Supplementary Data

THYROSIM App for Education and Research Predicts Potential Health Risks of Over-The-Counter (OTC) Thyroid Supplements (April 2016 issue)

Han, Eisenberg, Larsen, DiStefano III

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I. Software Particulars

Web Client-Server Implementation and Interactions

The THYROSIM implementation is different than software applications that run independently on a single computer or device. Different components of the web application are distributed over two computers: the client-user machine – via the client browser – and on a remotely located server accessible only via the client browser. The actual simulation computations are run on the remote server. Interactions among the different open-source application software components listed in Table S1 are summarized in the flowchart in Figure S1, illustrating what runs where and how components communicate.

Online Code Repository

In keeping with the spirit of reproducible research and open source software, the complete codebase of THYROSIM is available to students, researchers, clinicians or any other interested party in an online code repository accessible here: https://bitbucket.org/DistefanoLab/thyrosim/. This provides users the opportunity for working with, modifying or extending this simulation model on their own computers. The basic model – without the web interface – is included in the repository in addition to the complete codebase, in the form of stand-alone Octave and Matlab scripts, as well as in SBML[1] (Systems Biology Markup Language) format under the "resource" folder. SBML is a portable programming format for representing computational models in a common language (1). This model can be read and run using many popular software programs, including Matlab (2) and COPASI (3).

II. Additional Model Validations Noted in Results

Figure S2 includes two simulated experiments from data in figs. 2 and 3b in LeBoff *et al*. (4). (See the Results section.) The data (not shown in fig. S2) are tracked very well by these simulations.

The *green* simulation curves follow a simulated 75 μg T3 dose at time zero. The *blue* simulation curves follow a simulated combination dose of 75 μg T3 + 275 μg T4 measured in the five grain thyroglobulin dose given the subjects described in (4).

Figure S3 includes simulated responses to two combined doses of T3 and T4 given to subjects in Snyder and Utiger (5). Simulated responses are from the 1st to 28th day of daily dosing treatment. Responses are about the same each day after several days, both shown in fig. 2 in (5). Peak values reached are about the same as in the figure data.

The *blue* curves represent responses to 15 μg T3 + 60 μg T4 daily, and the *green* curves represent responses to 30 μg T3 + 120 μg T4 daily, all over 28 days.

Figure S4 depicts the simulated responses that very closely track the seven-day dosing studies and measurement data shown in fig. 2 of Wenzel *et al*. (6). The original figure is also reproduced in Figure S5.

III. Model Equations and Parameters

See pages 7-9 in Supplementary Data.

Equation and Parameter Notes

(1) $u_1(t)$ and $u_4(t)$ in the first and fourth equations are exogenous IV inputs of T4 and T3. They are normally zero, but are needed for structural identifiability analysis and IV simulation studies.
(2) Parameters k_{03} and k_{06} are shown in red in the equations. They are assumed to be approximately zero in adult and children models: degradation (other than to T3) and elimination of T4 in slow compartments (mostly muscle) are assumed negligible compared to T4 conversion to T3.
(3) ICs on the ODEs are not included here. To get ICs, run the model to steady state with normal SR_3 and SR_4 values and use the results to set ICs and the first $\tau = 8$ h results for representing the 8-h delay in the loop. For parameter estimation, the ICs are functions of the parameters to be estimated. These need to be evaluated and used as constraints on the search.

