

Leucine Incorporation into VLDL

Case Study

- How to create tracee and tracer models
- How to create the tracer-tracee ratio as the measurement variable
- How to use a forcing function to simulate the leucine system

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Leucine Incorporation into VLDL

Prerequisites

The prerequisite for this case study is having worked through the SAAM II introductory tutorial, “Getting Started with SAAM II Compartmental.” It will be useful to have worked through the Using SAAM II tutorial “Using Forcing Functions.”

What you will learn in this case study

The general approach to modeling lipoprotein kinetics using stable isotopes including:

- How to create tracee and tracer models.
- How to create the tracer-tracee ratio, the measurement variable, from the model.
- How to use forcing functions to simulate the leucine system.

Files Required

Data:

The data file for this case study is

Leu_VLDL.dat

This data file is a text file. The contents of this file are included at the end of this case study.

Introduction

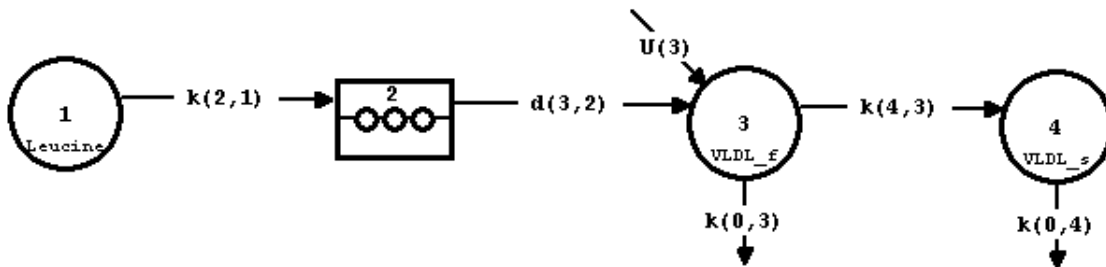
A popular way to study lipoprotein kinetics is to use stable isotopes. The protocol calls for introducing a label such as “ ^{13}C ” on an amino acid such as leucine. Labeled leucine is introduced as a bolus, an infusion or a primed infusion.

In this case study¹, labeled leucine is incorporated into VLDL (very low density lipoprotein) apolipoprotein B (apoB), a major protein associated with VLDL. Serial plasma samples of both leucine and VLDL apoB are obtained, and the tracer-tracee ratio for apoB is calculated using a method of Cobelli et. al^{2,3}. Labeled leucine was infused using a primed infusion format. The infusion lasted for 10 hours; the experiment lasted 107 hours. Thus there was a washout phase for the labeled leucine during which time it rapidly disappeared from plasma.

1. Parhofer, KG, Barrett, PHR, D Dier and G Schonfeld. Lipoproteins containing the truncated apolipoprotein apoB-89 are cleared from human plasma more rapidly the apoB-100 containing lipoproteins. *J. Clin. Invest.* 89:1931-1937, 1992.
2. Cobelli, C, G Toffolo, DM Bier and R Nosadini. Models to interpret kinetic data from stable isotope tracer studies. *Am. J. Physiol.* 1987, E551-E564, 1987.
3. Cobelli, C, D Foster and G Toffolo. Tracer Kinetics in Biomedical Research. Kluwer Academic/Plenum Publishers, New York, 2000.

Part 1. Create the tracee and tracer model

1. **Start the SAAM II Compartmental** application. The **SAAM II Compartmental** main window will open.
2. Create the following system model on the **Drawing Canvas**.



In creating this model, you will need to open the **Compartment Attributes** dialog box by double-clicking on Compartment **1**, typing “Leucine” in the **Reference Name** box, and clicking **Done**. The process will be repeated for VLDL_f (the fast turning over VLDL compartment) and VLDL_s (the slow turning over VLDL compartment). Remember to create **U(3)**, with the Flux tool active, you click first on the **Drawing Canvas**, and then Compartment **3**.

3. Specify the **Delay** attributes.
 - a. Double-click Delay **2** to open the **Delay Attributes** dialog box.
 - b. Type “Synthesis” in the **Reference Name** box.
 - c. Type “Synthesis_t” in the **Delay Time** box (this will be the time of the delay due to synthetic incorporation of leucine into VLDL).
 - d. Enter “5” in the **Number of Compartments** box. The **Delay Attributes** dialog box will appear as follows:

Delay Attributes

Delay Number: (1 to 9999)

Reference Name:

Delay Time:
(Value or Variable)

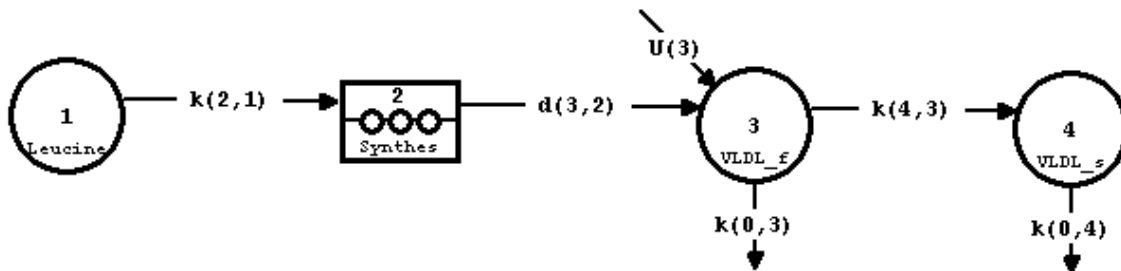
Number of compartments:

Transfer Coefficient:

Delay Width:

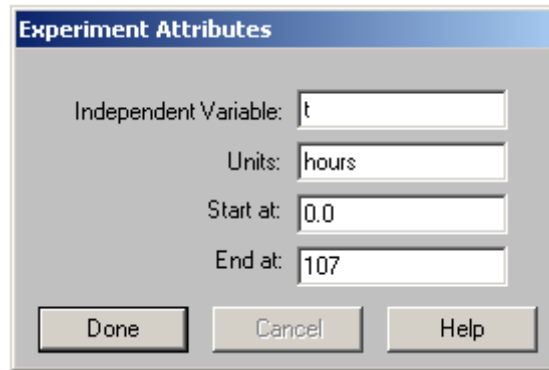
$d(3,2) = 1.0$

e. Click **Done**. Your model will appear as follows:

