A dried whole blood spot thyroglobulin assay for use in monitoring iodine deficiency

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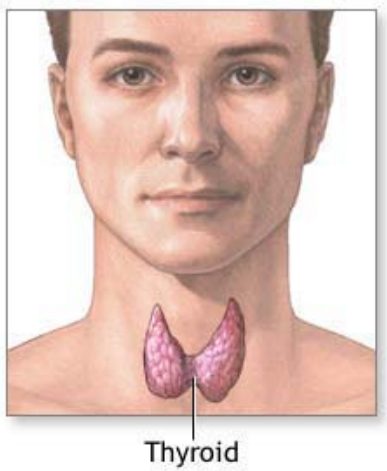
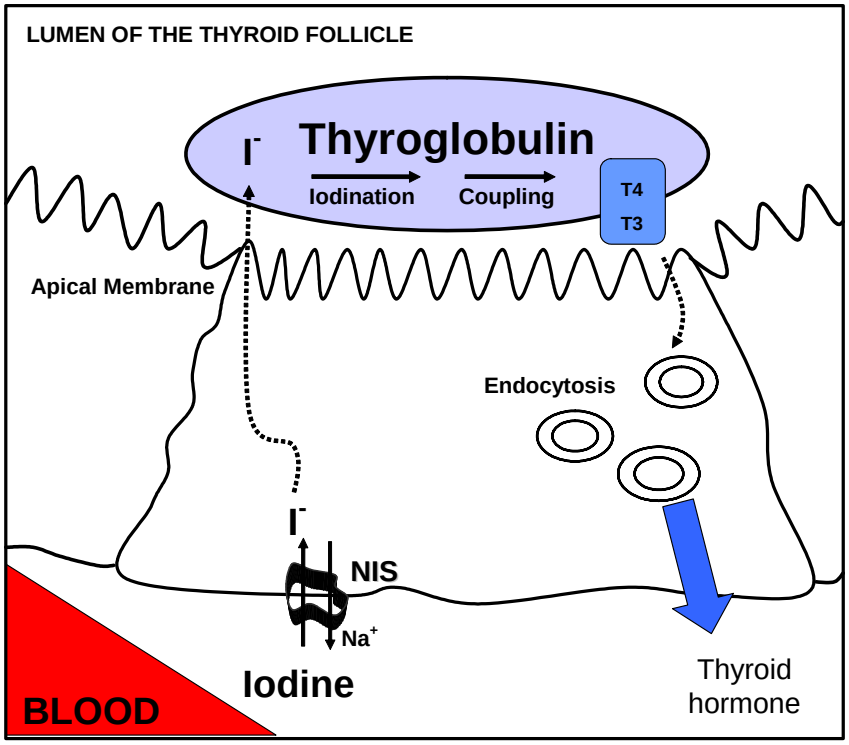
Background

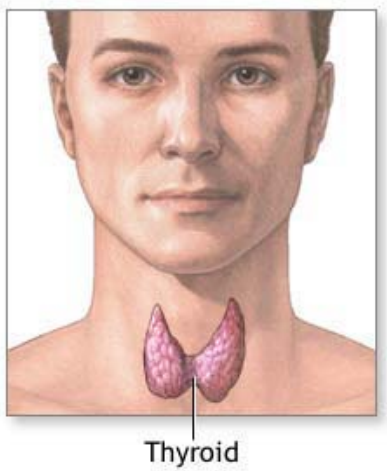
- Limitations of available indicators of iodized salt impact:
  - Urinary iodine: a sensitive indicator of recent intake, but not thyroid function
  - Goiter: a poor indicator for mos/yrs, as thyroid size decreases only slowly after iodization
  - TSH: sensitive only in the newborn period
- An indicator of thyroid status, sensitive to recent changes in iodine intake, would be valuable