IV. References

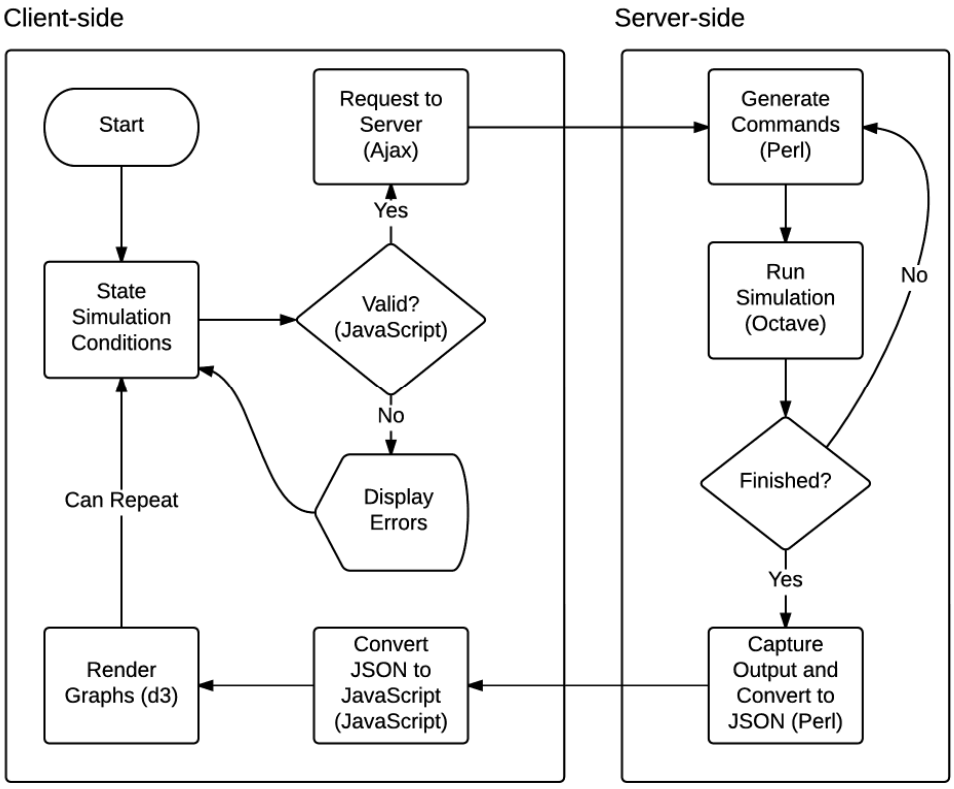
1. sbml.org/Basic_Introduction_to_SBML (accessed June 26, 2013).
2. Matlab 2012 MATLAB and Statistics Toolbox Release 2012b. The MathWorks, Inc., Natick, MA.
3. Hoops S, Sahle S, Gauges R, Lee C, Pahle J, Simus N, Singhal M, Xu L, Mendes P, Kummer U 2006 COPASI – a COmplex PAthway SImulator. Bioinformatics **22**:3067-3074.
4. LeBoff MS, Kaplan MM, Silva JE, Larsen PR 1982 Bioavailability of thyroid hormones from oral replacement preparations. Metabolism **31**:900-905.
5. Snyder PJ, Utiger RD 1972 Inhibition of thyrotropin response to thyrotropin-releasing hormone by small quantities of thyroid hormones. J Clin Invest **51**:2077-2084.
6. Wenzel KW, Meinhold H 1974 Evidence of lower toxicity during thyroxine suppression after a single 3-mg L-thyroxine dose: comparison to the classical L-triiodothyronine test for thyroid suppressibility. J Clin Endocrinol Metab **38**:902-905.

[1] Drs. Sarah Keating (European Bioinformatics Institute, Hinxton, Cambridgeshire, UK) and Mike Hucka (Computing and Mathematical Sciences, California Institute of Technology, Pasadena, CA) assisted with translating the Octave ODE solver code into SBML, so the THYROSIM model code also can be distributed fully in a universal format. For SBML details, see http://sbml.org/Basic_Introduction_to_SBML.