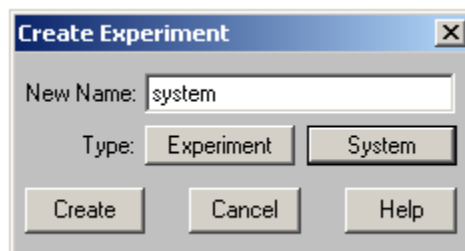


4. Create the trace experiment (steady state VLDL apoB) model.

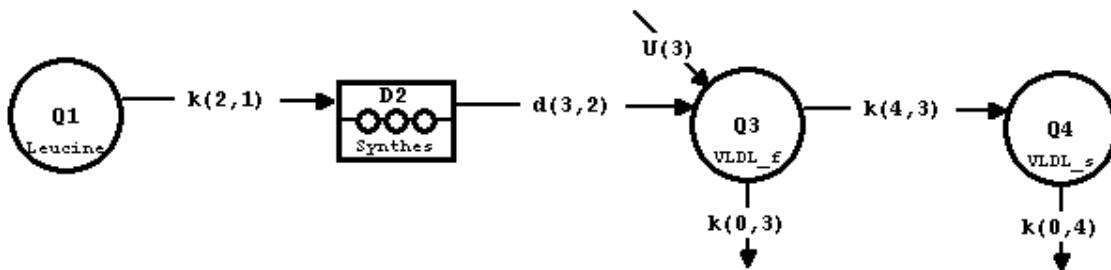
- In the **SAAM II Toolbox**, click **Experiment**. The **Experiment Attributes** dialog box will open.
- Type “hours” in the **Units** box.
- Enter “107” in the **End At** box. The **Experiment Attributes** dialog box will appear as follows:



- d. Click **Done**. The **Create Experiment** dialog box will open. Click **System** to make this the **Type**. The **Create Experiment** dialog box will appear as follows:



- e. Click **Create**. Your model will appear as follows:



Remember your system model is designed to deal with your tracee data. In this case, it is required because, as described below, the measurement variable is the tracer-tracee ratio. In SAAM II, the tracee model compartments are designated by capital “Q” as opposed to the tracer which is designated by small “q”.

- f. Double-click **Q3** to open the **Compartment Attributes** dialog box.
- g. Type the equation “Q3 = Vmass – Q4” in the **Equation** pane. The **Compartment Attributes** dialog box will appear as follows:

Compartment Attributes

Name: Q3

Reference Name: VLDL_f

Units: Forcing Function...

Equations:

Q3 = Vmass - Q4

Done Cancel Help



Specifying steady state mass. V_{mass} is the total VLDL apoB mass; this will be entered as a fixed parameter as it is measured in the experiment.

Remember for the steady state solution, for every unknown $U(i)$ there must be a known mass. The known mass can be the mass of a single compartment $C(j)$ or a sum of masses of compartments.

In this case, the total VLDL apoB mass is known, V_{mass} . Thus “ $V_{mass} = Q(3) + Q(4)$ ”. Since SAAM II does not deal with implicit equation, you must write with “ $Q(3) = V_{mass} - Q(4)$ ” or, associated with Compartment **Q4**, “ $Q4 = V_{mass} - Q(3)$ ”.



- h. Click **Done**.
5. Create the tracer experiment model.
 - a. In the **SAAM II Toolbox**, click **Create**. The **Create Experiment** dialog box will open. Click **Experiment** to be sure this is the **Type**. The **Create Experiment** dialog box will appear as follows:

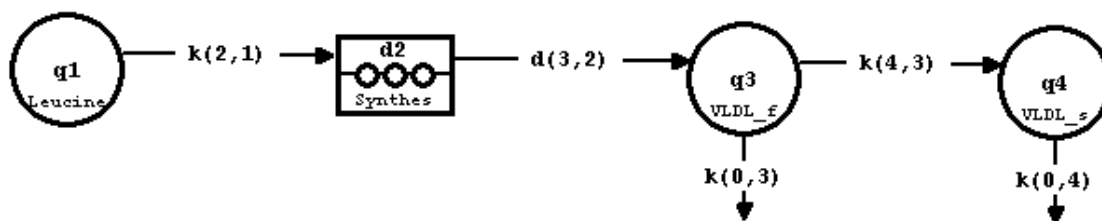
Create Experiment

New Name:


Type: Experiment System

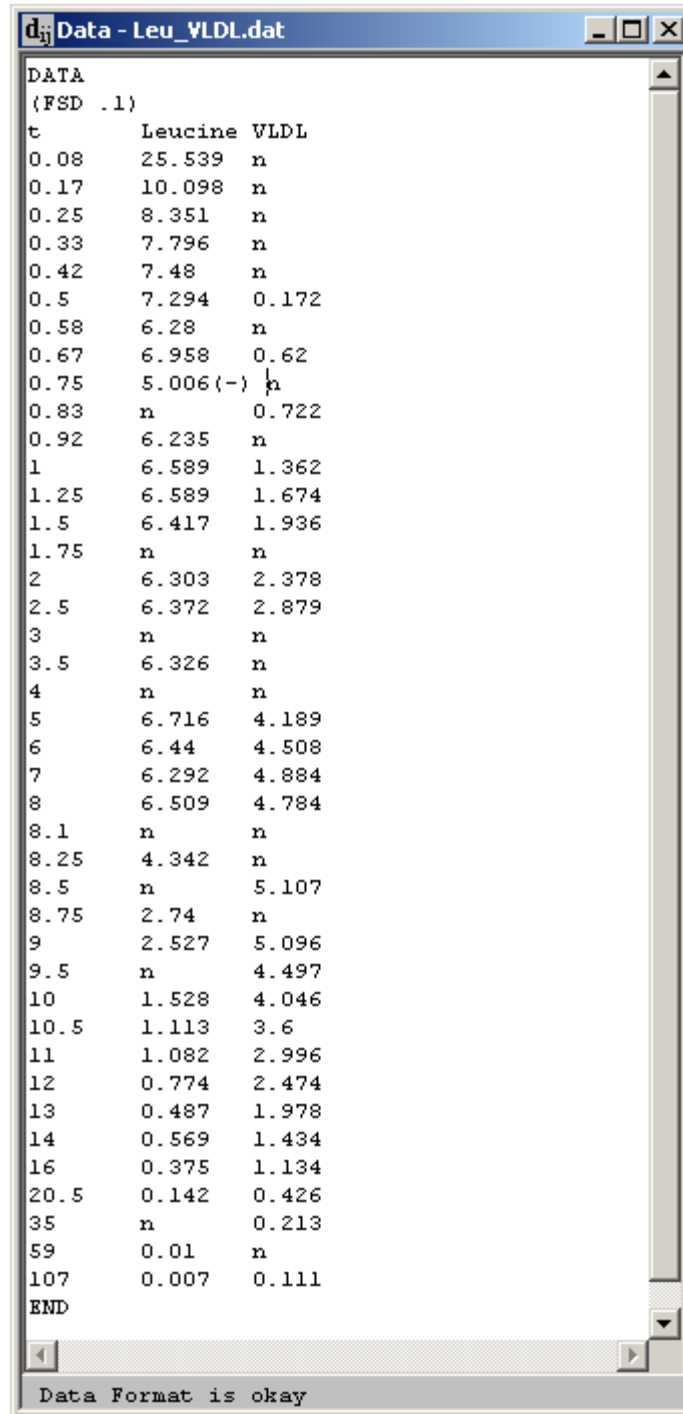
Create Cancel Help

- b. Click **Create**. Your tracer model will appear as follows:



6. Add the data to your model.

- On the **Show** menu, click **Data**, or alternatively click **Data**  on the **SAAM II Toolbar**. The **Data** window will open.
- On the **File** menu, click **Open**. The file **Leu_VLDL.dat** should appear in the list (if it does not, find the folder where you have installed this data file).
- Double-click **Leu_VLDL.dat**. The data in this file will appear in the **Data** window as follows:



t	Leucine	VLDL
0.08	25.539	n
0.17	10.098	n
0.25	8.351	n
0.33	7.796	n
0.42	7.48	n
0.5	7.294	0.172
0.58	6.28	n
0.67	6.958	0.62
0.75	5.006 (-)	n
0.83	n	0.722
0.92	6.235	n
1	6.589	1.362
1.25	6.589	1.674
1.5	6.417	1.936
1.75	n	n
2	6.303	2.378
2.5	6.372	2.879
3	n	n
3.5	6.326	n
4	n	n
5	6.716	4.189
6	6.44	4.508
7	6.292	4.884
8	6.509	4.784
8.1	n	n
8.25	4.342	n
8.5	n	5.107
8.75	2.74	n
9	2.527	5.096
9.5	n	4.497
10	1.528	4.046
10.5	1.113	3.6
11	1.082	2.996
12	0.774	2.474
13	0.487	1.978
14	0.569	1.434
16	0.375	1.134
20.5	0.142	0.426
35	n	0.213
59	0.01	n
107	0.007	0.111

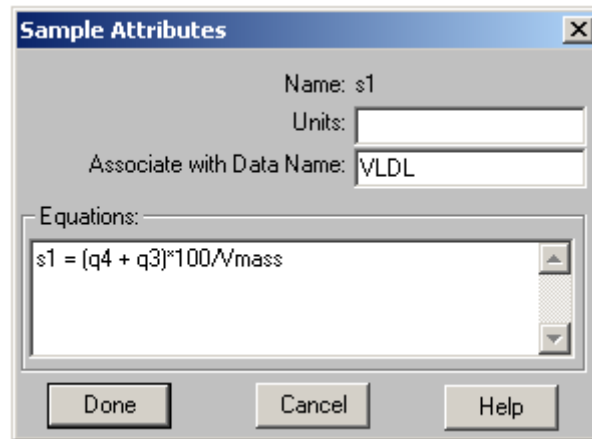
DATA
(FSD .1)
END

Data Format is okay

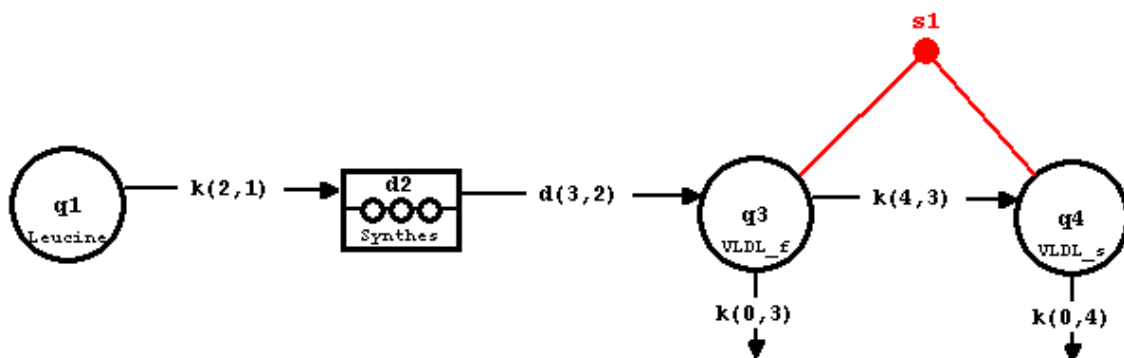
Notice there are two columns of data, one for leucine and the other for the VLDL apoB.

- d. Close the **Data** window.
7. Create the VLDL apoB sample.

- In the **SAAM II Toolbox**, click **Sample**.
- Click compartments **q3** and **q4**, then click on the **Drawing Canvas**. The sample **s1** will appear.
- Double-click **s1** to open the **Sample Attributes** dialog box.
- Type “VLDL” in the **Associate with Data Name** box.
- Edit the sample equation “ $s1=q4 + q3$ ” to read “ $s1=(q4 + q3)*100/Vmass$ ” in the **Equations** box. The “100” in the above equation converts the tracer-tracee ratio into a percent which are the units of the data. Your model and **Sample Attributes** dialog box will appear:



- Click **Done**. Your model will appear as follows:





The measurement equation in stable isotope experiments. The measurement equation “ $s1 = (q4 + a3) * 100 / V_{mass}$ ” can be interpreted as the ratio of the amount of tracer VLDL apoB divided by the steady state amount of VLDL apoB; it is called the tracer-tracee ratio. This is the correct analogue to specific activity as opposed to enrichment or isotope abundance, two commonly measured values. For more information, you are encouraged to read the two references to Cobelli et al given in the Introduction.

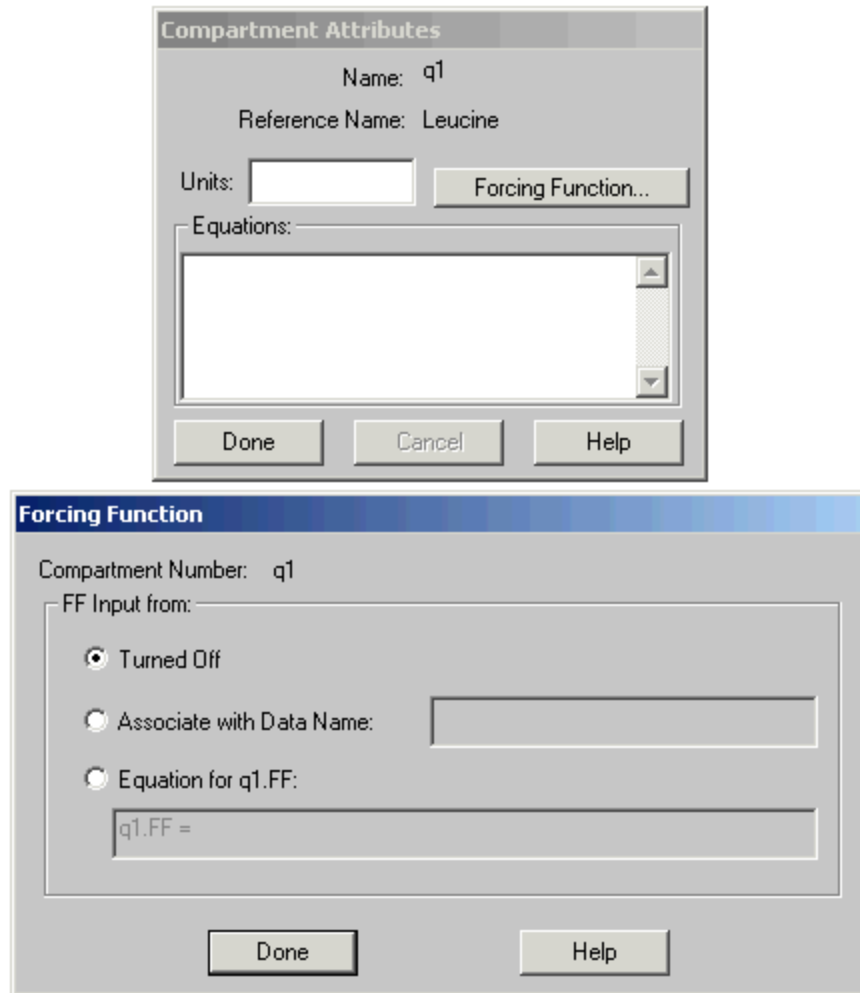
You should also note that the measurement equation could have been written “ $s1 = (q4 + q3) * 100 / (Q4 + Q3)$ ” since, by definition, “ $V_{mass} = Q3 + Q4$ ”.



