ENDOCRINE SYSTEMS MODELING

TOWARDS PERSONALIZED TREATMENT OF THYROID DISEASES

by

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ABSTRACT

Thyroid hormones are fundamental in the development and function of the human body. They are produced by the thyroid and their concentration is regulated by a negative feedback loop involving also the hypothalamus and the pituitary. Many studies have shown that each individual has a unique HPT axis set-point, which means that everyone has his or her own personal level of thyroid hormones. However, when the thyroid is affected by a disease, such as hypothyroidism, a change in the thyroid function modifies the levels of thyroid hormones, therefore they do not match the patient's set-point anymore. Hence, the aim of this thesis is to investigate the relationship between the thyroid hormones TSH and FT4 in order to predict each individual's set-point. Moreover, the variation in time of the two hormones in patients under medication is studied as well, which allows to determine around which values the concentrations of TSH and FT4 will stabilize and, if these are not matching the set-point, to adjust the patient's medication dosage. This research was conducted using patients' datasets from different hospitals. In particular, datasets of hypothyroid and thyroidectomized patients were included in this study. The analysis of hypothyroid patients' measurements shows that TSH can be expressed as a negative exponential function of FT4. Furthermore, the individual set-point can be predicted as the point of maximum curvature of that function and this has been validated using data from thyroidectomized patients. Finally, a model of coupled differential equations, taking into account the effects of medication, has been developed to describe how the concentrations of the hormones are changing in time after a patient starts the treatment.

CONTENTS

1

INTRODUCTION

Thyroid hormones are fundamental in the development and function of the human body. They are produced by the thyroid and their concentration is regulated by a negative feedback loop, which involves also the hypothalamus and the pituitary. This configuration of the feedback loop is called hypothalamus-pituitary-thyroid (HPT) axis [1]. Many studies have shown that each individual has a unique HPT axis set-point, which means that everyone has its own personal level of thyroid hormones. However, when the thyroid is affected by a disease, such as hypothyroidism, a defect of the thyroid function modifies the levels of thyroid hormones, therefore they do not match the proper needs of that individual anymore.

Hypothyroidism is a disease that affects the thyroid and it is characterised by very low levels of thyroid hormones. It is one of the most common disorders in the general population and currently it is treated with tablets of levothyroxine (LT4). However, 35-60% of patients treated with levothyroxine do not reach the reference range of TSH. Furthermore, it is estimated that 5-10% of patients still present persistent complaints, despite their hormone concentrations being within the normal reference ranges [2]. In particular, in the Netherlands there are already 480.000 patients taking levothyroxine and 15% of them still have symptoms, even if their thyroid hormone levels are within the reference ranges. One of the reasons why this happens might be because the hormone levels of these patients do not match their HPT axis set-point. This is why this topic is an important issue and it has received increasing attention during the last years. Hence, it is fundamental to find a mathematical description of the HPT axis, together with a prediction of the individual set-point. In this way, the medication dosage can be adjusted for every patient such that each individual can reach its own set-point.

The modeling of the HPT axis started more than sixty years ago. However, not all proposed models are valid and applicable. In particular, the first models involved many parameters that could not be estimated. In the last years, the modeling of the HPT axis has focused more on population models. In fact, most of the HPT axis models developed until now are based on a statistical approach. This means that they involve and combine data from multiple patients. However, this approach implies that the hormone levels of

one individual can influence the hormone values of someone else, which does not happen in reality. Furthermore, population models cannot be applied to single individuals, hence they cannot be used to estimate the specific set-point of a patient.

However, recently, a patient-specific mathematical model of the HPT axis has been developed. This is expressed as a negative exponential function between the concentrations of thyroid-stimulating-hormone (TSH) and free thyroxine (FT4). Furthermore, in this model the set-point is established as the point of maximum curvature of the exponential function [3]. Thus, the purpose of modeling the HPT axis from a mathematical perspective is to gain insight into the mechanism of the negative feedback loop and improve the diagnostic process and the treatment of thyroid diseases. The set-point computed in this way can be used to adjust the therapy of thyroid disorders for each individual, such that the conditions of many patients can improve.

Therefore, the goal of this thesis project is to validate the existing exponential model for the HPT axis and, possibly, further improve and optimize it. This represents a fundamental step towards a personalized treatment of thyroid diseases. The acceptance of this theory in the medical society will help to improve the condition of many thyroid patients that still present complaints or have an inaccurate diagnosis. Another focus of the thesis is the study of the optimal path leading to the set-point, so that patients can reach their set-points in a shorter time. This will allow patients to feel better after a few weeks from the start of the treatment, and not after months or years, as it is currently happening.

The remainder of this thesis will be organized as follows. Chapter 2 provides a medical background about the HPT axis, with an explanation of all the terms needed to understand its functioning. Chapter 3 presents a brief review of the work published so far, which includes population models and the newly developed exponential model. Chapter 4 includes the problem description, with the research questions formulated to work on this project. In Chapter 5, the datasets used to work on the project are introduced. Chapter 6 includes the results obtained from the study of the mathematical relationship between FT4 and TSH, together with the reconstruction of the set-point. In Chapter 7, a validation of the set-point theory is carried out using the available datasets. Chapter 8 describes how the set-point can be reached in an optimal way. Finally, conclusions and further research suggestions are reported in Chapter 9.

2

MEDICAL BACKGROUND

2.1. GENERAL CONCEPTS

The thyroid gland is located below the larynx, anterior to the trachea. It is divided into two lobes, one on each side of the trachea [1, 4], as shown in Figure (2.1). It is one of the largest endocrine glands and one of its main functions is to produce two major hormones, thyroxine (T4) and triiodothyronine (T3). These hormones increase the metabolic rate of the body and without them the chemical reactions of the body would become very slow [1]. Thyroid hormones are fundamental for a correct development, therefore a lack of them would cause an inhibited growth [4]. Another important effect of thyroid hormones is to facilitate the growth and development of the brain [1]. The thyroid gland produces for the most part T4, so only a minimal amount of the hormones produced by the thyroid consists of T3. However, both hormones are functionally important because a significant portion of T4 is converted to T3. In order to produce normal quantities of T4, it is needed to ingest enough iodine. About half of T4 is then slowly deiodinated to form additional T3 [1]. Only 20% of the circulating T3 is produced by the thyroid gland, while the remaining amount is produced by peripheral conversion of T4 [5, 6]. The production of these thyroid hormones is controlled by the thyroid-stimulating hormone (TSH), also called thyrotropin, which is produced by the anterior pituitary.

The pituitary is a small gland situated in a cavity at the base of the brain. It is connected to the hypothalamus and it is divided into anterior and posterior pituitary. The anterior pituitary produces TSH, whose function is to stimulate the synthesis and secretion of the thyroid hormones T4 and T3. The hypothalamus is a small region in the brain, located close to the pituitary gland. Despite its small size, it is responsible for many fundamental activities, including the release of hormones. In particular, the hypothalamus produces thyrotropin-releasing hormone (TRH), whose major function is to stimulate the secretion of TSH [1] and function as a reference value for FT4 in the pituitary.

A feedback mechanism through the hypothalamus and the pituitary is operated in order to control the secretion of thyroid hormones. This is essential to guarantee the correct level of thyroid hormones needed to maintain a normal metabolic activity. TSH

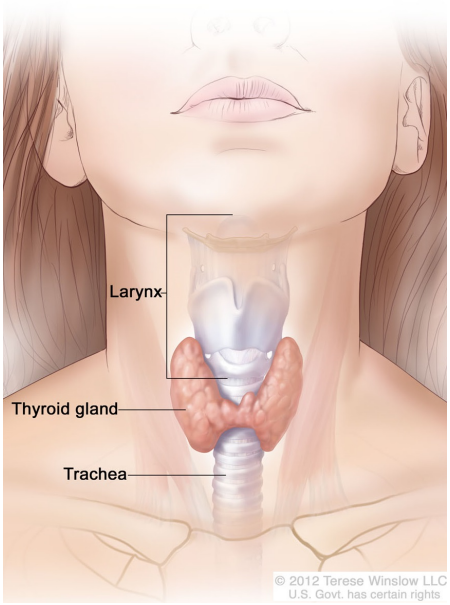


Figure 2.1: Schematic view of the thyroid anatomy.

is produced by the anterior pituitary and it stimulates the secretory activities of the thyroid cells. The first early effect of TSH is to cause the release of T4 and T3 in the blood stream within the next minutes. All the other effects require hours or days to develop completely. The secretion of TSH by the anterior pituitary is controlled by a hormone produced by the hypothalamus, the TRH [1]. This hormone is delivered to the anterior pituitary in order to increase the synthesis and release of TSH [4]. An increased level of thyroid hormones in the body decreases the production of TSH. When the rate of secretion of thyroid hormones is around 1.75 times more than the normal rate, the secretion rate of TSH becomes almost zero, until a following activation condition is initiated. An increased level of FT4 inhibits the production of TSH by affecting the anterior pituitary directly. The goal of the feedback is to keep almost a constant concentration of thyroid hormones [1]. Figure (2.2) shows a representation of the hypothalamus-pituitary-thyroid (HPT) axis with its feedback loop.

Another physiological aspect that affects the HPT axis is clock time. In fact, the HPT axis is regulated by a circadian cycle [8, 9]. The circadian system is an internal process that repeats every 24 hours and regulates all physiological and behavioural processes, including the release of hormones. The secretion of TSH follows a daily rhythm. In fact, during the late afternoon or early evening, the TSH concentration starts to rise, until it reaches its peak at the beginning of the sleep period. After that, TSH concentration declines again during the remaining part of the sleep period in order to reach the low daytime level [8, 9]. The 24-hours cycle of the TSH secretion is stable and robust and it is not influenced by age or sex. A diurnal variation in thyroid hormones T3 and T4,

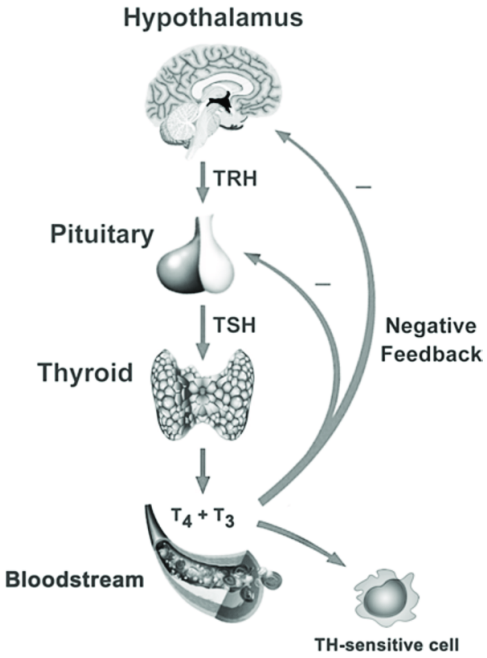


Figure 2.2: HPT axis negative feedback loop. Taken from [7].

however, is not obvious since different studies report discording results [8, 9].

Thyroid hormones are transported in the blood by specific proteins. This is one of the reasons why total T4 and T3 are measurements with relative large variations, making them not enough reliable measurements to study changes in the thyroid function. Free T4 (FT4) and free T3 (FT3) refer to the respective thyroid hormones not bound to serum proteins. They are considered to be more precise indicators of thyroid dysfunctions [4]. In order to recognise a thyroid disorder, a thyroid function test (TFT) is conducted. In the current clinical practice, as a first screening, the level of TSH is checked and, if it is abnormal, the concentration of FT4 is then measured as well [10].

2.2. HYPOTHYROIDISM

A widespread thyroid disorder is hypothyroidism. Hypothyroidism is a pathological condition that refers to thyroid hormone deficiency [2] and it affects around 10% of the global population. Hypothyroidism affects more often women, Caucasian individuals, older people and patients with autoimmune diseases [5]. Since the symptoms and clinical manifestations can vary between different individuals, the definition of hypothyroidism is based on reference ranges of the fundamental biochemical concentrations, namely TSH and FT4 [2, 11]. Reference ranges depend on the assay used and the population analyzed. An assay is the investigative procedure that can determine the presence of a substance and its amount. The reference ranges are defined between the 2.5th and 97.5th percentiles of a healthy population [2]. The most common used reference

range for TSH is $0.4 - 4.0$ mU/L, while for many laboratories the normal range for FT4 is $10.0 - 20.0$ pmol/L [12]. However, the reference ranges have been a matter of debate in the last years. In fact, some studies show that in particular the upper limit of the TSH reference range tends to increase in older people. Nevertheless, there is not enough evidence to adjust the reference ranges according to age [11].

Hypothyroidism can be divided into different categories: primary (or overt) and subclinical (or mild) hypothyroidism. Primary hypothyroidism is defined as a TSH concentration above the reference range and an FT4 concentration below the normal range [2], while subclinical hypothyroidism is defined as TSH above the population range and FT4 within the reference range [2, 11]. Another possible classification of hypothyroidism is into primary, secondary, tertiary and peripheral hypothyroidism. Primary hypothyroidism is caused by thyroid hormone deficiency. Secondary hypothyroidism is due to TSH deficiency, while tertiary hypothyroidism is caused by a shortage of TRH; these two categories are usually grouped together and referred to as central hypothyroidism. Finally, peripheral hypothyroidism is due to extra-thyroidal causes [2].

As anticipated, clinical presentation of hypothyroidism can vary significantly between patients, ranging from life-threatening to no symptoms at all. Even though life-threatening conditions are very rare nowadays, it is still fundamental to recognise hypothyroidism in its early stages, in order to start immediately with the treatment [2]. The most common symptoms are weight gain, cold intolerance, fatigue, shortness of breath, change of voice, constipation, dry skin, hair loss, deterioration of kidney function, impaired memory and mood. However, these symptoms can vary with age and sex, therefore none of them can be used to identify patients subject to hypothyroidism [2, 11]. Thus, in the current clinical practice, reference ranges are the only instruments used to identify hypothyroidism.

The standard treatment of hypothyroidism is levothyroxine (LT4) monotherapy, taken in solid formulation on an empty stomach [2]. Changing different levothyroxine products is not recommended because differences in brand contents can provide unexpected results due to the narrow therapeutic ranges [5]. In particular, in older patients or in those patients with a low body weight, a small change in the medication can cause large effects on TSH concentrations [2]. This is why after the start of medication or after the adjustment of the dosage, the concentration of TSH should be checked more frequently until it is stabilised [2, 11]. Both under-treatment and over-treatment should be avoided, because they can cause serious problems. The current treatment target consists in normalizing the level of TSH and resolving all the complaints. Nevertheless, it is estimated that 35-60% of the patients does not reach the reference target for the TSH level. There could be some reasons that prevent patients from reaching their target, such as prescription of wrong dosage, non-adherence to the therapy, interaction with other medication. Around 5-10% of the patients still have persistent complaints, despite being biochemically euthyroid [2, 11], where euthyroidism is the condition of having a thyroid gland that functions normally.

One of the reasons that explains why this happens might be that the concentrations of circulating thyroid hormones are regulated by the HPT axis through an individual set-point, which allows a smaller intra-individual variability than the inter-individual one [13]. The concept of HPT axis set-point will be explained further in the next sections.

Therefore, the level of TSH required to reach the concentrations of thyroid hormones needed by an individual can vary significantly between different patients. This explains also why patients with similar values of TSH respond differently to the treatment [2, 11]. Population-based reference ranges are not able to define the thyroid status in an individual, because average results based on a population cannot be satisfactorily applied to all the members of the population [14]. Another possible explanation to the persistence of symptoms in some patients might rely on some flaws of the levothyroxine monotherapy itself. Furthermore, it should be taken into account that the clinical interpretation of a normal level of TSH represents euthyroidism at the level of the pituitary, not in all tissues [2]. From the previous considerations, it is clear that the current situation causes dissatisfaction both in patients and in doctors. One way of trying to solve this problem consists in reconstructing the individual set-point, which can give better outcomes than population-based reference ranges [14]. Hence, this will be the focus of the thesis.

2.3. MEASUREMENTS DETAILS OF TSH, FT3 AND FT4

Thyroid function tests are employed to distinguish hyperthyroid and hypothyroid states from the euthyroid one. In order to do this, direct measurements of the concentrations of TSH and total or free T4 and T3 are employed. However, measurement of FT3 is only used on a limited basis [15]. In fact, in hypothyroid individuals T3 is the last value to become abnormal, hence many hypothyroid patients still show normal levels of T3 [15]. Reference ranges of TSH and FT4 are established by each laboratory through a statistical distribution of TSH and FT4 levels, measured from a group of healthy patients belonging to the general population [16]. The measurement of free hormones (FT4 and FT3) is usually accepted as an appropriate indicator of the thyroid functional state. Hence, for diagnostic purposes, the focus should be on free hormones rather than on the total ones [17, 18].

Generally, clinical research analyzes data from a population with a statistical approach. Hence, uncertainties and measurement inaccuracies do not play a relevant role, since most of the variability is cancelled out when considering aggregated data. However, when reconstructing the hypothalamus-pituitary (HP) curve for a specific individual, only measurements from the same laboratory, conducted using the same technique and with the same calibration accuracy should be used. This is because the way of reporting laboratory results and their interpretation changes between different laboratories, causing a big impact on the reconstruction of the HP characteristic. In fact, for example, in many laboratories the level of FT4 is reported as an integer value, which implies an uncertainty of the real value. When rounding to the nearest integer, an absolute uncertainty of ± 0.5 pmol/L is expected, while, when truncating the decimal digits, the uncertainty of FT4 becomes ± 1 pmol/L. However, in other laboratories, the value of FT4 is rounded by keeping one decimal digit. In addition, it is fundamental that the measurements of TSH and FT4 of a patient are always taken at a fixed time of the day, 24 hours after the last taken dosage and before the daily dose of LT4, in order to prevent pharmacodynamic effects that might interfere with the measurements [16].

In this thesis project, data that is already available will be used, hence measurements might come from different laboratories or they may be performed with different techniques. However, for future projects, it will be important to take also into account how

the data is collected, hence it is fundamental to consider measurements coming from the same laboratory, taken with the same method and at the same time of the day.

Thyroid hormones are available in the blood in extremely small quantities, hence it is very hard to measure their concentrations. However, in the 1970s, a very sensitive method for the measurement of hormones, called radioimmunoassay, was developed [1]. A radioimmunoassay consists of making a known quantity of an antigen radioactive and then use it to detect the amount of the same antigen in the patient's blood sample. The radioimmunoassay method was first introduced in 1959 by Yalow and Berson to measure the level of insulin in the blood. After that, methods for measuring the concentrations of TSH, T4 and T3 were soon developed [17]. Since then, there have been many improvements in assays for TSH, FT4 and FT3 [10]. Nowadays, immunoassays are the most common methods to measure the levels of TSH, FT4 and FT3 [17, 19]. An immunoassay measures the concentration of a substance by using an antibody or an antigen. A problem with immunoassays is specificity. In fact, it can happen that when measuring the concentration of FT4, also a small amount of FT3 can be included in the measurement because the specificity of immunoassays is not optimal. Furthermore, this technique might have an impact on the diagnosis and treatment of hypothyroidism. In fact, sometimes it can happen that samples from hypothyroid patients have thyroid hormone levels below the detectable limits of immunoassays [19].

Recently, research has focused on mass spectrometry methods for the simultaneous analysis of TSH, T4 and T3. Therefore, mass spectrometry methods now represent a valid alternative to immunoassays and the instrumentation is becoming widespread in clinical laboratories [19]. A mass spectrometry can measure the mass of a molecule after having transformed it into a gas-phase ion. Mass spectrometry methods have successfully dealt with problems related to many immunoassays for thyroid hormones because they present higher specificity and accuracy. This can improve diagnostic capabilities because measurements of thyroid hormones are more reliable. Therefore, mass spectrometry is now regarded as the new gold standard and, in the near future, it has the potential to be applied in clinical assessment routine, in particular for FT4 and FT3 [18].

3

RELATED WORK

3.1. MATHEMATICAL MODELING OF THE HPT AXIS

As described in the previous section, the well-being of many patients is still suboptimal, despite their TSH and FT4 values being within the reference ranges. This explains the need for individualised treatment [3] and it is the reason why, in the last years, the mathematical modeling of the HPT axis set-point has received increasing attention.

The first model was elaborated in 1956 by Danziger and Elmegreen [20]. Their contribution is fundamental because it points out the importance of mathematical modeling in endocrine control system. The authors implemented a system of non-linear differential equations, which can be linearized and eventually solved, if the parameters of the system are known or can be estimated. Furthermore, the authors emphasised the difficulty in obtaining measurements, hence the parameters are not known or are known with little accuracy [20]. Another important contribution was given by DiStefano and Stear in 1968 [21]. They improved the previous model and presented it in the framework of feedback control system theory. They developed a system with two coupled differential equations with 11 parameters and variables. However, not all of them are measurable, which makes their model not usable in practice [21]. Next, the publication of Wilkin et al. in 1977 [22] finally mentioned the importance of the loop gain in the representation of the HPT axis through control-loop theory. The paper from Leow [23], published in 2007, pointed out the necessity of a simple mathematical model, where only measurable parameters are involved. Furthermore, this study finally led the way in the implementation of an exponential model between TSH and FT4 [23]. In 2010, Hoermann et al. published a study [24] in which they compare the linear model between log(TSH) and FT4 with a non-linear model based on the error function (erf) between log(TSH) and FT4. This new non-linear model proved to fit better the data. However, the models were tested on aggregated data and not on single patients, so this implies a mutual influence between all the individuals included in the population. Finally, in 2014, Goede et al. [3] implemented a negative exponential parameterized model between TSH and FT4 and tested it on datasets belonging to single individuals, not on population data. In fact their

model is valid on an individual level and the values of the parameters differ from one patient to another. This model will be explained further in the next section.

There are probably many ways to model the HPT axis with a more complex approach, however simpler mathematical models are more attractive because easier to understand. Furthermore, some highly accurate models may not be applicable in practice, because they might require values of quantities, like TRH, whose measure is either not available or not reliable. In addition, even these accurate and complicated models cannot include all the factors influencing the level of hormones. That is why a minimal model including only measurable and observable variables is to be preferred. Any model including many variables might be too complicated for normal use, despite being definitely more accurate. On the contrary, a simpler model would be less realistic, but more understandable and easier to apply, even by non-mathematicians. Hence, it is not necessary that a mathematical model includes all the relevant factors under consideration, as long as the assumptions and the limitations are correctly understood and taken into account [23].

3.2. TSH-FT4 EXPONENTIAL MODEL

The relationship between TSH and FT4 has been modelled as a negative exponential in 2014 by Goede et al. [3] and this has remained the standard until now. Even though T3 is the main active hormone, this mathematical model only includes the relationship between TSH and FT4. In fact, since a model with two degrees of freedom is adopted, the influence of FT3 can be subsumed within the two model parameters [3]. Generally, the level of FT3 is kept constant under a large variety of conditions. Hence, a more comprehensive model including also FT3 would not contribute to a better model, because the constant level of FT3 is directly couple with the set-point value of FT4.

In this model, the hypothalamus-pituitary (HP) complex is considered as a master regulator unit, which calibrates its level of TSH according to the concentration of FT4. The HP curve should be analyzed in an open loop, without the influence of a healthy operating thyroid [3]. In fact, in an euthyroid individual, the closed feedback loop is operating correctly, so every measurement of TSH and FT4 represents the HPT axis set-point. Therefore it is not possible to derive the HP characteristic. However, this is not happening in patients affected by thyroid diseases since their feedback loop is not working properly, so their values of FT4 and TSH change after every adjustment of their medication dosage.

It can be noticed that TSH varies inversely with FT4, in a non-linear way. In particular, small changes in FT4 can lead to large changes in TSH. When TSH and FT4 are both represented on a linear scale, the relationship between them resembles a hyperbolic, sigmoid or exponential decay curve. Hence, through the years, a log-linear model between TSH and FT4 has been developed, where TSH has been represented in a logarithmic scale and FT4 in a linear one. If both TSH and FT4 are expressed in a linear scale, then their relationship can be modelled through a negative exponential function with two independent parameters [3].