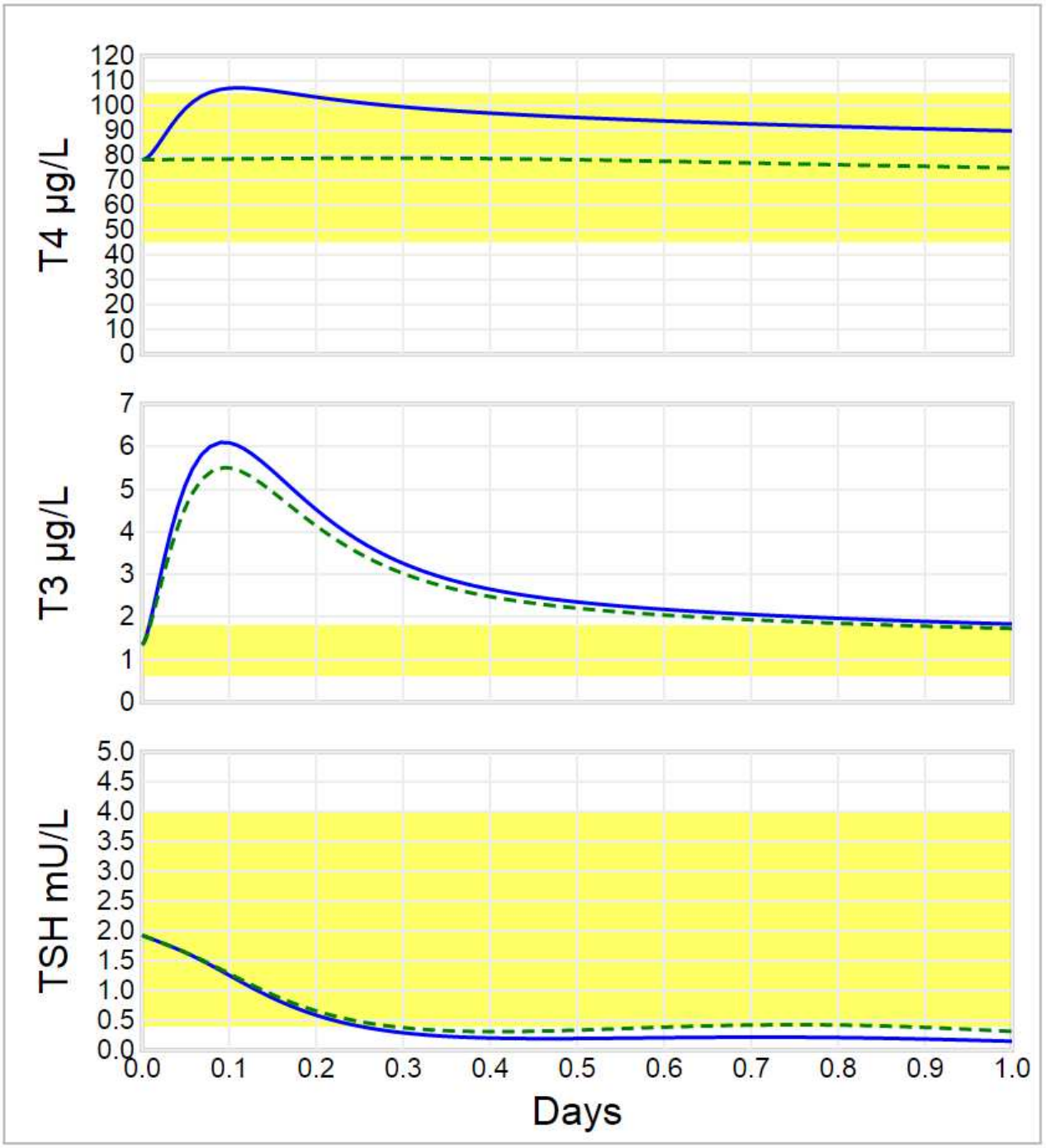
Supplementary Table S1. Software Components Used in Programming and Hosting the Web Application

Component	Version	Description	Reference
Client-side			
HTML		Markup language for writing web pages.	www.w3.org/TR/html401/
CSS		Style sheet language that describes the visual representation of HTML.	www.w3.org/TR/CSS2/
JavaScript (JS)		Language for dynamic content, such as adding or deleting inputs.	www.w3schools.com/jsref/
jQuery	1.10.2	JS library that simplifies programming in JS.	jquery.com
jQuery UI	1.10.1	JS library for user interface interactions, such as scrollbars.	jqueryui.com
d3	3.4.6	JS library for graphing.	d3js.org
Ajax		Protocol for sending user-input asynchronously to the server and receive calculation results.	www.adaptivepath.com/ideas/ajax-new-approach-web-applications
JSON		Data format used to transfer calculation results between the server and browser.	tools.ietf.org/html/rfc4627
Server-side			
Linux OS	Ubuntu 12.04 LTS	Operating system on the server.	help.ubuntu.com/12.04/
Apache HTTP Server	2.2.22	Web server software that delivers the web page to the user.	httpd.apache.org/docs/2.2/index.html
Perl	5.14.2	Language used to process user-inputs, execute the ODE solver, receive solver results, convert results to display units and send results back to the browser.	www.perl.org/about.html
JSON::Syck	1.21	Perl package (library) to convert Perl data structures to JSON.	search.cpan.org/~toddr/YAML-Syck-1.27/lib/JSON/Syck.pm
CGI	3.52	Perl package that handles HTTP requests.	perldoc.perl.org/CGI.html
CPAN	1.57	Perl package repository where the above mentioned packages are retrieved.	www.cpan.org
Octave	3.2.4	Language for numerical computations.	www.gnu.org/software/octave/
odepkg	0.8.2	Octave plugin for solving ODEs.	octave.sourceforge.net/odepkg/

HTML, CSS and JavaScript are browser-dependent and version numbers are omitted. Ajax and JSON do not have version numbers.