Part 2. Create the Forcing Function for leucine

In this part of the case study, you will create the forcing function for plasma leucine which will be used to “drive” the VLDL apoB system

1. Double-click Compartment **q1** to open the **Compartment Attributes** dialog box. Click **Forcing Function...** The two dialog boxes will appear as follows:



2. In the **Forcing Function** dialog box, click **Associate with Data Name**. The **Associate with Data Name** pane will become available. Type "Leucine" in the **Associate with Data Name** pane. The **Forcing Function** dialog box will appear as follows:

Forcing Function

Compartment Number: q1

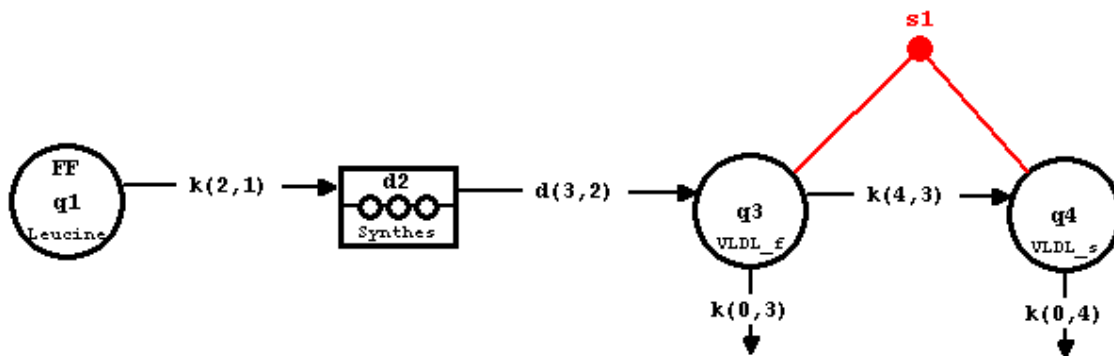
FF Input from:

Turned Off

Associate with Data Name:

Equation for q1.FF:

3. Click **Done** to close the **Forcing Function** dialog box, and then click **Done** to close the **Compartment Attributes** dialog box. Your model will appear as follows:

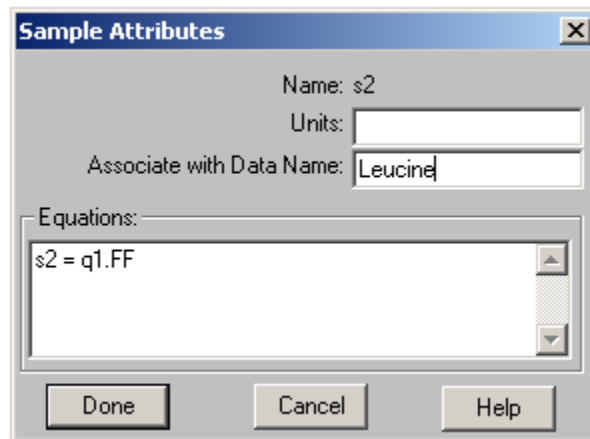


The forcing function. The forcing function will be created using the leucine data by linearly extrapolating between sequential data. The option is to specify an equation for the forcing function. This is commonly done by fitting a sum of exponentials to the data. Using the data themselves and letting SAAM II create the function via linear interpolation is a convenience that should be used then the forcing function data themselves are relatively noise-free.

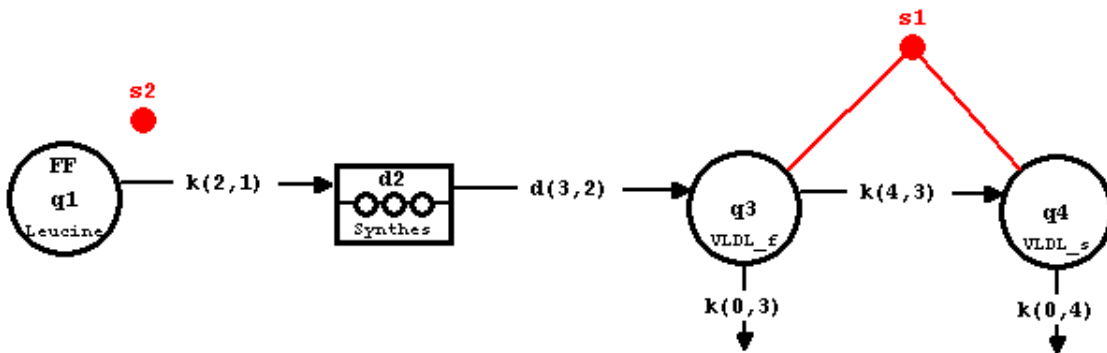


4. Define the second sample for the forcing function.
- In the **SAAM II Toolbox**, click **Sample**.
 - Click **q1**, and then on the **Drawing Canvas**. The sample **s2** will appear.

- Double-click **s2** to open the **Sample Attributes** dialog box.
- Type “Leucine” in the **Associate with Data Name** box.
- Type the sample equation “ $s2=q1.FF$ ”. The **Sample Attributes** dialog box will appear:



- Click **Done**. You will notice that **s2** appears solid, but no longer connected to **q1**. Your model will appear as follows:




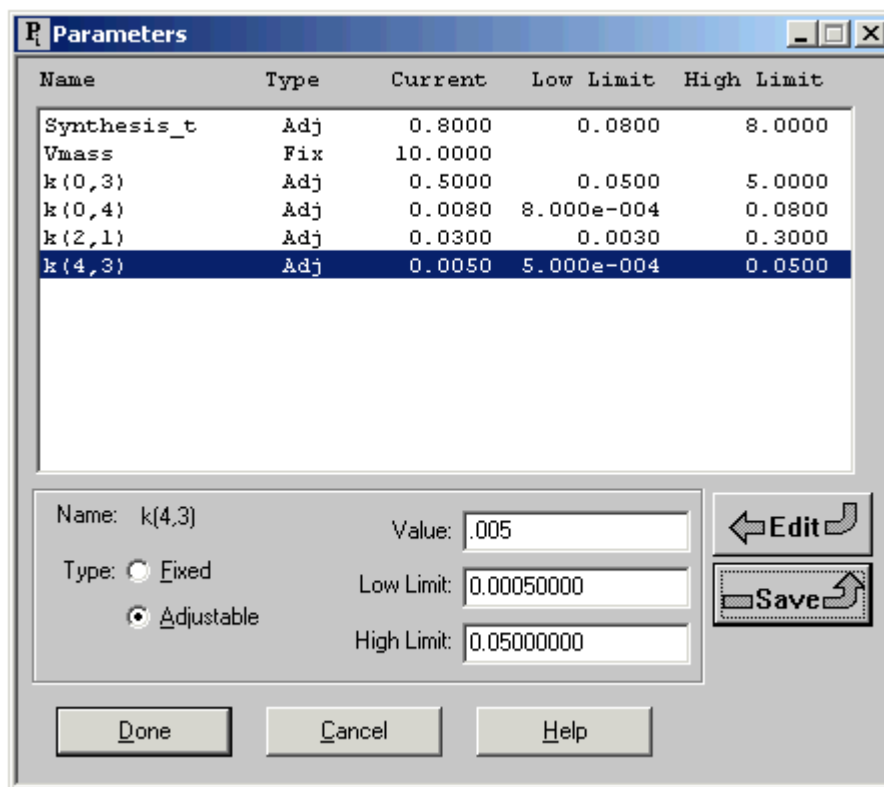
Samples. The Sample tool is a very powerful tool in SAAM II. Normally it is used to create samples that link a compartment, or compartments, with data. In this case, we are interested in viewing the forcing function. When we entered the equation “ $s2=q1.FF$ ”, **q1** no longer appeared in the sample equation, hence the line linking the sample to **q1** was severed.

Remember the name for the forcing function in SAAM II is $\text{cpt}\#\text{.FF}$ where $\text{cpt}\#$ is the number of the compartment with which the forcing function is associated.



Part 3. Work with the model.

1. Enter the parameter estimates.
 - a. On the **Show** menu, click **Parameters**, or alternatively click **Parameters**  on the **SAAM II Toolbar**. The **Parameters** dialog box will open. Enter the parameter values as shown below.



Name	Type	Current	Low Limit	High Limit
Synthesis_t	Adj	0.8000	0.0800	8.0000
Vmass	Fix	10.0000		
k(0,3)	Adj	0.5000	0.0500	5.0000
k(0,4)	Adj	0.0080	8.000e-004	0.0800
k(2,1)	Adj	0.0300	0.0030	0.3000
k(4,3)	Adj	0.0050	5.000e-004	0.0500

Name: k(4,3) Value: .005


Type: Fixed Low Limit: 0.00050000

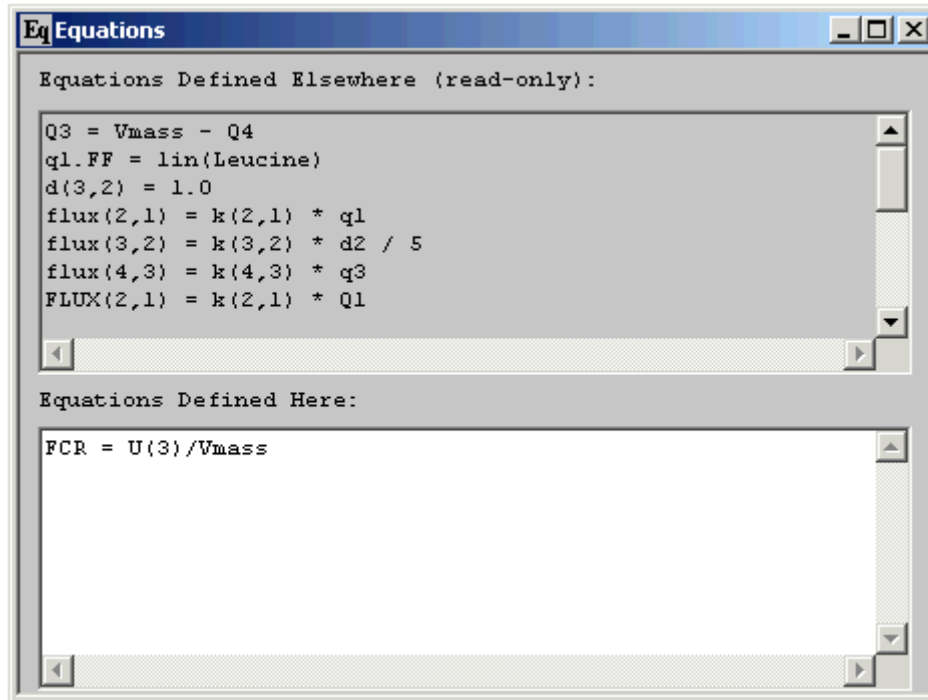
Adjustable High Limit: 0.05000000


Buttons: Edit, Save, Done, Cancel, Help

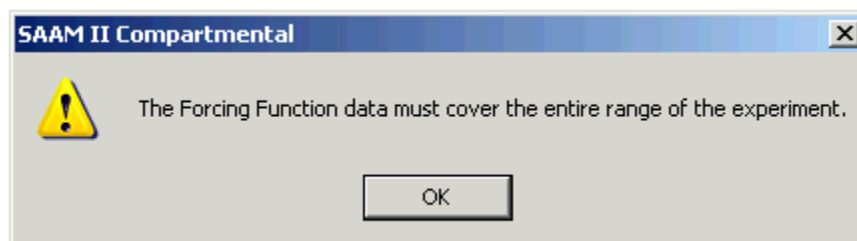
- b. Click **Done**.
2. Estimate the fractional clearance rate of VLDL apoB

Two of the most important parameters estimated in lipoprotein studies such as this is the synthesis of the lipoprotein, in this case represented by **U(3)**, and the fractional clearance rate. The formula for the fractional clearance rate, *FCR*, is given by “ $\text{FCR} = \text{U}(3)/\text{Vmass}$ ”.

- a. On the **Show** menu, click **Equations**, or alternatively click the **Equation**  tool on the **SAAM II Toolbar**. The **Equations** dialog box will open.
- b. In the **Equations Defined Here** pane, type “FCR = U(3)/Vmass”. The **Equations** dialog box will appear as follows:



- c. Close the **Equations** dialog box.
3. Solve the model, and view the solution.
- a. On the **Compute** menu, click **Solve**, or alternatively click **Solve**  on the **SAAM II Toolbar**. The following message will appear:






Forcing Functions. When you are using data to define a forcing function, the data must cover the entire range of the experiment. This means there must be a datum at time zero, and a datum at the last time of the experiment. Usually there is not a datum available at time zero, so you will have to add a dummy value, usually close to the first datum, so the solution can proceed.