The mathematical model is as follows:

$$[\text{TSH}] = Se^{-\varphi[\text{FT4}]}.$$

In this thesis, the square brackets are used to represent the concentration of the hor-

mones. The model has two degrees of freedom, S, the multiplier, and φ, the slope of the exponential coefficient. Variations of S, with fixed φ, translates the HP curve horizontally on the FT4 axis. Variations of φ folds or unfolds the shape of the HP curve around a chosen point. S and φ are a set of parameters that describes the HP curve of a specific individual. In particular, S is associated with the FT4 value of the set-point, while φ is an individual physiological constant related to the TSH set-point value. Exponential functions are completely determined by two different sets of coordinates. Therefore, it is possible to recover S and φ when two or more measured points are available and distinct. If $([\text{TSH}]_1, [\text{FT4}]_1)$ and $([\text{TSH}]_2, [\text{FT4}]_2)$ are two distinct measurements belonging to the same individual, S and φ have then the following expressions:

$$\varphi = \frac{1}{[\text{FT4}]_1 - [\text{FT4}]_2} \ln\left(\frac{[\text{TSH}]_2}{[\text{TSH}]_1}\right)$$

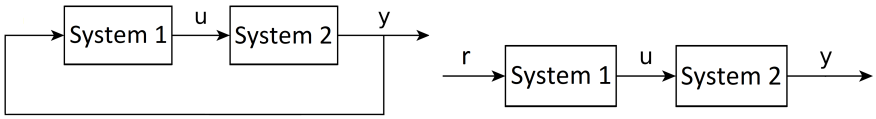
$$S = [\text{TSH}]_1 e^{\varphi[\text{FT4}]_1} = [\text{TSH}]_2 e^{\varphi[\text{FT4}]_2}.$$

The validation of the model is based on an individual application of the model. This is fundamental, since the HPT axis physiology of every individual is uniquely defined by S and φ. Therefore, this model should not be applied on aggregated random FT4-TSH data from a population. In particular, this model has been validated by Goede et al. with two datasets from two different hospitals. Its validity ranges are $5-40$ pmol/L for FT4 and $0.05-100$ mU/L for TSH. The model validation is performed on the dataset available for each individual and then repeated for every patient. This is because the HPT axis is uniquely defined for each person [3].

3.3. CONTROL THEORY BACKGROUND

The term control can have different meanings. According to [25], control refers to the use of algorithms and feedback in engineered systems. A modern controller measures the output of a system, compares it to the desired behaviour, computes the corrections needed and actuates the system in order to carry out the desired changes. This basic feedback loop is the fundamental concept in control theory [25]. The term feedback refers to a situation in which two or more systems are connected together in such a way that each branch of the feedback loop can influence the other [25]. Hence, the main idea is that the output of a system can be measured, fed back to a controller and then used to influence the system itself [26].

Control systems can be classified in closed loop and open loop systems. Figure (3.1) shows how these two different kinds of systems can be represented. A closed loop system is a system in which the components are interconnected in a loop, so, according to Figure (3.1a), the output of system 1 is the input of system 2 and the output of system 2 is the input of system 1 [25]. Hence, in a closed loop system, the controlled output signal is measured and fed back in the control computation. A closed loop system can also be called feedback control [26]. On the contrary, if the connection between the two systems is not present, the system is defined as open loop. According to Figure (3.1b), the interconnection between system 2 and system 1 is removed [25]. Therefore, in an open loop system, the controller does not use the system output in control computation [26].



(a) Closed loop (b) Open loop

Figure 3.1: Representation of an open loop system and a closed loop system

Figure (3.2) shows a simple representation of a feedback loop. P represents the plant, which consists of the central component of the feedback system, whose output is controlled. C is the controller, which is the component that actually computes the desired control signal. The controller computes the difference between the reference signal and the controlled output signal and uses it as a measure of the system error [26]. The output

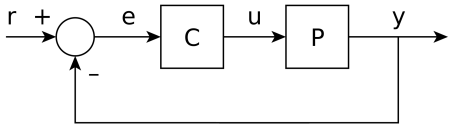


Figure 3.2: Simple feedback loop

of the system, y, is fed back to a comparator, together with the reference value r. After that, the controller uses e, defined as the difference between the reference value r and the output y, in order to change the input u of the plant P, which is the system under control. If the plant P and the controller C are linear and time-invariant then the system can be analysed using the Laplace transform on the variables, so the following relations hold:

$$Y(s) = P(s)U(s)$$
$$U(s) = C(s)E(s)$$
$$E(s) = R(s) - Y(s).$$

The first two relations can be re-written as

$$E(s) = \frac{U(s)}{C(s)}$$
$$U(s) = \frac{Y(s)}{P(s)}$$

and, if combined, they yield to

$$E(s) = \frac{Y(s)}{P(s)C(s)}.$$

This expression of $E(s)$ can be substituted in the last relation of $Y(s)$, obtaining

$$\frac{Y(s)}{P(s)C(s)} = R(s) - Y(s)$$
$$\Rightarrow Y(s) = \frac{P(s)C(s)}{1+P(s)C(s)} R(s).$$

$|P(s)C(s)|$ is called loop gain. When $|P(s)C(s)| \gg 1$, then $Y(s) \simeq R(s)$. This is why the loop gain should be greater than 1, because in this case the plant and the controller can still communicate with each other. This concepts is fundamental in the next section, where the HPT axis is modelled as a closed loop system.

In the following section, the HPT axis will be represented through a closed-loop system. In that case, the controller and the plant, respectively the HP unit and the thyroid, will be time-invariant but not linear. Hence, the product of the differential gain transfer function of the pituitary and of the thyroid is necessary to express the loop gain. The system function blocks will then be described through linear relations, which will be valid over a limited range of the input and output values [12]. In fact, when the devices are nonlinear, the input can be considered only over a small range of values, such that the output can be assumed linear.

3.4. HPT FEEDBACK CONTROL

The HPT axis can be modelled through control theory, in particular using a negative feedback loop configuration. The system loop is divided into function blocks, such that every block is distinguished by its own nonlinear transfer characteristic valid over the total range of the input and output signals. In this way, the HPT negative feedback loop can be analyzed through mathematical considerations. Hence, the main components of the loop, HP and thyroid blocks, are both characterised by distinct relationships between TSH and FT4 [12]. According to the terms introduced in the previous section, the HP is the controller, while the thyroid is the plant, because it just satisfies the secretory demands [27]. Figure (3.3) shows the negative feedback loop of the HPT axis. S_{FT4} is the internal set point value of FT4. In fact, the normal operation of a negative feedback loop implies the existence of a set-point intrinsic to the system. Hence, any alteration of TSH and FT4 causes a disequilibrium that influences the HP block in order to restore the system towards the set-point [27].

The HP complex is modelled with FT4 as the primary input signal and TSH as the output one. As explained previously, TSH can be expressed as a negative exponential function of FT4

$$[\text{TSH}] = Se^{-\varphi[\text{FT4}]}.$$

The HP differential gain factor G_{HP} is defined as the derivative of TSH with respect to FT4 [12, 27]:

$$G_{HP} = \frac{d[\text{TSH}]}{d[\text{FT4}]} = \frac{-\varphi S}{e^{\varphi[\text{FT4}]}} = -\varphi[\text{TSH}].$$

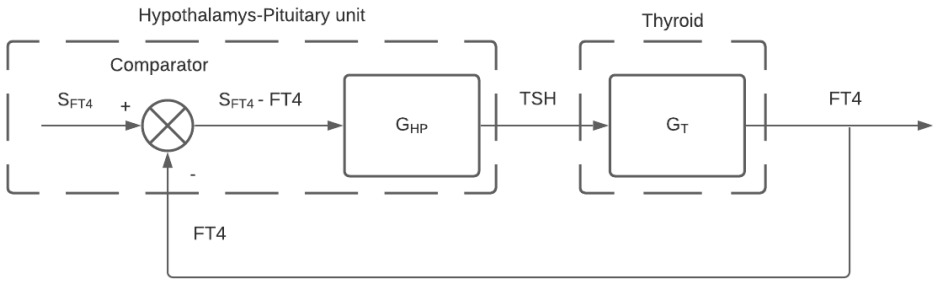


Figure 3.3: Generalized feedback loop of the HPT axis [12]

The thyroid characteristic can be modelled according to an adaptation of the Michaelis-Menten kinetics, which is very popular in enzyme kinetics, and it results in:

$$[\text{FT4}] = A\left(1 - e^{-\alpha[\text{TSH}]}\right).$$

where α determines the steepness of the thyroid characteristic and A represents the maximum secretory value of T4 and T3, and consequently of FT4. α and A are specific for each individual [27]. The thyroid differential gain factor G_T is defined as the first derivative of FT4 with respect to TSH [27]:

$$G_T = \frac{d[\text{FT4}]}{d[\text{TSH}]} = A\alpha e^{-\alpha[\text{TSH}]}$$

The loop gain G_L is given by [12, 27]:

$$G_L = |G_{HP} G_T|,$$

so its expression is [27]:

$$G_L = \left|-\varphi[\text{TSH}]\, A\alpha e^{-\alpha[\text{TSH}]}\right| = \varphi[\text{TSH}]\, A\alpha e^{-\alpha[\text{TSH}]}.$$

For an optimal and stable control, the loop gain G_L should always be greater than 1. When G_L becomes smaller than 1, the interaction between the HP unit and the thyroid is lost, resulting in an open loop situation [12]. The loop gain is a function of TSH, thus it is possible to study its maximum by computing the derivative of G_L with respect to TSH and set it equal to 0:

$$\frac{d\, G_L}{d[\text{TSH}]} = \frac{\varphi\alpha A - \varphi\alpha^2 A[\text{TSH}]}{e^{\alpha[\text{TSH}]}} = 0.$$

This is verified only when $[\text{TSH}] = \dfrac{1}{\alpha}$, so if the set-point value of TSH is known, it is possible to set

$$\alpha = \frac{1}{[\text{TSH}]_{\text{setpoint}}}.$$

According to the thyroid model, the set-point value of FT4 is then

$$[\text{FT4}]_{\text{setpoint}} = A\left(1 - e^{-1}\right),$$

which allows to calculate the value of A. Finally, the maximum loop gain becomes:

$$G_{L_{\max}} = \frac{A\varphi}{e}.$$

It is clear that the thyroid parameters depend on the set-point values. Furthermore, the thyroid operates in such a way that the loop gain is at its maximum value at the set-point. Furthermore, the expression of the loop gain G_L provides a simple criterion to determine the open loop condition because G_L has to be larger than 1 for closed loop operation [12, 27].

3.5. HPT-AXIS SETPOINT

The term homeostasis refers to the maintenance of almost constant conditions in the internal environment. All organs and tissues of the body perform functions in order to maintain these conditions in a small stable range [1]. The HP characteristic contains the set of all possible points of homeostasis [3]. In fact, the normal reference ranges of TSH and FT4 fall within the knee region of the HP curve, which corresponds to the most pronounced bend of the negative exponential curve, hence this leads to think that the set-point should be in that interval [27]. The knee region of the HP function includes also the point of maximum curvature, which is the point characterised by the minimum radius of curvature [3]. Thus, it is natural to assume that the set-point of the HPT axis corresponds to the point of maximum curvature of the HP function [27]. Figure (3.4) shows the HP curve with its set-point corresponding to the point of maximum curvature. The red lines are the limits of the reference ranges for TSH and FT4, which are used in the graph in order to delimit the knee region of the exponential curve. It can be noticed that the set-point is located within the knee region of the HP curve, as expected. Furthermore, it should be noticed that this graph was realized by keeping the same scale on both the TSH and FT4 axes, in order to clearly identify the knee region.

The curvature K is defined as $K = \dfrac{1}{R}$, where R is the radius of the curvature circle. For the HP curve the curvature K is

$$K = \frac{\dfrac{d^2[\text{TSH}]}{d[\text{FT4}]^2}}{\left(1 + \left(\dfrac{d[\text{TSH}]}{d[\text{FT4}]}\right)^2\right)^{3/2}}.$$

When computing the derivatives, the curvature becomes

$$K = \frac{\varphi^2 S e^{-\varphi[\text{FT4}]}}{\left(1 + \varphi^2 S^2 e^{-2\varphi[\text{FT4}]}\right)^{3/2}}.$$

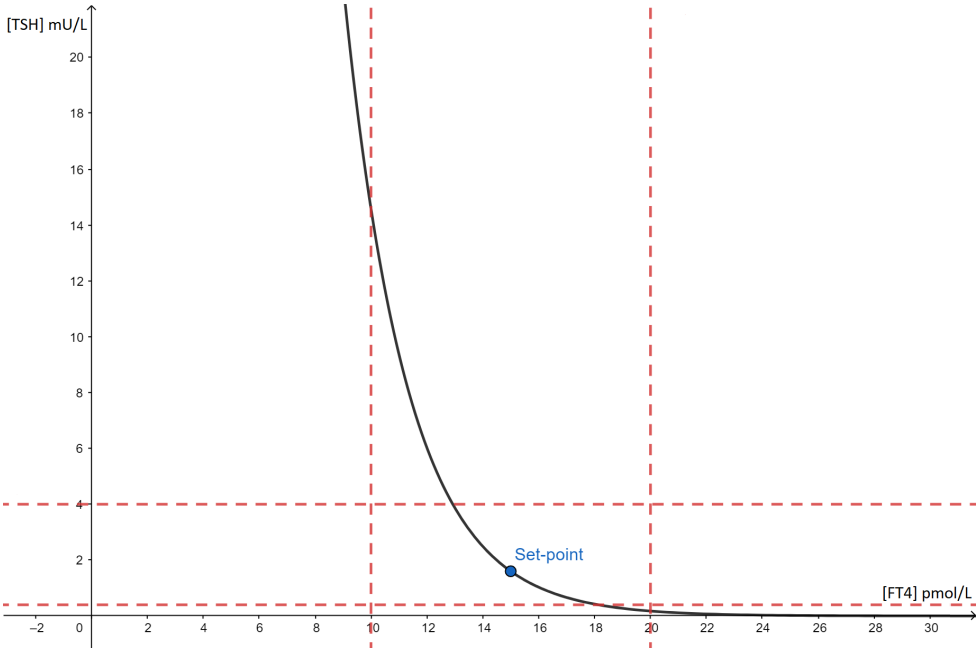


Figure 3.4: The black solid line represents the exponential HP curve. The red dashed lines represent the limits of the reference ranges of TSH and FT4, corresponding to the knee region of the exponential curve. The blue point, defined as the set-point, corresponds to the point of maximum curvature

In order to study when the curvature is maximum, it is possible to compute the derivative of K with respect to FT4 and set it equal to 0:

$$\frac{dK}{d[\mathrm{FT4}]} = \frac{\varphi^3 S e^{-\varphi[\mathrm{FT4}]}\left(1+\varphi^2 S^2 e^{-2\varphi[\mathrm{FT4}]}\right)^{1/2}\left(2\varphi^2 S^2 e^{-2\varphi[\mathrm{FT4}]}-1\right)}{\left(1+\varphi^2 S^2 e^{-2\varphi[\mathrm{FT4}]}\right)^3} = 0.$$

This leads to

$$2\varphi^2 S^2 e^{-2\varphi[\mathrm{FT4}]} - 1 = 0,$$

which results in

$$[\mathrm{FT4}] = \frac{\ln\left(\varphi S\sqrt{2}\right)}{\varphi}.$$

The corresponding value of TSH is

$$[\mathrm{TSH}] = \frac{1}{\varphi\sqrt{2}}.$$

Hence, these are the coordinates corresponding to the set-point of the HPT axis [27].

Goede et al. tried to develop another independent mathematical proof in order to show that the HPT axis set-point corresponds to the point of maximum curvature [27]. As presented in the previous section, the HPT axis can be modelled through control theory, in particular using a negative feedback loop configuration, where each function block is distinguished by its own nonlinear transfer characteristic. For the HPT axis, the main components of the loop are the HP and the thyroid blocks. The HP complex is modelled through an exponential decay,

$$[\text{TSH}] = Se^{-\varphi[\text{FT4}]},$$

while the thyroid function is

$$[\text{FT4}] = A\left(1 - e^{-\alpha[\text{TSH}]}\right).$$

As described previously, the loop gain of this system is maximum at the set-point. In fact, given the loop gain

$$G_L = \varphi[\text{TSH}]A\alpha e^{-\alpha[\text{TSH}]},$$

its maximum can be computed by setting

$$\frac{d\,G_L}{d[\text{TSH}]} = \frac{\varphi\alpha A - \varphi\alpha^2 A[\text{TSH}]}{e^{\alpha[\text{TSH}]}} = 0.$$

This is verified when

$$\alpha = \frac{1}{[\text{TSH}]_{\text{setpoint}}}.$$

Using the expression of the thyroid model, it is also possible to derive

$$A = \frac{[\text{FT4}]_{\text{setpoint}}}{\left(1 - e^{-1}\right)}.$$

Based on the relations for α and A, the thyroid function becomes

$$[\text{FT4}] = \frac{[\text{FT4}]_{\text{setpoint}}}{\left(1 - e^{-1}\right)}\left(1 - e^{-\frac{[\text{TSH}]}{[\text{TSH}]_{\text{setpoint}}}}\right).$$

In order to plot the thyroid function on the same graph as the HP curve, it is necessary to invert it:

$$[\text{TSH}] = -[\text{TSH}]_{\text{setpoint}} \ln\left(\frac{[\text{FT4}]_{\text{setpoint}} - [\text{FT4}]\left(1 - e^{-1}\right)}{[\text{FT4}]_{\text{setpoint}}}\right).$$

The set-point is computed as the point of maximum curvature of the HP exponential function. If the set-point is available, it is then possible to derive the corresponding HP curve that has that point as point of maximum curvature. Furthermore, when evaluating the thyroid function at [FT4]=$[\text{FT4}]_{\text{setpoint}}$, it is possible to obtain

$$\begin{aligned}[\text{TSH}] &= -[\text{TSH}]_{\text{setpoint}} \ln\left(\frac{[\text{FT4}]_{\text{setpoint}} - [\text{FT4}]_{\text{setpoint}}\left(1 - e^{-1}\right)}{[\text{FT4}]_{\text{setpoint}}}\right) = \\ &= -[\text{TSH}]_{\text{setpoint}} \ln(1 - 1 + e^{-1}) = [\text{TSH}]_{\text{setpoint}}.\end{aligned}$$

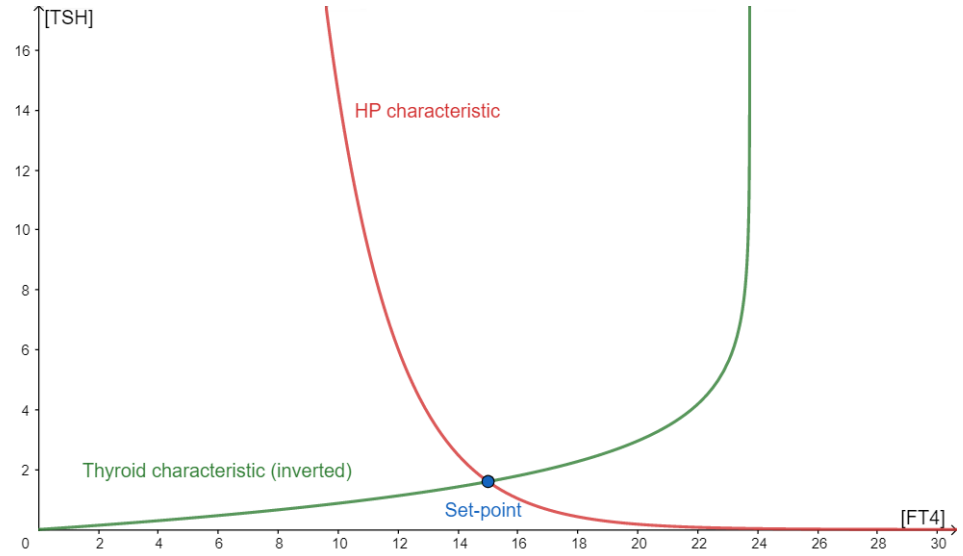


Figure 3.5: The HP and the inverted thyroid functions are intersecting in the point of maximum curvature

According to Goede et al. [27], this proves that the HP curve and the inverted thyroid function are intersecting in the point of maximum curvature, thus the set-point [27].

However, this mathematical proof presents some pitfalls. In fact, it seems that actually the inverted thyroid characteristic can intersect the HP curve even if the set-point is predicted in a different way and not as the point of maximum curvature. The previous calculations only prove that the inverted thyroid characteristic is intersecting the HP curve in the point ($[FT4]_{setpoint}$, $[TSH]_{setpoint}$) and not specifically in the point of maximum curvature of the HP function. Furthermore, the analysis of the loop gain only determines that the loop gain is maximum in the set-point value of TSH and there is no reference to the set-point value of FT4.

3.6. POPULATION MODELS

The majority of models for the HPT axis proposed until now present a statistical approach. A population curve is obtained from a cross-sectional study, involving multiple patients, and it is the result of simultaneous plots of TSH and FT4 values belonging to a large number of individuals [28]. Thus, cross-sectional studies include data from different individuals, where each of them presents a different HPT axis set-point [29]. HP and thyroid curves of single individuals, as described in previous sections, are independent from each other. However, in a population context, different HP and thyroid curves can influence each other [28]. Furthermore, it is not clear how population models can be applied to single individuals, since each person has different HP and thyroid functions, with a different set-point for the HPT axis [29]. Therefore, since population models de-

scribe the behaviour of a population from a statistical perspective, individual measurements are not relevant in this context. Thus, population models cannot describe the behaviour of single individuals, because they are supposed to be valid over the entire population.

Population models are widespread in clinical research and biomedicine [30]. However, this approach considers that all the individuals in a population have an influence on each other. In particular, in this case it means that the TSH concentration of an individual can affect the FT4 concentration of someone else and vice versa, which is not reasonable [31]. Biomedicine relies on advanced statistical techniques, even though a statistical approach is not always adequate to describe physiological processes and, on the contrary, its results could even be misleading [30]. In fact, population-based analysis, which relies on a statistical approach, is subject to amalgamation problems, so it is better to adopt a patient-specific approach instead [32].

In 1990 Spencer et al. introduced a population-based model where the relationship between TSH and FT4 is considered log-linear [33],

$$\log[\text{TSH}] = a + b[\text{FT4I}].$$

This model was derived by analysing a population of individuals with different states of the thyroid function, ranging from hypo- to hyperthyroidism. It was possible to derive this relationship because of some improvements in the immunoassays used to measure TSH and FT4 [33]. In fact, the increased sensitivity of the new TSH assay allowed them to grasp new insights regarding the mechanism of the HPT axis. Therefore, Spencer et al. were able to confirm some previous studies regarding the log-linear relationship between TSH and FT4 and extend this result also to subnormal levels of TSH [33].

Hence, from this study, the population model of the HPT axis started to be considered log-linear. However, some years ago, new cross-sectional studies analyzed the relationship between log(TSH) and FT4 and came to the conclusion that it can be better described by non-linear models. This is probably due to the improvements in TSH and FT4 measurements, which are now more precise and provide more reliable results. Therefore, some studies have proposed more complex models, based in particular on the error function, on negative sigmoid functions and on higher order polynomials.

In 2010, Hoermann et al. proposed a population model between log(TSH) and FT4 based on the error function [24],

$$\log[\text{TSH}] = \frac{\sqrt{\pi}k}{2q}\,\text{erf}(q([\text{FT4}]-a)) + d([\text{FT4}]-a) + b,$$

where erf represents the error function, which has the following expression

$$\text{erf}(z) = \frac{2}{\sqrt{\pi}}\int_0^z e^{-t^2}\,dt.$$

In 2012, Clark et al. conducted a cross-sectional study of the thyroid function in an older population and found that the relationship between log(TSH) and FT4 is better described by a fourth order polynomial [34]. The authors investigated the relationship between log(TSH) and FT4 with non-linear models based on higher order polynomials,

up to the fourth power of FT4, and compared it with the common linear model. The results of this study showed that the relationship between log(TSH) and FT4 is non-linear and that it can be better described by a fourth order polynomial of FT4. Clark et al. suggest that a more complex model for the relationship log(TSH)-FT4 may be needed.

In 2013, Hadlow et al. proposed a different non-linear model, based on two sigmoid curves, in order to describe the relationship between log(TSH) and FT4 [35]. In this study, the relationship between TSH and FT4 presented non-linear properties even after the logarithmic transformation of TSH. Hence, a negative sigmoid curve of the following form

$$\log[\text{TSH}] = A + \frac{B}{1 + e^{-(C-[\text{FT4}])/D}}$$

was adopted. In particular, the sigmoid curve was used in two stages, which means that the relationship between log(TSH) and FT4 can be described by two sigmoid curves, implying a discontinuity in the relationship between TSH and FT4. Therefore, it was also necessary to determine a threshold value for the concentration of FT4 in order to define the validity of the two curves.