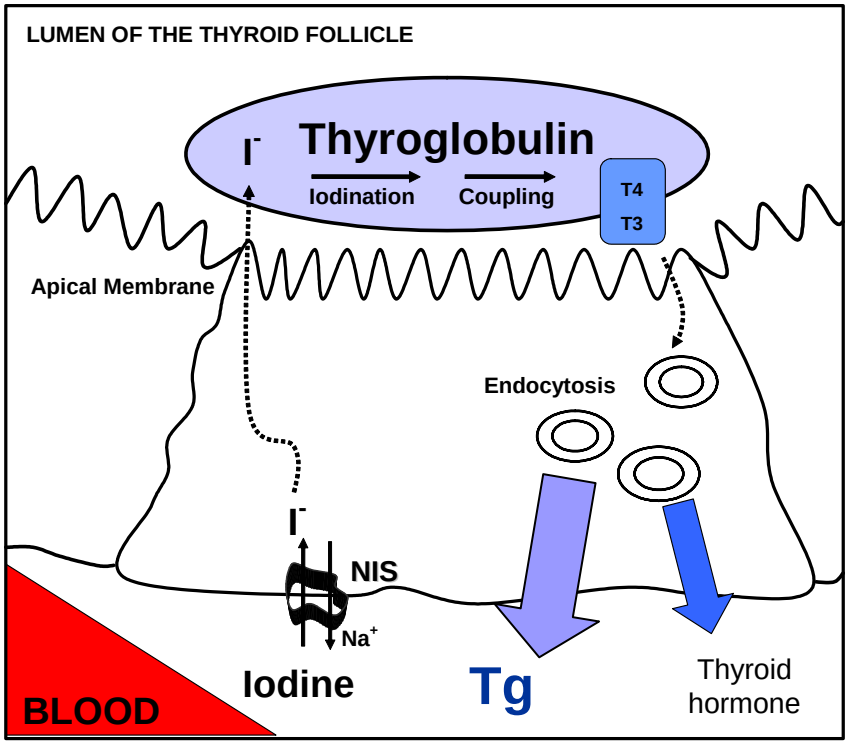
- Thyroglobulin (Tg)
  - Tg, a thyroid-specific glycoprotein precursor to thyroid hormone





- Thyroglobulin (Tg)
  - Tg, a thyroid-specific glycoprotein precursor to thyroid hormone
  - In iodine deficiency, transcytosis of Tg-containing endosomes results in Tg release into circulation





- Thyroglobulin (Tg)
  - In areas of IDD and endemic goiter, elevated serum Tg reflects TSH hyperstimulation and thyroid hyperplasia
  - Tg may be a sensitive marker of thyroid dysfunction in areas of endemic goiter

However….

- Commercially-available assays measure Tg in serum; require venipuncture, centrifuge and frozen transport, difficult in remote areas

- Requires an international reference range and standardized reference material

Objectives

- optimize and validate a **Tg assay on dried whole blood spots (DBS)**
- develop a reference standard
- establish an international reference range for DBS-Tg in iodine-sufficient children
- test the standardized assay and reference range in an intervention trial to evaluate Tg as indicator of response to iodized salt

DBS Thyroglobulin

- A 2-site DELFIA (dissociation enhanced lanthanide fluorescent immunoassay) for serum Tg was adapted for use on blood spots and validated in Swiss children

DBS Tg – Assay Performance

DBS Tg assay

- Median CV in controls (n=36) 6.3 %

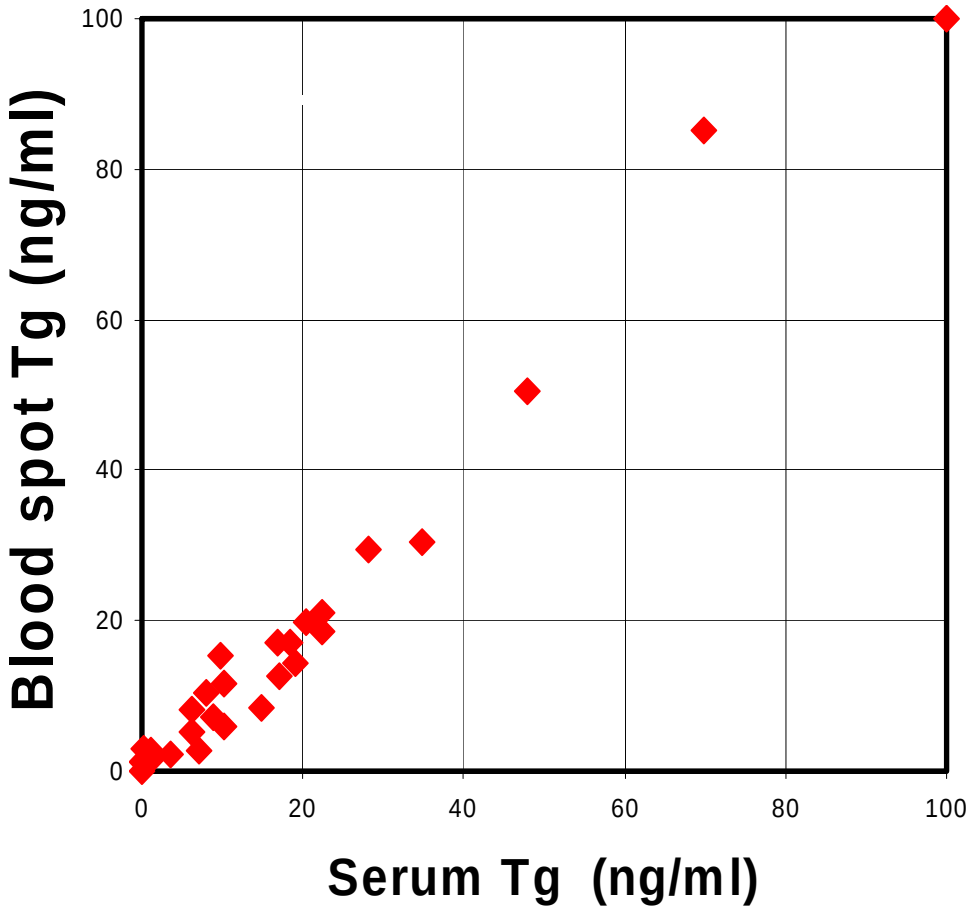
- Median CV in duplicate samples (n=489) 14.4 %
  - < 10 µg/l 16.7 %
  - >10 µg/l 13.4 %

