

# SecDec Manual

**Version 2010-03-01**

**SecDec is a web application for the calculation of insulin secretion by C-peptide deconvolution developed at Institute of Biomedical Engineering of National Research Council, Padua, Italy**

SecDec is available at <http://webmet.pd.cnr.it/dec/>

# SecDec General Information

## Before you start

Before you start reading about SecDec, you should be aware of some characteristics of the system and the deconvolution procedure.

Deconvolution (and therefore SecDec) is a complex procedure; to use SecDec you need to understand how deconvolution works. To ensure accurate results it is necessary to analyze the individual data manually. We have designed SecDec to perform this task in a flexible and efficient way, avoiding oversimplifications. SecDec is not a one-click application. To obtain best results with SecDec, you need to learn how to use the various options. SecDec is difficult or impossible to use without understanding the principles on which the analysis is based. Deconvolution and the SecDec options are explained later in this manual.

SecDec has a graphical interface that requires a recent computer system but no proprietary software. The SecDec graphical interface is based on Java; you must thus have an updated version of Java installed on your computer to work with SecDec. Java loads more slowly than ordinary web pages; this is particularly evident when the Java graphical interface is activated for the first time.

The SecDec site has been developed with the aim to provide a widely accessible service. However, because your data must be sent to the SecDec server, access requires registration with username and password, so that your data are accessible only to you. Accounts are given upon request and are free for non-profit organizations. To obtain an account, we just need to know your identity and you have to agree on some basic conditions for use.

SecDec is designed to hold your data in a database so that you can work interactively and retrieve the results in a file at completion of the analysis. SecDec implements the necessary safety measures to protect your data, as described in detail in the following sections. Before you request a SecDec account, check if our policy is adequate for your purposes.

## Access policy

We do not allow free access to SecDec because SecDec includes a permanent database which holds your data. Once we have authorized your access to SecDec, you will be free to work with SecDec without restrictions. Your data will remain in the SecDec database for unlimited time. However, you must accept the following facts:

- We cannot guarantee unlimited life to SecDec; we may need to shut SecDec down if the conditions do not allow us to continue its maintenance. However, because this event is predictable, you will be notified in advance at your email address.
- We cannot ensure constant availability of SecDec. SecDec may be temporarily unavailable, also for a relatively long time, due to the limited number of users that can access SecDec simultaneously. In addition, SecDec may be closed due to server and software maintenance.
- We cannot protect the user against loss of data due to exceptional events. You can use the SecDec backup function to create a backup copy of your data.

- Your data are visible to you and to the SecDec administrator but are not accessible to other SecDec users. We have adopted safety measures to protect your data, as described in detail in a following section.
- Your password (except the initial one) is encrypted and is not visible to the SecDec administrator.

Your contact data will be kept strictly confidential and will be exclusively used for communications concerning SecDec.

### **Obtaining an account**

To request an account, write us using the contact form available at the SecDec home page. We will send you a printable form to fill out with your name and address. The form also illustrates the conditions for access, which you need to accept. Complete the form and return it to us by email or fax. Free access to SecDec is granted only to non-profit organizations.

Once your request has been accepted, you will receive at your email address a temporary password for access to SecDec. For security reasons, at the first login you should change your password.

If you want to be removed from SecDec users, just let us know using the contact form.

- ⚠ When you use the contact form at the SecDec home page, be sure to write your email address correctly. If your address is incorrect, we cannot send you back the account request form. Write again in case you don't receive a reply in a week or so. The email address you insert in the request form will be used to communicate with you in case of problems with SecDec. Use a permanent address; otherwise, you may not receive important notifications concerning SecDec.

### **Data protection**

To use SecDec you must submit your data to our server and the data will remain on the server until you decide to delete them. We have adopted the following measures to protect your data:

- Access to your data through the internet is possible only knowing the password of your account. Your password is known only to you as it is encrypted in the SecDec database.
- The SecDec database on our server is not accessible from outside the firewall protecting the server with means other than the SecDec login system.
- No one except the SecDec administrator has access to the SecDec database on the server.
- The data stored in the SecDec database include only the information that is strictly necessary to perform deconvolution. These data are of little value for people who do not know the experimental conditions in which they are obtained.
- At any time you can create a backup copy of your database for future reuse and delete your data from the server.
- The result and backup files that you download from the server, though accessible through the internet, are given a random name that cannot be guessed and are deleted from the server after 10-20 min from their creation.
- Access to SecDec is possible with an https encrypted connection, in addition to the simple http protocol (just use <https://webmet.pd.cnr.it/dec>). Although the https connection is

safer, we cannot provide a certificate to guarantee our identity; access with the secure protocol thus gives a warning in your browser.

### **System requirements**

To run SecDec you need an internet browser with Javascript and cookies enabled and Java installed. Most recent browsers have this configuration as a standard. SecDec may not work on computers that run old internet software.

Before you start using SecDec, it is advisable that you check if your computer has an appropriate version of Java installed and if it works properly. See the Java installation section at the end of this manual.

SecDec has a graphical interface that requires a modern color screen with a reasonable size and resolution. Low size screens may require scrolling to display the whole graphical interface.

## SecDec Outline

This section gives an outline of the steps necessary to calculate insulin secretion using SecDec. The details of each step are explained in the User's guide. To understand the procedure, the basic concepts concerning deconvolution should be known.

Once you have an account, you can access SecDec with your username and password. When you start SecDec for the first time, you will see a simple main menu<sup>1</sup>. In the menu, there is an option for creating a new study. SecDec can work with data from multiple studies with different characteristics, such as a 4-hour meal test and a 2-hour hyperglycemic clamp. You can keep several studies simultaneously in the SecDec database. To upload new data you must first create a new study.

To create a new study, you must provide some information on the time grid which is used to calculate insulin secretion and on the method used to assign a weight to each C-peptide point (C-peptide weights are explained in the User's guide). These settings apply to all the data of the study, but can differ between studies.

Once you have created your first new study, SecDec shows below the main menu a single row table showing some information on the study. This table is called the *Studies table*. The table will increase as you create new studies. One of the table columns shows the number of subjects included in the study, which is initially zero.


User: **test** New Study Backup Account Logout

study	N	done	smoothing	briskness	
clamp	3	0	-	no	
ivgtt	3	1	-	no	
meal	3	1	20	yes	

Fig 1. The Studies table. The user *test* has created three studies.

The Studies table displays several icons to perform actions. The action performed when the icon is clicked is described by a yellow tooltip that appears when the mouse pointer is on the icon.

To load your data, you must first prepare a data file as described in the User's guide. Each data file may contain multiple subjects. When your data file is ready, click on the add icon.


 The *add* icon.

This displays a page with which you can upload your file. If your data are in the correct format, they are inserted in the SecDec database and the number of loaded subjects is


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<sup>1</sup> If you have requested a SecDec account pre-loaded with the test data shown in this manual, the Studies table (described below) is displayed after login.


displayed in the table (column “N” in Fig 1). If there are errors in the data, the whole data set contained in the file is rejected and the data errors are displayed. You can view the errors in your submitted data at any time by clicking on the errors icon.

 The *errors* icon.

At any time, you can add more data or replace existing data. You can also delete the whole study and all the data.

 The *replace* (left) and *delete* (right) icons.

Once you have loaded your data, click on the view icon to see all the loaded subjects and proceed with the analysis.

 The *view* icon.

By clicking on the view icon, a new table, called the *Subjects table*, is displayed with all the loaded subjects.

User: **test** Study: **meal** Studies Logout








ID	sex	weight	height	age	diabetic	done	smoothing		
1	M	73.1	1.79	33	no	yes	20		
2	M	74.6	1.76	30	no	no			
3	M	85.1	1.89	25	no	no			

Fig 2. The Subjects table that displays the subjects of the *meal* study.

Next to each subject there are two icons, one for analyzing the data or editing the stored results and one for deleting the subject.

 The *edit/analyze* (left) and *delete* (right) icons.

When you click on the edit/analyze icon, SecDec displays a graphical interface for data analysis.