4

PROBLEM DESCRIPTION

After these introductory chapters presenting the medical background and the related work on the HPT axis available so far, it is clear that there are still many patients with thyroid disorders that present complaints, even if their biochemical situation seems euthyroid. Therefore, a mathematical description of the HPT axis might be helpful in order to improve the diagnosis and medication of patients suffering from thyroid disorders. In particular, it is important to develop a model that can be applied to each single individual and that does not involve a statistical approach including many unrelated individuals. To this purpose, the research questions presented in the following sections have been analysed in order to work on this thesis project.

4.1. RESEARCH QUESTIONS

This thesis project is based on the following research questions:

- How can the HPT axis be modelled from a mathematical perspective? How can the existing model be improved?

In order to answer these questions, the exponential model by Goede et al. [3] will be considered as the starting point. It will be implemented and tested with the available data. After that, it would be possible to determine if the model is a good fit for the available datasets or if it can be somehow improved.

- How can the set-point of an individual be predicted?

Once the relationship between TSH and FT4 has been modelled, a prediction of the set-point should be found. If the model is the exponential one, then it should be verified that the set-point corresponds to the point of maximum curvature. Otherwise, if the exponential model has been modified and improved, it should be determined which point of the curve represents the HPT axis set-point. It could also be proved why, from a physiological perspective, it is reasonable to assume that that specific point of the curve corresponds to the set-point.

- Once a prediction of the set-point is available, how can it be proved that it corresponds to the actual set-point?

If any data from the pre-disease period is available, it should be verified if the predicted set-point matches the real one. This question might be answered using data of patients that underwent thyroidectomy, since in most of the cases a couple of measurements for pre-surgery TSH and FT4 are available. However, in this case it is important to interpret the data in the correct way. It should be noted that it might not be possible to verify this, it depends on the available data.

- How can the optimal path leading to the desired HPT set-point be found? How much time is needed to reach the set-point?

A healthy person is in a situation of dynamic equilibrium, hence the measurements of TSH and FT4 will not vary much during time. The situation is completely different in case of a hypothyroid patient, when the concentration of FT4 decays in time. According to the current guidelines, it can take several months before the concentrations of TSH and FT4 reach again normal levels. Therefore, this last question regards the study of the instationary situation. Since time in the treatment of a disease is important, it is fundamental to study the optimal path to reach the new desired HPT state. Hence, the goal is to predict how a patient can reach a new equilibrium, corresponding to the set-point, and how long this process will take.

5

DATA

5.1. DATASETS

Different datasets are used to work on this project. In particular, one dataset of hypothyroid patients is specifically used to study the relationship between TSH and FT4, while other two datasets of thyroidectomized patients, one from the University Radboud Medical Centre in Nijmegen and one from the Erasmus Medical Center in Rotterdam, are used for validating the set-point theory.

5.1.1. HYPOTHYROID PATIENTS

A dataset containing measurements of 28 hypothyroid patients is used to investigate the relationship between TSH and FT4 and its set-point. This dataset contains information of patients belonging to different hospitals. Therefore, general reference ranges for TSH and FT4 have to be adopted when analyzing this dataset, because the ones characterising each hospital are not specified. Hence, the reference range considered for TSH is 0.4-4.0 mU/L, while the one for FT4 is 10.0-20.0 pmol/L. Multiple measurements are available for every patient, but the dates and times in which the measurements were collected are not available. Furthermore, for all the patients included in the dataset, the TSH measurements are expressed with one or two decimal digits. However, this is not happening for the FT4 values. In fact, in some cases, the FT4 measurements are reported with one decimal digit, which is good enough in terms of accuracy, while in other cases these measurements are expressed with integers, which causes a higher inaccuracy. However, even though this dataset might not be optimal, it is still fine for the purpose of studying the relationship between TSH and FT4.

5.1.2. NIJMEGEN DATASET

A dataset from the Radboud hospital in Nijmegen is used to validate the set-point theory. This dataset contains the measurements of 20 thyroidectomized patients. For each patient, at least one pre-thyroidectomy measurement and several post-thyroidectomy TFTs are available. 12 patients are women, while the remaining 8 patients are men. All

the patients underwent thyroidectomy between 2014 and 2015. The available TFTs include a measurement for TSH and one for FT4. The reference ranges adopted for this dataset changed during time. In fact, until the 10th of September 2015, the reference range for TSH was 0.40-4.00 mU/l, while the one for FT4 was 8-22.0 pmol/L. After the 10th of September 2015, the reference ranges became 0.27-4.20 mU/L for TSH and 10.0-23.0 pmol/L for FT4. In this dataset, the TSH values are expressed with two decimal digits, while the FT4 levels with one decimal digit. The time interval between the measurements is variable. In some cases, the available pre-thyroidectomy measurements are conducted a couple of months before the surgery, while in other cases the measurements date back to several months prior to the operation. Even when multiple pre-operative measurements are available, the time stamp between two consecutive measurements is not fixed. Regarding the post-thyroidectomy measurements, in some cases, the first available measurement is conducted a few weeks after the surgery, but for other patients it is dated months after the operation. Furthermore, in just a few patients the time stamp between consecutive measurements is maintained almost constant, around 2-4 months. In the majority of cases, the time interval between consecutive measurements is variable. It should also be noticed that for most of the patients, the measurements are always taken at different times during the day.

5.1.3. ERASMUS MEDICAL CENTER DATASET

A dataset of thyroidectomized patients from the Erasmus Medical Center is used for multiple purposes. The dataset contains information about 30 patients. However, only 11 patients present pre- and post-thyroidectomy measurements, the remaining 19 patients present only post-operative TFTs. Therefore, only the 11 patients with pre-surgery TFTs can be used to validate the set-point theory, while the other patients can be used to investigate the TSH-FT4 relationship. When available, the pre-thyroidectomy measurements are one or two, while the post-thyroidectomy ones are at least 3 for every patient. The reference ranges adopted by the Erasmus Medical Center are 0.4-4.3 mU/L for TSH and 11-25 pmol/L for FT4. The date and time in which the measurements were conducted are not reported. It is just known that all the 30 patients included in the dataset have been diagnosed with thyroid cancer between 2013 and 2017. In this dataset, TSH is expressed using 3 decimal digits, while FT4 measurements are reported with one decimal digit.

5.2. OUTLIERS

Before starting to study the relationship between TSH and FT4 and its set-point, it is important to determine if a dataset contains any outlier. An outlier is an observed point that clearly differs from the other measurements. This is a quite vague definition, because it is up to who analyses the data to determine if a certain measurement does not follow the behaviour of all the other data points and, therefore, should be considered as an outlier. After the outliers have been detected, it is necessary to remove them from the analysed dataset because they can influence the model by providing poor results.

However, in this project, the aim is to determine the fewest possible outliers in each dataset. In fact, it is important not to remove too many measurements, in order to pre-

serve the general behaviour of the dataset. Furthermore, it was noticed that the selection of different outliers can lead to quite different results. That is why a method to detect outliers has been developed, so that the choice of outliers is not totally dependent on who is working on the dataset but there are at least some guidelines to follow.

First of all, it is suggested to plot all the measurements belonging to one patient on the same graph. In this way it is possible to get an idea of the general behaviour of the measurements. Usually it is better to consider as outliers all the measurements with FT4 values smaller than the reference range, for example those with FT4<8-10 pmol/L. In fact, these measurements are usually isolated, therefore it is hard to determine if their corresponding TSH values are valid. Another step that can be immediately performed is checking if there are measurements with TSH levels that are too high or too low for their corresponding FT4. If this is the case, it is possible to directly remove these measurements from the dataset. An example of this situation is presented in Figure (5.1), where it is clear that two measurements have TSH values too low for their corresponding FT4 levels, therefore these measurements can directly be considered outliers.

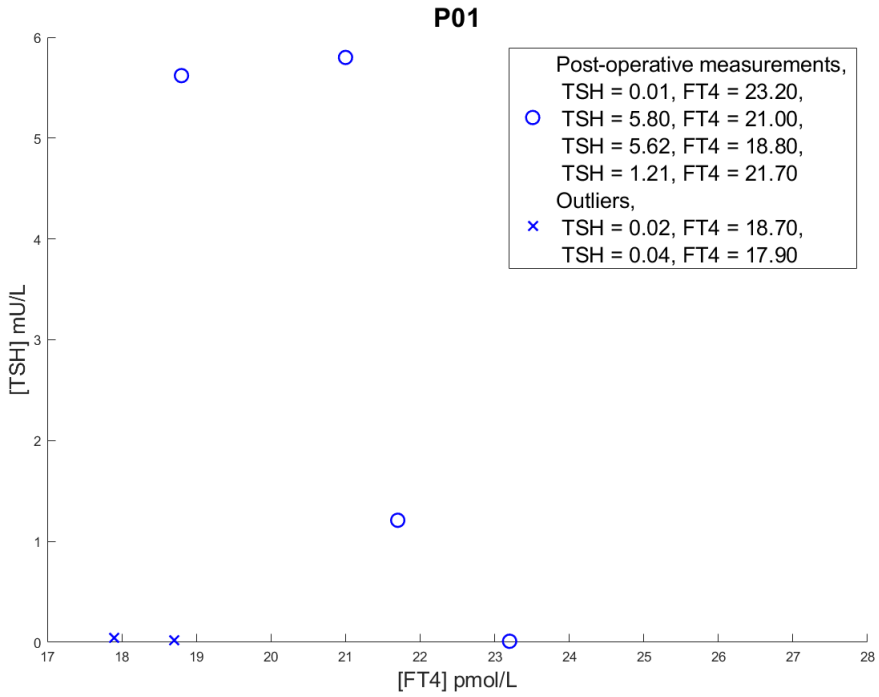


Figure 5.1: Plot of the TFTs of Patient 1 from the Nijmegen dataset. It is evident that two of the measurements are outliers, because their TSH levels are too small for their corresponding FT4 values.

After this initial procedure, it is possible to perform a more accurate outlier detection

to determine if there are other outliers. This consists in fitting the data with the appropriate function, using a robust method instead of the usual least-squares method. In particular, it is suggested to use the Least Absolute Residual (LAR) method.

The aim of least-square methods is to find the parameters of a function that best fit the data. The ordinary least-squares method aims to find the optimal parameter values by minimizing S, the sum of the squared residuals [36],

$$S = \sum_{i=1}^{n} \big(y_i - f(x_i)\big)^2 .$$

(x_i, y_i) represent the observed values, so in this specific case they correspond to the measurements $([\text{FT4}]_i, [\text{TSH}]_i)$, while f is the function that should fit the observed data. Therefore, the term residual indicates the difference between the actual observation and the value predicted by the model function [36]. The LAR method is an alternative to the common least-squares approach, in the sense that it is robust to outliers. The LAR method finds a curve that minimizes S, which in this case is defined as the sum of the absolute values of the residuals [37],

$$S = \sum_{i=1}^{n} \big|y_i - f(x_i)\big|.$$

With this approach, extreme values have a smaller influence because the LAR method gives equal importance to all the observations, which is not happening with the ordinary least-squares method. In fact, by squaring the residuals, more emphasis is given to large residuals, which might actually correspond to outliers.

Therefore, after fitting the data with the LAR method, it is possible to detect immediately the measurements that do not follow the behaviour of the fitted curve and are very far from the model. It is possible to determine these outliers by using a visual approach or by computing the distance between the measurements and the fitted curve. The distance between a data point ([FT4], [TSH]) and a curve $y = f(x)$ can be computed in the following way:

$$\begin{aligned} D &= \sqrt{(x - [\text{FT4}])^2 + (y - [\text{TSH}])^2} = \\ &= \sqrt{(x - [\text{FT4}])^2 + (f(x) - [\text{TSH}])^2}. \end{aligned}$$

Since a distance is always positive and the square root is an increasing function, it is possible to study D^2 instead of D. Therefore, it is possible to compute the derivative of D^2 with respect to x and set it equal to 0,

$$\frac{dD^2}{dx} = 2(x - [\text{FT4}]) + 2f'(x)(f(x) - [\text{TSH}]) = 0,$$

in order to find the value of x for which the distance will be minimum. Once the value of x is available, it can be substituted back into the expression for D in order to find the value of the distance between the point and the curve.

It is possible to define a threshold for the distance according to the dataset analyzed, so if the distance between one measurement and the model is larger than the specified

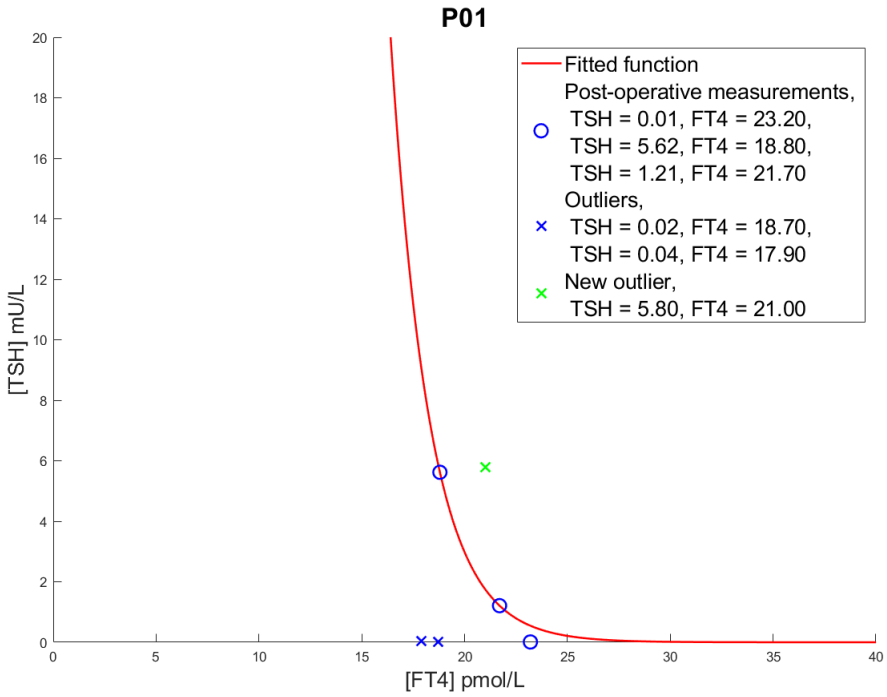


Figure 5.2: Plot of the TFTs of Patient 1 from the Nijmegen dataset with the fitted function. In addition to the outliers detected previously, it is clear, even with a visual approach, that the dataset contains one more outlier, marked in green.

threshold, that measurement will be considered an outlier. Figure (5.2) continues the example of Patient 1 from the Nijmegen dataset presented previously. After having already excluded the two previous outliers, it is clear that one more measurement is quite far from the fitted curve, hence it can be considered an outlier and it is marked with a green "X".

6

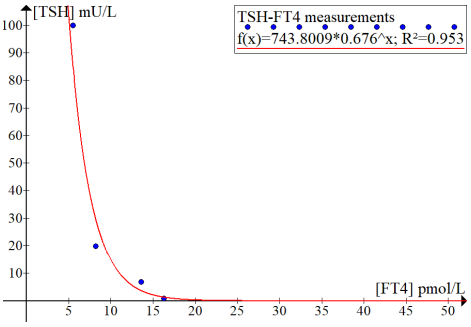
HP CURVE AND ITS SET-POINT

The first goal of this research is to investigate the relationship between TSH and FT4. For this purpose, two datasets can be used, the one of hypothyroid patients and the one from the Erasmus Medical Center, as presented in the previous chapter. The dataset of hypothyroid patients includes measurements collected in different hospitals, therefore general reference ranges for TSH and FT4 are used, in particular 0.4-4.0 mU/L for TSH and 10.0-20.0 pmol/L for FT4. All 28 patients' datasets are taken into account to study and define the relationship between TSH and FT4. Regarding the Erasmus dataset, the 19 thyroidectomized patients that do not present pre-operative measurements are used to study the relationship between TSH and FT4. For this dataset, the reference ranges are 0.4-4.3 mU/L for TSH and 11-25 pmol/L for FT4. The results obtained through the two datasets can be presented together because they are comparable and do not show any substantial difference.

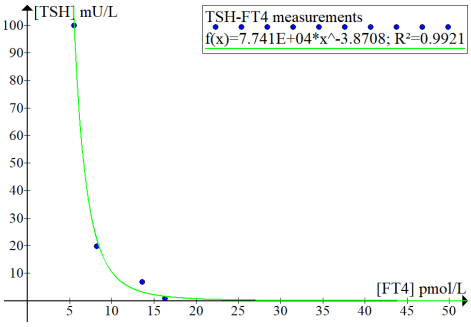
As a first step, each patients' dataset is fitted with different models, such as the exponential, logarithmic, polynomial, power functions. In this way, it is possible to check how different models approximate the TSH-FT4 relationship. In this phase, it is not necessary to remove any outlier from the patients' datasets because the aim is to find a general model that describes the TSH-FT4 relationship, therefore there is no need to study each individual dataset too accurately. The results of some of these experiments are shown in Figure (6.1). In this initial step, the software Graph 4.4.2 was used because it is very intuitive and visual, therefore it is useful for plots and for analysing the relationship between TSH and FT4.

It is already clear from Figure (6.1) that some of the functions used for fitting the data are not representing the behaviour of the data in an accurate way. From a mathematical perspective, different models can be compared by computing their goodness-of-fit R^2, which is defined as

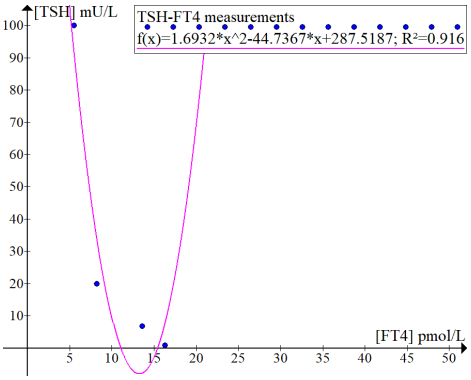
$$R^2 = 1 - \frac{SSE}{SST}.$$



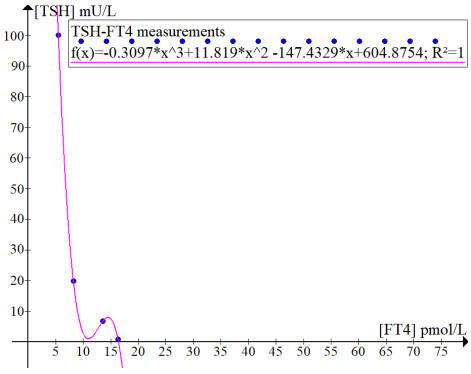
(a) Exponential function [TSH] = $ae^{b[\mathrm{FT4}]}$.



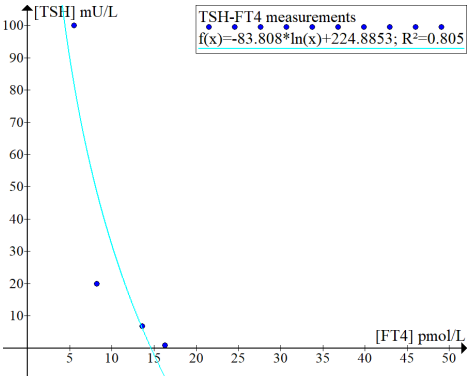
(b) Power function [TSH] = $a[\mathrm{FT4}]^b$.



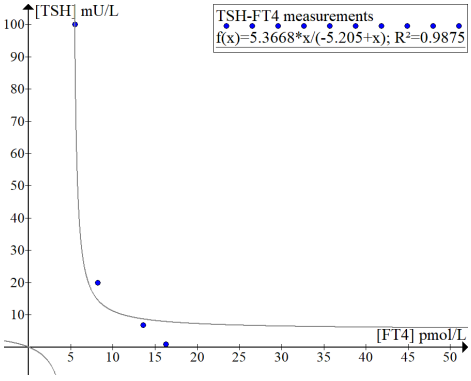
(c) Second-order polynomial [TSH] = $a[\mathrm{FT4}]^2 + b[\mathrm{FT4}] + c$.



(d) Third-order polynomial [TSH] = $a[\mathrm{FT4}]^3 + b[\mathrm{FT4}]^2 + c[\mathrm{FT4}] + d$.



(e) Logarithmic function [TSH] = $a\ln[\mathrm{FT4}] + b$.



(f) Saturation-growth rate [TSH] = $\frac{a[\mathrm{FT4}]}{b+[\mathrm{FT4}]}$.

Figure 6.1: Measurements belonging to the hypothyroid patient AP05 fitted with different functions.

SSE is the residual sum of squares and it is defined as

$$\text{SSE} = \sum_i (y_i - f_i)^2,$$

while the total sum of squares SST is

$$\text{SST} = \sum_i (y_i - \overline{y})^2,$$

where y_i are the observed measurements from the dataset, f_i are the fitted values and $\overline{y}$ is the mean of the observed values. In this case, y_i correspond to the TSH measurements, while f_i correspond to the TSH values approximated through the model. The best case happens when R^2=1, because it means that the fitted values match the observed ones.

However, even if from a mathematical perspective some models seem accurate because of a high goodness-of-fit, they actually might not be good approximations of the measurements. Referring to Figures (6.1c) and (6.1d), it is clear that fitting the data points with polynomial functions does not provide good results. In fact, even though the values of R^2 are high for both cases, the fitted functions do not represent the behaviour of the measurements in an accurate way. Furthermore, in order to fit the data with a second-order polynomial it is necessary to have at least 3 TFTs, for a third-order polynomial at least 4 measurements should be available and so on. However, sometimes only a couple of measurements might be available for a patient, hence it is better to prefer a model requiring less data points.

From Figure (6.1e), it is evident that also the logarithmic function is not representing the behaviour of the measurements accurately. Furthermore, the [TSH] values computed through the logarithmic model assume negative values for [FT4]>14.63 pmol/L, which is not realistic because the concentration of TSH should always be positive.

The saturation-growth rate model presented in Figure (6.1f) has some problems as well. At first sight it might seem acceptable, however the model is characterised by the presence of two asymptotes, one vertical and one horizontal. Therefore, the range of values that the model can take in the first quadrant, which is the one of interest because the concentrations should always be positive, is reduced. In this particular example, there is a vertical asymptote for [FT4]=5.205 pmol/L, therefore the TSH levels corresponding to FT4 values in the range 0-5.205 pmol/L are negative, which is not acceptable. Similarly, there is a horizontal asymptote at [TSH]=5.3668 mU/L, hence negative FT4 values provide TSH concentrations in the interval 0-5.3668 mU/L. Hence, this model has to be discarded. Similar results are obtained also with hyperbolic models, $[\text{TSH}] = a + \dfrac{b}{[\text{FT4}]}$, and reciprocal functions, $[\text{TSH}] = \dfrac{1}{a[\text{FT4}] + b}$, therefore these models are not appropriate to describe the TSH-FT4 relationship either.

As a result of the experiments conducted on all the analyzed patients, two models have to be preferred, the exponential function and the power function, presented in Figures (6.1a) and (6.1b), respectively. The exponential function has the following expression

$$[\text{TSH}] = Se^{-\varphi[\text{FT4}]},$$

where $S > 0$ and $\varphi > 0$, while the power function is

$$[\text{TSH}] = a[\text{FT4}]^b,$$

where $a > 0$ and $b < 0$. First of all, it is important to notice that both models require at least two measurements of TSH and FT4 for each individual, which is a positive aspect because both models can be applied to all the available datasets, since all of them consists of at least two measurements.

When two TFTs are available for a patient, $([\text{FT4}]_1, [\text{TSH}]_1)$ and $([\text{FT4}]_2, [\text{TSH}]_2)$, the model parameters of the exponential model can be computed as

$$\varphi = \frac{1}{[\text{FT4}]_1 - [\text{FT4}]_2} \ln\left(\frac{[\text{TSH}]_2}{[\text{TSH}]_1}\right),$$

$$S = [\text{TSH}]_1 e^{\varphi[\text{FT4}]_1} = [\text{TSH}]_2 e^{\varphi[\text{FT4}]_2},$$

while for the power function the parameters are defined as

$$b = \frac{\ln\left(\frac{[\text{TSH}]_2}{[\text{TSH}]_1}\right)}{\ln\left(\frac{[\text{FT4}]_2}{[\text{FT4}]_1}\right)}$$
$$a = \frac{[\text{TSH}]_1}{[\text{FT4}]_1^b} = \frac{[\text{TSH}]_2}{[\text{FT4}]_2^b}.$$

When more than two TFTs are available for a patient, one way to determine the model parameters consists in using a least-squares approach, through which it is possible to estimate the parameters that provide the best fit of the measurements.