Supplementary Figure S1. Flowchart of the Simulation Process



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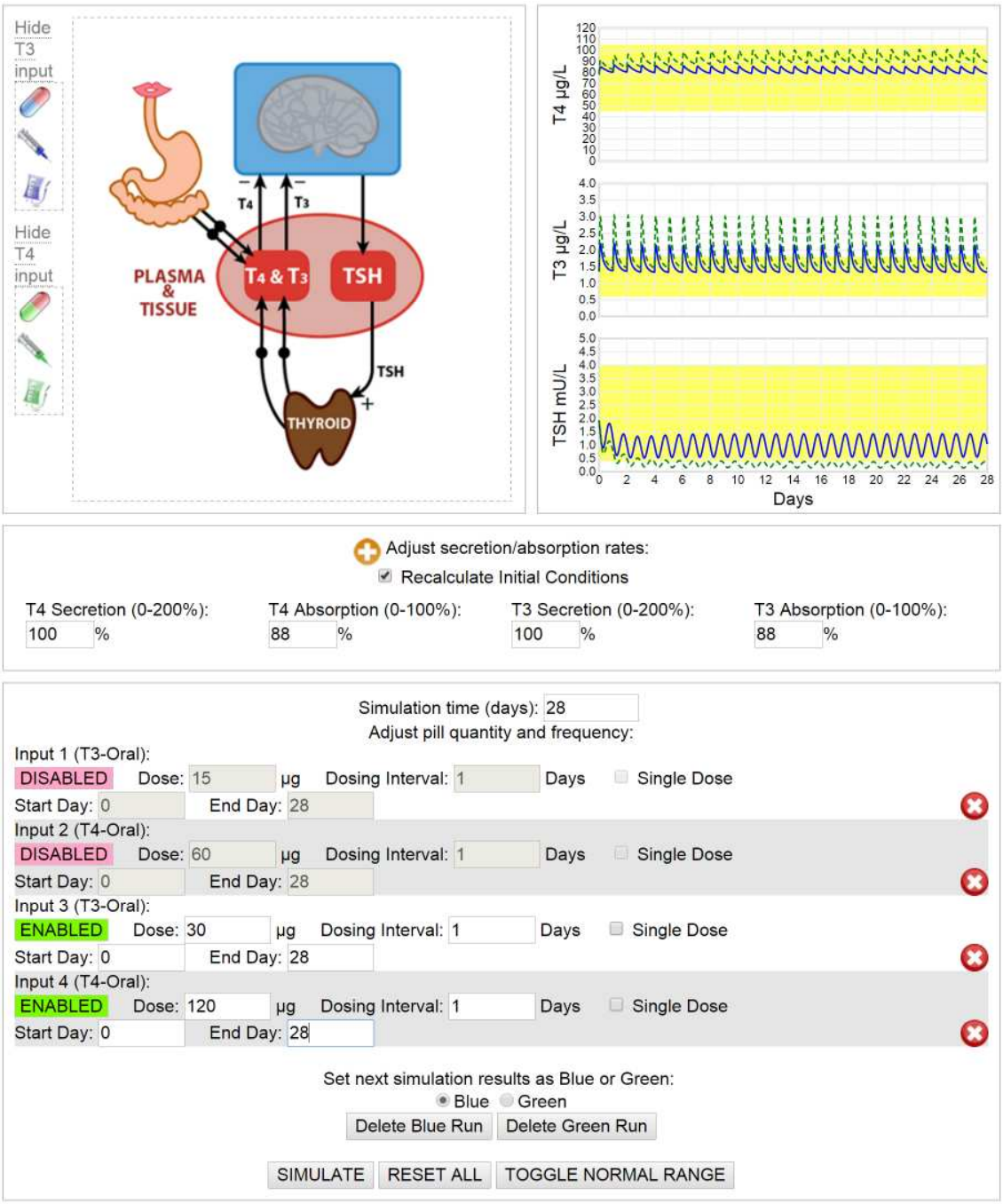