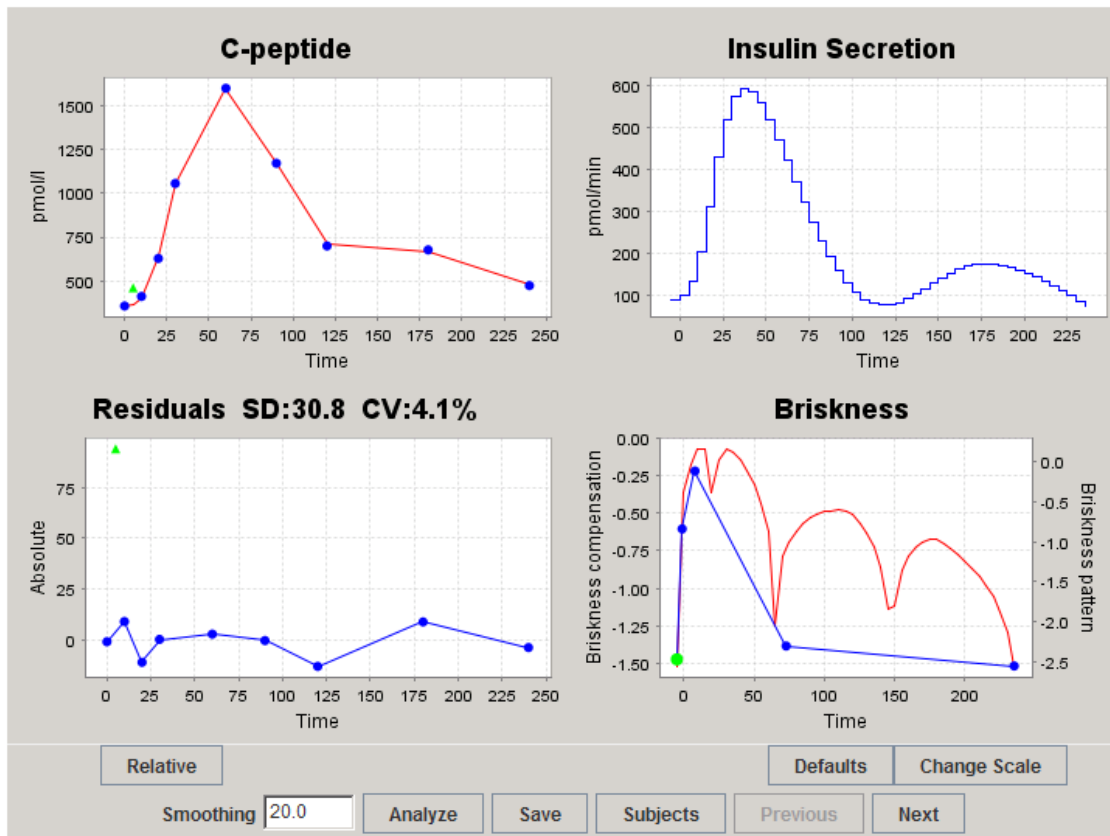



Fig 3. The SecDec graphical interface.

The SecDec graphical interface has four panels with graphs. The C-peptide data and the model residuals are shown in the left panels; insulin secretion is plotted in the top right panel. The bottom right panel provides a tool for determining the smoothing constraint on insulin secretion, as explained in detail in the User's guide. When the analysis window is displayed for a subject that has not been analyzed previously, the results are determined using default smoothing options.


 When you click on the edit icon to activate the SecDec graphical interface for the first time in your session, Java is started. This may require considerable time to load the Java software. Successive activation of the Java interface is faster.

In the analysis window, the input box at the bottom provides the basic method for controlling the insulin secretion smoothing. The number in the box is a smoothing factor; the higher is the smoothing factor the smoother is insulin secretion and the higher the C-peptide residuals. Insert a new smoothing factor value and click the Analyze button to recalculate insulin secretion using the new smoothing factor. After some seconds, the updated results are shown in the analysis window. The advanced smoothing options provided in lower right panel are described in the User's guide.

Once you have determined the optimal smoothing method and obtained satisfactory results, you can save the results by clicking the Save button. You can then proceed to the next subject

by clicking the Next button. To return to the Subjects table, click the Subjects button or the Subjects link on top of the window.

Once you have analyzed all subjects, you can download the insulin secretion results by returning to the Studies table (using the main menu) and clicking the download icon for the study.

 The *download* icon (see Fig 1).

You can suspend the analysis of your data at any time and return to SecDec later. Be sure you have saved the results of the subject you have analyzed last. To resume your analysis, login into SecDec again and click the view icon of the study you were working with. When the Subjects table is displayed, click the edit icon of the first subject not yet analyzed. This redisplay the analysis window.



# SecDec User's Guide

## Understanding deconvolution with SecDec

This section gives an outline of the procedures used to perform deconvolution with SecDec and of the available options. The section on the graphical interface below describes in detail how to use the SecDec controls to perform the actions illustrated here.

Deconvolution is the procedure by which the unknown input to a linear dynamical system (in this case insulin secretion) is determined from a measured output (C-peptide concentration) knowing the system dynamics (C-peptide kinetics) (Fig 4).

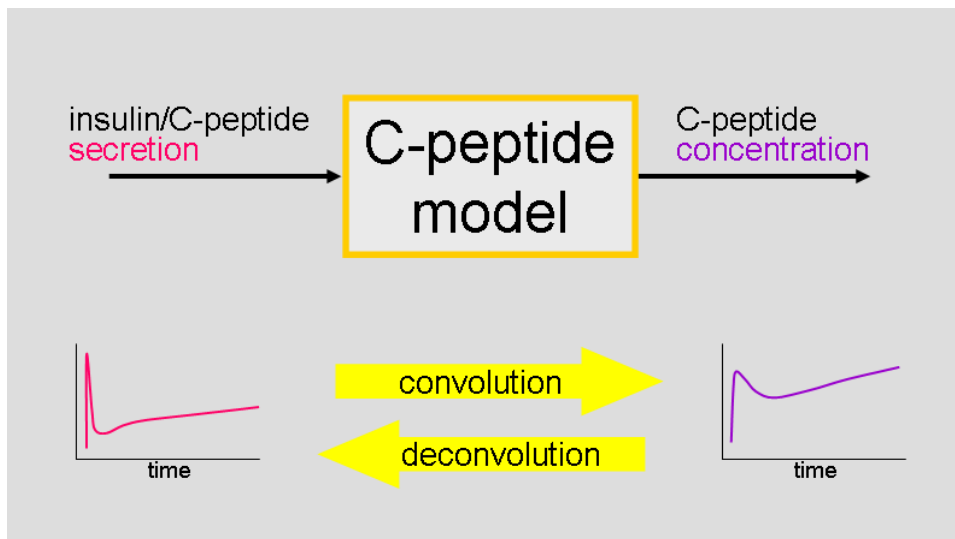


Fig 4. Convolution and deconvolution.

Calculation of insulin secretion from C-peptide deconvolution is described in (1). SecDec employs the C-peptide kinetics model by van Cauter et al. (2), with which the C-peptide kinetics parameters are individualized according to the subject's anthropometric data. Insulin secretion is calculated in pmol/min; in the results file, however, it is saved in pmol/min per square meter of estimated body surface area, as typically reported in the publications. The body surface area values used for normalization are also stored in the results file; thus, insulin secretion in pmol/min can be also calculated.

To perform deconvolution it is necessary to represent insulin secretion, which is a continuous function of time, in some discrete form. SecDec represents insulin secretion as a piecewise-constant function of time. This means that insulin secretion is assumed to take constant values in small time intervals, defined by the user (Fig 5).

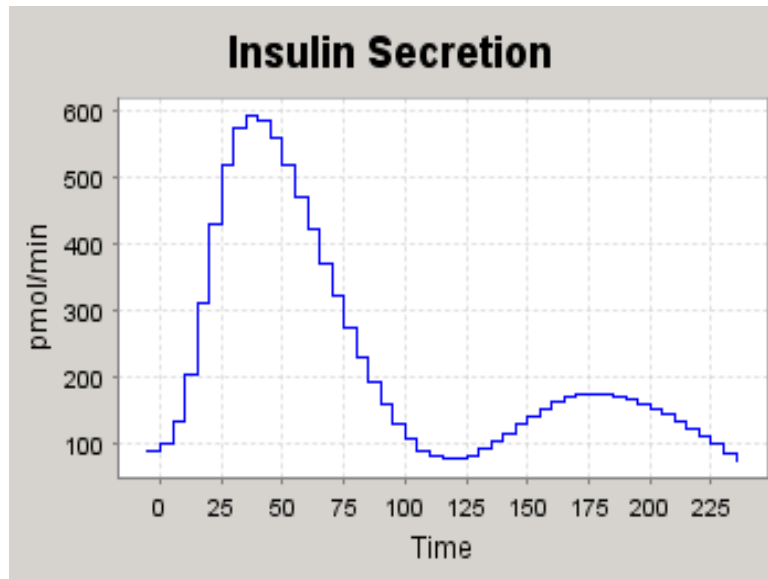


Fig 5. Piecewise-constant function representing insulin secretion.