The software package Matlab can be used to study in depth the differences between these two models. In order to fit the measurements with the two different models in Matlab, it is possible to use the built-in function `fit`, because, through a least-squares method, it returns the parameters that provide the best fit of the data.

For the majority of the patients' datasets, both the exponential and power functions present high values for the goodness-of-fit R^2, usually more than 90%. In fact, in these cases, the values of R^2 are very similar to each other, so it is impossible to determine which of the two models is better by basing the decision only on the values of R^2. An example of this situation is presented in Figure (6.2), where, also visually, it is difficult to distinguish the two functions because they are very similar to each other. In this case, the goodness-of-fit for the exponential function is 99.98%, while for the power function is 99.87%, hence both models present extremely high values of the goodness-of-fit.

However, there are some datasets that present lower values of R^2. In some cases, it is possible to detect some outliers through the procedure described previously and remove them from the dataset. When the outliers are removed, the goodness-of-fit might improve. An example of this situation is presented in Figure (6.3). For both the exponential and the power function models presented in Figure (6.3a), the goodness-of-fit is around 61%, which is a quite low value. Therefore, it is necessary to detect some outliers

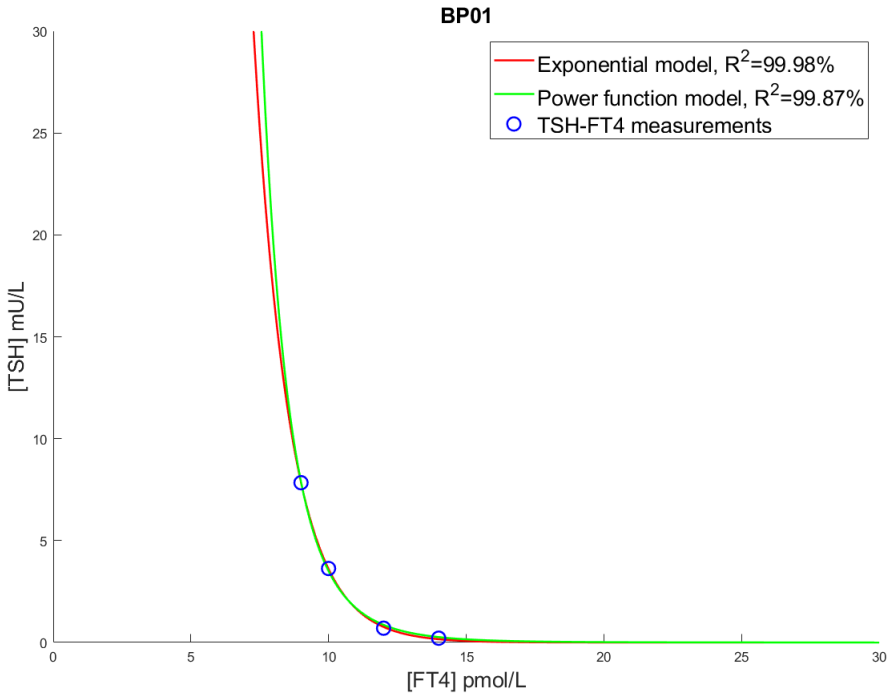
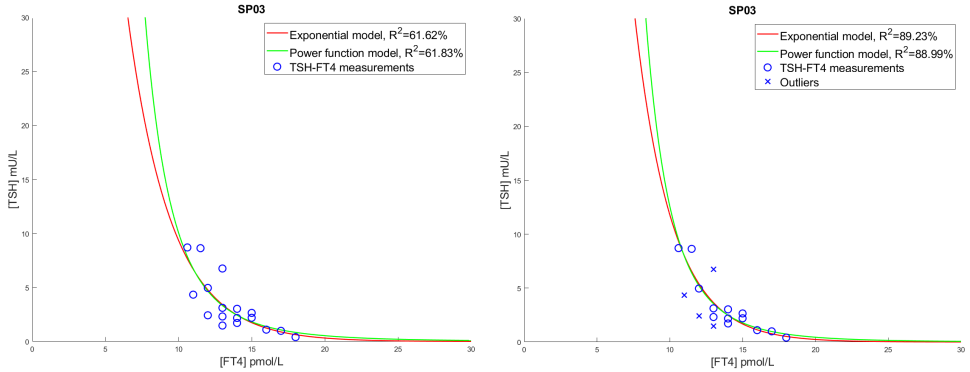


Figure 6.2: Plot of the measurements of the hypothyroid patient BP01, fitted with the exponential model (in red) and the power function (in green).

in order to improve the goodness-of-fit for the two models. Figure (6.3b) presents the case where the outliers have been removed. In this case the goodness-of-fit for both the exponential and power functions is much higher, around 89%. As it can also be appreciated in the figures, after removing the outliers, the two models are visually even more similar.

In other cases, however, it can happen that there is not any outlier, or, even if the outliers are removed, the goodness-of-fit does not improve. This might be due to several reasons. In fact, if the measurements are mostly expressed using integers, this might have an influence on the accuracy of the model. Furthermore, it can happen that measurements with different values of FT4 present the same value for TSH and vice versa, which is not realistic. However, this happens because the measurements are actually approximations of the real values. Furthermore, this is more likely to happen if there are many measurements available for a single individual. This situation is presented in Figure (6.4). In this example, the goodness-of-fit for the exponential function is 51%, while for the power model is 45%. Both values of R^2 are quite low, however the goodness-of-fit cannot be improved in any way, hence the poor performance of both models might be due to the low quality of the measurements. Furthermore, the two curves are quite



(a) Exponential and power functions fitted considering all the measurements.

(b) Exponential and power functions fitted removing the outliers.

Figure 6.3: Measurements belonging to the hypothyroid patient SP03 fitted with the exponential and power functions, with and without outliers.

different from each other.

The exponential and power functions can also be inspected visually, in order to check which of the two models simulates better the behaviour of the TSH-FT4 measurements. By doing so, in some cases it can be noticed that the exponential model is better than the power function. This can be seen in the example reported in Figure (6.5). In fact, the exponential model represents the curvature of the TSH-FT4 measurements in a better way. The goodness-of-fit for both models is very high, nevertheless, as it can be seen in the figure, the exponential function represents better the behaviour and the curvature of the measurements, while the power function, even if it still provides a high goodness-of-fit, is above the measurements and it does not model their curvature in a precise way. Therefore, the two curves are quite different from each other.

However, it is hard to determine which model is better based only on the results of this visual approach. Therefore, an idea would be to compute also the set-points of the two models and compare the results in order to determine which function is more appropriate to describe the TSH-FT4 relationship.

For both models, the set-point is computed as the point of maximum curvature of the function. According to Goede et al. [3], the set-point of the exponential function is defined as the point of maximum curvature. This is because it corresponds to the point where the sensitivity for any change around this point is maximal. The same reasoning can be applied to the power function, so also in that case the set-point can be computed as the point of maximum curvature.

The general expression for the curvature of a function is

$$K = \frac{\frac{dy^2}{dx^2}}{\left(1+\left(\frac{dy}{dx}\right)^2\right)^{3/2}},$$

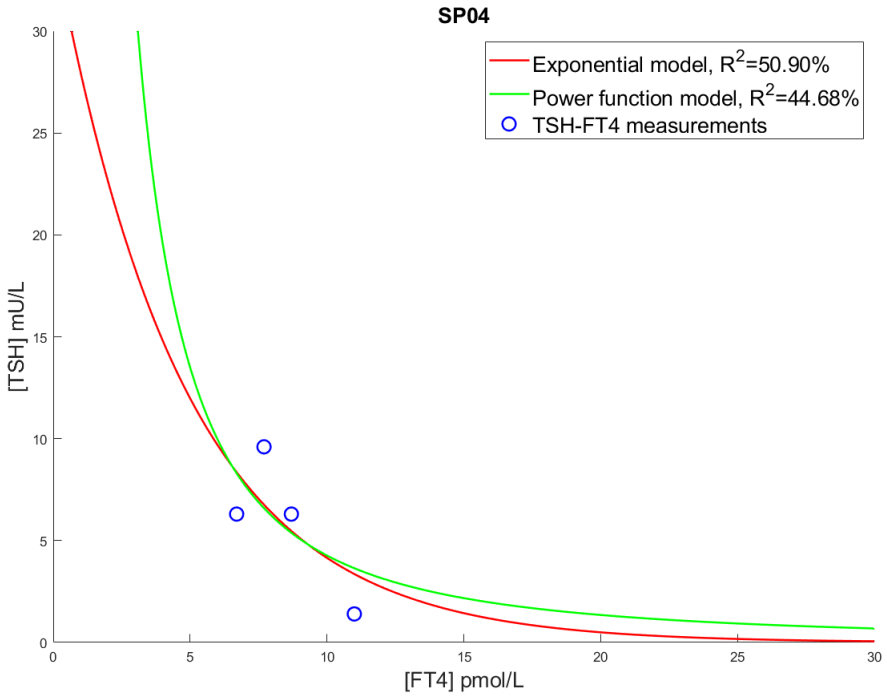


Figure 6.4: Plot of the measurements of the hypothyroid patient SP04, fitted with the exponential model (in red) and the power function (in green).

hence for the exponential model $[\text{TSH}] = Se^{-\varphi[\text{FT4}]}$ the curvature is

$$K = \frac{\varphi^2 S e^{-\varphi[\text{FT4}]}}{\left(1+\varphi^2 S^2 e^{-2\varphi[\text{FT4}]}\right)^{3/2}},$$

while for the power function $[\text{TSH}] = a[\text{FT4}]^b$ the curvature has the following expression

$$K = \frac{ab(b-1)[\text{FT4}]^{b-2}}{\left(1+(ab[\text{FT4}]^{b-1})^2\right)^{3/2}}.$$

In order to study when the curvature is maximum, it is necessary to solve

$$\frac{dK}{d[\text{FT4}]} = 0.$$

By doing so, it is possible to derive the coordinates of the set-point. For the exponential

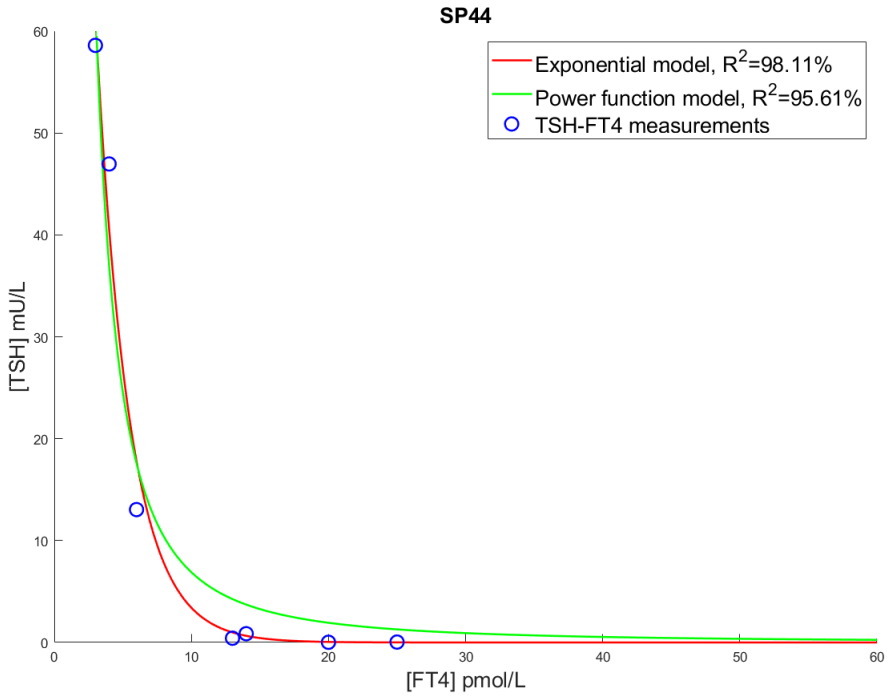


Figure 6.5: Plot of the measurements of the hypothyroid patient SP44, fitted with the exponential model (in red) and the power function (in green).

model, the set-point is defined by

$$[\mathrm{FT4}]_{\text{set-point}} = \frac{\ln\left(\varphi S\sqrt{2}\right)}{\varphi}$$
$$[\mathrm{TSH}]_{\text{set-point}} = \frac{1}{\varphi\sqrt{2}}.$$

For the power function, the coordinates of the set-point are

$$[\mathrm{FT4}]_{\text{set-point}} = \left(\frac{2-b}{a^2b^2-2a^2b^3}\right)^{1/(2b-2)}$$
$$[\mathrm{TSH}]_{\text{set-point}} = a\left(\frac{2-b}{a^2b^2-2a^2b^3}\right)^{b/(2b-2)}.$$

Therefore, comparing the set-points of the two models might give more insights into determining which of the two functions describes better the relationship between TSH and FT4.

In many cases, especially if the power and exponential functions have a very similar behaviour, as shown in Figure (6.6), the two set-points are almost indistinguishable

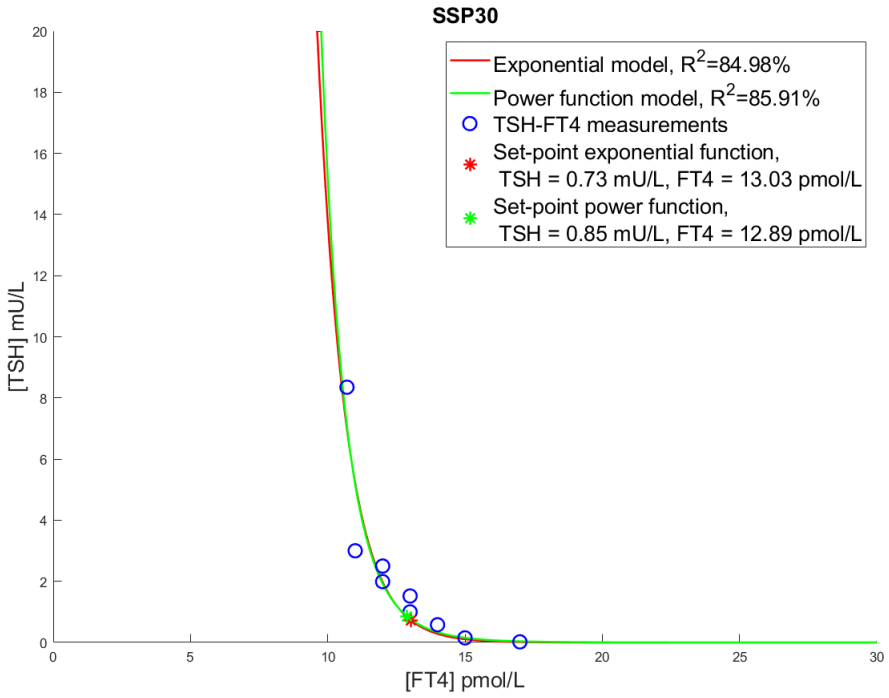


Figure 6.6: Plot of the measurements of the hypothyroid patient SSP30, fitted with the exponential model (in red) and the power function (in green).

from each other. However, other datasets give more interesting results. First of all, this experiment shows that for the exponential function all the datasets, except one, present the set-point within the reference ranges. However, for the power the function, many datasets present a set-point outside the normal reference ranges, in particular because of a high value of TSH. Figure (6.7) presents an example in which the set-point of the exponential function is within the reference ranges, while the set-point of the power function is outside these reference ranges. In particular, the TSH value of the set-point of the power function is much larger than the reference range. Furthermore, it can visually be noticed that the exponential function simulates better the behaviour of the data points also in this case. After this analysis, it is finally possible to conclude that the TSH-FT4 relationship is best modelled by a negative exponential function, because it provides a more reasonable prediction of the set-point, which should be found within the reference ranges.

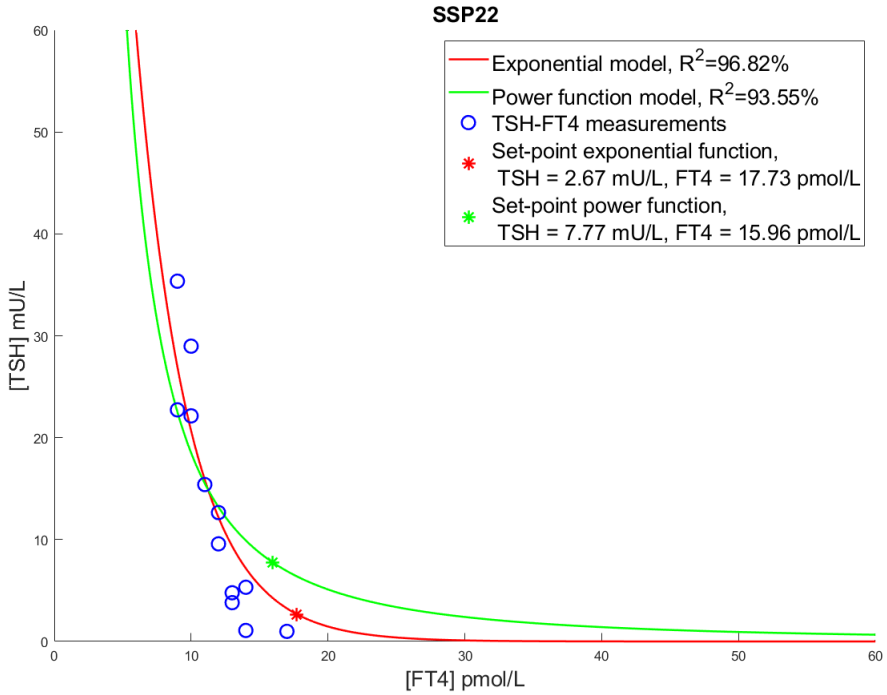


Figure 6.7: Plot of the measurements of the hypothyroid patient SSP22, fitted with the exponential model (in red) and the power function (in green).

7

VALIDATION OF THE SET-POINT THEORY

After having established that the TSH-FT4 relationship can be represented through a negative exponential function, it is fundamental to validate the set-point theory. This means that it is required to prove that the set-point of the HPT axis actually corresponds to the point of maximum curvature of the HP curve. This can be done by using measurements of patients that underwent a thyroidectomy. In fact, in these cases, TFTs are conducted both before and after the removal of the thyroid. Therefore, this allows a comparison between the pre- and post-thyroidectomy set-points, in order to verify the validity of the latter.

First of all, it should be reminded that a healthy thyroid produces only 20% of the total amount of T3, while the remaining amount is produced by a peripheral conversion of T4 [5, 6]. Hence, in thyroidectomized patients, it is necessary to increase the dose of LT4 in order to compensate also for the amount of T3 that is normally produced by the thyroid. In particular, around 25% of the originally secreted T4 should be added to the compensating dose of LT4 in order to obtain the same level of pre-operative FT3. Therefore, the post-thyroidectomy level of FT4 is expected to be 25% larger than the pre-operative one. On the other hand, there is no evidence to believe that the TSH level of the set-point should change before and after a thyroidectomy.

The first step in the process of validating the set-point theory is to consider the post-operative data and use it to reconstruct the post-operative HP curve. This can be done by fitting the measurements with the negative exponential function presented previously. After the post-thyroidectomy HP curve is available, it is possible to compute its set-point as the point of maximum curvature of the exponential function, using the formulas presented in the previous chapter. After this, it is necessary to compute a prediction of the pre-operative set-point. According to the previous considerations, it is well established that the level of TSH before and after the thyroidectomy should remain the same, while the post-thyroidectomy level of FT4 should be 25% larger than the pre-operative one.

Hence, the predicted pre-operative set-point is

$$[\text{TSH}]_{\text{predicted}} = [\text{TSH}]_{\text{post-operative}}$$
$$[\text{FT4}]_{\text{predicted}} = \frac{[\text{FT4}_{\text{post-operative}}}{1.25}.$$

After this step, two different approaches can be adopted to validate the set-point theory. The first method consists in directly comparing the patients' pre-operative measurements with the predicted set-points. According to Andersen et al. [13], one TFT describes the individual set-point with a precision of ±25% for FT4 and ±50% for TSH. Therefore, in this case, the set-point theory is considered validated if the predicted TSH and FT4 set-point values are found within a distance of 50% and 25% from the pre-operative measurements of TSH and FT4, respectively.

On the contrary, the second approach consists in reconstructing the pre-operative HP curve and its set-point. In fact, this method does not consider the pre-surgery measurements as indicators of the real pre-operative set-points because, even if they are within the reference ranges, they might still deviate from the real set-point. As explained previously, TSH is considered constant before and after the thyroidectomy. Therefore, the TSH level of the pre-thyroidectomy set-point should be equal to the post-operative one. Hence, using the expression of the set-point value of TSH, $[\text{TSH}] = \frac{1}{\varphi\sqrt{2}}$, it can be concluded that the model parameter φ of the pre-operative HP curve is actually the same as the parameter φ in the post-thyroidectomy HP curve. This means that the only parameter changing in the model is S. In order to determine S in the pre-operative exponential function, it is necessary to use the pre-operative measurements in the following way:

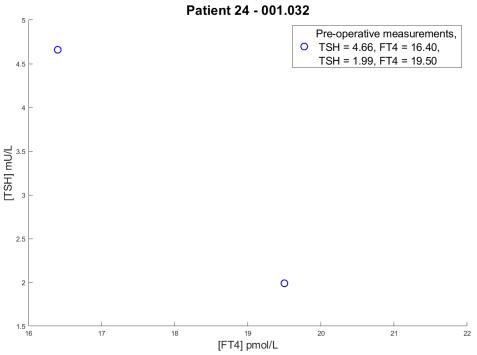
$$S = [\text{TSH}]_{\text{pre}} e^{\varphi [\text{FT4}]_{\text{pre}}}.$$

Once the pre-operative model is available, it is possible to compute its set-point as the point of maximum curvature and compare it with the predicted one. The TSH level of the pre-thyroidectomy set-point is equal to the one of the predicted set-point. Therefore, it is necessary to compare only the predicted and actual pre-thyroidectomy levels of FT4. The set-point theory is considered validated for a patient if the actual pre-thyroidectomy FT4 concentration is maximum 10% larger or smaller than the predicted FT4 level.

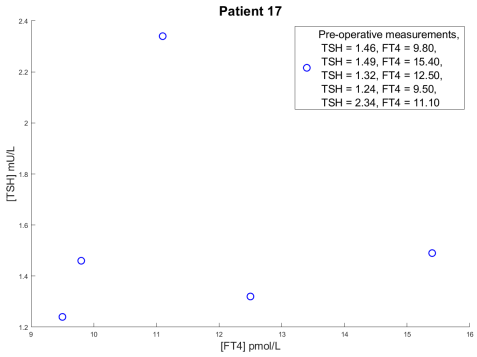
In order to validate the set-point theory with both approaches, two datasets have been used, the one from the Nijmegen hospital and the one from the Erasmus Medical Center.

7.1. COMPARISON WITH THE PRE-OPERATIVE MEASUREMENTS

The Nijmegen and the Erasmus dataset were analyzed with the first approach, consisting in a direct comparison between the predicted set-point and the pre-operative measurements. In particular, the Nijmegen dataset consists of measurements belonging to 20 patients, however only 16 of them could be used, because the other 4 patients' dataset do not allow to reconstruct the post-operative HP curve. On the other hand, all the 11 patients' dataset of the Erasmus Medical Center could be taken into account to validate the set-point theory.



(a) Pre-operative measurements belonging to Patient 24 - 001.032 from the Erasmus dataset.

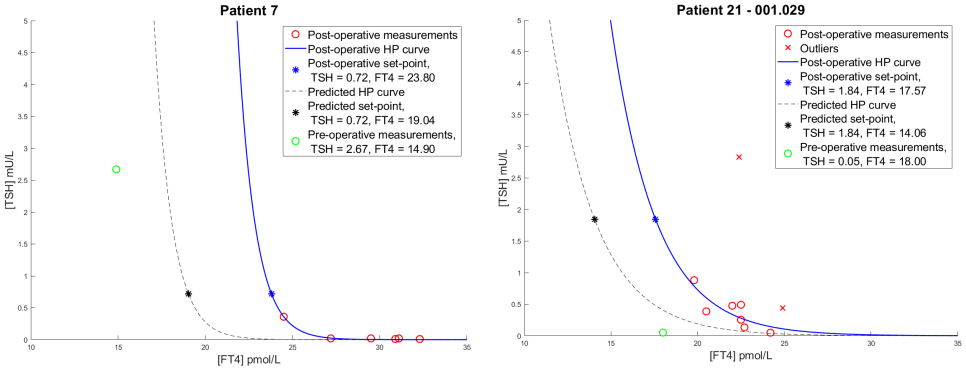


(b) Pre-operative measurements belonging to Patient 17 from the Nijmegen dataset

Figure 7.1: Examples of pre-operative measurements that are clearly different from each other.