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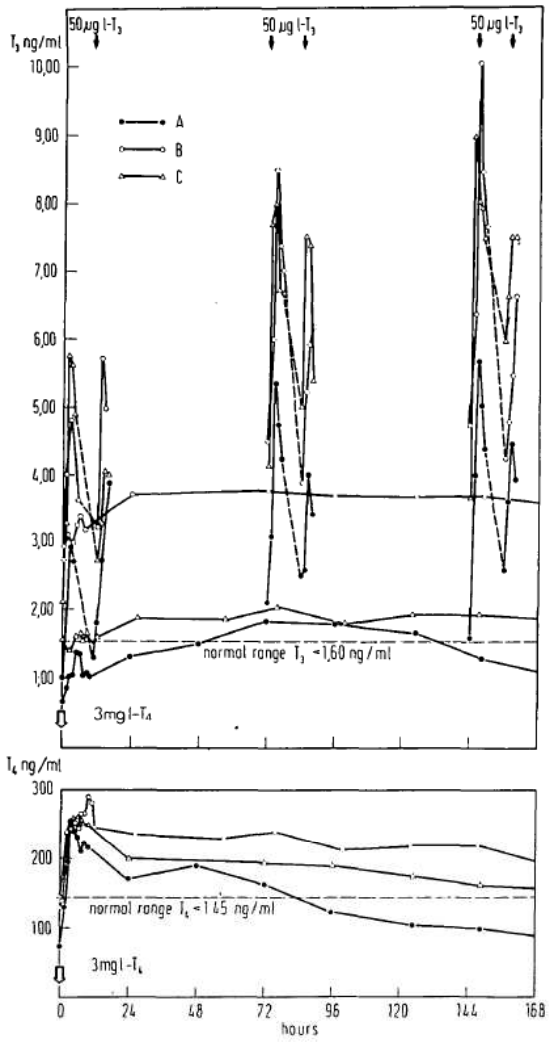

FIG. 2. Comparison of T_3 and T_4 suppression test. One normal subject (A); patient I.R. with T_3-thyrotoxicosis (B); patient S.F. with euthryroid endocrine ophthalmopathy (C). The small arrows indicate the time of T_3 applications, the large empty arrow the single T_4 dose. Note the high peaks after T_3 ingestion and the lower and less variable serum levels of T_3 after T_4 intake.

40% after L-T_3 of the subjects had complaints, the mean frequency of registered adverse symptoms being 12% ± 7 SD after L-T_4 and 35% ± 17 SD after L-T_3 (t test $p < 0.001$).

In addition, we were able to confirm that in patients with autonomously functioning thyroid 3 mg L-thyroxine is tolerated better than the multidosed L-triiodothyronine test. Even during the high initial serum T_4 peaks up to 390 ng/ml

TABLE 1. Effect of L-T_3 and L-T_4 suppression test on 4 subjects with autonomously functioning thyroid gland

Subject	Test	Before test		During highest hormone peaks		8th Day of the test		Thyroid status
		Pulse rate	Blood pressure	Pulse rate	Blood pressure	Pulse rate	Blood pressure	
I.D.	T_3-suppression	84	130/80	132	140/70	120	140/70	Compensated autonomous adenoma
	T_4-suppression	80	125/80	84	130/85	88	130/80	
S.F.	T_3-suppression	68	115/80	96	—	88	120/85	Euthyroid endocrine ophthalmopathy
	T_4-suppression	76	125/85	84	—	84	120/80	
I.R.	T_3-suppression	120	140/60	148	140/50	148	145/60	T_3-thyrotoxicosis
	T_4-suppression	128	150/70	124	—	132	140/70	
L.S.	T_3-suppression	80	130/80	140	140/70	116	150/80	T_3-thyrotoxicosis
	T_4-suppression	88	120/80	92	125/80	84	130/85	

Supplementary Figure S5. Wenzel *et al.* fig. 2.

Thyroid H-P-T-Axis Simulator Equations For Hypothyroid, Euthyroid & Mildly-Hyperthyroid Human Adults & Children

(13 ODE's, 12 algebraic terms, 3 outputs & ~56 parameters)

Notes: All mass units are μmol, time units are hours = h, volumes liters = L, unless otherwise noted;
DE ≡ differential equation