To calculate the insulin secretion values from the known C-peptide concentrations it is also necessary to impose a smoothing constraint on insulin secretion. The smoothing constraint prevents insulin secretion from exhibiting spurious fluctuations, which is a problem specific of deconvolution.

Although a smoothing constraint is necessary to prevent spurious fluctuations with deconvolution, the kind of constraint that gives optimal results is problem-dependent. For this reason, a large variety of smoothing schemes exist; each requires the user to specify in some form the degree of smoothing appropriate for the problem. Therefore, deconvolution is a somewhat subjective procedure.

The use of a smoothing constraint implies that when the calculated insulin secretion profile is used as an input to the C-peptide kinetics model, the predicted C-peptide concentrations do not coincide with the measured values. For each time point of the C-peptide time series there is a difference between the observed and the model-predicted C-peptide value, called residual. The residuals are dependent on the degree of smoothing of insulin secretion; typically, the smoother is insulin secretion the higher are the residuals (Fig 6).

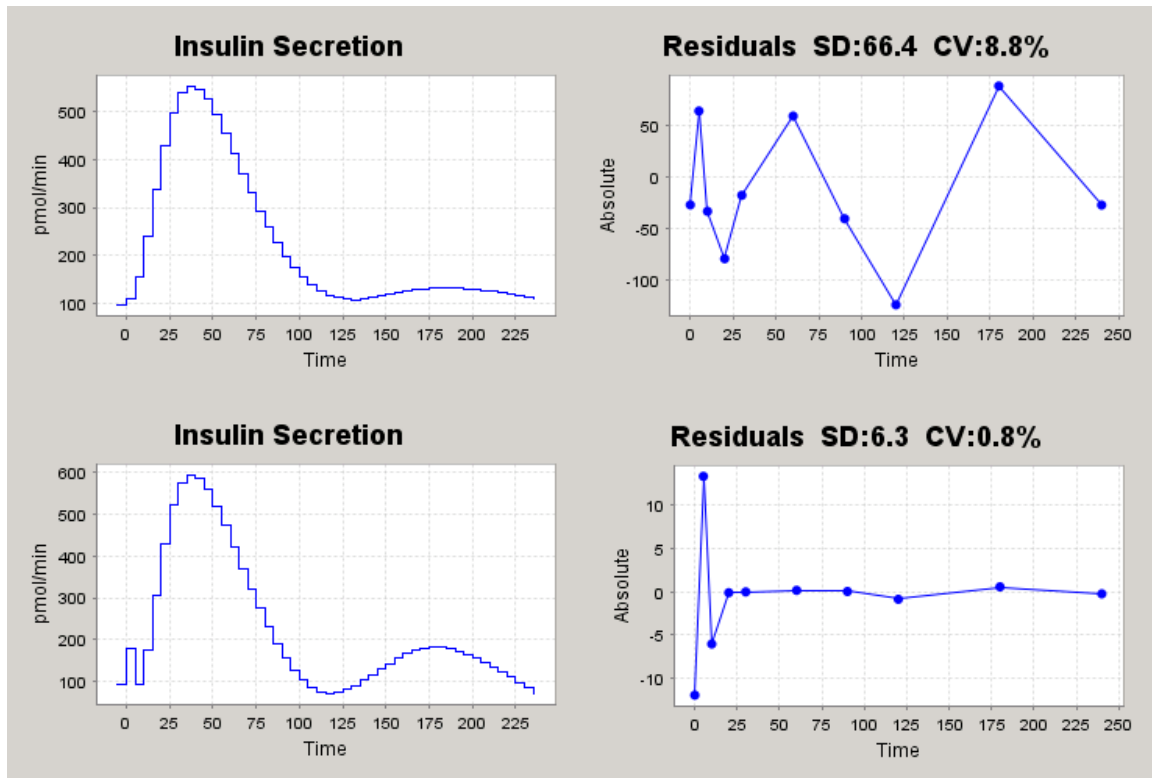


Fig 6. Effects of insulin secretion smoothing (left) on the C-peptide residuals (right). The smoother is insulin secretion the higher are the C-peptide residuals. Note the coefficients of variation of the C-peptide residuals shown on top of the right panels.

Even if insulin secretion were known exactly, non-zero residuals would be expected because the C-peptide measurement has some degree of imprecision. Thus, a typical procedure with deconvolution is to tune the smoothing so that the standard deviation of the residuals is close to that expected based on the measurement error. Some deconvolution methods, in fact, chose the degree of smoothing so that the standard deviation of the residuals (or most often their coefficient of variation) equals some pre-specified value.

SecDec does not follow this scheme for several reasons. First, and most importantly, it cannot be expected that the residuals are solely due to measurement error and that their coefficient of variation is a constant. In fact, other sources of variability besides measurement error contribute to the C-peptide variability. As a consequence, data sets from the same study may exhibit a different degree of variability, to the extent that expecting the same coefficient of variation in all cases seems inappropriate (Fig 7). When data sets have a different degree of variability, assuming a fixed coefficient of variation may yield unbalanced results in the individual subjects.

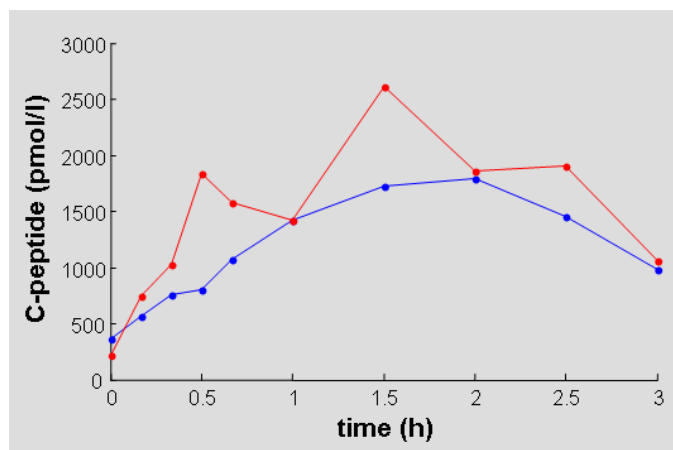


Fig 7. C-peptide concentration in two subjects from the same study (3-h OGTT) showing different variability.

Second, algorithms aimed at attaining a fixed coefficient of variation are slower. Because every case must be checked for the appropriateness of the results and often re-analyzed choosing a different target coefficient of variation, in our experience this procedure does not offer advantages over a direct control on the smoothing. Therefore, SecDec gives the user control on the smoothing and the user chooses the smoothing scheme that best describes the C-peptide data.

In its simplest form, SecDec allows the user to control the smoothing through a number, denoted as the *smoothing factor*. The higher is the smoothing factor the smoother is the insulin secretion profile. Maximum smoothing is obtained with a very high smoothing factor (theoretically infinite) and causes insulin secretion to be a straight line. With minimum smoothing (a zero smoothing factor), insulin secretion has typically an erratic time course. With this simple form for smoothing control, the user chooses the smoothing factor that maintains insulin secretion reasonably smooth and yields residuals that look random and with a coefficient of variation in agreement with the expected C-peptide error.

While the simple smoothing factor is the basic method for smoothing control, it is not sufficient to solve satisfactorily problems in which the degree of insulin secretion smoothness is not constant during the experiment. A typical case is the hyperglycemic clamp, in which first-phase insulin secretion is a brisk peak while second-phase secretion exhibits a gradual rise. In this situation, the degree of smoothness is different for first and second phase; with a single smoothing factor it is not possible to describe satisfactorily both first and second phase. Therefore, using just the simple smoothing factor option, the first-phase insulin secretion peak is blunted, while second-phase secretion tends to have spurious oscillations. In parallel, the C-peptide residuals are higher than they should be during first phase, and lower during second phase (Fig 8).