The results obtained through the two datasets are comparable, hence they can be presented together. The first issue that can be noticed is when multiple pre-thyroidectomy measurements are available. There is a total of 14 datasets that present multiple pre-operative measurements. In only 5 datasets the pre-operative measurements are quite similar, so in these cases it is possible to carry out the comparison with the predicted set-point. However, in most of the cases, the measurements are very different from each other, therefore it is impossible to choose the pre-thyroidectomy measurement that should be compared to the predicted set-point. Hence, in such cases, it is not possible to verify the validity of the set-point theory. Figure (7.1) shows two examples in which the pre-operative measurements are not similar. Figure (7.1a) is extracted from the Erasmus Medical Center dataset. In this case there are two pre-operative measurements, which have both the FT4 and TSH levels very different from each other. Figure (7.1b) shows a more extreme example, taken from the Nijmegen dataset. In this case, the difference between the smallest and largest TSH concentrations is more than 1 mU/L, which is quite significant considering the typical scale of values for TSH. Similarly for FT4, the difference between the smallest and largest concentrations is almost 6 pmol/L, which also in this case represents a quite remarkable dissimilarity.

Therefore, it is possible to take into account only the datasets presenting one pre-operative measurement and the few ones with multiple pre-thyroidectomy measurements similar to each other. In the majority of cases, the discrepancy between the predicted set-point and the pre-operative measurements is larger than the accepted difference presented previously. Figure (7.2) presents two examples in which the predicted set-point is very different from the pre-operative TFT. For 7 patients, it happens that either the predicted FT4 or TSH values are within the accepted interval of the corresponding pre-operative levels, however the other quantities are not matching. Figure (7.3a) shows an example in which the predicted TSH level is similar to the one of the pre-operative measurement, while the FT4 values are different. Figure (7.3b) presents the



(a) In Patient 7 from the Nijmegen dataset, the predicted set-point is different from the pre-operative measurement.

(b) In Patient 21 from the Erasmus dataset, the predicted set-point is different from the pre-operative measurement.

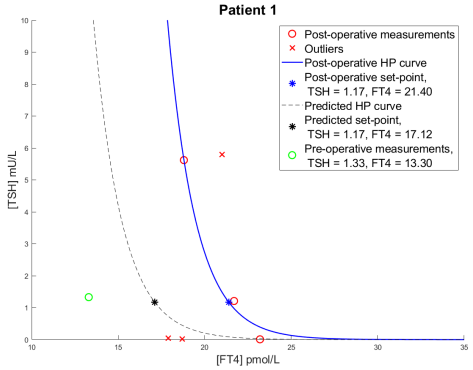
Figure 7.2: Two datasets in which the predicted set-point is not matching the pre-operative TFT.

opposite situation, so the FT4 concentration of the pre-operative measurement and the predicted one are very similar, but the TSH levels are not matching. In only a few datasets the predicted concentrations of TSH and FT4 are both similar to the corresponding pre-thyroidectomy measurements. Figure (7.4) presents two cases in which the set-point theory seems validated according to this approach.

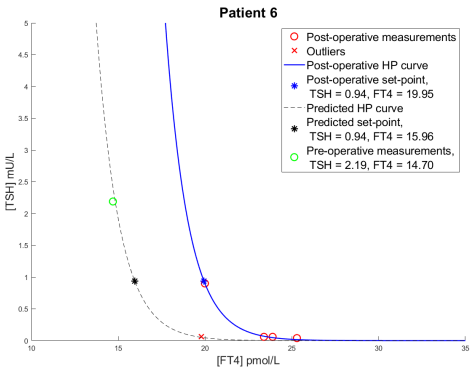
These results lead to two possible conclusions: the prediction of the set-point might be wrong, therefore the set-point cannot be computed as the point of maximum curvature, or the preoperative measurements cannot be considered as set-points. However. as it is shown also in the figures presented previously, the analysis of the datasets provide a large variety of different results. In fact, none of the datasets presents similar behaviours that might allow to derive a different prediction of the set-point. For example, in some cases pre-operative TSH is larger than the predicted one, while in other cases it is smaller. The same happens with FT4 as well. Therefore, what can be concluded from this approach is that the pre-thyroidectomy measurements might not always coincide with the real set-point. Moreover, the fact that multiple pre-operative measurements are usually very different from each other is another element in support of this thesis.

7.2. RECONSTRUCTION OF THE PRE-OPERATIVE HP CURVE AND SET-POINT

The datasets of thyroidectomized patients from the Nijmegen hospital and the Erasmus Medical Center have then been analyzed with the second approach, consisting in reconstructing the pre-operative set-point and comparing it with the predicted one. The total amount of patients included in the two datasets is 31, however not all of them could be used to validate the set-point theory. In fact, in a few cases the post-thyroidectomy measurements do not allow to reconstruct the HP curve, so these datasets have to be ex-

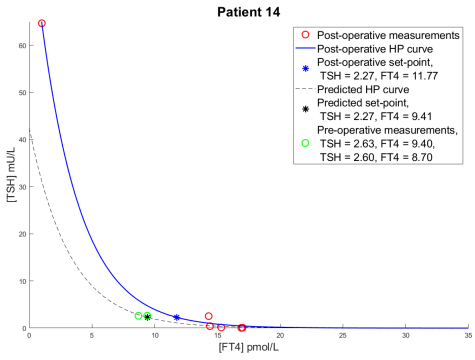


(a) In Patient 1 from the Nijmegen dataset, the predicted TSH level is similar to the TSH concentration of the pre-operative measurement, while the FT4 concentrations are very different.

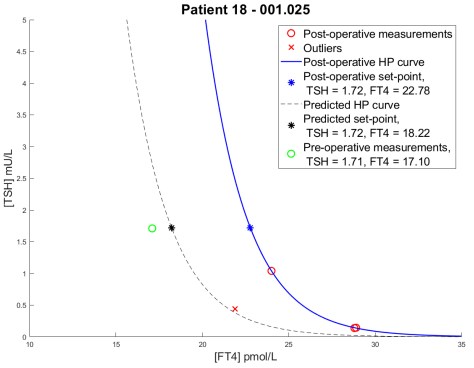


(b) In Patient 6 from the Nijmegen dataset, the predicted FT4 level is similar to the FT4 concentration of the pre-operative measurement, while the TSH concentrations are very different.

Figure 7.3: Two datasets in which either the predicted FT4 or TSH values are similar to the corresponding pre-operative levels.

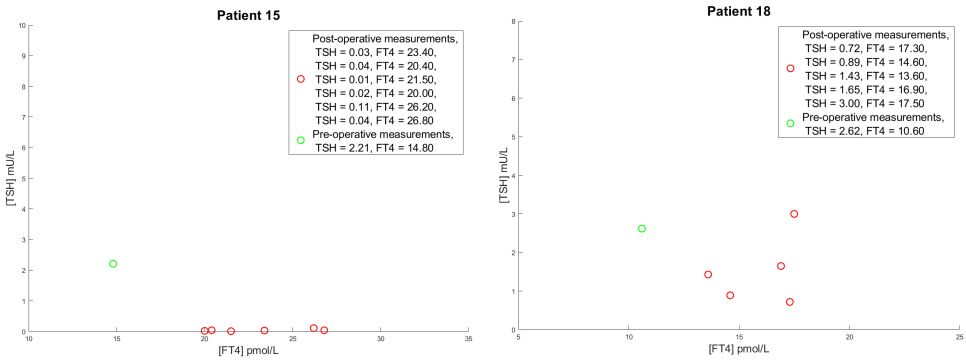


(a) In Patient 14 from the Nijmegen dataset, the predicted set-point and the pre-operative measurements are very similar.



(b) In Patient 18 - 001.025 from the Erasmus dataset, the predicted set-point and the pre-operative measurement are very similar.

Figure 7.4: Two datasets in which the predicted set-point coincides with the pre-operative measurements.



(a) Patient 15 should be excluded because its post-operative TFTs do not allow to reconstruct the negative exponential HP curve.

(b) Patient 18 should be excluded because its post-operative TFTs do not allow to reconstruct the negative exponential HP curve.

Figure 7.5: Two of the datasets from the Nijmegen hospital that have to be excluded.

cluded. The results obtained with this approach are encouraging. In fact, for about half of the datasets the set-point theory is validated because the predicted FT4 level and the actual pre-thyroidectomy FT4 concentration are matching almost perfectly. Regarding the datasets in which the set-point theory is not validated, there are multiple reasons that might explain why this happens. In some cases, it might be because of the pre-thyroidectomy measurements, which might cause an inaccurate reconstruction of the HP curve and, consequently, of the set-point. In other cases, the reason might be due to an autonomous production of FT3 [38]. In fact, this causes the pre-thyroidectomy FT4 to be lower than expected because an extra source of T3 inhibits the production and release of TRH, which results in a lower FT4 set-point value. Moreover, this is also supported by the fact that, when the set-point theory is not validated, the predicted FT4 value is always larger than the reconstructed one.

A more detailed analysis of the results obtained with the Nijmegen and Erasmus datasets is presented in the following sections. Furthermore, the Appendix contains the code implemented to verify the set-point theory and an overview of the results obtained with each individual patient.

7.2.1. NIJMEGEN DATASET

The Nijmegen dataset contains measurements of 20 thyroidectomized individuals, however not all of them can be used to validate the set-point theory. 5 datasets cannot be taken into account because their post-operative measurements do not allow to reconstruct the HP curve. Two examples of the excluded patients are depicted in Figure (7.5). The other 3 datasets have a very similar behaviour.

For the remaining 15 datasets, the goodness-of-fit of the post-thyroidectomy HP curve is always higher than 93%, and in the majority of cases it is even larger than 97%. The results from the comparison between the predicted and reconstructed pre-operative FT4 concentrations show that for 7 patients they are very similar, while in the remaining

8 datasets there is a discrepancy between the two values. Figure (7.6) shows an example of one of the 7 cases in which the set-point is validated. As it can be seen in the figure,

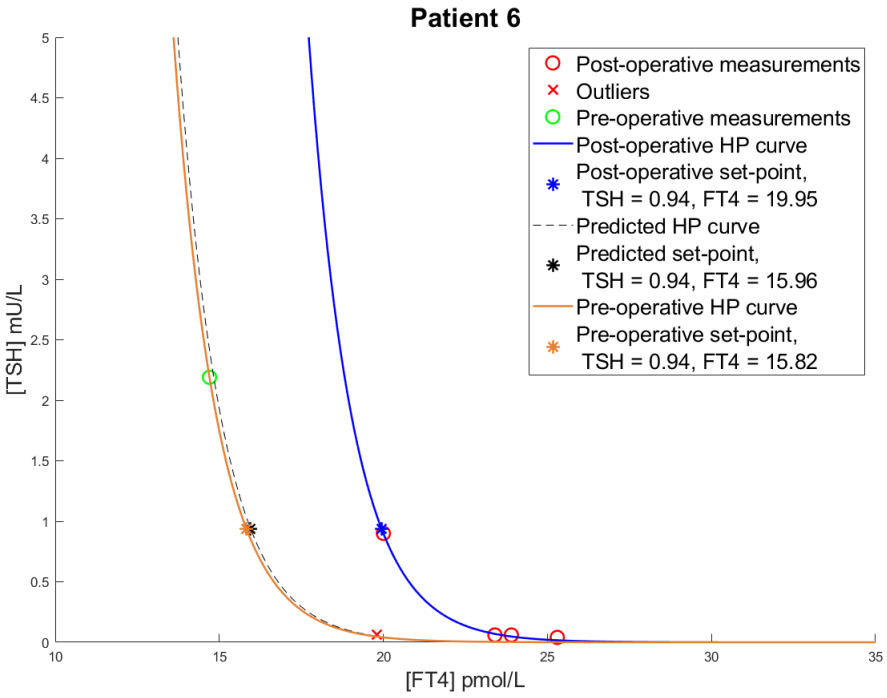


Figure 7.6: In Patient 6 the predicted and reconstructed pre-operative set-points are matching perfectly.

the predicted and reconstructed set-points are matching almost perfectly. One reason to explain the difference between the predicted and actual pre-thyroidectomy set-points in the other 8 datasets might be an autonomous production of T3. In fact, many patients contained in this dataset present pre-operative FT4 measurements with very small values, towards the lower boundary of the reference range, combined with low-normal levels of TSH. This might be a hint for an unknown source of T3, which causes a lower concentration of FT4. Figure (7.7) presents an example of a dataset in which the set-point theory is not validated.

7.2.2. ERASMUS MEDICAL CENTER DATASET

As already presented in Section 5, the Erasmus dataset contains measurements belonging to 30 thyroidectomized patients, however only 11 of them present pre-thyroidectomy TFTs. Therefore, these are the datasets that can be used to verify the set-point theory. Of the 11 patients that can be taken into account, only 9 of them can actually be used. In fact, the measurements of the two excluded datasets do not seem reliable, therefore

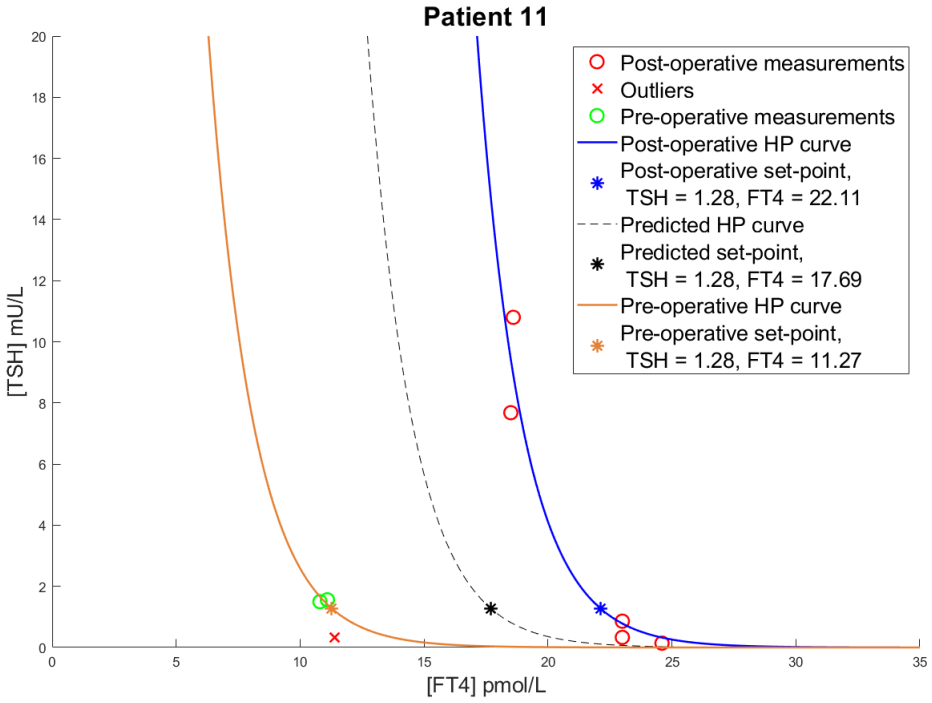
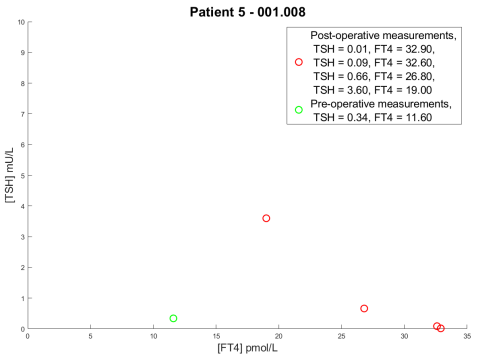


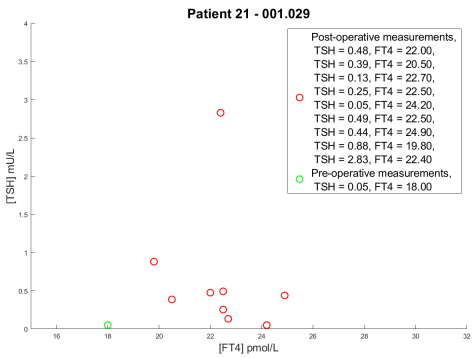
Figure 7.7: In Patient 11 the predicted and reconstructed pre-operative set-points are very different.

this does not allow a comparison between the pre- and post-thyroidectomy set-points. Figure (7.8) presents the two datasets that should be excluded from the analysis. In particular, for Patient 5 - 001.008, Figure (7.8a), the pre-thyroidectomy measurement of TSH is outside the reference range adopted by the hospital. Patient 21 - 001.029, Figure (7.8b) should not be included because its pre-operative TFT presents a very low level of TSH and the post-thyroidectomy measurements do not resemble an exponential behaviour.

When reconstructing the post-thyroidectomy HP curves, all the datasets present a goodness-of-fit higher than 90%, with the majority of datasets having a goodness-of-fit larger than 97%. The results obtained from the comparison between the predicted and reconstructed pre-thyroidectomy set-point show that in 7 cases the set-point theory is validated. In fact, in these datasets the two set-points are very similar to each other. In Figure (7.9) the predicted and reconstructed pre-operative set-points are matching perfectly, hence this is an example in which the set-point theory is validated. In the remaining two cases, the predicted FT4 level of the set-point is much larger than the pre-operative one. This might be caused by a pre-operative unknown source of T3, which can influence the level of FT4 of the set-point. Figure (7.10) is one of the two cases in which the set-point theory is not validated because the predicted set-point is very differ-



(a) Patient 5 - 001.008 should be excluded because the pre-operative TFT is outside the reference range.



(b) Patient 21 - 001.029 should be excluded because of the behaviour of the post-operative TFTs.

Figure 7.8: Datasets from the Erasmus Medical Center that have to be excluded.

ent from the reconstructed one. In particular, the predicted set-point is larger than the reconstructed one. As explained in the previous sections, this might happen for different reasons. It might be caused by an inaccurate post-operative HP curve. In fact, in this case this was reconstructed using only 3 measurements that do not present a high variability, therefore the resulting exponential curve might not be precise. Another possible reason might be the autonomous production of pre-operative T3, which might cause a reduction of the pre-thyroidectomy FT4 level.

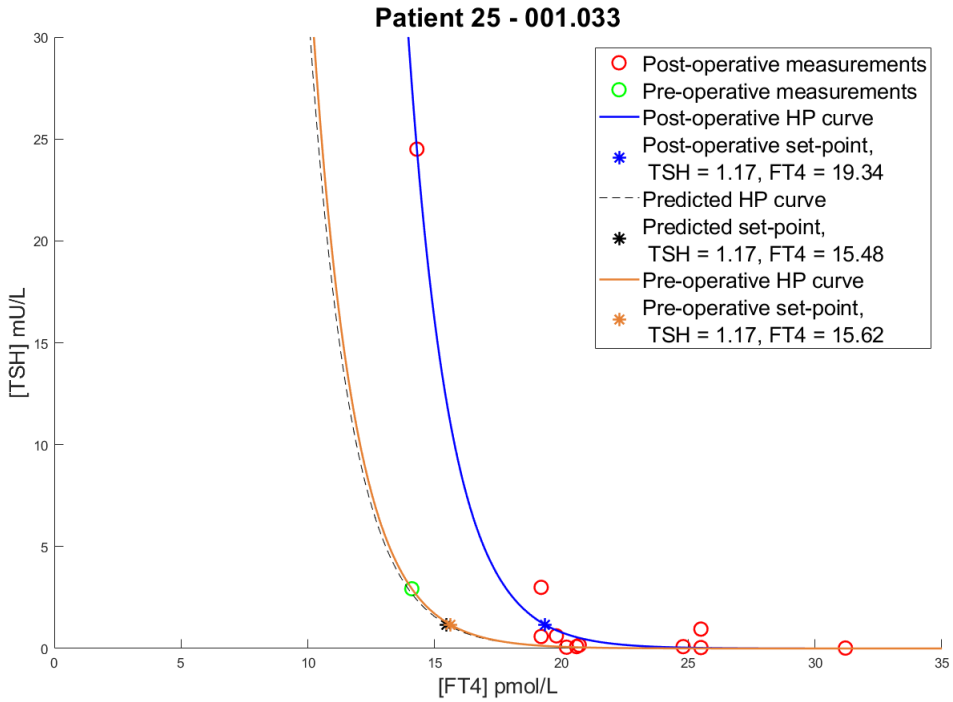


Figure 7.9: In Patient 25 - 001.033 the predicted and reconstructed pre-operative set-points are matching perfectly.

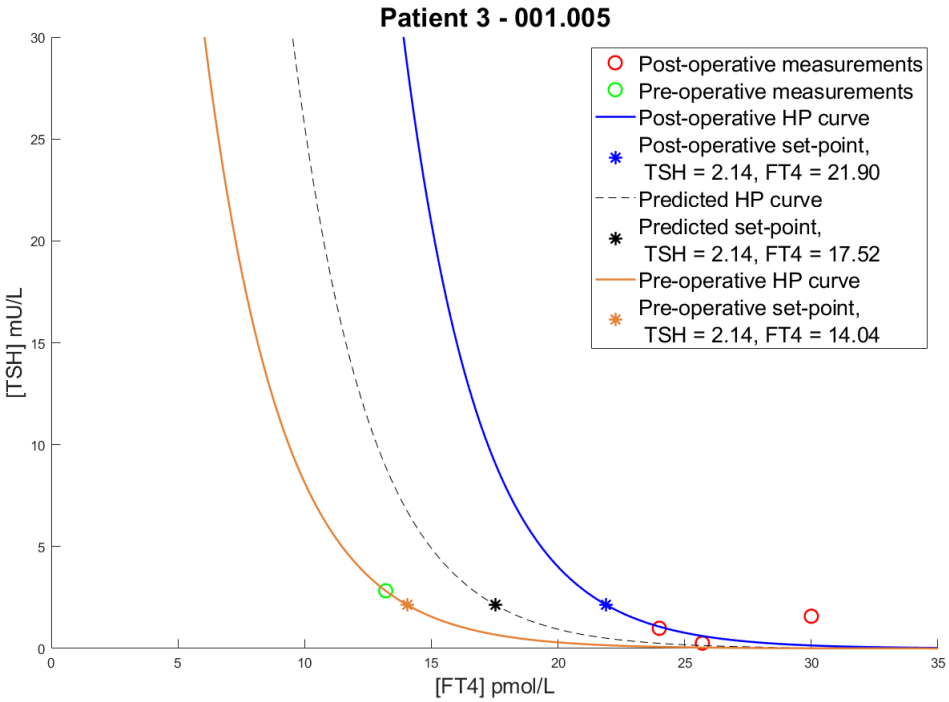


Figure 7.10: In Patient 3 - 001.005 the predicted and reconstructed pre-operative set-points are different.

8

OPTIMAL PATH TOWARDS THE SET-POINT

8.1. RELATED WORK

Some work regarding the optimal path towards the set-point has already been developed by Goede et al. [39]. Before presenting it, it is necessary to introduce the concept of half-life. Every molecule in the human body subjected to metabolism is characterised by a specific half-life, which is defined as the time needed to decrease its concentration by 50%. The notion of half-life is associated with a negative exponential decay process,

$$A(t) = A_0 e^{-\frac{t}{\tau}}$$

or

$$A(t) = A_0 e^{-\delta t},$$

where $\tau = \frac{1}{\delta}$ [39]. Since the initial concentration is A_0, the half-life $t_{1/2}$ can be found in the following way:

$$\begin{aligned} \frac{1}{2} A_0 &= A_0 e^{-\frac{t_{1/2}}{\tau}} \\ \Rightarrow -\frac{t_{1/2}}{\tau} &= \ln \frac{1}{2} \\ \Rightarrow t_{1/2} &= \tau \ln 2. \end{aligned}$$

It is also possible to re-write the expression for $A(t)$ as

$$A(t) = A_0 2^{-\frac{t}{\tau \ln 2}},$$

and, since $t_{1/2} = \tau \ln 2$, it becomes

$$A(t) = A_0 2^{-\frac{t}{t_{1/2}}}.$$

The appearance process of LT4 can be studied by considering incremental discrete steps of 24 hours, so t assumes integer values because it represents the days. It is known that T4 has an average half-life $t_{1/2}$ of 7 days [39], so it follows that for T4

$$\tau = \frac{t_{1/2}}{\ln 2} = \frac{7}{\ln 2} \simeq 10 \text{ days}$$

and

$$\delta = \frac{1}{\tau} = \frac{1}{10} = 0.1 \ \frac{1}{\text{days}}.$$

It is possible to consider an example in which the daily administered dose of LT4 is equal to 100 μg. Therefore, based on the previous considerations, the initially absorbed amount of 100 μg of LT4 taken at the beginning of the day results in a remaining amount of $100e^{-0.1\cdot 1} = 90.48$ μg after 24 hours. However, the next day a new dose of 100 μg is added and so forth, until the dose equilibrium has been reached. The dose equilibrium is defined as the steady-state level A_e and it occurs when the daily administered dose is equal to the metabolic loss over 24 hours [39]. From this definition, the steady-state A_e can be derived as follows:

$$\begin{aligned} &\text{Daily dose} = A_e(1 - e^{-\delta}) \\ \Rightarrow\ &A_e = \frac{\text{Daily dose}}{1 - e^{-\delta}}. \end{aligned}$$

In the previous example characterised by a daily dose of 100 μg of LT4, the steady-state would be

$$A_e = \frac{100}{1 - e^{-0.1}} = 1050\ \mu g.$$

It is also possible to derive a generalized form of the equation for the steady-state A_e, where it is assumed that the medication is based on periodical doses administered every n days:

$$A_e = \frac{\text{Periodical dose in } \mu\text{g over n days}}{1 - e^{n\delta}}.$$

Figure (8.1) shows that the accumulation of T4 behaves as an asymptotically accumulating characteristic, saturating at a steady-state level from which no higher accumulation will occur. The continuous time function for the accumulated T4 can be derived as

$$\begin{aligned} A_e(t) &= D_d + (A_e - D_d)(1 - e^{-\delta t}) = \\ &= A_e + (D_d - A_e)e^{-\delta t}, \end{aligned}$$

where D_d represents the daily dose, while A_e is the steady-state [39]. Figure (8.2) presents a plot of this function for $D_d = 100$ μg and $A_e = 1050$ μg.