Thyroid Secretion & D&E Submodel Equations (Eisenberg *et al.* 2008)	
$\dot{q}_1(t) \equiv \dot{T}_{4P}(t) = SR_4(t) + k_{12}q_2(t) + k_{13}q_3(t) - \left(k_{31}^{free} + k_{21}^{free}\right)FT_{4P}(t) + k_4^{absorb}T_4^{GUT}(t) + u_1(t)$	DE for Plasma T_4 (T_{4P}) (μmol/h)
$\dot{q}_2(t) = k_{21}^{free}FT_{4P}(t) - \left(k_{12} + k_{02} + \frac{v_{\max}^{D1\,fast}}{K_m^{D1\,fast} + q_2(t)}\right)q_2(t)$	DE for Fast tissue T_4 (μmol/h)
$\dot{q}_3(t) = k_{31}^{free}FT_{4P}(t) - \left(k_{13} + k_{03} + \frac{v_{\max}^{D1slow}}{K_m^{D1slow} + q_3(t)} + \frac{v_{\max}^{D2slow}}{K_m^{D2slow} + q_3(t)}\right)q_3(t)$	DE for Slow tissue T_4 (μmol/h)
$\dot{q}_4(t) \equiv \dot{T}_{3P}(t) = SR_3(t) + k_{45}q_5(t) + k_{46}q_6(t) - \left(k_{64}^{free} + k_{54}^{free}\right)FT_{3P}(t) + k_3^{absorb}T_3^{GUT}(t) + u_4(t)$	DE for Plasma T_3 (μmol/h)
$\dot{q}_5(t) = k_{54}^{free}FT_{3P}(t) + \frac{v_{\max}^{D1\,fast}q_2(t)}{K_m^{D1\,fast} + q_2(t)} - \left(k_{45} + k_{05}\right)q_5(t)$	DE for Fast tissue T_3 (μmol/h)
$\dot{q}_6(t) = k_{64}^{free}FT_{3P}(t) + \frac{v_{\max}^{D1slow}q_3(t)}{K_m^{D1slow} + q_3(t)} + \frac{v_{\max}^{D2slow}q_3(t)}{K_m^{D2slow} + q_3(t)} - \left(k_{46} + k_{06}\right)q_6(t)$	DE for Slow tissue T_3 (μmol/h)
$FT_{3P}(t) = \left(a + bT_{4P}(t) + cT_{4P}^2(t) + dT_{4P}^3(t)\right)T_{3P}(t)$	free T_3 in plasma (μmol)
$FT_{4P}(t) = \left(A + BT_{4P}(t) + CT_{4P}^2(t) + DT_{4P}^3(t)\right)T_{4P}(t)$	free T_4 in plasma (μmol)
$SR_3(t) = S_3TSH_P(t-\tau) \qquad SR_4(t) = S_4TSH_P(t-\tau)$	TH secretion rates (μmol/h), τ is hrs time-delay (Mpy by fractions to adjust secretion rates)

Brain-Pituitary Submodel Equations (Eisenberg *et al.* 2010)	
$\dot{q}_7(t) \equiv \dot{TSH}_P(t) = SR_{TSH}(t) - f_{\deg}^{TSH}TSH_P(t)$	DE for Plasma *TSH* (μmol/h)
$\dot{q}_8(t) \equiv \dot{T}_{3B}(t) = \frac{f_4}{T_{4P}^{EU}}T_{4P}(t) + \frac{k_3}{T_{3P}^{EU}}T_{3P}(t) - k_{\deg}^{T_{3B}}T_{3B}(t)$	DE for T_3 in the brain (μmol/h)
$\dot{q}_9(t) \equiv \dot{T}_{3B}^{LAG}(t) = f_{LAG}\left(T_{3B}(t) - T_{3B}^{LAG}(t)\right)$	DE for lagged T_3 in brain (μmol/h)
$SR_{TSH}(t) = \left(B_0 + A_0 f_{CIRC}\sin\left(\frac{2\pi}{24}t - \phi\right)\right)e^{-T_{3B}^{LAG}(t)}$	TSH secretion rate (μmol/h) $f_{\text{circ}} \equiv 1$ for eu- & hyperthyroid model
$f_{\deg}^{TSH} = k_{\deg TSH}^{HYPO} + \frac{V_{\max}^{TSH}}{K_{50}^{TSH} + TSH_P(t)}$	Nonlinear *TSH* degradation rate function (h^{-1})
$f_{LAG} \equiv k_{LAG}^{HYPO} + \frac{2T_{3B}^{11}(t)}{K_{LAG}^{11} + T_{3B}^{11}(t)}$	Nonlinear lag-time function for T_{3B} in brain (h^{-1})
$f_4 \equiv k_3 + \frac{5k_3}{1 + e^{2T_{3B}(t)-7}}$	Nonlinear rate function for T_4 transport into & conversion of T_4 to T_3 in brain (h^{-1})

$$f_{CIRC} \equiv 1 + \left(\frac{A_{\max}}{A_0 e^{-T_{3B}^{LAG}(t)}} - 1 \right) \left(\frac{1}{1 + e^{10T_{3B}^{LAG}(t) - 55}} \right)$$