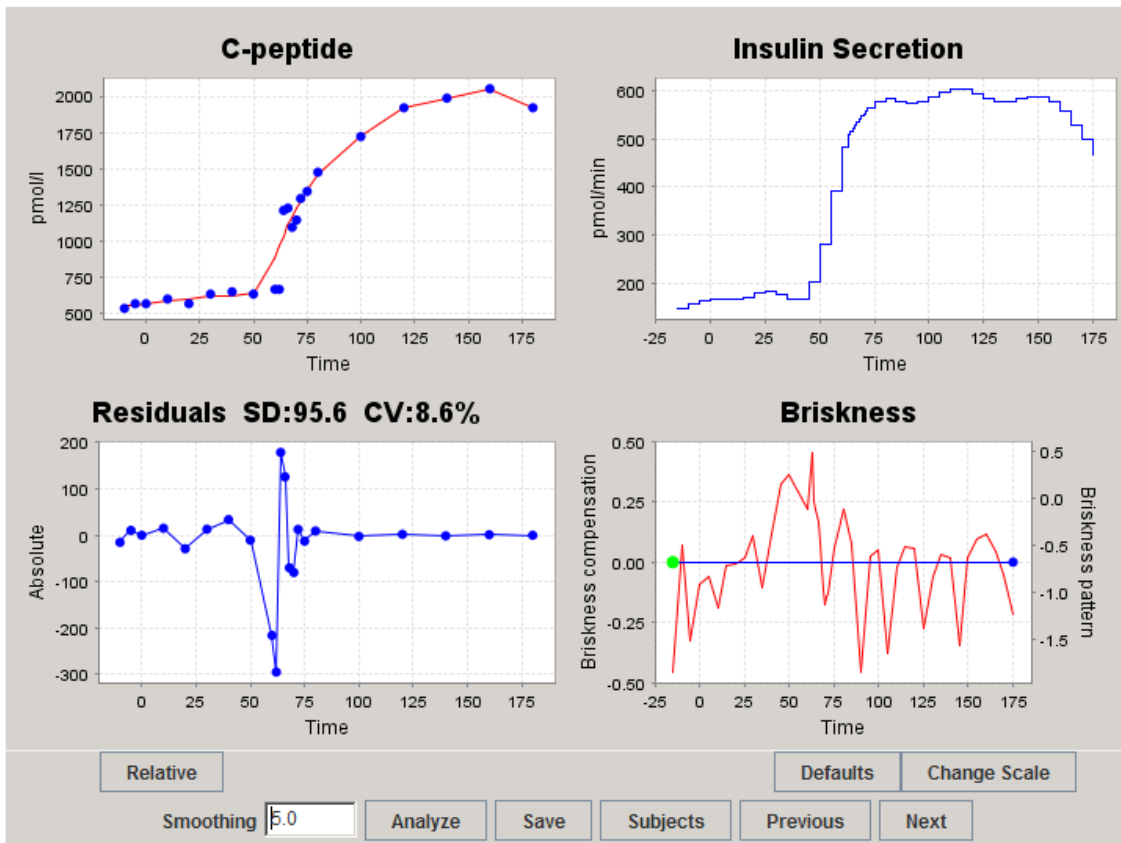


Fig 8. Insulin secretion (top right) and residuals (bottom left) in the 1<sup>st</sup> and 2<sup>nd</sup> phase of a hyperglycemic clamp (started at 60 min). Initial calculation using a simple smoothing factor and no briskness compensation.

To solve this problem, SecDec has a graphical tool to balance the smoothing during the periods of the experiment that require a different degree of smoothness, as in the hyperglycemic clamp. The tool is based on the following principles.

When a subject is analyzed, SecDec displays in the lower right panel a graph representing the *briskness pattern* of the calculated insulin secretion (Fig 8, lower right panel, red line). As it can be appreciated from Fig 8, rapid changes in insulin secretion are associated with a higher briskness: the red line in Fig 8 has a peak during first-phase secretion (around 60 min).

To balance the smoothing between the brisker first phase and the smoother second phase, SecDec allows the user to build a line that follows the briskness pattern, using the mouse (Fig 8, lower right panel, blue line). The details for drawing this line, denoted as the *briskness compensation line*, are explained in a following section. Reanalyzing the data using an appropriate briskness compensation line produces an insulin secretion profile that approximates the expected pattern and more balanced residuals (Fig 9).

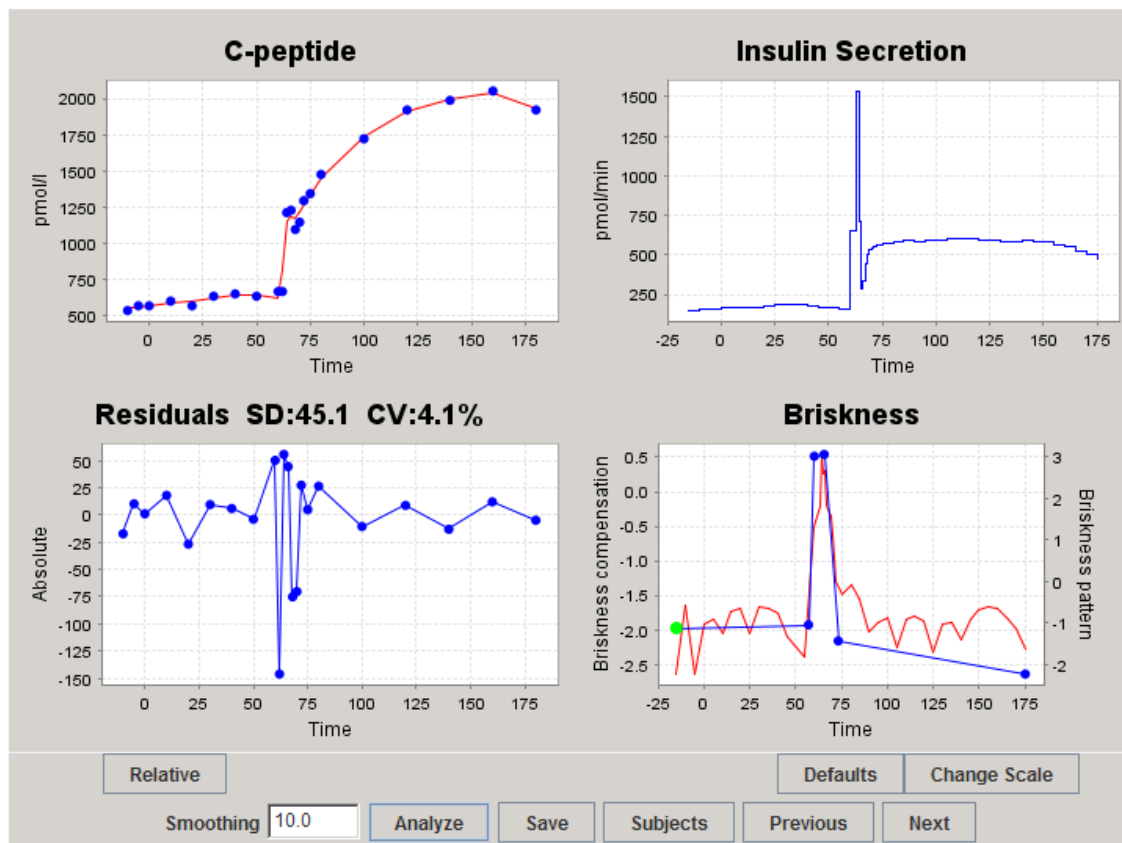


Fig 9. Insulin secretion during the 1<sup>st</sup> and 2<sup>nd</sup> phase of a hyperglycemic clamp recalculated using an appropriate briskness compensation line (bottom right panel, blue line), which follows the briskness pattern.

The briskness compensation line should not follow the details of the briskness pattern but only distinguish between phases with different secretion patterns, as in Fig 9. The use of the briskness compensation line interferes somewhat with the smoothing factor. When the briskness compensation line is changed to a substantial extent, it may be necessary to modify the smoothing factor to obtain the desired results. In this case, some iterations involving changes in the smoothing factor and the compensation line may be required. Note that in Fig 9 the smoothing factor has been doubled compared to Fig 8.

When the insulin secretion pattern is a mixture of brisk and smooth phases and the pattern is rather heterogeneous between subjects, it may be quite demanding to analyze the data accurately. In fact, the briskness compensation line and the smoothing factor need to be iteratively adjusted to match the individual patterns. In particular, the presence of brisk peaks needs a careful design of the briskness compensation line so that insulin secretion is allowed to vary sharply at the peak position, while maintaining the appropriate degree of smoothing in the periods preceding and following the peak. Thus, if the peak position varies among subjects, this fine tuning may need to be repeated for every subject.

To facilitate the application of an appropriate briskness compensation line and smoothing factor, SecDec allows the user to save both of them as default deconvolution parameters. Default parameters are used to perform deconvolution when the subject's data are loaded for

the analysis the first time. Thus, if the briskness pattern is rather homogeneous among the subjects of the study, the time required to tune the smoothing parameters is reduced.