It should be pointed out that it is possible to achieve the desired steady-state faster by increasing the daily dose of FT4. In fact, still considering the previous example, the

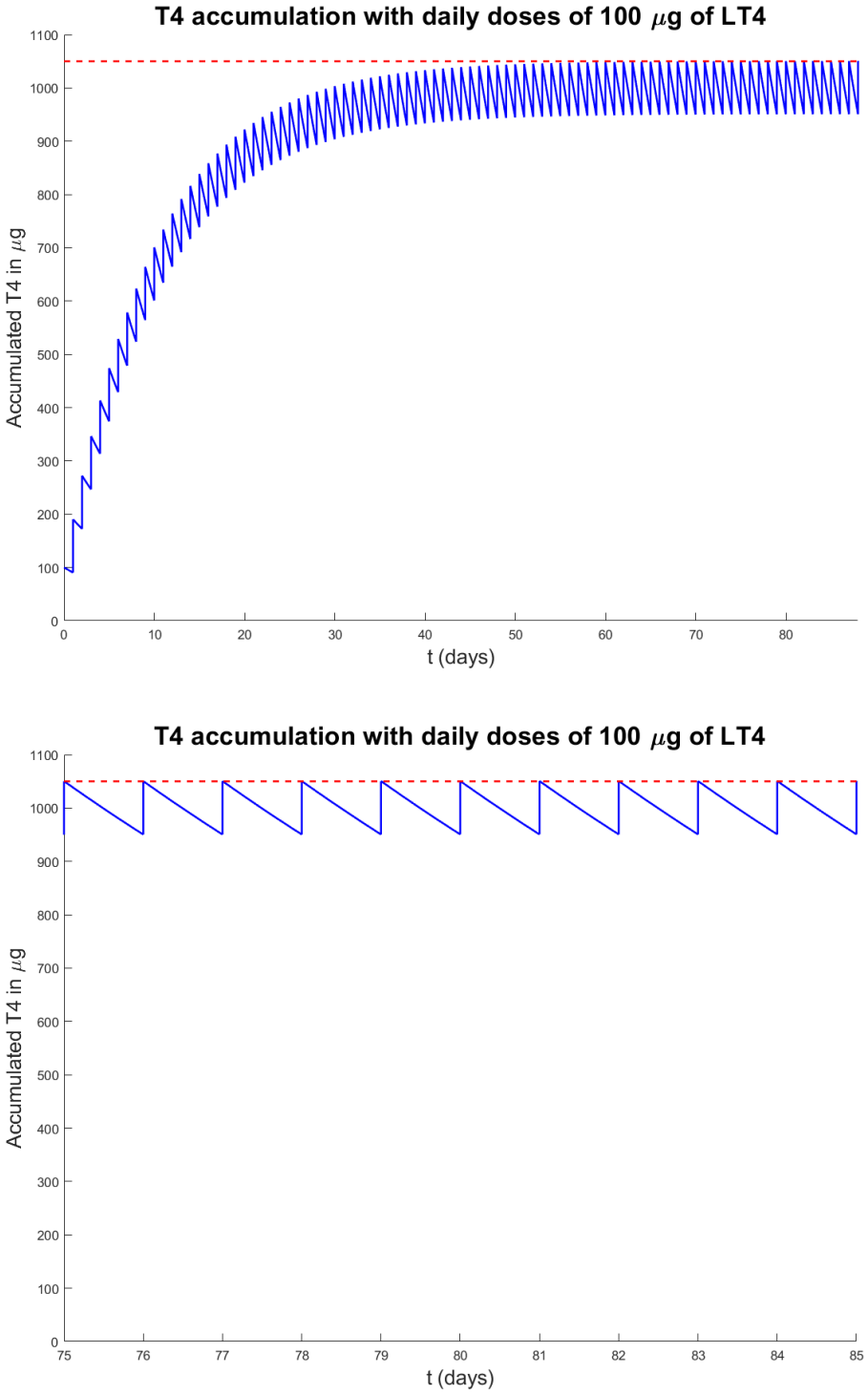


Figure 8.1: Accumulation process of T4 based on a daily dose of 100 μg of LT4, with a zoom of the equilibrium situation.

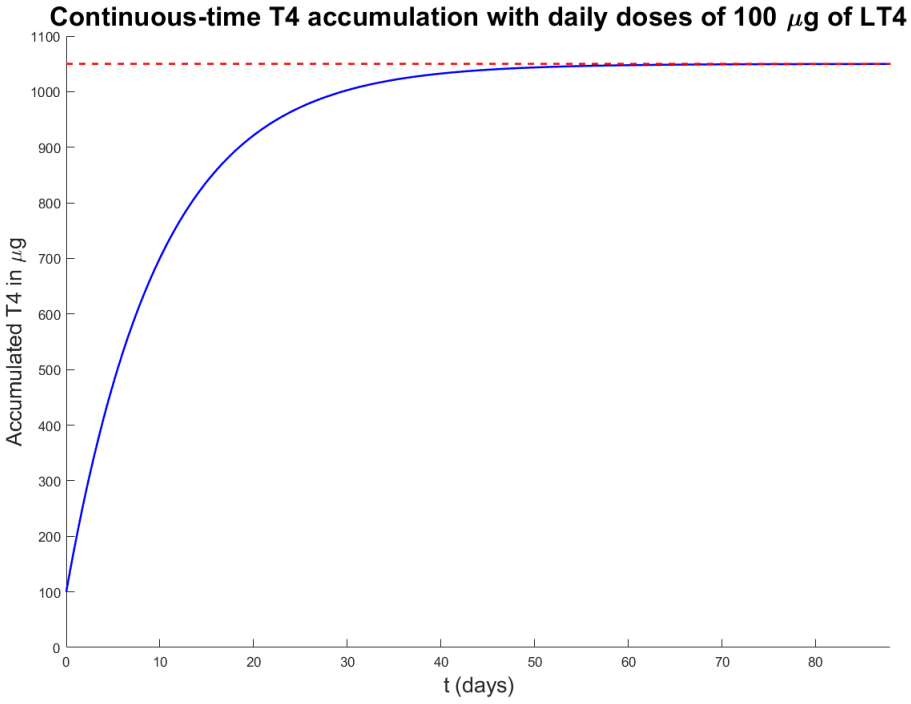


Figure 8.2: Continuous-time accumulation process of LT4 based on a daily dosage of 100 μg, with a steady-state of 1050 μg.

steady-state of 1050 μg, based on a daily dose of 100 μg of LT4, can be reached in a shorter period when the medication dosage on the first days is allowed to be increased up to 300 μg of LT4 per day. In fact, starting with a first dose of 300 μg of LT4 on day 0, a second dose on day 1 and so on, it is possible to find out that after only four days the steady-state level is basically reached. At the beginning of day 4 it is necessary to administer a dose of just 110 μg of LT4 and, from the next day, the medication should be continued with 100 μg of LT4 on a daily basis. Figure (8.3) depicts the rapid accumulation to the desired steady-state level with four daily doses of 300 μg of LT4, starting at day 0, followed by the normal daily dosage of 100 μg of LT4 once the steady-state has been reached. The benefit of this procedure is to reduce the period required to reach the steady-state, which can help resolving hypothyroid sympoms faster. However, this approach should always be adopted in consultation with the physician, in order to analyse all the possible contra-indications to high doses of LT4. Moreover, if such a strategy is followed, it is important to appropriately monitor the health status of the patient [39].

On the other hand, in some situations it might also be necessary to achieve a reduced steady state. Therefore, if that is the case, it is required to stop the medication for a few days until the new steady-state has been reached. The current steady-state is defined as

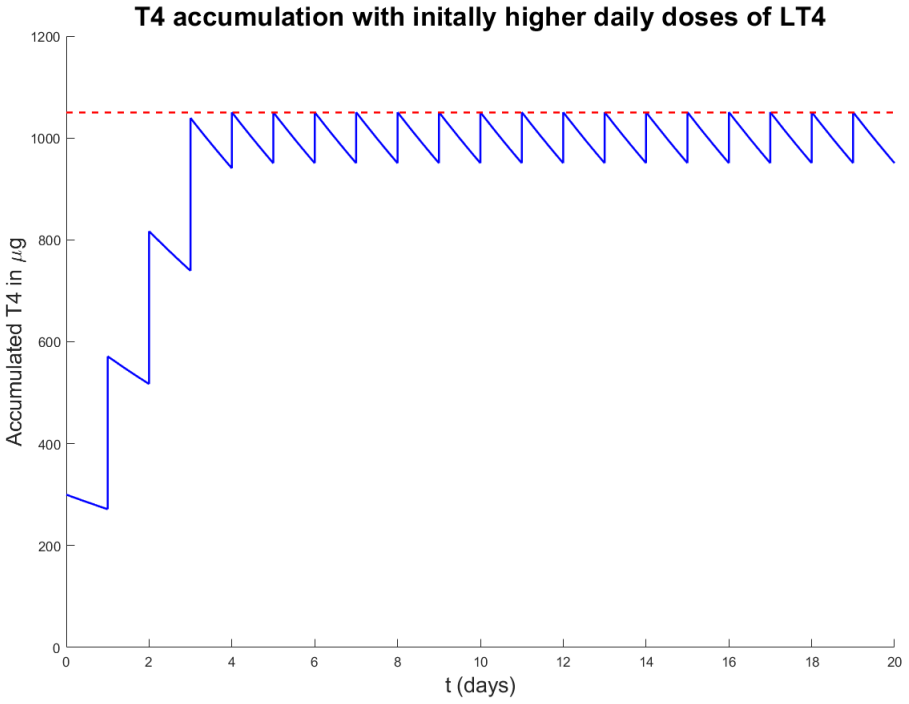


Figure 8.3: Accumulation process of LT4 based on a daily dosage of 300 μg from day 0 to day 3, of 110 μg on day 4 and continuing with a daily dose of 100 μg from day 5.

A_1, while the reduced steady-state is A_2. As presented previously, the natural decay is

$$A(t) = A_1 e^{-\delta t}.$$

Therefore, since it is necessary to reach A_2, which is smaller than A_1, the following holds

$$A_2 = A_1 e^{-\delta t}.$$

From this equation, it is possible to derive for how many days it is required to suspend the medication as

$$t = \left\lceil -\frac{1}{\delta} \ln\left(\frac{A_2}{A_1}\right) \right\rceil.$$

It should be noticed that t should be approximated to the nearest higher integer, because t represents the days, therefore it must be expressed by an integer value. The state reached after t days will be slightly lower than A_2 because of the approximation to the higher integer value, therefore the next day it will be necessary to compensate this difference by ingesting a dose of LT4 equivalent to

$$\Delta A = A_2 - A_2',$$

where $A_2' = A_1 e^{-\delta t}$, computed using the t found in the previous formula [39]. From the next day, the daily dose that should be ingested in order to keep the steady-state at A_2 is

$$\text{Daily dose} = A_2(1 - e^{-\delta}).$$

Figure (8.4) shows an example in which the reduced steady-state of 700 μg is reached starting from an initial steady-state of 1050 μg. In this example, it is necessary to suspend the treatment for 5 days and, after that, the medication can be resumed with a dose of 63 μg of LT4 on the first day and continued with a daily dose of 66 μg of LT4 for the following days. As in the previous case, this approach should be adopted in consultation with the physician, in order to evaluate all the possible benefits and contra-indications.

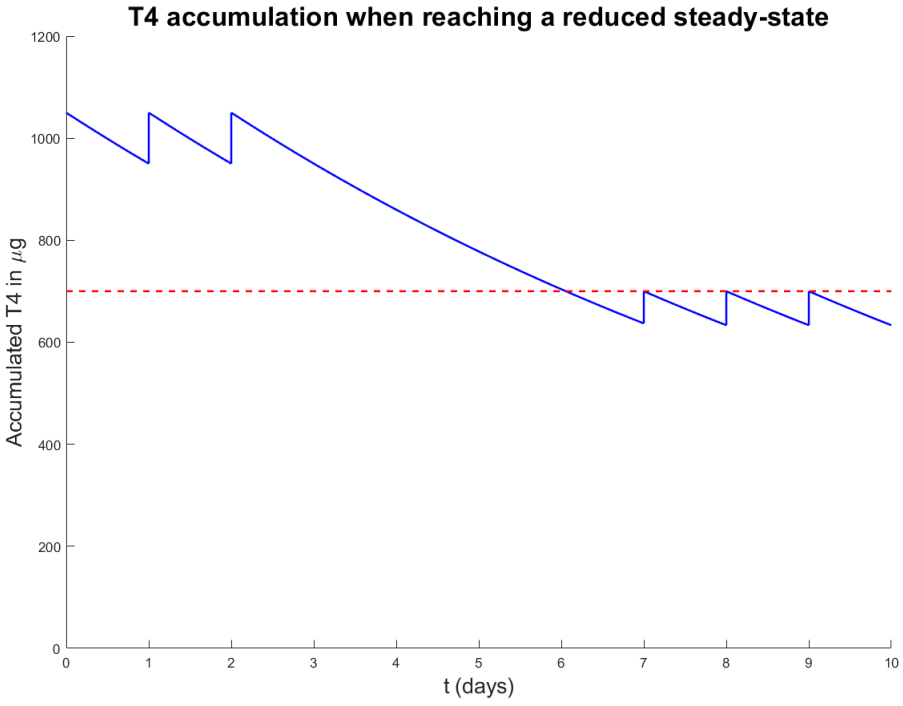


Figure 8.4: Accumulation process of LT4 when it is required to reach a reduced steady-state.

8.2. FT4 AS A FUNCTION OF TIME

After the analysis of the work that has already been published by Goede et al. [39], the aim is to expand it to investigate an alternative method to express FT4 as a function of time, in order to study its behaviour when a certain amount of medication is administered. Based on the previous considerations, when the administered dose of LT4 is

constant over a certain period, the behaviour of FT4 over time is similar to the one of the accumulated T4 presented in the previous section. In fact, first of all, it is important to underline that T4 and FT4 have the same half-life. The average half-life of T4 is around 7 days, but it varies from person to person in a range around 5-9 days. Furthermore, as accumulated T4 tends to its steady-state presented previously, also FT4 tends to a certain saturation level, which should correspond to the set-point value when the administered dosage of LT4 is correct. An example of the expected behaviour of FT4 over time is presented in Figure (8.5).

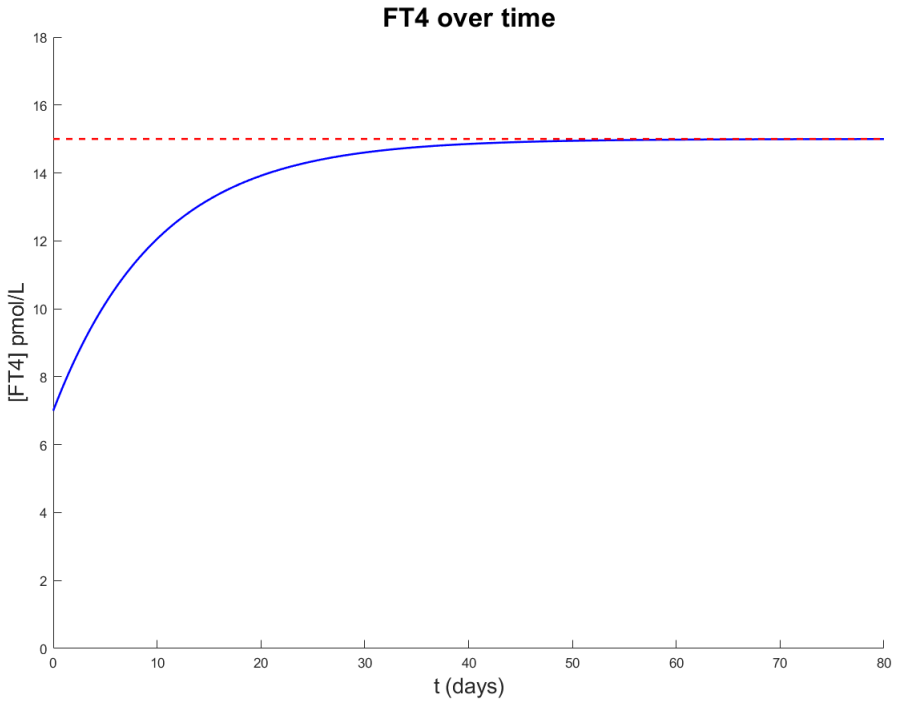


Figure 8.5: FT4 behaviour over time when the treatment for hypothyroidism is carried out.

Hence, similarly to the continuous-time function for the aggregated T4, $A_e(t) = A_e + (D_d - A_e)e^{-\delta t}$, FT4 can be represented as a function of time with the following expression

$$[\text{FT4}](t) = a + be^{-ct},$$

which requires to determine the parameters a, b and c. These parameters depend on the FT4 saturation level, on the residual activity of the thyroid and on the half-life of FT4. The parameter c is equivalent to the parameter δ used in the formula for the accumulated T4, because T4 and FT4 have the same time constant, therefore this allows to compute the specific half-life of FT4 of the individual taken into account. Furthermore, when $t = 0$ the

concentration of FT4 is equal to the residual FT4 still produced by the thyroid before the start of the treatment. When $t \to \infty$, the concentration of FT4 approaches its saturation level. So, after these considerations, the formula of FT4 over time can also be written with the following expression:

$$[\text{FT4}](t) = [\text{FT4}]_{\text{saturation}} + ([\text{FT4}]_0 - [\text{FT4}]_{\text{saturation}})e^{-\delta t},$$

where $[\text{FT4}]_0$ represents the residual FT4 produced by the thyroid before the start of the treatment. It should be pointed out that when the administered dose of LT4 is correct for the specific individual taken into account, the saturation level of FT4 is targeted to be equal to the set-point level of FT4.

Since it is required to compute 3 parameters, it is necessary to have at least 3 distinct measurements of FT4 conducted at different times in order to reconstruct the FT4 function. As pointed out before, it is mandatory to conduct a measurement right before the start of the treatment in order to establish the start value of FT4, necessary to reconstruct the curve. Furthermore, the TSH concentrations should be measured as well, because this allows to compute the set-point of the patient using the negative exponential function. In this way, it is possible to compare the FT4 level of the set-point to the asymptotic value of the FT4 function. In fact, if the medication is not administered in an appropriate dosage, the FT4 curve will not approach the set-point value but a higher value, if the administered dose is larger than needed, or a smaller value, if the daily dose is lower than what it should be. Therefore, this analysis allows to understand if the medication is adequate or if it should be adjusted. Furthermore, once the parameter δ is calculated, it is also possible to compute the half-life of T4 as

$$t_{1/2} = \frac{\ln 2}{\delta}.$$

It follows an example to display this theory in practice. It is assumed that an individual is administered a daily dose of 100 μg of LT4 and that 3 TFTs are conducted, one before the treatment is started and the other two after 7 and 14 days from the beginning of the treatment, where the measurements are presented in the following table:

t (days)	[FT4] pmol/L	[TSH] mU/L
0	6.6	35.06
7	10.0	8.90
14	11.1	5.52

First of all, it is better to start fitting the (FT4, TSH) measurements with the negative exponential function in order to derive the set-point, as done in Figure (8.6). In this case, the set-point is FT4 = 13.99 pmol/L and TSH = 1.74 mU/L. Next, the FT4 measurements should be plotted against time and fitted with the model presented previously,

$$[\text{FT4}](t) = a + be^{-ct}.$$

This can be easily done through the fitting tool in Matlab. In this way, the parameters a, b and c can be approximated and, for this specific case, they have the following values:

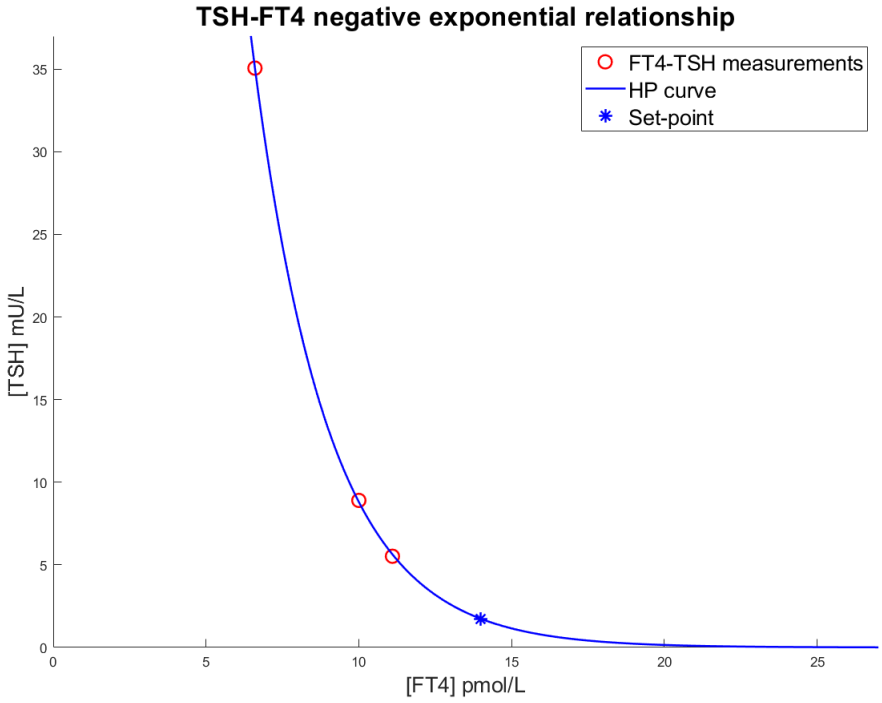


Figure 8.6: HP curve and set-point.

$a = 11.63$ pmol/L, $b = -5.026$ pmol/L and $c = 0.1612 \frac{1}{\text{days}}$. As explained previously, c corresponds to δ, therefore it is possible to derive the half-life of T4 specific of the individual:

$$t_{1/2} = \frac{\ln 2}{\delta} = \frac{\ln 2}{0.1612} = 4.3 \text{ days}.$$

The parameter a corresponds to the saturation level of the FT4 function, therefore it can be compared with the set-point FT4 value to check whether the medication dosage is appropriate for the individual. In this case, the saturation level of FT4 is 11.63 pmol/L, while the set-point FT4 value is 13.99 pmol/L. This means that the daily dosage of LT4 should be increased, so that the patient can reach its own set-point.

