to help their motor skills development. Children who were taking drugs that may induce weight gain were excluded.
2-Regarding CK, our study observed elevated CK values (> 90 IU/L) in 7 out of 23(30.4%) cases of OD group and one case (130 U/L) in NOD, four of them (95,123,193, 130U/L) could be attributed to hypothyroidism related myopathy, another explanation for such mild elevation are infectious myopathy secondary to viral infection (Kumar et al., 2011) strenuous physical activity (Totsuka et al., 2002), racial variations (Gledhill et al., 1988). In addition, muscle breakdown should not be ignored. Genetic causes as a reason for exaggerated increase in CK levels in response to exercise in DS children could be a subject for further study.

Thanks for reviewers for their helpful comments. We hope that our answers and modifications be informative and accepted.

On behalf of authors:

DR/ Amany El-Hawary