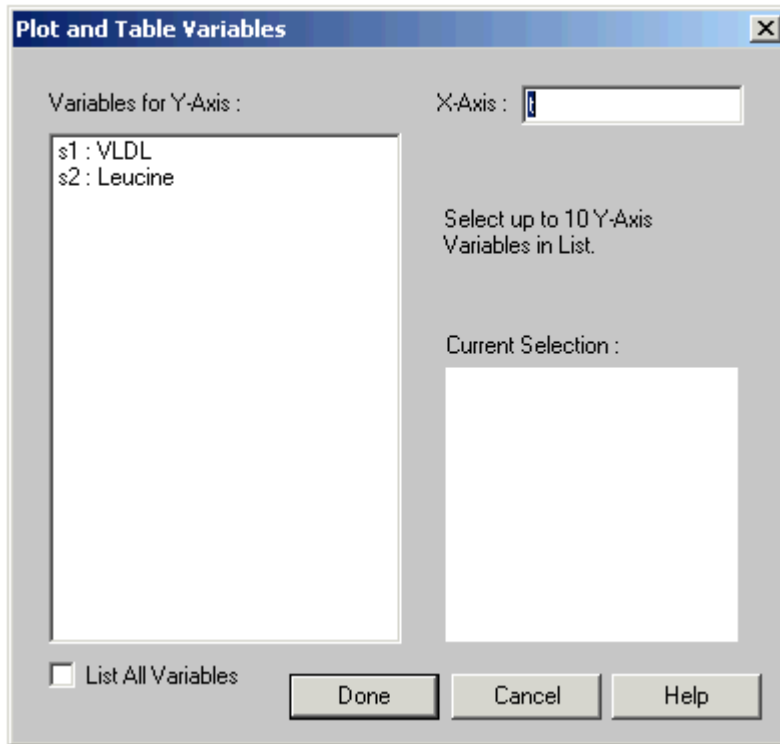


- b. Click **OK**.
- c. Open the **Data** window. Enter a zero time value for Leucine of 26. The first lines of your data file will appear:

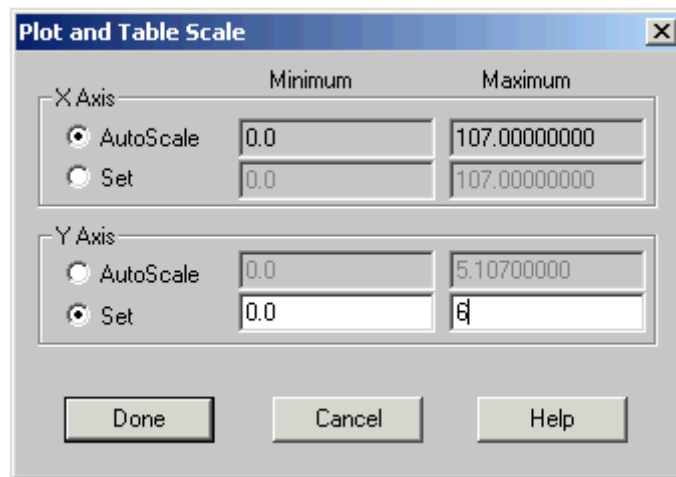
t	Leucine	VLDL
0	26	n
0.08	25.539	n
0.17	10.098	n
0.25	8.351	n

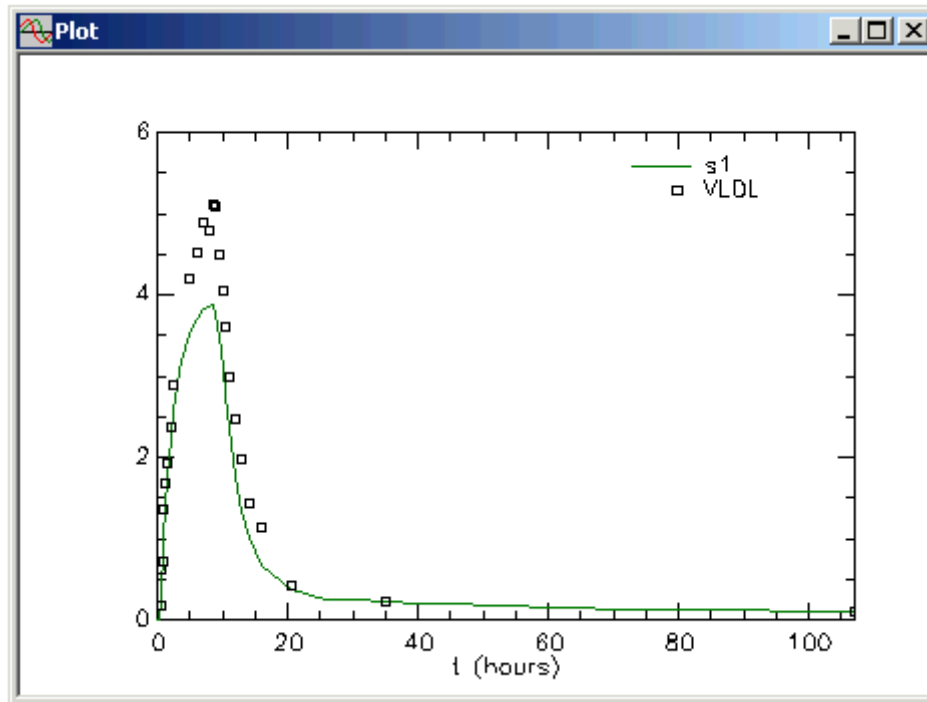
Close the **Data** window.

- d. Re-Solve the model.
- e. On the **Show** menu, click **Plot**, or alternatively click **Plot**  on the **SAAM II Toolbar**. The **Plot and Table Variables** dialog box will open as follows:




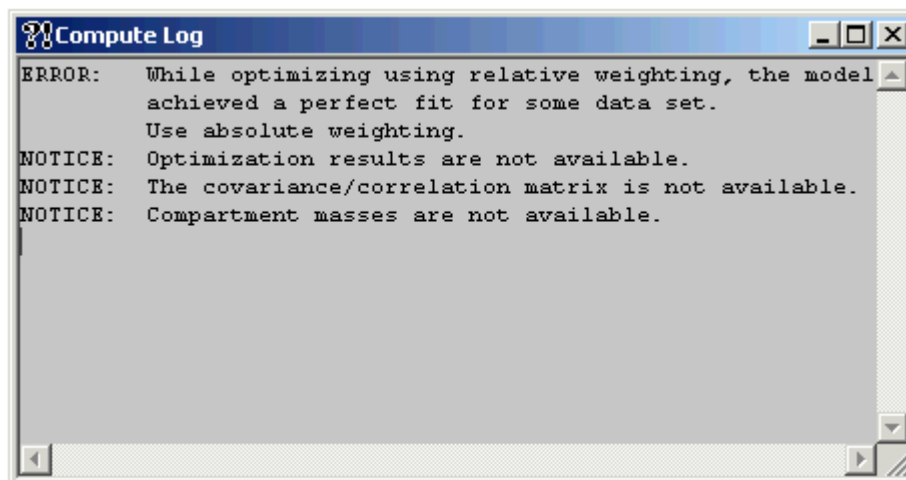
- f. Click **s1:VLDL** to move this to the **Current Selection** pane.
- g. Click **Done**. The plot should appear in linear mode. To obtain the following plot, the **Plot and Table Scale** was changed as follows:





Close the **Plot** window.