Reconstructing brisk secretion peaks may be difficult; spurious oscillations in insulin secretion may occur or the C-peptide data fit may be unsatisfactory. Secretion may also be negative, as SecDec does not use a positivity constraint to avoid bias. This is not only due to the deconvolution algorithm, but also to the use of standardized parameters with the C-peptide kinetics model (2), which may be inappropriate for some subjects. To overcome, at least in part, these problems, it is suggested to use a fine time grid for insulin secretion and to tune the briskness compensation line accurately by trial and error (cf. Fig 9).

### Creating a study

SecDec is designed to store and work with data from different studies, such as a 2-hour hyperglycemic clamp, a 4-hour meal test and a 24-hour multiple meal experiments. Because each study has its own characteristics, before you load your data you need to create a new study and set the options appropriate for it.

You create a new study by clicking on the New Study link on the menu of the Studies table (Fig 1). This displays a page with the study settings (Fig 10).

User: test

Studies Logout

Study

Weights

No weights

Weighting function

File weights override settings

Time grid

Uniform step  min

Custom

Create Cancel

Fig 10. Study settings.

When you create a new study you have to assign a name to it, so that it can be identified (top input box in Fig 10). You then need to set some configuration parameters for the study. In particular, you need to specify how the C-peptide residuals are weighted (mid box in Fig 10) and which time grid is used to calculate insulin secretion (bottom box in Fig 10). These options are described in detail below.

### *C-peptide weights*

Roughly speaking, deconvolution minimizes the sum of the squared C-peptide residuals, subject to the smoothness constraint. One would expect that the magnitude of each C-peptide residual reflects the standard deviation of the C-peptide measurement error expected for that point. To obtain a residual pattern consistent with the C-peptide measurement error, it is necessary to weight each residual by the inverse of its expected standard deviation. Residuals that are associated with a higher standard deviation should be weighted less than those associated with a lower standard deviation.

SecDec offers two weighting options. With the first option, no weights (or equivalently, all weights equal to 1) are used, i.e., all residuals are weighted in the same way. With the second option, the user can specify a standard deviation function for determining the weights, as  $\text{weight} = 1 / (\text{standard deviation})$ . This is the suggested option.

The standard deviation function expression must be inserted in the input box if this option is selected. The expression must be a mathematical function of a single independent variable representing C-peptide concentration. A typical expression is for instance:

$$0.05 * x + 10$$

This expression represents a standard deviation that is a linear function of C-peptide concentration. Similarly, the expression  $0.05 * x$  represents a constant coefficient of variation of 5%.

The standard deviation function expression can also be more elaborate. The independent variable needs not to be denoted with “x”; another name, such as “cpep”, can also be used. Thus, the expressions  $0.05 * x$  and  $0.05 * \text{cpep}$  are equivalent. The expression can contain parentheses and other mathematical operators and function. Valid expressions are for instance,  $0.001 * x^2 + 0.02 * x + 10$ , representing a 2<sup>nd</sup> degree polynomial (the symbol “^” represents the power),  $100 / (10 + x)$  (a Michaelis-Menten function) and  $100 * (1 - \exp(-0.01 * x))$ . The mathematical functions you can use in an expression are: sqrt, square root; exp, exponential function; log and log10, logarithms in base e and 10; sin, cos, tan, sine, cosine and tangent, respectively; sinh, cosh, tanh, hyperbolic sine, cosine and tangent, respectively.

The standard deviation function expression is checked when you click the Create button (Fig 10) to create a new study; if the expression is incorrect an error is given. However, be sure that you have inserted the appropriate function expression; otherwise, deconvolution may give unexpected results.

With both weighting options, by checking the box “File weights override settings” C-peptide weights are retrieved from the data file when the subject’s data are loaded (see the following section); the weights available in the file override those calculated according to the user’s options. If you want to use your own weights, select the “No weights” option, check the box and include the weights in your data files.



### *Secretion time grid*

As discussed above, secretion is represented as a piecewise constant function over a user-specified time grid. The secretion time grid can be specified in two ways: as an equally-spaced time grid or as an arbitrary set of times (i.e., an unequally-spaced time grid).

With an equally-spaced time grid, you need to specify only the time step in minutes. If you chose a time step of 2 min, secretion is calculated every 2 min from the time corresponding to the first available C-peptide point until 2 min before the last available C-peptide (secretion is not calculated at the last C-peptide point because its value would influence model-predicted C-peptide concentration only *after* the last C-peptide point).

With an unequally-spaced time grid, the user can define an arbitrary set of time points using the syntax:

```
0 10 20:2:30 35:5:60 80 100 120
```

With this syntax, successive time points are indicated as a space-separated list, and the expression 20:2:30 denotes a uniformly-spaced time grid from 20 to 30 with a time step of 2 min. Thus, the expression above corresponds to the time grid: 0, 10, 20, 22, 24, 26, 28, 30, 35, 40, 45, 50, 55, 60, 80, 100 and 120 min. The time grid must include at least two time points and times must be in ascending order. The time grid is checked when you click the Create button to create a new study; if the time grid is incorrect an error is given.

With an unequally-spaced time grid it is important to check that the grid is appropriate for all the subjects of a study. If a subject has a shorter data set (e.g. if in the example above the last C-peptide data point is at 100 min), the time grid is truncated accordingly and the results are as expected. But if a subject has a longer data set (e.g. the last C-peptide data point is at 180 min), it is assumed that secretion holds its last value until the end of the experiment (in the example, secretion would be constant from 120 to 180 min). If this assumption is not appropriate, the time grid must be extended to work satisfactorily with the longest data set.

With both choices for the time grid, it is always assumed that secretion has some non-zero value from minus infinity up to the first point of the time grid (basal secretion). Thus, the first time grid point is the instant at which insulin secretion starts to change from its basal value.

Depending on the time grid options and the data, the individual time grids may not be homogeneous. This is not a problem for SecDec. When insulin secretion results are downloaded, however, the results are provided on the same time grid; the subjects with a shorter experimental length have missing secretion values.

The study settings can be changed until you save the results of one subject; after the first save, the study settings cannot be edited.

### **The Studies table**

Once you have created the first study in your SecDec account, the study is shown in the *Studies table*.





























study	N	done	smoothing	briskness	
clamp	3	0	-	no	      
ivgtt	3	1	-	no	      
meal	3	1	20	yes	      

Fig 11. The Studies table.

The Studies table shows the following information on a study (the order in the list corresponds to the order of the columns in the table of Fig 11):

- The name of the study.
- The number of subjects whose data have been loaded in the SecDec database.
- The number of subjects whose data have been analyzed.
- The default smoothing factor used in the analysis (initially undefined).
- Whether a default briskness compensation line has been defined (a yes/no field).

In addition to the information on the study, the Studies table displays icons to perform actions on the study, as shown below.

	Study settings
	View study subjects
	Add data
	Replace data
	Show errors
	Delete study
	Download study results

When the mouse pointer is over one of these icons, a tooltip is shown to remind the function of the icon.

The study settings are not shown in the Studies table. To see the study settings, click on the study settings icon. This displays a page in which you can both see and edit the settings. Study settings cannot be edited once you have saved the results of the analysis of one subject.

### Loading data for a study

Once you have created a study, you can load your data in the SecDec database. To load the data, keep in mind the following principles:

- Data can be loaded from Excel or text tab-delimited files.
- Data must be submitted using a template file, available at the SecDec Help & files page (link at the SecDec login page).


- Data can be submitted in a single file or in multiple files.
- Submitted data are checked for errors. If errors are found, all data contained in the file are rejected (including the correct data) and a list of errors is displayed. In this case, the errors must be corrected in the data file and the file resubmitted. Only when no errors are found in a data file the data are loaded in the SecDec database.
- Data cannot be edited directly in the SecDec database; however, they can be deleted or replaced.
- To replace data, prepare a separate file with the complete data of all subjects whose data need be replaced. Submit the file to SecDec using the replace function, which is distinct from that used to load new data. Data replacement follows the same rules as the submission of new data.
- Data can be deleted from the SecDec database by clicking on the delete icons in the tables. The delete icon in the Studies table deletes the data of all subjects of a study and the study itself. The delete icon in the Subjects table (described below) deletes the data of a single subject.

The rules for loading data are described in detail below.