Circadian rhythm saturation function (h^{-1})
$f_{circ} \approx 1$ for eu- & mild hyper model

2-Compartment Gut Input Submodels (Mak *et al*. 1973; Eisenberg *et al*. 2008)

$$\dot{q}_{10}(t) \equiv \dot{T}_4^{PILL}(t) = -k_4^{dissolve} T_4^{PILL}(t), \qquad T_4^{PILL}(0) \equiv T_4 Dose$$

DE for L-T_4 pill dissolution in gut (μmol/h)

$$\dot{q}_{11}(t) \equiv \dot{T}_4^{GUT}(t) = k_4^{dissolve} T_4^{PILL}(t) - \left(k_4^{excrete} + k_4^{absorb} \right) T_4^{GUT}(t)$$

DE for absorbable L-T_4 in gut (μmol/h)

$$\dot{q}_{12}(t) \equiv \dot{T}_3^{PILL}(t) = -k_3^{dissolve} T_3^{PILL}(t), \qquad T_3^{PILL}(0) \equiv T_3 Dose$$

DE for L-T_3 pill dissolution in gut (μmol/h)

$$\dot{q}_{13}(t) \equiv \dot{T}_3^{GUT}(t) = k_3^{dissolve} T_3^{PILL}(t) - \left(k_3^{excrete} + k_3^{absorb} \right) T_3^{GUT}(t)$$

DE for absorbable L-T_3 in gut (μmol/h)

3 Measured Output Equations

$$y_{T_{4P}}(t) \equiv y_1(t) \equiv T_{4P}(t)/V_P = q_1(t)/V_P \ \mu\text{mol/L} \rightarrow 777 q_1(t)/V_P \ \mu\text{g/L}$$

Plasma T_4 concentration

$$y_{T_{3P}}(t) \equiv y_4(t) \equiv T_{3P}(t)/V_P = q_4(t)/V_P \ \mu\text{mol/L} \rightarrow 651 q_4(t)/V_P \ \mu\text{g/L}$$

Plasma T_3 concentration

$$y_{TSH_P}(t) \equiv y_7(t) \equiv TSH_P(t)/V_{TSH} = q_7(t)/V_{TSH} \ \mu\text{mol/L} \rightarrow 5.6 q_7(t)/V_{TSH} \ \text{mU/L}$$

Plasma *TSH* concentration

Parameter Values for 3 Conditions: (1) Adult Eu- & Mildly Hyper- (same #s); (2) Adult Hypo-; and (3) Child Hypothyroid

***Note*:** Unless otherwise specified, parameter values are for both children and adults

Parameter	*Units*	*Estimate*	*% CV*	*Source*
ϕ	*h*	-3.71	1.04	Eisenberg 2008
A_0	*µmol/h*	581	61.4	Eisenberg 2008
B_0	*µmol/h*	1166	60.7	Eisenberg 2008
$k_3 = k_4$	*µmol/h*	0.118	6.43	Eisenberg 2008
f_4 range	h^{-1}	0.118-0.708	--	Eisenberg 2010
$k_{\text{deg}}^{T_{3B}}$	h^{-1}	0.037	12.6	Eisenberg 2008
V_P *(kids)*	*L*	1	-	Ben-Shachar 2011
V_P *(adults)*	*L*	3.2	-	Eisenberg 2008
V_{TSH} *(kids)*	*L*	2.5	-	Ben-Shachar 2011
V_{TSH} *(adults)*	*L*	5.2	-	Eisenberg 2008
$K_m^{D1\,fast}$	*µmol*	2.85	-	Eisenberg 2008 fixed
K_m^{D1slow}	*µmol*	95	-	Eisenberg 2008 fixed
K_m^{D2slow}	*µmol*	0.075	-	Eisenberg 2008
$v_{\max}^{D1\,fast}$	*µmol/h*	9.99×10^{-3}	30.6	Eisenberg 2008
$v_{\max}^{D1slow}$	*µmol/h*	6.63×10^{-4}	6.27	Eisenberg 2008
$v_{\max}^{D2slow}$	*µmol/h*	7.46×10^{-4}	6.27	Eisenberg 2008
S_3	h^{-1}	3.36×10^{-4}	6.49	Eisenberg 2008
S_4	h^{-1}	1.74×10^{-3}	7.4	Eisenberg 2008
τ	*h*	8	-	Eisenberg 2008
k_4^{absorb}	h^{-1}	0.88	2.2	Eisenberg 2008
$k_4^{dissolve}$	h^{-1}	1.3	-	Eisenberg 2008
$k_4^{excrete}$	h^{-1}	0.12	16.3	Eisenberg 2008

