CIRCADIAN RHYTHM OF SALIVARY CORTISOL IN OBESE CHILDREN WITH CLINICAL SIGNS OF HYPERCORTISOLISM

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INTRODUCTION

- The increased prevalence of childhood obesity (OB) is nowadays associated to a greater referral of obese children to investigate endocrine diseases causing or contributing to OB [1].
- Weight gain, associated to delayed growth, is the classical presentation of true hypercortisolism (HF) in children that are still growing [2]. In older children, the clinical features may overlap with those observed in exogenous OB [3].
- The loss of the cortisol (F) nocturnal nadir constitutes the biochemical hallmark of HF regardless of its cause. Late night salivary F is easy to perform in children, however, the cut-off of 4 nmol/L, suggestive of Cushing Syndrome (CS), was obtained in adults [3,4].
- The circadian rhythm (CR) of salivary cortisol may provide useful information on HF in obese children with clinical signs of F overproduction.

AIMS

1- To describe possible disturbances in the circadian rhythm of salivary cortisol in obese children with clinical signs of hypercortisolism.

2- To evaluate the 99th centile of SAF at night obtained in obese children as a potential cut-off for assessment of hypercortisolism states in pediatric settings.

SUBJECTS & METHODS

Design: Cross-sectional, age-matched, observational, prospective study.

Obese (OB) Inclusion criteria: BMI≥ 97th centile and clinical signs suggestive of HF:

- Concomitant weight gain and delayed growth or,
- Obesity associated to ≥2 of the following:

moon face, red striae, headache, hypertension, hypertricosis/hirsutism, insulin resistance, acanthosis nigricans, ovarian dysfunction, dyslipidemia.

Exclusion criteria: syndromic obesity, iatrogenic hypercortisolism.

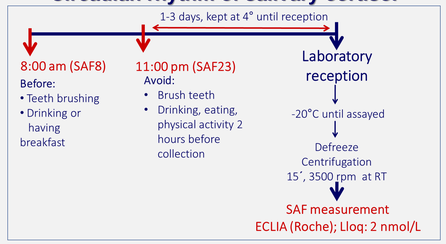
- Clinical follow up of at least 2 years and appropriate biochemical tests.

Cushing Syndrome (CS) diagnosis was based on clinical signs, biochemical tests and MRI [3].

Normal weight (NW) Inclusion criteria: 10th<BMI <85th centile, absence of acute or known chronic disorders at the time of the study.

Elimination criteria for both groups: hematic, incomplete or insufficient sampling, erroneous time collection, corticoid therapy or medication that may influence cortisol secretion.

Circadian rhythm of salivary cortisol



Absolute Change of SAF at night

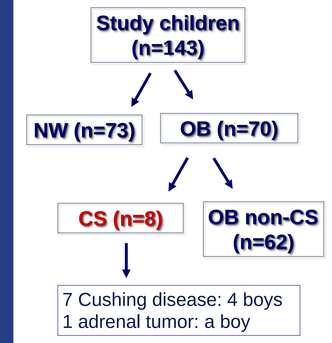
Ach(nmol/L)= (SAF8 – SAF23)

Relative Change of SAF at night:

RCh(%)= [(SAF8 – SAF23) / SAF8] x 100

Written consent and assent were obtained.
The study was approved by the local Institutional Review Board of Dr. Ricardo Gutiérrez Children's Hospital.

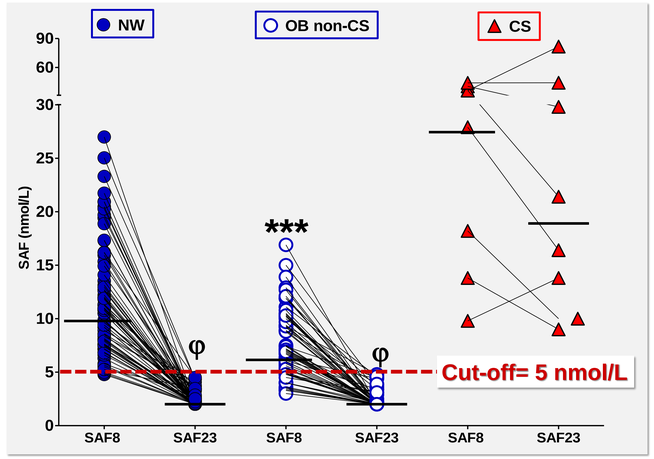
RESULTS



Clinical features of OB group

OB group	%
Weight gain+growth delay	12.9
Moon face	28.5
Red striae	14.3
Headache	6.9
Hypertension	19
Hypertricosis/hirsutism	39.7
Insulin resistance	60.3
Acanthosis nigricans	65.1
Ovarian disfunction	17.4
Dyslipidemia	80