### *Necessary data*

For each subject, the following data are required:

- A subject ID, i.e., a number or a string that identifies the subject uniquely. Duplicate IDs cannot exist within the same study.
- The subject's sex, coded with the letter "M" for male and "F" for female.
- The subject's age, in years (allowed range 1-120 years).
- The subject's height, in meters or centimeters (allowed range 0.5-2.5 m).
- The subject's weight, in kilograms (allowed range 5-300 kg).
- A flag indicating if the subject is a type 2 diabetic subject. If the cell with the flag is left blank, the subject is non-diabetic. If the cell is marked with any sign (e.g. an "x"), the subject is diabetic. Warning: do not insert a code like "no", as it has the opposite effect (as the cell is non-blank). The diabetes status is needed for the standardized C-peptide kinetics model.
- A column with times at which C-peptide is sampled. Times must be in minutes, without missing data and in ascending order.
- A column with the C-peptide values corresponding to the given times. C-peptide values must be in pmol/l and must be positive. Missing C-peptide values (blank cells) are allowed. Every C-peptide value must have a corresponding time. There must be at least 4 non-missing C-peptide values.
- An optional column with the C-peptide weights. These data are used only if the checkbox "File weights override settings" in the study settings is checked and can be entirely missing. In this case, missing weights are replaced with those calculated according to the study settings. Every weight value must have a corresponding time.

 It's your responsibility to use IDs complying with the privacy and security rules concerning the subjects' data.

### *Preparing a data file*

Data can be submitted as an Excel file or a text tab-delimited file. The text tab-delimited format is provided for use with software other than Excel that cannot export data in an Excel format. When using software for editing tables, remember the following important points:

- Excel files are not always readable by SecDec. If an Excel file cannot be read, try saving it in Excel 5/95 format (or earlier), or, as a last chance, as a text tab-delimited file.
- The use of country-specific decimal point separators should not be a problem with Excel files. However, text tab-delimited files must use the point (and not the comma) as a decimal separator (e.g., 1.23, not 1,23).
- Missing numeric values, when allowed, are represented in Excel as blank cells or as cells containing non-numeric values. With text tab-delimited files, blank cells must correspond to two successive tabs, as obtained by saving an Excel file in this text format. If you produce a text tab-delimited file with software other than Excel, check that this is the format actually produced. If this is not the case, use a non-numeric code to indicate a missing value.
- Only Excel files, with extension “.xls”, or text tab-delimited files, with extension “.txt” can be read by SecDec. If you are using a Mac that does not have file extension by default, append the appropriate extension to the file before you submit it to SecDec.

A data file contains the data of one subject in a data block spanning three columns and several rows. Data items are identified by a label. Scalar data are inserted to the right of the label, column data are inserted in columns below the column headings.

The top of the subject’s data block (spanning two columns) contains the subject ID, the anthropometric parameters and the flag for diabetes (Fig 12).

<b>ID</b>	S1
<b>sex</b>	M
<b>height</b>	1.875
<b>weight</b>	87.10
<b>age</b>	25.53
<b>diabetic</b>	

Fig 12. The data block with the anthropometric parameters.

The bottom of the subject’s data block (spanning three columns) contains the C-peptide data and the optional weights (Fig 13).

<b>time</b>	<b>C-peptide</b>	<b>cpw</b>
-30	508	
-15	501	
60	233	
90	237	
...	...	

Fig 13. The data block with C-peptide and the optional column for weights.

To prepare a data file, download the template in the format suitable for your software. Templates are available at the Help & files page, accessible from the SecDec login page. The template contains three data blocks as a reference (Fig 14).

<b>ID</b>		
<b>sex</b>		
<b>height</b>		
<b>weight</b>		
<b>age</b>		
<b>diabetic</b>		
<b>time</b>	<b>C-peptide</b>	<b>cpw</b>

Fig 14. Labels of a complete empty data block from the Excel template.

Copy a complete data block to the right of the existing blocks to hold data for more than three subjects. Insert the scalar data in the top blocks, to the right of the labels. Insert the C-peptide times and values (and optionally the weights) below the column headings of the lower block.

To prepare a data file with data of many subjects, keep in mind the following rules:

- To add more subjects, insert more data blocks to the right by copying the labels.
- Data blocks may be spaced by a variable number of empty columns; empty columns between data blocks may improve clarity, but are irrelevant for the submission of data.
- Keep corresponding labels of the various data blocks aligned on the same row (i.e., don't place a subject ID in cell B1 and the next ID in cell F2) and do not alter the relative position of the labels within a data block. Files with misaligned or altered labels are rejected. However, provided that the data labels are aligned, the file can contain extra space around and between the data blocks.
- You can insert other data, such as comments or other information, outside the data block area, but not below the C-peptide column data. However, you must not use labels that are present in the template.
- If you use Excel, you can add formatting to the file as you need. Formats are irrelevant for data submission.
- If you have stored your data in an Excel workbook, only the active (visible) worksheet is read by SecDec. Be sure that the worksheet with your data is the active worksheet when you submit the file.
- A valid file must contain at least a data block for one subject.
- All data blocks must be complete.
- The C-peptide data for the subjects can have different lengths and also sampling times.
- No data must be inserted below the C-peptide data.
- All subjects IDs must be non-missing and distinct.

### *Submitting a data file*

To add data to a study, click the add icon for that study in the Studies table. This displays a form with which you can browse your documents to select the appropriate file. Once you have selected the data file, click the submit button to send the data to SecDec.

SecDec checks the file for errors. The following checks are performed, in the order of the list below. If these checks are not passed, the file is rejected and error messages are displayed explaining the errors.

1. The file must be readable and have the appropriate format.
2. All subject IDs must be non-missing and distinct.
3. None of the subject IDs must already exist in the database.
4. All the individual data must be correct.

If steps 1 and 2 are passed, in case of errors in the following steps SecDec shows a detailed list explaining the errors, which will help the user to correct the problems. For instance, if the user submits data with subject IDs that are already in the SecDec database, the duplicate IDs are shown in the list. Similarly, if some anthropometric data are missing or incorrect, the subject IDs with incorrect data and the data problems are displayed.

The error list, shown upon data submission if errors are present, remains available to the user also after the window with the error list is closed; click the errors icon for the desired study in the Studies table to see the errors. This helps the user to correct the problem at any time. See the section Correcting data errors for more details.

When you submit data and errors are found, no data are loaded in the SecDec database even if the errors concern data of a single subject. This keeps the submission procedure simpler, as you can resubmit the whole file after correction.

### **Replacing existing data**

If you realize that the submitted data are inappropriate you can modify them by submitting a new data file. This is the only way to modify data, as SecDec does not allow direct data editing.

You can submit modified data at any time. However, if you have already analyzed the data you are going to modify and saved the results, the results will be lost.

To replace data, prepare a file with the complete data of the subjects whose data you want to change, following the same rules used to submit new data. You must include only the data of the subjects whose data you want to replace, as all the subjects included in the file will be replaced in the SecDec database with this operation.

Once the data file with the subjects to replace is ready, click the replace icon in the Studies table corresponding to the desired study. This displays a form with which you can browse your documents to select the appropriate file. Once you have selected the data file, click the submit button to send the data to SecDec. The form for submitting data to replace is the same as that for submitting new data. However, a warning message is displayed if you are replacing data when the results of the analysis for some subjects have already been saved.

Errors are checked as for the submission of new data, but checking for IDs already existing in the SecDec database is obviously not performed. If you submit data for new subjects together with data of subjects already existing in the SecDec database using the replace function, all subjects are added or replaced in the database (provided they are correct).

## Deleting a study

To delete a study and all its data, click the delete icon of the study in the Studies table. Before the data are actually removed from the SecDec database, a confirmation message is displayed. Deletions are irreversible.

## Correcting data errors

The errors found in the data files submitted for a study are permanently stored in a list of errors. The list is displayed by clicking the errors icon of the study in the Studies table. The errors in the list are sorted according to the date and time of submission of the data file, in inverse order. Thus, the most recent errors are shown on top. The errors relative to a given data file have the same date and time, which is the date and time of the file submission. The error list shows, in addition to the date and time, the ID of the subject whose data are incorrect and a message explaining the error.

User: **test** Study: **clamp** Studies Logout

run date	ID	error
2009-06-15 10:56:37	3	ID already exists in database
2009-06-15 10:56:37	3	Weight is missing or out of range
2009-06-15 10:56:37	a	C-peptide or C-peptide weight have no corresponding times
2009-06-15 10:56:37	b	Age is missing or out of range

Fig 15. The Errors table for the study *clamp*.