k_3^{absorb}	h^{-1}	0.88	7.2	Eisenberg 2008
$k_3^{dissolve}$	h^{-1}	1.78	32.0	Eisenberg 2008
$k_3^{excrete}$	h^{-1}	0.12	7.2	Eisenberg 2008
A	*unitless*	0.000289	--	Eisenberg 2006
B	$mmol^{-1}$	0.000214	--	Eisenberg 2006
C	$mmol^{-2}$	0.000128	--	Eisenberg 2006
D	$mmol^{-3}$	-8.83×10^{-6}	--	Eisenberg 2006
a	*unitless*	0.00395	--	Eisenberg 2006
b	$mmol^{-1}$	0.00185	--	Eisenberg 2006
c	$mmol^{-2}$	0.000610	--	Eisenberg 2006
d	$mmol^{-3}$	-0.000505	--	Eisenberg 2006
k_{02} *(kids)*	h^{-1}	0.0114	17.0	Ben-Shachar 2011
k_{12} *(kids)*	h^{-1}	0.523	19.2	Ben-Shachar 2011
k_{13} *(kids)*	h^{-1}	0.0514	28.8	Ben-Shachar 2011
k_{21}^{free} *(kids)*	h^{-1}	2275	14.4	Ben-Shachar 2011
k_{31}^{free} *(kids)*	h^{-1}	255	36.0	Ben-Shachar 2011
k_{02} *(adults)*	h^{-1}	0.0189	25.7	Eisenberg 2006
k_{12} *(adults)*	h^{-1}	0.868	18.3	Eisenberg 2006
k_{13} *(adults)*	h^{-1}	0.108	12.4	Eisenberg 2006
k_{21}^{free} *(adults)*	h^{-1}	1503	31.2	Eisenberg 2006
k_{31}^{free} *(adults)*	h^{-1}	584	16.6	Eisenberg 2006
k_{54}^{free}	h^{-1}	2043	--	Eisenberg 2006
k_{64}^{free}	h^{-1}	127	--	Eisenberg 2006
k_{05}	h^{-1}	0.207	12.8	Eisenberg 2006
k_{45}	h^{-1}	5.37	16.3	Eisenberg 2006
k_{46}	h^{-1}	0.0689	4.79	Eisenberg 2006
A_{max}	*mmol/h*	2.37	61.4	Eisenberg 2010
$k_{\deg TSH}^{HYPO}$	h^{-1}	0.53	--	Eisenberg 2010
K_{LAG} *(kids)*	*unitless*	6.5	--	Ben-Shachar 2011
K_{LAG} *(adults)*	*unitless*	5	--	Eisenberg 2010
k_{LAG}^{HYPO}	h^{-1}	0.0034	5.87	Eisenberg 2010
K_{50}^{TSH}	μmol	23	--	Eisenberg 2010
T_{3P}^{EU} *(kids)*	*nmol/L*	2.6	--	Elmlinger 2001
T_{4P}^{EU} *(kids)*	*nmol/L*	100.8	--	Elmlinger 2001
T_{3P}^{EU} *(adults)*	μmol	0.006	--	Eisenberg 2010
T_{4P}^{EU} *(adults)*	μmol	0.29	--	Eisenberg 2010
$V_{\max}^{TSH}$	h^{-1}	0.037	12.6	Eisenberg 2010
Next 4 params	*are for*	*linearized*	*model*	
k_{21}	h^{-1}	0.544	31.2	Eisenberg 2006
k_{31}	h^{-1}	0.211	16.6	Eisenberg 2006
k_{54}	h^{-1}	9.24	13.7	Eisenberg 2006
k_{64}	h^{-1}	0.573	6.20	Eisenberg 2006