- Minimal detectable concentration 1.42 µg/l

- Agreement of serum and DBS Tg assays
  - Pearson correlation coefficient r = 0.98
  - 95% CI for the lower limit -8 µg/l
  - 95% CI for the upper limit 11.9 µg/l

Zimmermann et al. Am J Clin Nutr 2003

DBS vs serum Tg assay



Tg in Swiss children (n=29) without known thyroid disease and with negative Tg-Ab

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DBS-Tg reference standard

- Serum Tg reference material of the European Community Bureau of Reference (CRM-457) was adapted for DBS and its stability tested over one year
- Stability of the CRM-457 Tg reference standard on DBS over 1 y of storage at -20° and -50°C was acceptable
- Reference material for DBS-Tg now available from Zürich, and soon from WHO

DBS-Tg international reference range

- DBS-Tg was determined in an international sample of 5-14 y-old children (n=700)
  - euthyroid
  - anti Tg antibody-negative
  - residing in areas of long-term iodine sufficiency
  - five major ethnic groups: African, Asian, Arabic, Caucasian, Hispanic

Reference range: sample characteristics

Site	n	Age (y)	M/F ratio	Urinary iodine (μg/L)
Bahrain	142	10.1 (5.9-14.1)	1.03	177 (43-701)
Peru	125	10.0 (5.0-12.0)	1.66	161 (15-860)
South Africa	127	9.7 (6.0-13.1)	1.02	266 (38-758)
China	230	9.0 (6.0-12.0)	1.05	234 (0-672)
Switzerland	86	10.0 (7.0-14.0)	1.53	130 (6-390)
Total	**710**	**9.6 (5.0-14.1)**	**1.18**	**198 (0-860)**

Zimmermann et al. J Clin Endocrinol Metab Dec 2006

Reference range: thyroid function and Abs

Site	n	TSH (mU/L)	TT4 (pmol/L)	Tg-Ab (U/mL)
Bahrain	142	1.1 (0.3-3.6)	102±17	0.3 (0.3-6.1)
Peru	125	1.0 (0.5-3.4)	97±16	0.3 (0.3-7.8)
South Africa	127	0.9 (0.5-2.8)	108±22	0.3 (0.3-2.9)
China	230	1.4 (0.4-3.7)	114±19	0.3 (0.3-9.0)
Switzerland	86	0.6 (0.2-1.2)	90±22	0.3 (0.3-7.3)
Total	**710**	**1.1 (0.2-3.7)**	**105±21**	**0.3 (0.3-9.0)**

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Reference range: DBS-Tg results

	DBS-Tg (µg/L)	
Site	**Median (25%, 75%)**	**97%**
Bahrain	19.3 (12.6, 27.6)	48.9
Peru	11.6 (7.0, 19.2)	36.1
South Africa	18.4 (13.2, 26.0)	40.7
China	13.3 (8.9, 21.2)	35.6
Switzerland	11.2 (7.0, 15.9)	24.8
Total	**14.5 (9.4, 22.7)**	**40.2**