Use the error list to identify the incorrect data in your files and to correct them. Once you have corrected the data, you may delete the errors from the error list by clicking the Delete errors button at the bottom of the list. If you clear the error list before you submit a data file, after the file submission the error list will contain only the errors relative to the last submitted file, if any. Otherwise, to identify the errors present in your last submission you have to look at the date and time of the error.

## The Subjects table

Once you have loaded the data for a study in the SecDec database, you may start the analysis. To start, click the view icon in the Studies table. This displays a table showing the subjects' data, denoted as *Subjects table*.







ID	sex	weight	height	age	diabetic	done	smoothing		
1	M	73.1	1.79	33	no	yes	20		
2	M	74.6	1.76	30	no	no			
3	M	85.1	1.89	25	no	no			

Fig 16. The Subjects table that displays the subjects of the *meal* study.

The Subjects table displays the following information on the subjects (the order in the list corresponds to the order of the columns in the table of Fig 16):

- The subject's ID.
- The subject's anthropometric parameters (sex, weight, height, age and the diabetes status).
- Information on whether the subject has been analyzed (column "done").
- The smoothing factor used.

In addition to the information on the subjects, the Subjects table shows two icons to perform actions, as shown below.



Analyze/edit subject



Delete subject

When the mouse pointer is over one of these icons, a tooltip is shown to remind the function of the icon.

Click on the analyze/edit icon to analyze a subject for the first time or edit the analysis. This displays the SecDec graphical interface, which is explained in detail in the following section.

Click the delete subject if you want to delete a specific subject. Before the data are actually removed from the SecDec database, a confirmation message is displayed. Deletions are irreversible. If you delete a subject by mistake, you can reload the data as explained above.

### The SecDec graphical interface

When you click the analyze/edit icon in the Subjects table, if the subject has not been yet analyzed, deconvolution is performed using default parameters; if the subject's has already been analyzed, the results of the analysis are loaded. In both cases, SecDec displays a graphical interface, which show the results and allows the user to edit the analysis.



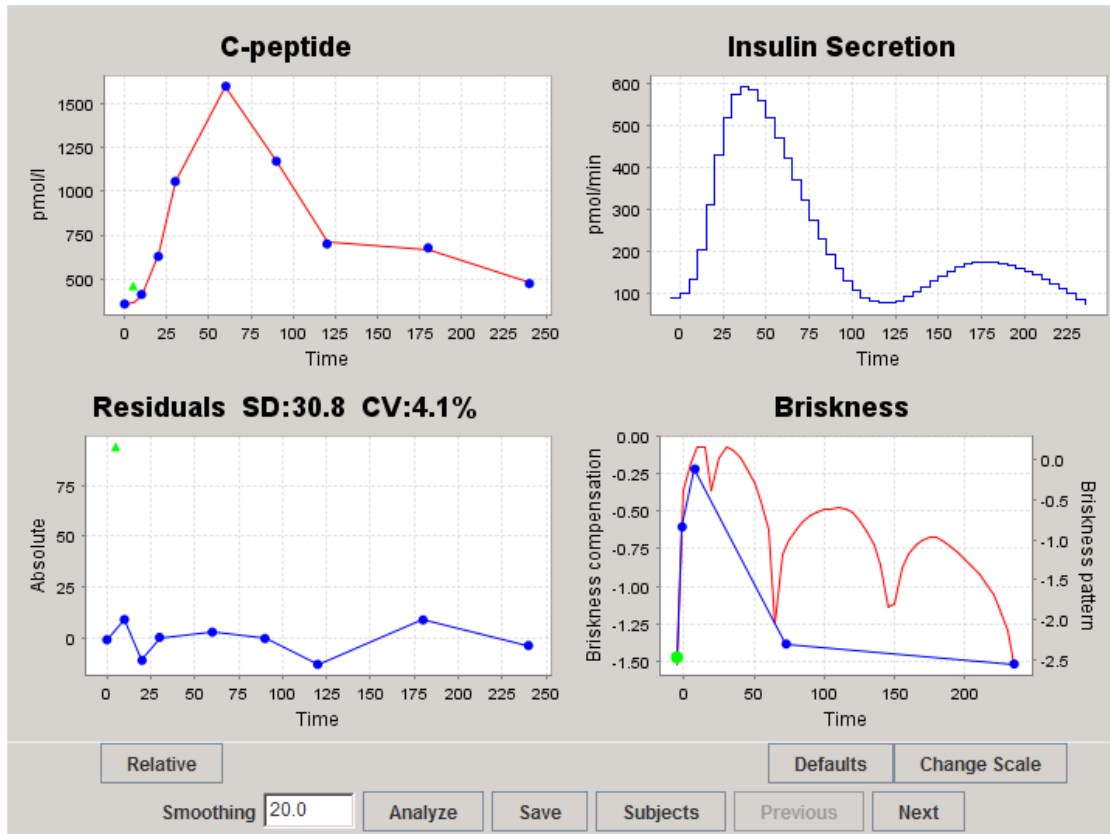


Fig 17. The SecDec graphical interface.

The SecDec graphical interface displays on top of the graphical window the user, study and subject's ID on the left and a navigation menu on the right, four plots in the central part and several action buttons below the plots. The purpose of these elements is described below.

#### *C-peptide plot*

The C-peptide plot (Fig 17, top left) shows the C-peptide data (as blue circles) and the data fit obtained with the current deconvolution parameters (as a continuous red line). The model-predicted C-peptide values are calculated only at the data points; the line is a simple linear interpolation.

On the C-peptide plot it is possible to exclude points from the analysis. To exclude a point, click on the small blue circle representing the point. The blue circle turns into a green triangle when the point is excluded (as in Fig 17, left). To re-include the point, click on the point again.

#### *Residuals plot*

The residuals plot (Fig 17, bottom left) shows the C-peptide residuals as blue circles connected by a blue line. When the graphical interface is activated, the absolute residuals are shown. By clicking the Relative button at the bottom of the plot, the residuals toggle from absolute to relative. The plot title shows the standard deviation (SD) and the percent

coefficient of variation (CV) of the residuals; excluded points are not taken into account when these parameters are calculated.

On the residuals plot it is possible to exclude points as in the C-peptide plot by clicking on the symbols.

### *Secretion Plot*

The secretion plot (Fig 17, top right) shows insulin secretion, in pmol/min. This plot has no interactive functions.


### *Briskness plot*

The briskness plot (Fig 17, bottom right) shows the insulin secretion briskness pattern as a red line; the scale for this line is the right-hand side scale.

The blue line is the briskness compensation line; the line connects two or more breakpoints, shown as blue circles. Initially, the default briskness compensation line is a horizontal straight line with just two breakpoints. The user can modify the line by adding breakpoints and changing their position. The aim is to reproduce the main features of the briskness pattern (the red line), as in Fig 9 or 17.

To move a breakpoint of the briskness compensation line, click first on the point to select it. A selected point is shown in green rather than in blue. Points can also be selected in sequence using the left and right arrow keys. Once a point is selected, it is moved to another place by clicking on the graph at the desired position. Note however that the extreme point can be moved only vertically and that the abscissa of a point cannot be moved beyond the abscissa of the preceding and following point. Note that moving the extreme points may sometimes be difficult because you need to click right above or below the point (as you cannot move the abscissa). If you have difficulties, try to zoom in the x-axis to enlarge the scale and obtain a more accurate pointing. Zooming is described below.

To add a breakpoint, hold down the control key and click on the white area of the panel at the desired position. To delete a breakpoint, select the point and press the delete/cancel key. The extreme points cannot be deleted.

 Warning: in typical keyboards the delete/cancel key is that in the key group above the arrow keys, not that in the main keyboard above the enter key.

The Change scale button below the briskness plot allows the user to change the scale of the briskness compensation line (the left scale of the plot). This may be relevant initially, when the briskness compensation line is a simple horizontal line with a default ordinate. Setting a scale for the briskness compensation line that matches that of the briskness pattern (the scale on the right) may help to design a line that reproduces the features of the briskness pattern. When the Change scale button is clicked, a small pop-up window is shown in which the y-axis limits can be set manually (Fig 18, left).