The new amount of medication that should be administered can be computed in the following way. If 100 μg of LT4 allow the patient to reach a FT4 steady-state of 11.63 pmol/L, then x amount of medication is needed to reach 13.99 pmol/L:

$$100 : 11.63 = x : 13.99$$
$$\Rightarrow x = \frac{13.99 \cdot 100}{11.63} = 120.3 \, \mu g.$$

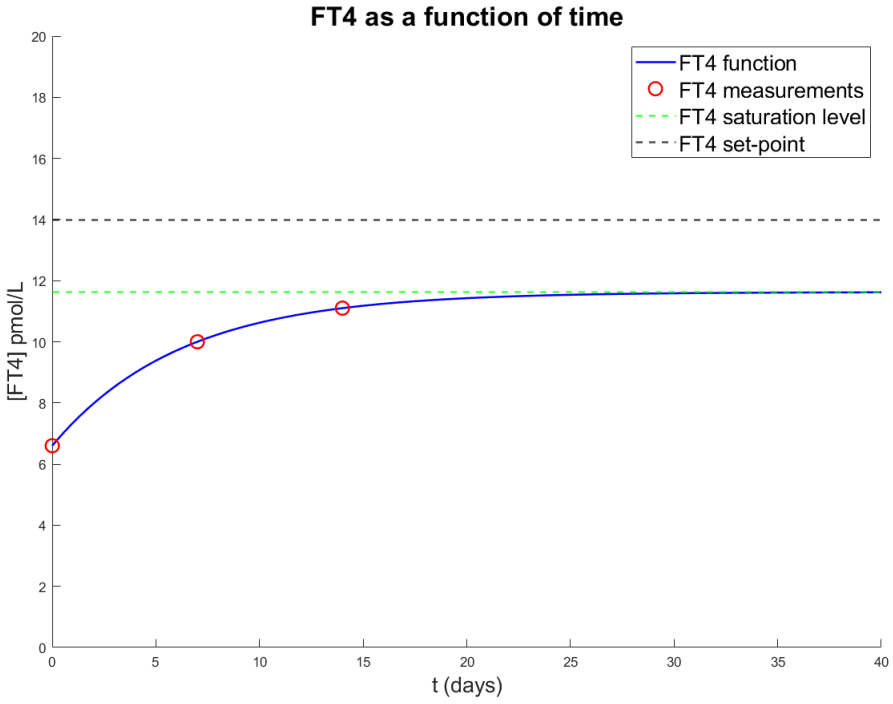


Figure 8.7: FT4 as a function of time, with the comparison between the set-point and the saturation level.

This formula can be generalized as

$$\text{New daily dose} = \frac{[\text{FT4}]_{\text{set point}} \cdot \text{Current daily dose}}{[\text{FT4}]_{\text{saturation}}}.$$

There can be different ways to reach the new state. One option is to reach the new state slowly, therefore, from the next day, the dose can be increased up to 120 μg of LT4, so this allows to reach the correct steady state in about 30/40 days. Another approach is to administer 100 + 120 = 220 μg on the 15th day and then continue the medication with a daily dose of 120 μg. In this way, the new steady state is reached within a day. Obviously, the specialist should consider all the contra-indications and benefits of these approaches and choose the best one for the patient.

8.3. MODEL WITH DIFFERENTIAL EQUATIONS

Another approach to study the optimal path towards the set-point consists in developing a model of coupled differential equations that simulates how the concentrations of TSH and FT4 are changing in time after a hypothyroid patient starts the treatment. Therefore, the idea is to take into account only TSH and FT4 as variables, because they are the two

quantities that have been considered so far.

8.3.1. LOTKA-VOLTERRA MODEL

The first model taken into account to study the time behaviour of TSH and FT4 is a system of Lotka-Volterra equations, based on the paper published by Koós [40]. This approach is general and not specific for the HPT axis, therefore it can be applied to all the glands included in the endocrine system. In this case, the two glands considered to build the model are the pituitary and the thyroid, which are producing TSH and FT4, respectively. Therefore, in this situation, the Lotka-Volterra system becomes

$$\begin{aligned} \frac{d[\text{TSH}]}{dt} &= (A - B[\text{FT4}])[\text{TSH}], & [\text{TSH}](t_0) &= [\text{TSH}]_0, \\ \frac{d[\text{FT4}]}{dt} &= (-C + D[\text{TSH}])[\text{FT4}], & [\text{FT4}](t_0) &= [\text{FT4}]_0, \end{aligned}$$

where the model parameters A, B, C and D are positive.

The model presents two equilibrium states. The first one is at $[\text{TSH}] = \frac{C}{D}$, $[\text{FT4}] = \frac{A}{B}$, representing the levels at which the hormone concentrations stabilizes after a few weeks from the start of the medication. The second equilibrium state is at $[\text{TSH}] = 0$, $[\text{FT4}] = 0$, which does not have a physiological meaning and therefore does not seem reasonable. A stability analysis around the equilibrium points can help to determine if different choices for the model parameters can provide solutions with different behaviours. In order to do so, the system has to be linearised around the equilibrium states as

$$\begin{pmatrix} \frac{d[\text{TSH}]}{dt} \\ \frac{d[\text{FT4}]}{dt} \end{pmatrix} = \begin{pmatrix} A - B[\text{FT4}]_{\text{eq}} & -B[\text{TSH}]_{\text{eq}} \\ D[\text{FT4}]_{\text{eq}} & -C + D[\text{TSH}]_{\text{eq}} \end{pmatrix} \begin{pmatrix} [\text{TSH}] - [\text{TSH}]_{\text{eq}} \\ [\text{FT4}] - [\text{FT4}]_{\text{eq}} \end{pmatrix},$$

where $\left([\text{FT4}]_{\text{eq}}, [\text{TSH}]_{\text{eq}}\right)$ represents the equilibrium state.

When considering the equilibrium point $(0, 0)$, the linearised matrix becomes

$$\begin{pmatrix} A & 0 \\ 0 & -C \end{pmatrix}.$$

Its eigenvalues are $\lambda_1 = A$ and $\lambda_2 = -C$, so, since all the model parameters are positive, one of the eigenvalues is negative, while the other one is positive. This does not depend on the specific values of the parameters. Therefore, the equilibrium point $(0, 0)$ is always unstable.

When considering the other equilibrium point, $\left(\frac{C}{D}, \frac{A}{B}\right)$, the linearised matrix becomes

$$\begin{pmatrix} 0 & -\frac{BC}{D} \\ \frac{AD}{B} & 0 \end{pmatrix}.$$

In this case, the eigenvalues are $\lambda_{1,2} = \pm i\sqrt{AC}$. Since the real part of the eigenvalues is 0, it is not possible to conclude anything about the non-linear system from this analysis. It is then necessary to derive the first integral, which is

$$F([\text{TSH}],[\text{FT4}]) = -C\ln([\text{TSH}]) + D[\text{TSH}] - A\ln([\text{FT4}]) + B[\text{FT4}].$$

$F([\text{TSH}],[\text{FT4}])$ is a Morse-function in a neighbourhood of $\left(\frac{C}{D},\frac{A}{B}\right)$, so it can be expanded as

$$F([\text{TSH}],[\text{FT4}]) = F\left(\frac{C}{D},\frac{A}{B}\right) + \frac{D^2}{2C}\left([\text{TSH}] - \frac{C}{D}\right)^2 + \frac{B^2}{2A}\left([\text{FT4}] - \frac{A}{B}\right)^2 + \ldots$$

This shows that the orbits around the equilibrium point $\left(\frac{C}{D},\frac{A}{B}\right)$ are closed, so it follows that the solutions of TSH and FT4 are periodic. From this analysis it is clear that the solution presents an oscillatory behaviour, which, once again, is not dependent on the value of the parameters. This model might be more appropriate to describe the daily behaviour of the negative feedback loop instead of describing the change of FT4 and TSH in a hypothyroid person taking medication.

Nevertheless, it follows an example in which the system has been solved by selecting some arbitrary values for the parameters. They have been chosen in such a way that the equilibrium state is [FT4] = 15 pmol/L, [TSH] = 1 mU/L. The values of the equilibrium state have also been chosen in an arbitrary way, hence they do not have a specific meaning but they just represent a plausible set-point. Therefore, the values selected for the parameters are

$$\begin{aligned} A &= 15\ \frac{1}{\text{day}} \\ B &= 1\ \frac{\text{L}}{\text{pmol day}} \\ C &= 0.1\ \frac{1}{\text{day}} \\ D &= 0.1\ \frac{\text{L}}{\text{mU day}}. \end{aligned}$$

Furthermore, the initial values are set arbitrarily at [FT4] = 6 pmol/L, [TSH] = 40 mU/L. Even if the parameters and the initial conditions have different values, the behaviour of the solution is still the same. The system has been numerically solved using the symplectic Euler method [41], defined as

$$\begin{cases} u_{n+1} = u_n + hf(u_{n+1}, v_n) \\ v_{n+1} = v_n + hg(u_{n+1}, v_n). \end{cases}$$

The solution obtained from the system using these values is presented in Figure (8.8). As explained previously, this is not the expected behaviour, because the solution is not supposed to show these oscillations. Therefore, this model based on the Lotka-Volterra equations can be discarded.

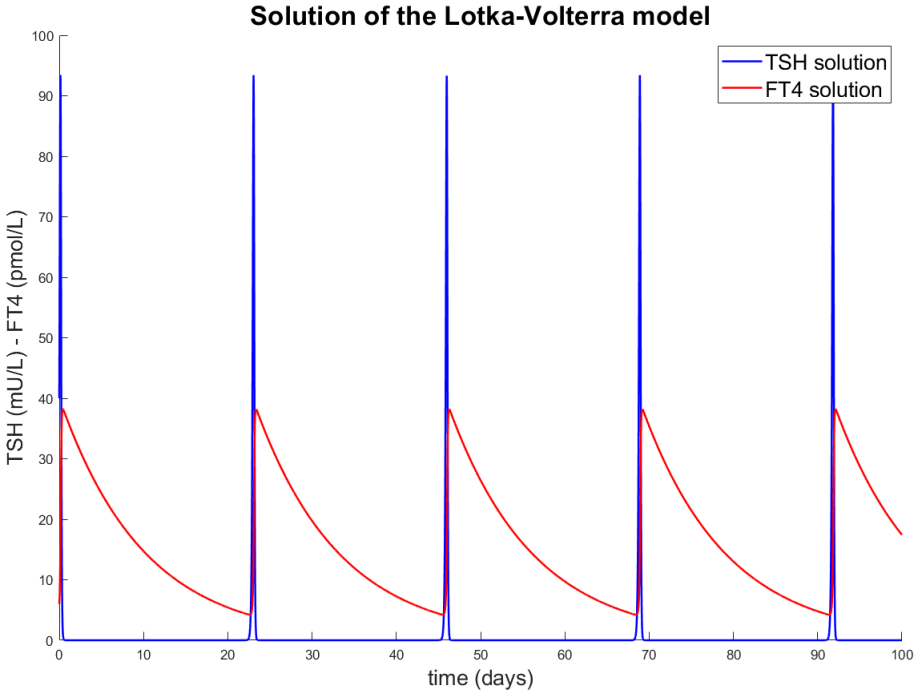


Figure 8.8: Solution of the Lotka-Volterra system.

8.3.2. MODEL BY PANDIYAN ET AL.

In 2011, Pandiyan proposed a mathematical model that analyses the negative feedback loop of the HPT axis in patients affected by the Hashimoto thyroiditis, which is a disease affecting the size and function of the thyroid [42]. This mathematical model involves four clinical variables: TSH, FT4, anti-thyroid peroxidase antibodies (TPOAb) and the functional size of the thyroid (T). Anti-thyroid peroxidase antibodies are a kind of antibodies that are mainly detected in patients affected by the Hashimoto disorder, while the functional size of the thyroid refers to the size of the active and operating part of the thyroid [42, 43]. This model consists of a system of four differential equations describing how the four variables change in time:

$$
\begin{aligned}
&\frac{d[\mathrm{TSH}]}{dt} = k_1 - \frac{k_1[\mathrm{FT4}]}{k_a + [\mathrm{FT4}]} - k_2[\mathrm{TSH}], && [\mathrm{TSH}](t_0) = [\mathrm{TSH}]_0,\\
&\frac{d[\mathrm{FT4}]}{dt} = \frac{k_3\mathrm{T}[\mathrm{TSH}]}{k_d + [\mathrm{TSH}]} - k_4[\mathrm{FT4}], && [\mathrm{FT4}](t_0) = [\mathrm{FT4}]_0,\\
&\frac{d\mathrm{T}}{dt} = k_5\left(\frac{[\mathrm{TSH}]}{\mathrm{T}} - N\right) - k_6[\mathrm{TPOAb}]\mathrm{T}, && \mathrm{T}(t_0) = \mathrm{T}_0,\\
&\frac{d[\mathrm{TPOAb}]}{dt} = k_7[\mathrm{TPOAb}]\mathrm{T} - k_8[\mathrm{TPOAb}], && [\mathrm{TPOAb}](t_0) = [\mathrm{TPOAb}]_0.
\end{aligned}
$$

The rates of changes of both TSH and FT4 are equal to the difference between the secretion rates and excretion rates of TSH and FT4, respectively. Secretion refers to the release of a substance, while excretion refers to the process of waste removal from the body. In the differential equation for TSH, the secretion rate is expressed by two terms, where the first term k_1 represents the maximum secretion rate of TSH in the absence of FT4 from the blood, while the other term, $\frac{k_1[\text{FT4}]}{k_a+[\text{FT4}]}$, describes the inhibition rate of TSH and it is modelled through Michaelis-Menten kinetics. The excretion rate of TSH is proportional to the concentration of TSH itself, hence it is modelled as $k_2[\text{TSH}]$ [42]. For FT4, the secretion rate is considered proportional to the functional size of the thyroid and it is modelled through Michaelis-Menten kinetics as $\frac{k_3[\text{TSH}]\text{T}}{k_d+[\text{TSH}]}$. Also in this case, the excretion term of FT4 is assumed proportional to the concentration of FT4. [43]. The rate of change of the functional size of the thyroid is modelled as the difference between the growth rate and the destruction rate of the thyroid. Finally, the last differential equation describes the rate of change of the anti-thyroid peroxidase antibodies as the difference between the production rate and the loss rate of TPOAb [43].

8.3.3. MODEL ADAPTED TO A GENERAL SITUATION

The next step is to use the model developed by Pandiyan et al. [42, 43] as a starting point and adapt it to the more general situation studied in this thesis. In particular, the model should be generalized, so that it can be applied to all hypothyroid patients and not only to those affected by Hashimoto thyroiditis. Furthermore, it would be reasonable to have a model including only the variables that have been considered so far, namely the concentrations of TSH and of FT4.

Therefore, the model by Pandiyan et al. [42, 43] presented in the previous section can be modified by removing the last two differential equations. The equation for the antithyroid antibodies TPOAb should be removed because these antibodies are only typical for people affected by the Hashimoto disorder. The differential equation for the functional size of the thyroid should be removed because the thyroid's size cannot be measured in practice and it is a variable that has not been considered so far. However, the size of the thyroid appears also in the differential equation for FT4, hence in this case it can be considered a constant. In this way, its value can be incorporated into the parameter k_3. Therefore, the model becomes

$$\begin{aligned}
\frac{d[\text{TSH}]}{dt} &= k_1 - \frac{k_1[\text{FT4}]}{k_a+[\text{FT4}]} - k_2[\text{TSH}], & [\text{TSH}](t_0) &= [\text{TSH}]_0,\\
\frac{d[\text{FT4}]}{dt} &= \frac{k_3[\text{TSH}]}{k_d+[\text{TSH}]} - k_4[\text{FT4}], & [\text{FT4}](t_0) &= [\text{FT4}]_0.
\end{aligned}$$

The initial conditions of the model correspond to the concentrations of TSH and FT4 right before the treatment is started. This model contains 6 parameters and they are all considered positive. However, not all of them need to be estimated every time the model is applied to a different patient. In fact, according to Pandiyan [42], some of the

parameters can be found in the literature, so it can be assumed that

$$k_1 = 5000 \ \frac{\text{mU}}{\text{L day}}$$
$$k_2 = 16.63 \ \frac{1}{\text{day}}$$
$$k_4 = 0.099 \ \frac{1}{\text{day}}.$$

k_1 refers to the maximum amount of TSH that can be secreted when [FT4] = 0 pmol/L, while k_2 and k_4 are related to the half-lives of TSH and FT4, respectively. The remaining parameters have to be computed specifically for every individual. Pandiyan et al. estimate the parameters by using the equilibrium values of TSH and FT4, which corresponds to the set-point of the individual taken into account [43].

Therefore, this implies that the set-point should be known before applying this model to a specific individual. However, it might be appropriate to find the parameters in a different way that does not involve the set-point. Hence, an idea would be to determine the parameters of the model through an optimisation problem that minimises the distance between the measurements of TSH and FT4 and the solution of the system of differential equations. A general formulation of the optimisation problem would be

Find the model parameters such that

$$\sum_i ||y(t_i) - y_i||_2^2$$

is minimized,

where $y(t_i)$ is the solution of the differential equation evaluated at t_i, while y_i represents the i^{th} measurement. In this case, the model consists of a system of two coupled differential equations, so $y(t)$ and y_i are vectors, hence the problem can be formulated as

Find the parameters k_a, k_d and k_3 such that

$$\sum_i \left|\left| \begin{pmatrix} [\text{TSH}](t_i) - [\text{TSH}]_i \\ [\text{FT4}](t_i) - [\text{FT4}]_i \end{pmatrix} \right|\right|_2^2$$

is minimized,

where [TSH](t), [FT4](t) is the solution of the system, while ($[\text{TSH}]_i$, $[\text{FT4}]_i$) are the measurements of the individual taken into account.

8.3.4. TEST WITH ARTIFICIAL DATASETS

The model has been first tested with two artificial datasets to check how the solution behaves and to make some improvements according to the results. The artificial datasets have been obtained by selecting two arbitrary exponential functions and extracting measurements from the curves. In particular, the first dataset is extracted from the exponential curve with set-point (point of maximum curvature) equal to [FT4] = 13 pmol/L,

t (days)	[FT4] pmol/L	[TSH] mU/L
0	8	40
1	8.5	24.09
2	8.9	18.16
5	9.9	8.95
10	11.1	3.83
15	11.9	2.18
20	12.3	1.64
30	12.7	1.24
40	12.9	1.07
50	12.96	1.03

Table 8.1: Artificial dataset 1

[TSH] = 1 mU/L. The measurements considered are presented in Table (8.1). The second dataset is extracted from the exponential curve with set-point FT4 = 15 pmol/L, TSH = 0.8 mU/L. Table (8.2) presents the measurements belonging to the second artificial dataset. For both cases, a total of 10 measurements was taken into account. In the first

t (days)	[FT4] pmol/L	[TSH] mU/L
0	10	66.44
3	11.3	21.06
6	12.2	9.50
9	12.9	5.12
12	13.5	3.01
15	13.9	2.12
18	14.2	1.62
21	14.4	1.36
24	14.5	1.24
27	14.7	1.04

Table 8.2: Artificial dataset 2

dataset, the time-stamp between consecutive measurements is variable, while for the second dataset it is always equal to 3 days.

The model presented in the previous section has been tested with the two artificial datasets. Figure (8.9) shows the solution of the system obtained using the first artificial dataset. All the ten measurements have been used and the values of the estimated parameters are $k_a = 0.34$ pmol/L, $k_d = 0.00$ mU/L and $k_3 = 1.50$ pmol/(L day).

The first thing that can be noticed in this experiment is that the solution for TSH is very steep and it does not reproduce precisely the behaviour of the TSH measurements. Therefore, the model should be adjusted in order to obtain a more accurate solution. In order to do so, the work proposed in two different studies based on the original model of Pandiyan should be taken into account [44, 45]. In both papers, the authors modify the

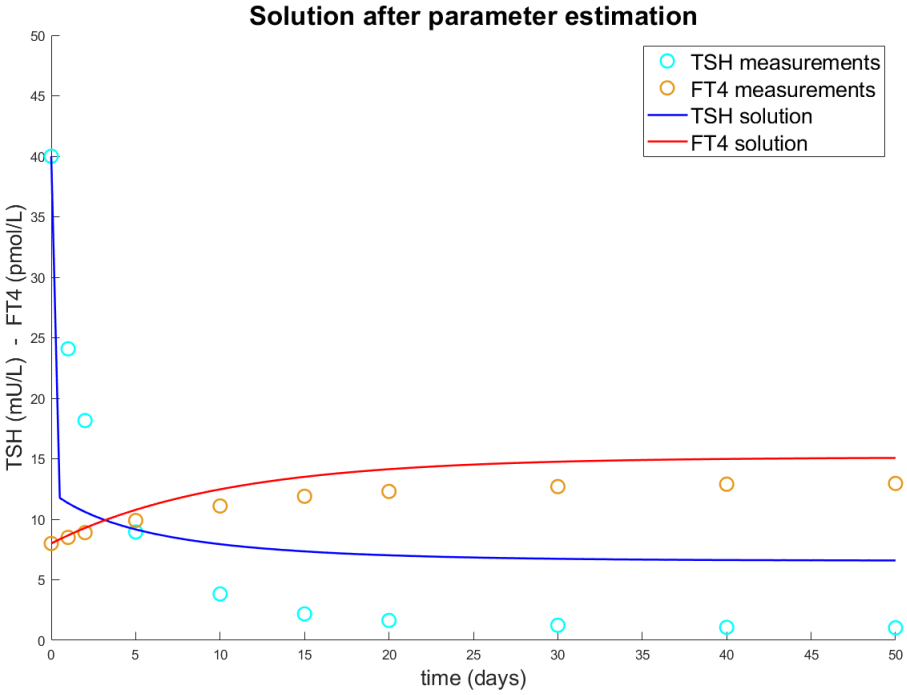


Figure 8.9: Solution of the model applied to the first artificial dataset.

Michaelis-Menten terms in the differential equations for TSH and FT4 as

$$\frac{d[\text{TSH}]}{dt} = k_1 - \frac{k_1[\text{FT4}]^{n_1}}{k_a + [\text{FT4}]^{n_1}} - k_2[\text{TSH}], \qquad [\text{TSH}](t_0) = [\text{TSH}]_0,$$
$$\frac{d[\text{FT4}]}{dt} = \frac{k_3[\text{TSH}]^{n_2}}{k_d + [\text{TSH}]^{n_2}} - k_4[\text{FT4}], \qquad [\text{FT4}](t_0) = [\text{FT4}]_0.$$

Therefore, the exponents n_1 and n_2 have to be estimated as well.

Since the goal is to study the time behaviour of the hormones after the start of treatment, the model should also include a term taking into account the effect of medication. Therefore, it is appropriate to add a constant k_5 to the equation of FT4, because the treatment is supposed to directly influence the concentration FT4. The model becomes then

$$\frac{d[\text{TSH}]}{dt} = k_1 - \frac{k_1[\text{FT4}]^{n_1}}{k_a + [\text{FT4}]^{n_1}} - k_2[\text{TSH}], \qquad [\text{TSH}](t_0) = [\text{TSH}]_0,$$
$$\frac{d[\text{FT4}]}{dt} = \frac{k_3[\text{TSH}]^{n_2}}{k_d + [\text{TSH}]^{n_2}} - k_4[\text{FT4}] + k_5, \qquad [\text{FT4}](t_0) = [\text{FT4}]_0,$$

where the parameters to be estimated are k_a, k_d, k_3, k_5, n_1 and n_2.