4. Fit the model and view the statistics.
 - a. Fit your model to your data. On the **Compute** menu, click **Fit**, or alternatively click **Fit**  on the **SAAM II Toolbar**. The following message will appear:



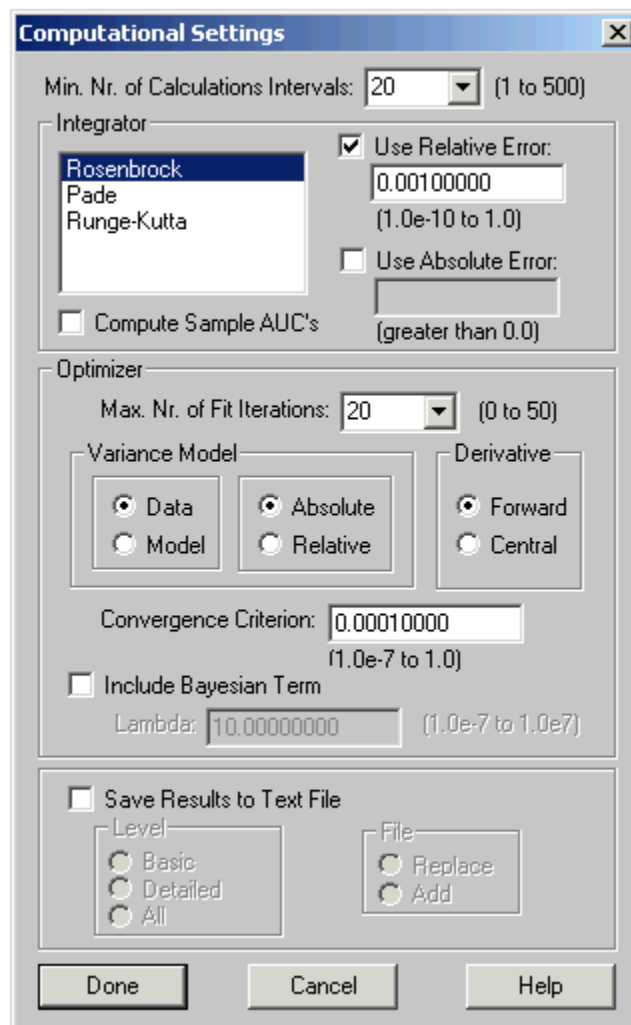
The message means that the data error model of “data-relative” is not appropriate for this model. We will follow the suggestion to set the model to “data-absolute”.

Close the **Compute Log** window.

b. Change the variance model.

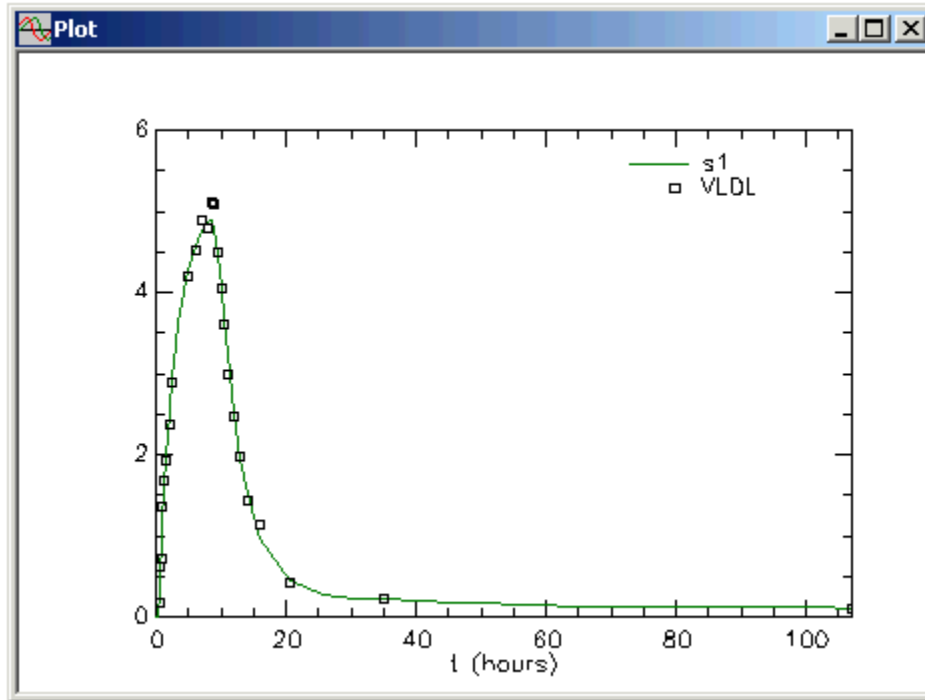
(1) On the **Compute** menu, click **Settings**. The **Computational Settings** dialog box will open.


(2) In the **Variance Model** pane, click **Absolute**. The **Computational Settings** dialog box will appear as follows:



(3) Click **Done**.

c. Re-Fit the model to the data. This time the fit is successful. The plot of **s1** and **VLDL** will appear as follows:



- d. On the **Show** menu, click **Statistics**, or alternatively, click **Statistics**  on the **SAAM II Toolbar**. The **Statistics** window will appear as follows:

Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
Synthesis_t	0.74419	2.74295e-002	3.68584e+000	0.68912	0.79925
Vmass	10.00000	** Fixed **	** Fixed **	** Fixed **	** Fixed **
k(0,3)	0.39122	2.15660e-002	5.51250e+000	0.34793	0.43452
k(0,4)	0.00340	2.65777e-003	7.82672e+001	-0.00194	0.00873
k(2,1)	0.03071	1.77663e-003	5.78433e+000	0.02715	0.03428
k(4,3)	0.00272	6.24541e-004	2.29401e+001	0.00147	0.00398
----- Derived Variables -----					
FCR	0.21865	6.15887e-002	2.81681e+001	0.09500	0.34229
<input type="radio"/> Correlation Matrix <input type="radio"/> Covariance Matrix <input checked="" type="radio"/> Objective					
		Objective	Scaled Data Variance		
s2 : Leucine		-1.543837e+000	1.000000e+000		
s1 : VLDL		-1.275233e+000	1.000000e+000		
Total objective		-2.819070e+000			
AIC		-4.043897e-001			
BIC		-3.155775e-001			

You can see the statistical information is quite acceptable. If you scroll to see the **Derived Variables**, and select **Correlation Matrix**, the **Statistics** window will appear as follows:

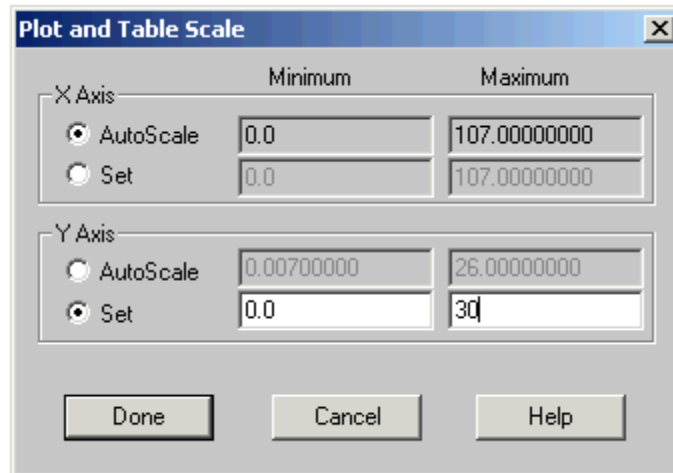
Statistics

Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
FLUX(2,1)	0.00000e+000	0.00000e+000	****	0.00000e+000	0.00000e+000
FLUX(3,2)	0.00000e+000	0.00000e+000	****	0.00000e+000	0.00000e+000
FLUX(4,3)	0.01511	7.05602e-003	4.66964e+001	9.44946e-004	0.02928
Q1	0.00000e+000	0.00000e+000	****	0.00000e+000	0.00000e+000
Q3	5.55022	1.43808e+000	2.59103e+001	2.66317	8.43726
Q4	4.44978	1.43808e+000	3.23179e+001	1.56274	7.33683
U(3)	2.18647	6.15887e-001	2.81681e+001	0.95003	3.42291
k(3,2)	6.71875	2.47643e-001	3.68584e+000	6.22159	7.21592

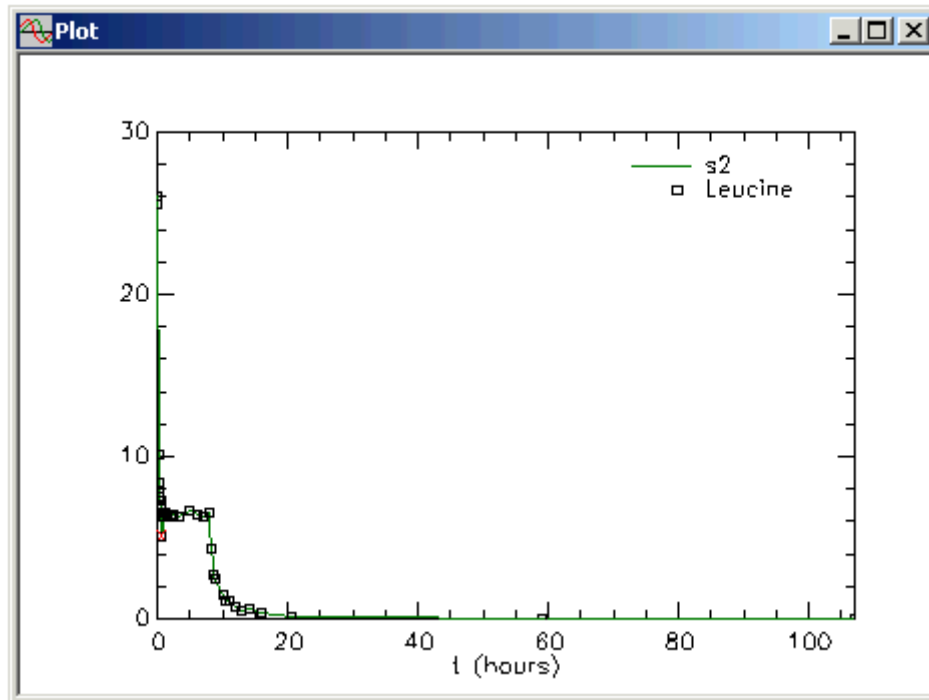
Correlation Matrix
 Covariance Matrix
 Objective

	Synthesis	Vmass	k(0,3)	k(0,4)	k(2,1)	k(4,3)
Synthesis	1.00000	***	0.71491	0.13718	0.77878	0.12101
Vmass	***	***	***	***	***	***
k(0,3)	0.71491	***	1.00000	0.33021	0.90261	0.36903
k(0,4)	0.13718	***	0.33021	1.00000	0.18695	0.90826
k(2,1)	0.77878	***	0.90261	0.18695	1.00000	0.16310
k(4,3)	0.12101	***	0.36903	0.90826	0.16310	1.00000

- f. Close the **Statistics** window; leave the **Plot** window open.
5. View the forcing function.
 - a. On the **Set** menu, click **Plot/Table Variables**. The **Plot and Tables Variables** dialog box will open.
 - b. Click **s2:Leucine** to move it to the **Current Selection** pane.
 - c. Click **Done**. You will have to change the **Plot and Table Scale** since it has been set for **s1:VLDL**.
 - d. On the **Set** menu, click **Plot/Table Scale**. The **Plot and Table Scale** dialog box will open.
 - e. Change the scale as shown in the following:



- f. Click **Done**. The plot of the forcing function and leucine data will appear:



The leucine data followed a primed constant infusion. You can see the very rapid disappearance of the priming dose. The infusion lasted 10 hours after which there was a washout of the labeled leucine.

- g. Close the **Plot** window.

Quit the **SAAM II Compartmental** application. You can save the study file if you wish.

Essential points to remember

- In stable isotope experiments, the measurement variable is the tracer-tracee ratio. It is easily simulated in SAAM II by creating the tracer model (which is possible since enrichment of the labeled leucine is almost 100%), and the tracee model for VLDL.
- The forcing function is a very convenient mechanism to model amino acid incorporation into a protein.

Data for this tutorial

DATA

(FSD .1)

t	Leucine	VLDL
0	26	n
0.08	25.539	n
0.17	10.098	n
0.25	8.351	n
0.33	7.796	n
0.42	7.48	n
0.5	7.294	0.172
0.58	6.28	n
0.67	6.958	0.62
0.75	5.006(-)	n
0.83	n	0.722
0.92	6.235	n
1	6.589	1.362
1.25	6.589	1.674
1.5	6.417	1.936
1.75	n	n
2	6.303	2.378
2.5	6.372	2.879
3	n	n
3.5	6.326	n
4	n	n
5	6.716	4.189
6	6.44	4.508
7	6.292	4.884
8	6.509	4.784
8.1	n	n
8.25	4.342	n
8.5	n	5.107
8.75	2.74	n
9	2.527	5.096
9.5	n	4.497
10	1.528	4.046
10.5	1.113	3.6
11	1.082	2.996
12	0.774	2.474
13	0.487	1.978
14	0.569	1.434
16	0.375	1.134
20.5	0.142	0.426
35	n	0.213
59	0.01	n

107 0.007 0.111
END