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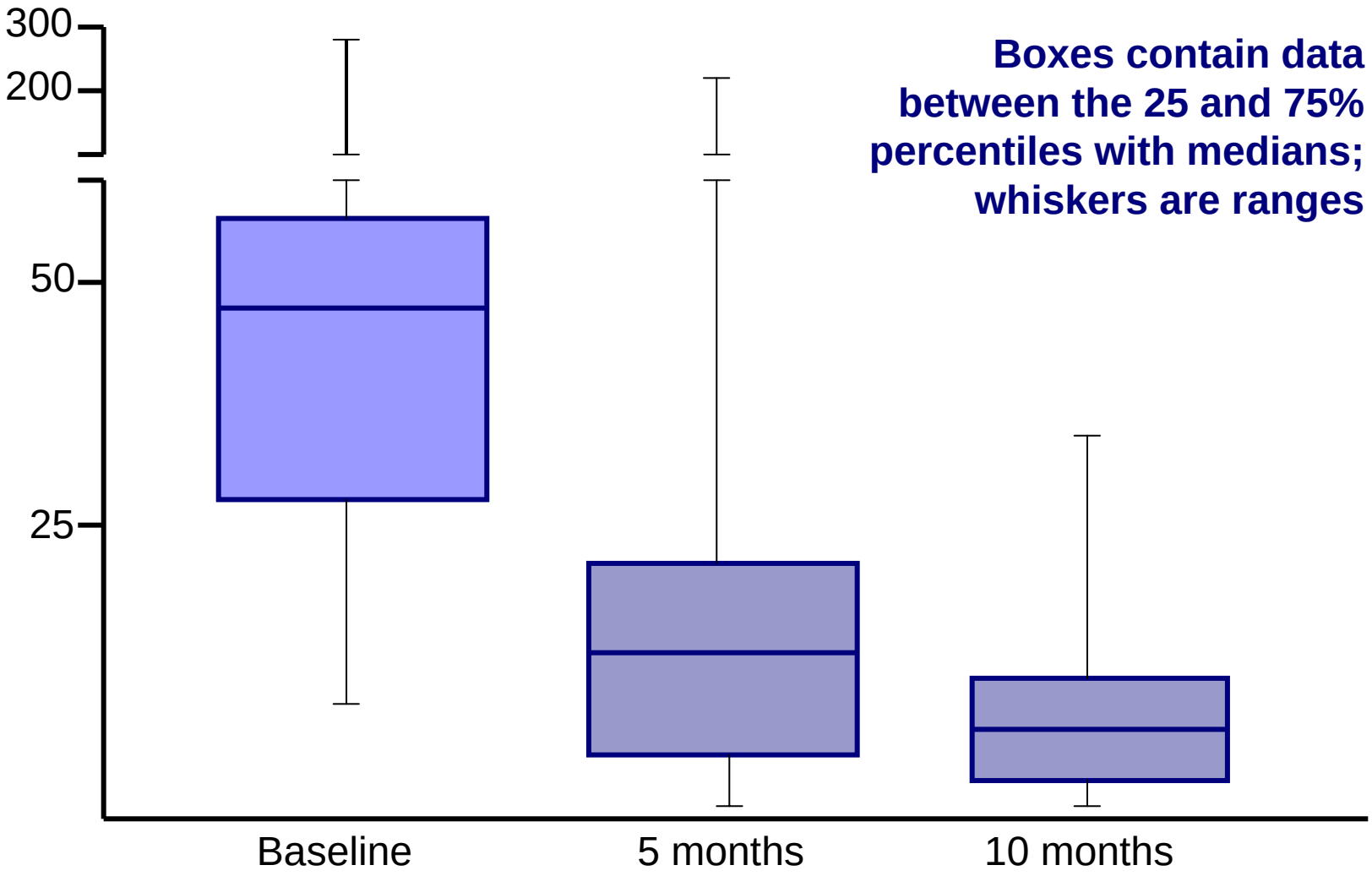
Intervention trial: DBS-Tg in children

- 10 month, prospective study in goitrous 6-15 yr-old Moroccan children

- DBS assay used to measure Tg before and after introduction of iodized salt

- UI, thyroid volume, TSH, TT4 measured and regression with Tg as dependent variable