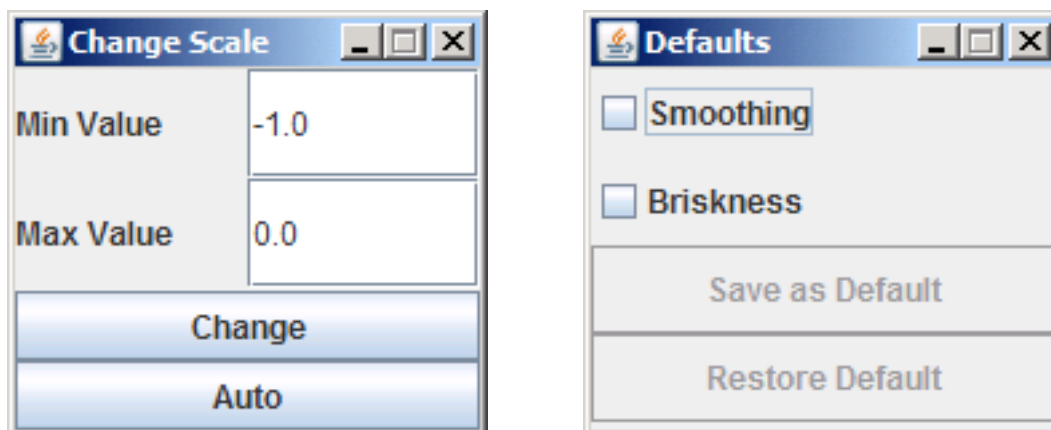


Fig 18. The Change scale (left) and Defaults (right) pop-up windows.

The Defaults button below the briskness plot opens a small pop-up window with which it is possible to set or retrieve default values for the smoothing factor or the briskness compensation line. The pop-up window (Fig 18, right) has two checkboxes to select the parameter (the smoothing factor or the briskness compensation line) and two action buttons (Save as default and Restore default).

### *Zooming*

In all plots, using the mouse it is possible to zoom in rectangular areas of the plot. Point to the upper left corner of the rectangle you want to zoom in, click and keep pressed the mouse button while dragging to the lower right corner and then release the button. To zoom out to the original scale, use the same method to draw a rectangle of any dimension in the opposite direction, i.e., from the lower right to the upper left corner.

The plot appearance can also be controlled with some options provided with pop-up menus (shown by clicking the right mouse button in Windows).

### *Action and navigation buttons*

The bottom of the graphical interface displays a number box and several buttons. The number box contains the smoothing factor. The Analyze button performs deconvolution using the smoothing factor entered in the box and the other parameters (i.e., the briskness compensation line and the exclusion of points, if any). After deconvolution is performed, the new results are displayed.

The Save button saves the results displayed. When you have made changes to the deconvolution parameters the Save button is dimmed and disabled, indicating that the analysis should be re-performed before saving.

The Subjects button displays the Subjects table. The Next and Previous buttons load the data of the subjects preceding or following the subject displayed. One of these buttons is dimmed when the first or last subject is displayed. Before you move to the next or previous subject, remember to save the current results.

## Downloading insulin secretion results

To download the calculated insulin secretion for a study, go to the Studies table and click on the download icon of the study. This shows a page with a Download button. Clicking on the button should display a dialog box with an option to save the file. The name of the results file is a randomly generated name; you can rename the file as you prefer. If this method fails, use the link that points to the results file below the download button. Do not click on the link as this will likely display the file in the browser window. Use the pop-up menu with the option for saving the link target (right-click on the link in Windows).

The results file is a text-tab delimited file with the calculated insulin secretion. The file can be directly opened by Excel and analogous programs. The structure of the file is shown in Fig 19.


SID:	S1	S2	S3
BSA:	1.912418	1.982418	1.780253
-5	47.10186	52.71129	70.2672
0	52.4369	60.36608	81.16632
5	70.084	70.17191	94.24587
10	106.974	104.2545	139.089
...	...	...	...

Fig 19. The results file with insulin secretion.

The top row shows the subject ID (SID). The second row contains the estimated body surface area (BSA) of the subjects, as insulin secretion is expressed in pmol/min per square meter of body surface area. The leftmost column, except for the headings in two top rows, contains the times (in minutes) at which insulin secretion is calculated (i.e., the time grid defined in the study settings). Insulin secretion (in pmol min<sup>-1</sup>m<sup>-2</sup>) is contained in the adjacent columns (one column per subject, one row per time instant). To obtain insulin secretion in pmol/min, the insulin secretion values reported in the columns must be multiplied by the subject's body surface area shown in the second row.

The first time instant for insulin secretion (-5 min in the example of Fig 19) corresponds to basal secretion and is defined by SecDec. The user-defined time grid begins from the second time point (0 min in the example of Fig 19).

It must be remembered that SecDec calculates secretion as a piecewise-constant function of time. Therefore, in the example of Fig 19, secretion for subject S1 has the value 47.10186 between -infinity to 0 min, 52.4369 between 0 and 5 min, 70.084 between 5 and 10 min, etc.

 The results text file uses a point (.) as decimal separator. If your program uses a different decimal separator, you should use an appropriate import function (available in the recent versions of Excel) or replace the decimal point with the decimal separator of your system using a text editor.

## SecDec database backup

To produce a file with a backup copy of your data in the SecDec database, use the Backup link in the menu of the Studies table. This displays a page with a Download button. Clicking

on the button should display a dialog box with an option to save the file. The name of the backup file is a randomly generated name; you can rename the file as you prefer. If this method fails, use the link that points to the backup file below the download button. Use the pop-up menu with the option for saving the link target (right-click on the link in Windows).

The backup file is a binary file that contains all your data as they are when you perform the backup. You cannot use this file to restore your data; you need to contact the SecDec administrator to perform this action.

Though the risk of loss of data from the SecDec database is small, keeping a backup copy is advisable. Making a backup copy is also appropriate before you delete your data from the SecDec database, in case you may need to restore your data in the future. Note that the backup file contains information on the deconvolution parameters besides the original data and the calculated insulin secretion.

### **Known problems**

There are a few known problems with the SecDec software. Most of them derive from difficulties inherent to the development of web applications. If the software problems prevent you from working with SecDec and this section does not provide a solution, try to contact the SecDec administrator (link at the SecDec login page).

#### *Java problems*

To work with SecDec, you need to have installed an updated version of Java on your computer. See the section below for more information about Java installation. Java is necessary for the SecDec graphical interface. Even if you have an appropriate version of Java on your computer, however, the SecDec graphical interface may occasionally not work. In this case, logout SecDec, try to close your browser and restart. On the Macintosh, the Java graphical interface may remain invisible until you click on it.

In the SecDec graphical interface, moving the extreme points of the briskness compensation line may sometimes be difficult because you need to click right above or below the point (as you cannot move the abscissa). Thus, you may experience that clicking does not produce the expected result. If you have this difficulty, try to zoom in the x-axis to enlarge the scale and obtain a more accurate pointing.

#### *Browser problems*

As explained in the system requirements, you need to have cookies and Javascript enabled in your browser. Depending on your browser settings, Javascript sometimes may cause the browser to block the Javascript actions and display an alert. You should allow the browser to execute these actions, which are completely safe.

The browser may in particular block the Javascript action when you use the contact form to send a message to the SecDec administrator. This may happen because Javascript tries to open a new window with the contact form. To use the form, tell the browser to allow Javascript to open the window.

SecDec has been designed according to the W3C standards and uses basic, well-established web programming methods. However, not all browsers comply with the W3C standards and the pages may occasionally be rendered inappropriately. In case of problems with page rendering, use a compliant browser.

### *SecDec server problems*

As explained in the general information, the SecDec calculation engine on the server can serve only a limited number of users simultaneously. This limitation concerns only some SecDec functions, such as performing the deconvolution analysis, which are executed by the calculation engine in a short time. Thus, the probability of exceeding the maximum number of users is low. However, this may happen occasionally. In this case, SecDec gives an error message and you need to retry you action later. SecDec may sometimes remain not accessible for relatively long time, in particular if the server is under maintenance. Try to login SecDec later; if the problem persist, contact the SecDec administrator.

### *Unknown problems*

If you encounter software problems with SecDec that are not described here, please report the problem to the SecDec administrator. You will help us to improve SecDec.

### **Java installation**

To verify if your computer has Java installed correctly, go to the site: [www.java.com](http://www.java.com). The site has options to verify if Java is installed on your computer and works properly. You can also download Java from [www.java.com](http://www.java.com), if it is not already installed on your computer.

### **References**

1. Hovorka R, Jones RH: How to measure insulin secretion. *Diabetes Metab Rev* 10:91-117, 1994
2. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368-377, 1992