Figure 1: Circadian Rythm of Salivary Cortisol



*** *p< 0.0001 versus NW (t-test);*

φ *p< 0.0001 versus SAF8 within each NW or OB non-CS (paired t-test)*

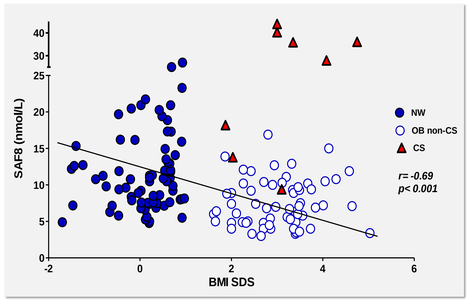
- SAF23 decreased significantly in NW and OB non-CS, *p< 0.0001*.
- A nocturnal decrease in SAF of at least 53% was observed in NW, *table 2*.
- SAF8, ACh and the RCh in OB non-CS were significantly lower than in NW, *table 2*.
- SAF23 in CS was always >99th centile cut-off obtained in OB non-CS (**5 nmol/L**, *dotted red horizontal line in figure 1*).

Table 2: Auxological and biochemical features of the study groups

	NW (n= 73)	OB non-CS (n= 62)	CS (n= 8)
Gender (F/M)	43/30	51/11	3/5
CA (years)	10.4 (2.2 – 18.0)	11.9 (2.5 - 18.0)	14.1 (7.8 - 16.9)
BMI-SDS	0.27 (-1.80 – 0.97)	1.88 (1.09 – 4.64)***	3.00 (1.69 – 4.29)
SAF8 (nmol /L)	**11.0 (5.1 – 24.7)**	**6.9 (3.3 – 15.1)*** **	31.9 (9.8 – 43.9)
SAF23 (nmol /L)	2.0 (2.0 – 4.3)	2.0 (2.0 – 4.6)	18.9 (9.0 – 81.7)
ACh (nmol/L)	**8.1 (2.8 – 20.4)**	**4.6 (1.3 – 12.2) *** **	10.5 (4.7 – 14.4) Δ
RCh (%)	**78 (53 – 91)**	**66 (31 – 86) *** **	41 (26 – 49)Δ

Data are expressed as the median (3rd - 97th range); *** *p< 0.0001 versus NW (t-test);* Δ *results in 4/8 CS patients. CA: Chronological age; F: females; M: males*

Figure 2: SAF8 versus BMI



- BMI-SDS was the strongest independent variable associated to lower SAF8 *(multiple regression study, figure 2).*
- Differences in SAF8, ACh and RCh between NW and OB non-CS were maintained after BMI-SDS adjustment *(p< 0.001)*.

A significant proportion of OB non-CS (17/62) presented RCh<53% *(3rd centile in NW)* as compare to NW (1/73), *Fisher t test p< 0.001.*

CONCLUSIONS

- The circadian rythm of cortisol was preserved in NW and obese children with clinical signs of hypercortisolism *(in whom hypothalamic-pituitary-adrenal axis disorders were ruled-out)*, by using simple and non-invasive saliva samples at 8 and 23 hs under standardized procedures.
- SAF23 cut-off of 5.0 nmol/L by ECLIA method is in range accordance with the one recommended threshold of Endocrine Society Guidelines (4 nmol/L) that was obtained by gold standard tandem mass spectrometry [3,4].
- In children with Cushing Syndrome, SAF23 was always higher than our proposed cut-off value that could be indicative of hypercortisolism in pediatrics and should be further confirmed according to clinical, biochemical and images studies.
- Obesity is a stressor and therefore, it may influence circadian rhythms. Lower SAF8 in obese could not be exclusively explained by increasing BMI. SAF reflects the biologically active free F fraction of cortisol and therefore, SAF8 differences between NW and OB non-CS groups are not associated to binding proteins status. The abnormal lipid profile observed in 80% of our obese non-CS cohort may suggest that other biomarkers of chronic inflammation could also induce changes in cortisol secretion, metabolism and/or excretion.

REFERENCES

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