Intervention trial: DBS-Tg in children



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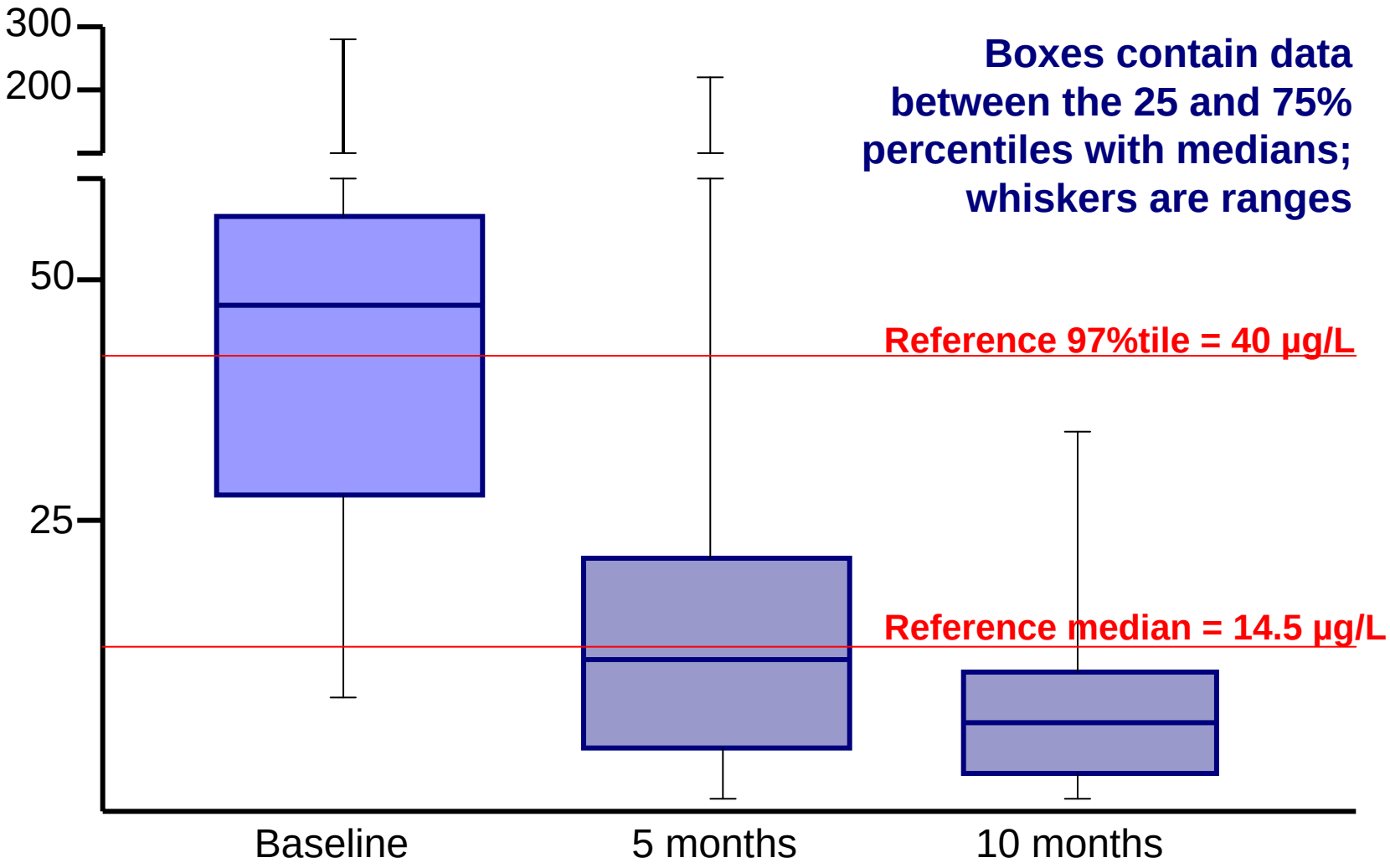
Intervention trial: DBS-Tg in children



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Correlations: DBS-Tg and other IDD indicators

	DBS Tg (µg/l)		
	Baseline	5 mos	10 mos
TSH (mU/l)	0.32[1]	0.08	0.09
TT4 (nmol/l)	-0.28[2]	-0.12	-0.10
UI (µg/l)	-0.41[1]	-0.19[2]	-0.16
Thy vol (ml)	0.47[1]	0.18[2]	0.19

[1] $p<0.001$; [2] $p<0.05$

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Conclusions

- Tg assay on DBS facilitates use for IDD monitoring
- Reference standard now available
- International reference range: **4-40 µg/L**; median **14.5 µg/L**

- **DBS-Tg**
  - recommended by WHO
  - short-term indicator of thyroid function, normalizes in several months after iodine repletion
  - complements use of UI to measure recent iodine intake and Tvol to assess long-term response

Co-workers

- Zürich
  - D Moretti, C Zeder, R Hurrell, T Torresani, L Molinari
- Bahrain
  - K Moosa, ZS Al-Dallal
- Peru
  - E Pretell, S Corigliano
- South Africa
  - P Jooste
- Morocco
  - N Chaouki
- China
  - Y Wei, ZP Chen

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MF Tuesday Poster Session
Posters on dried blood spot methods
T48-T50: Vitamin A status
T51: Erhardt et al.
VA, Fe, Infectious Disease Status