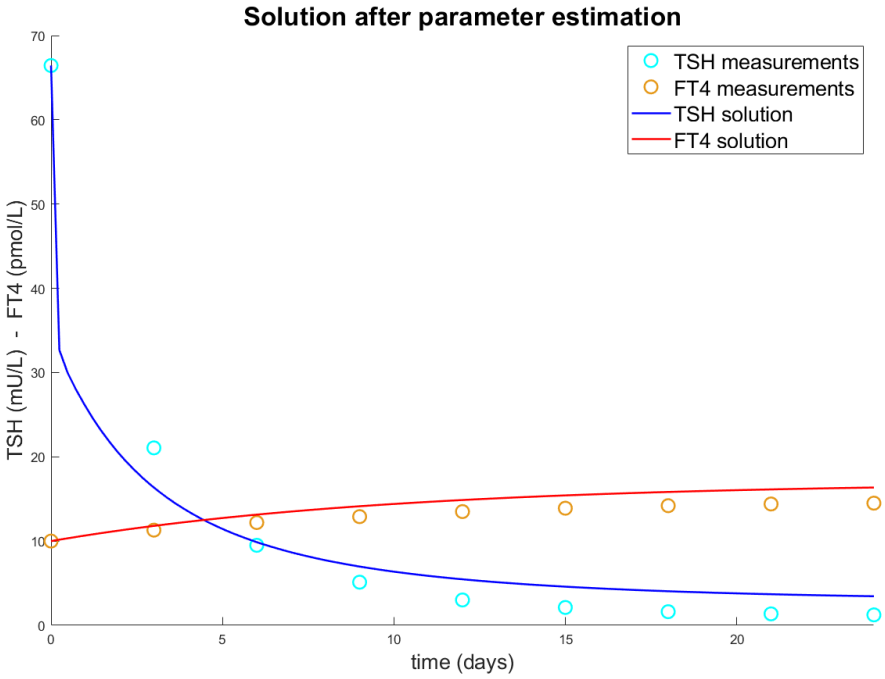
The results of new experiments run using both artificial datasets on the improved model show that the values of the estimated parameters change significantly according to the number of measurements included in the optimisation problem, which is not expected. An example is presented in Figure (8.10). In this case, two experiments have been conducted with the second artificial dataset, one considering the first 9 measurements from the dataset, while the other one takes into account all the 10 TFTs. The solutions of the model presented in Figure (8.10) are very similar from a visual perspective. However, the values of the estimated parameters are very different in the two cases. The values of the parameters estimated from 9 measurements are

$$\begin{aligned} k_a &= 9281.82 \ \frac{\text{pmol}}{\text{L}} \\ k_d &= 9.88 \ \frac{\text{mU}}{\text{L}} \\ k_3 &= 0.23 \ \frac{\text{pmol}}{\text{L day}} \\ k_5 &= 1.45 \ \frac{\text{pmol}}{\text{L day}} \\ n_1 &= 4.86 \\ n_2 &= 10.70, \end{aligned}$$

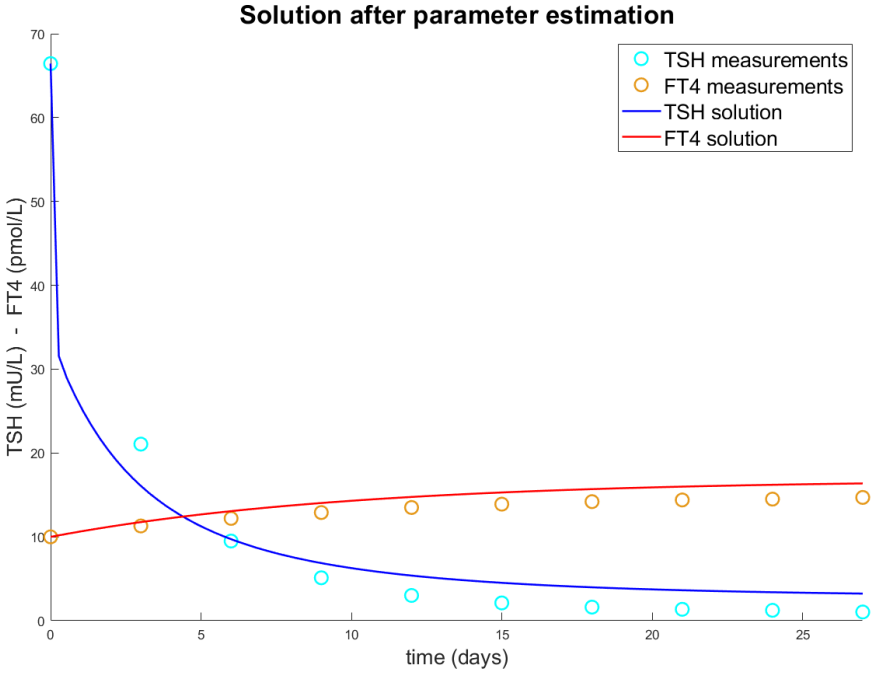
while the parameters obtained when considering the entire dataset are

$$\begin{aligned} k_a &= 10880.20 \ \frac{\text{pmol}}{\text{L}} \\ k_d &= 145.48 \ \frac{\text{mU}}{\text{L}} \\ k_3 &= 1.67 \ \frac{\text{pmol}}{\text{L day}} \\ k_5 &= 0.00 \ \frac{\text{pmol}}{\text{L day}} \\ n_1 &= 4.94 \\ n_2 &= 17.17. \end{aligned}$$

The only parameter remaining almost constant in all the experiments, even when considering the two different datasets, is n_1, with a value around 4. Therefore, it is possible to manually set $n_1 = 4$, so that this parameter does not need to be approximated. Another adjustment that can be made is to estimate the parameter k_2 for every individual instead of using the value from the literature. In fact, the solution for TSH is still very steep, as it can also be noticed in Figure (8.10), and does not reflect properly the behaviour of the measurements. Hence, an idea to fix this issue consists in estimating the parameter k_2 for every patient. Other experiments can then be conducted and the results show that, once n_1 has been manually fixed, n_2 assumes incredibly high values, which is not reasonable. Therefore, through a different approach, the parameter n_2 can be manually set equal to 1, so that the remaining parameters can be estimated. The experiments conducted in this setting show that the solution obtained with $n_2 = 1$ is still



(a) Solution of the model obtained using 9 measurements for the parameter estimation



(b) Solution of the model obtained using 10 measurements for the parameter estimation

Figure 8.10: Experiments conducted using a different number of measurements for the parameter estimation.

very accurate and similar to the one obtained when the parameter n_2 is approximated as well. However, a closer analysis show that, regardless of the values of the parameters, the first term in the equation of FT4, $\frac{k_3[\text{TSH}]^{n_2}}{k_d+[\text{TSH}]^{n_2}}$, is constant for every value of TSH, therefore this term can be removed from the equation because its contribution can be taken into account in the constant parameter k_5. Hence, the final model is

$$\begin{aligned}
\frac{d[\text{TSH}]}{dt} &= k_1 - \frac{k_1[\text{FT4}]^{n_1}}{k_a+[\text{FT4}]^{n_1}} - k_2[\text{TSH}], & [\text{TSH}](t_0) &= [\text{TSH}]_0,\\
\frac{d[\text{FT4}]}{dt} &= k_5 - k_4[\text{FT4}], & [\text{FT4}](t_0) &= [\text{FT4}]_0,
\end{aligned}$$

where $k_1 = 5000$ mU/(L day), $n_1 = 4$ and $k_4 = 0.099$ 1/day, so the only parameters that need to be estimated are k_a, k_2 and k_5. This implies that it is necessary to have only 3 TFTs of an individual in order to apply this model.

This final model presents one steady-state,

$$\begin{aligned}
[\text{FT4}]_{\text{eq}} &= \frac{k_5}{k_4}\\
[\text{TSH}]_{\text{eq}} &= \frac{1}{k_2}\left(k_1 - \frac{k_1[\text{FT4}]_{\text{eq}}^{n_1}}{k_a+[\text{FT4}]_{\text{eq}}^{n_1}}\right).
\end{aligned}$$

A stability analysis around the equilibrium point can be conducted in order to determine if different values of the parameters can lead to solutions with different behaviours. Therefore, the system has to be linearized around the steady-state as

$$\begin{pmatrix} \frac{d[\text{TSH}]}{dt} \\ \frac{d[\text{FT4}]}{dt} \end{pmatrix} = \begin{pmatrix} -k_2 & -\frac{k_1 k_a n_1 [\text{FT4}]_{\text{eq}}^{n_1-1}}{\left(k_a+[\text{FT4}]_{\text{eq}}^{n_1}\right)^2} \\ 0 & -k_4 \end{pmatrix} \begin{pmatrix} [\text{TSH}]-[\text{TSH}]_{\text{eq}} \\ [\text{FT4}]-[\text{FT4}]_{\text{eq}} \end{pmatrix}.$$

The eigenvalues of the linearized matrix are $\lambda_1 = -k_2$ and $\lambda_2 = -k_4$, so, since all the model parameters are positive, both eigenvalues are negative. This means that the equilibrium state is always stable.

Furthermore, the analytical solution of FT4 can easily be computed as

$$[\text{FT4}](t) = \left([\text{FT4}]_0 - \frac{k_5}{k_4}\right)e^{-k_4 t} + \frac{k_5}{k_4}.$$

The analytical solution for TSH can also be found by using a mathematical solver for differential equations.

8.3.5. TEST WITH REAL DATASETS

The next step is to apply the final model from the previous section to some of the available datasets. Therefore, it is first necessary to detect which datasets are suitable for this. The datasets of hypothyroid patients and the datasets from the Erasmus Medical Center have to be excluded because the measurements do not present the date in which

they were conducted and this does not allow to apply the parameter estimation procedure. Hence, the only suitable datasets are the ones from the Nijmegen Medical Center. However, not all of them can be used. In fact, some of them present measurements conducted after several months, which does not allow to reproduce accurately the behaviour of the measurements over time. Another issue is that the medication dosage taken by the patients is not known and it is not specified if it changed during time. In fact, the model of differential equations should be applied when the administered amount of LT4 is constant because, when it is adjusted, the value of the parameter k_5 changes.

Nevertheless, the model can be applied to 7 patients from the Nijmegen dataset. An example is presented in Figure (8.11), where the model is applied to Patient 9 from the Nijmegen dataset. The measurements and the date when they were conducted are presented in Table (8.3). Only the first 3 measurements are taken into account when applying the model and the parameter estimation procedure, because the other TFTs are conducted after a quite long period and the medication dose might have changed. The values of the estimated parameters are

Date	[TSH] mU/L	[FT4] pmol/L
27/08/2015	18.22	15
27/11/2015	1.54	27.5
25/01/2016	2.04	27.7
20/05/2016	0.01	38.1
01/07/2016	0.03	36.9
05/08/2016	0.01	34

Table 8.3: Measurements of Patient 9 from the Nijmegen dataset.

$$k_a = 269.24 \ \frac{\text{pmol}}{\text{L}}$$
$$k_2 = 1.30 \ \frac{1}{\text{day}}$$
$$k_5 = 2.73 \ \frac{\text{pmol}}{\text{L day}}.$$

The steady-state of TSH is 1.8 mU/L, while the steady-state of FT4 is around 27.5 pmol/L. The set-point of this patient, computed as the point of maximum curvature of the exponential function obtained by fitting the data, is [FT4] = 23.3 pmol/L and [TSH] = 3.68 mU/L, therefore in this case it might be better to decrease the daily dosage in order to reach the set-point.

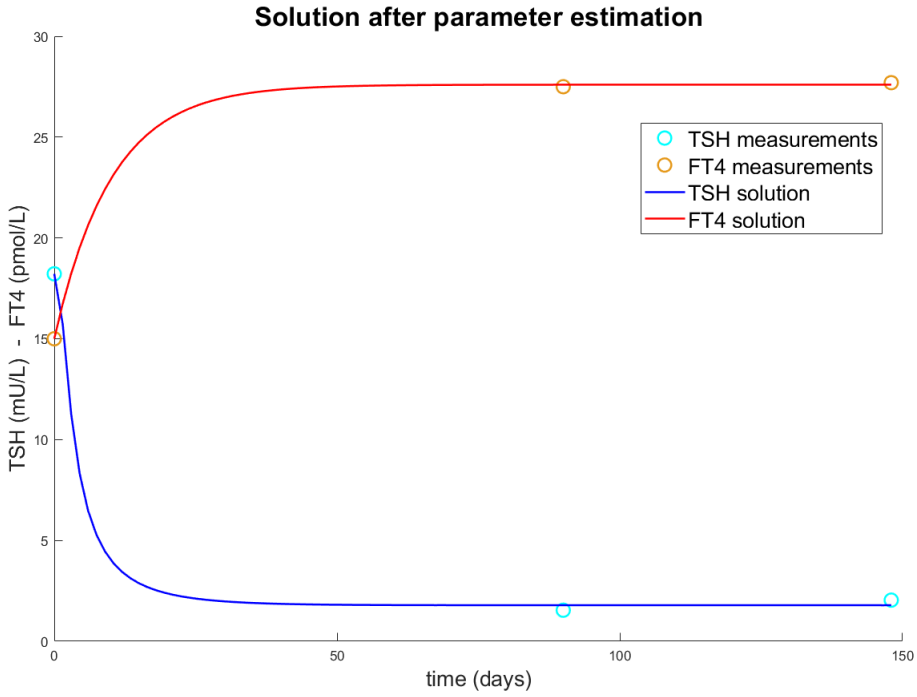


Figure 8.11: Solution of the model applied to Patient 9 of the Nijmegen dataset.

9

CONCLUSION

The aim of this thesis was to investigate and develop a mathematical model of the HPT axis that can be applied on an individual level to every hypothyroid patient. In particular, the focus was on the relationship between the hormones TSH and FT4 and the prediction of each patient's set-point. The optimal path leading to each patient's set-point was analysed as well. This was done by studying how the concentrations of TSH and FT4 are changing over time in hypothyroid patients taking medication, so that a shorter amount of time can be spent to reach every individual's set-point.

The relationship between TSH and FT4 was studied by fitting the measurements of each patient's dataset with different functions. Two models provide the best fit: the exponential and the power functions. However, a closer analysis, involving the goodness-of-fit R^2 and a visual approach, determines that the TSH-FT4 relationship is best described by a negative exponential curve, $[TSH] = Se^{-\varphi[FT4]}$. This result is in agreement with the previous work published by Goede et al. in 2014 [3].

After this, according to Goede et al. [3], the set-point of each individual was computed as the point of maximum curvature of the exponential function. This had never been verified before with real measurements, therefore the natural next step was the validation of the set-point theory. This was done using data belonging to thyroidectomized patients from the Erasmus Medical Center and from the Radboud hospital. In about half of the datasets analyzed, the set-point theory is validated because the predicted set-point is very similar to the reconstructed one. Therefore, this is a positive and encouraging result, supporting the theory that a patient's set-point corresponds to the maximum point of curvature of his exponential function. However, this set-point theory is not verified in all the analysed datasets. A reason that might explain the discrepancy between the predicted and reconstructed set-points is an autonomous production of T3, which results in a lower pre-operarive FT4 set-point level. Therefore, this issue still needs to be further explored.

Finally, the time behaviour of TSH and FT4 was studied by developing a model of differential equations. The final model allows to predict the steady-state at which the concentrations of TSH and FT4 will stabilize. This model is particularly useful when the

measurements are collected a few days after the start of the treatment. In fact, if the TFTs are conducted after 6-8 weeks from the start of the treatment, as it is currently done, their values already represent the steady-states, so in that case the model is not really useful. However, if the measurements are taken one or two weeks after the start of the treatment, it is possible to predict where the concentrations will stabilize and, in case these values do not coincide with the set-point, the medication can be adjusted in order to satisfy the patient's needs.

9.1. DATA LIMITATIONS

The data used in this thesis presents some limitations. In fact, the dataset of hypothyroid patients and the dataset from the Erasmus Medical Center do not provide the date and time at which the measurements were conducted, which is not optimal. The measurements included in the dataset from the Radboud hospital in Nijmegen present the date and time in which they were collected. This shows that, for every patient, the measurements are always conducted at different times during the day. This is not a good practice, because circadian effects can influence the measurements, causing the presence of outliers. Another negative aspect is that the LT4 dosage taken by the patient at the time of the measurement is not specified, which makes it difficult to apply the model with differential equations to the available datasets.

9.2. FUTURE RESEARCH

There are still several opportunities for further research and for improving the mathematical model of the HPT axis developed so far. First of all, for future experiments, it is suggested to collect measurements specifically for this scope, so that they can be as accurate as possible. In fact, it would be ideal to measure the concentrations of TSH and FT4 from blood samples collected always at the same time, in the morning, 24 hours after the last intake of levothyroxine. This is in order to avoid circadian effects that might influence the measurements. Furthermore, it is important to communicate the concentrations of FT4 using at least one decimal digit and not by approximating the value to the nearest integer, because this can cause some inaccuracies. Finally, the medication dosage taken by the patient when the measurements are collected should also be taken into account.

Even though the relationship between TSH and FT4 has been extensively studied and the results obtained in this thesis are confirmed by the research published by Goede et al. [3], it might still be beneficial to use data collected specifically for this purpose in order to study the influence of outliers.

An aspect of this research that still requires attention is the validation of the set-point theory. In particular, for this purpose it might be helpful to measure also the concentration of T3 before and after the thyroidectomy. In fact, this might provide some insights into the autonomous production of T3. After this, it is useful to conduct a trial in order to check whether patients actually feel better when the medication is administered to specifically satisfy their set-point.

Once it has been established that patients feel better when their TSH and FT4 concentrations correspond to their set-point, it might be appropriate to further explore the

time behaviour of TSH and FT4 after the start of the treatment. In fact, in order to prove the benefits of this model, it is important to collect a couple of measurements during the first two weeks from the start of the treatment. In fact, this allows to predict the steady-states at which TSH and FT4 will stabilize and, in case they do not coincide with the set-point, it is possible to adjust the medication dosage. Therefore, an improvement in this direction might show that, through this path, patients can feel better soon.

Finally, for future research, it might also be helpful to develop a model taking into account the circadian rhythms of TSH and FT4, so that it is also known how the set-point changes during the day. This will also allow to relate the measurements conducted at different times of the day with the set-point. However, it should be noted that the diurnal pattern of TSH and FT4 are fundamentally different in a healthy person or in a patient with a thyroid disorder. Furthermore, these results could be considered as an additional help for clinicians to improve the diagnostic process and as a guide to the optimal values of FT4 and TSH in patients treated for hypothyroidism.

ABBREVIATIONS

FT3 Free triiodothyronine

FT4I Free thyroxine index

FT4 Free thyroxine

HPT Hypothalamus-pituitary-thyroid axis

LT3 Liothyronine

LT4 Levothyroxine

T3 Triiodothyronine

T4 Thyroxine

TFT Thyroid function test

TPOAb Anti-thyroid peroxidase antibodies

TRH Thyroid-releasing hormone

TSH Thyroid-stimulating hormone

UNITS OF MEASURE

mU/L Unit of measure of TSH, corresponding to milliunits per litre. U is the international unit, which measures the amount of a substance.

pmol/L Unit of measure of FT4 and FT3, corresponding to picomole per litre.

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A

Code

Outliers detection

```
function [d] = outliers_detection(all_tft)

FT4 = all_tft(:,1);
TSH = all_tft(:,2);

% First plot the measurements to check the general behaviour and to
    detect the outliers that are clearly visible
figure(1)
scatter(FT4, TSH, 'Linewidth', 1)

% Fit the measurements with the required function, using a robust
    method --> Least Absolute Residual (LAR) method
[f, g] = fit(FT4, TSH, 'exp1', 'Robust', 'LAR');
x = 0:0.1:60;
hp_curve = f.a*exp(x*f.b);

% Plot of the fitted function
figure(2)
plot(FT4, TSH, 'o', 'Linewidth', 1)
hold on
plot(x, hp_curve, 'Linewidth',1)

% Compute the distance between the measurements and the model
syms X
for i = 1:length(FT4)
    x_solve = double(vpasolve( 2*(X-FT4(i)) + 2*f.a*f.b*exp(f.b*X).*(
        f.a*exp(f.b*X) - TSH(i)) == 0 ));
```

```
25      distance(i) = sqrt( (x_solve-FT4(i))^2 + (f.a*exp(f.b*x_solve) -
            TSH(i))^2 );
26  end
```

VALIDATION OF THE SET-POINT THEORY

```
close all
clear all
clc

% Pre-thyroidectomy TFTs
pre_data = [2.19 14.7];
TSH_pre = pre_data(:,1);
FT4_pre = pre_data(:,2);

% All the post-thyroidectomy TFTs
all_tft = [0.9 20; 0.04 25.3; 0.06 23.9; 0.06 23.4; 0.06 23.4; 0.06
    19.8];

% Outliers detection
d = outliers_detection(all_tft);

% Post-thyroidectomy measurements without outliers
post_data = [0.9 20; 0.04 25.3; 0.06 23.9; 0.06 23.4; 0.06 23.4];

outliers = [0.06 19.8];

TSH_post = post_data(:,1);
FT4_post = post_data(:,2);

% Fit the post-thyroidectomy measurements with the exponential
    function
[f, g] = fit(FT4_post, TSH_post, 'exp1');

S = f.a;
phi = - f.b;

x = 0:0.1:60;

HP_post = S*exp(-phi*x);

% Compute the set-point
FT4_sp_post = log(phi*S*sqrt(2))/phi;
TSH_sp_post = 1/(phi*sqrt(2));

% Predicted pre-thyroidectomy set-point
FT4_pred = FT4_sp_post/1.25;
TSH_pred = TSH_sp_post;

```

```
% Predicted pre-thyroidectomy HP curve
phi_pred = 1/(TSH_pred*sqrt(2));
S_pred = exp(phi_pred*FT4_pred)/(phi_pred*sqrt(2));

HP_pred = S_pred*exp(-phi_pred*x);

% Compare the predicted set-point to the pre-operative measurements
figure(1)
plot(FT4_post, TSH_post, 'ro', 'Linewidth', 1)
hold on
plot(outliers(:,2), outliers(:,1), 'rx', 'Linewidth', 1)
plot(FT4_pre, TSH_pre, 'go', 'Linewidth', 1)
plot(x, HP_post, 'b', 'Linewidth', 1)
plot(FT4_sp_post, TSH_sp_post, 'b*', 'Linewidth', 1)
plot(x, HP_pred, 'k--')
plot(FT4_pred, TSH_pred, 'k*', 'Linewidth', 1)
xlabel('[FT4] pmol/L', 'Fontsize', 16)
ylabel('[TSH] mU/L', 'Fontsize', 16)

% Actual pre-thyroidectomy HP curve
phi_pre = phi;
S_pre = mean(TSH_pre) * exp(phi_pre * mean(FT4_pre));

HP_pre = S_pre * exp(-phi_pre*x);

% Actual pre-thyroidectomy set-point
FT4_sp_pre = log(phi_pre*S_pre*sqrt(2))/phi_pre;
TSH_sp_pre = 1/(phi_pre*sqrt(2));

% Plot of the pre- and post-thyroidectomy HP curves with their set-
    points
figure(2)
plot(FT4_post, TSH_post, 'ro', 'Linewidth', 1)
hold on
plot(outliers(:,2), outliers(:,1), 'rx', 'Linewidth', 1)
plot(FT4_pre, TSH_pre, 'go', 'Linewidth', 1)
plot(x, HP_post, 'b', 'Linewidth', 1)
plot(FT4_sp_post, TSH_sp_post, 'b*', 'Linewidth', 1)
plot(x, HP_pred, 'k--')
plot(FT4_pred, TSH_pred, 'k*', 'Linewidth', 1)
plot(x, HP_pre, 'm', 'Linewidth', 1)
plot(FT4_sp_pre, TSH_sp_pre, 'm*', 'Linewidth', 1)

xlabel('[FT4] pmol/L', 'Fontsize', 16)
ylabel('[TSH] mU/L', 'Fontsize', 16)
```

```
86 title('Patient 6', 'Fontsize', 20)
87 legend('Post-operative measurements', 'Outliers', 'Pre-operative
       measurements', 'Post-operative HP curve', ...
88     sprintf('Post-operative set-point, \n TSH = %.2f, FT4 = %.2f',
           TSH_sp_post, FT4_sp_post), ...
89     'Predicted HP curve', sprintf('Predicted set-point, \n TSH = %.2f
           , FT4 = %.2f', TSH_pred, FT4_pred), ...
90     'Pre-operative HP curve', sprintf('Pre-operative set-point, \n
           TSH = %.2f, FT4 = %.2f', TSH_sp_pre, FT4_sp_pre), ...
91     'Location', 'northeast', 'Fontsize', 16)
92 axis([0 25 0 60])
93 set(gca,'box','off')
```

MODEL WITH DIFFERENTIAL EQUATIONS AND PARAMETER ESTIMATION

```
function Y = solve_system(param, t)

    % Solve the system of differential equations
    y0 = [40 8];
    [T, Yv] = ode45(@diff_eq, t, y0);

    % Model of differential equations for TSH and FT4
    function dY = diff_eq(t,y)
        dydt = zeros(2,1);
        dydt(1) = 5000 - 5000*y(2).^4./(param(1)+y(2).^4) - param(2)*
            y(1);
        dydt(2) = - 0.099*y(2) + param(3);
        dY = dydt;
    end

    Y = Yv;

end
```

```
close all
clear all
clc

% Time
t = [0; 1; 2; 5; 10; 15; 20; 30; 40; 50];

% Measurements
y = [40 8; 24.09 8.5; 18.16 8.9; 8.95 9.9; 3.83 11.1; 2.18 11.9; 1.64
     12.3; 1.24 12.7; 1.07 12.9; 1.03 12.96];

% Initial values for the parameters
param0 = [1 1 1];

% Least-squares to estimate the parameters
[param] = lsqcurvefit(@solve_system, param0, t, y, zeros(size(param0)
    ));

fprintf(1,'\tParameters:\n')
fprintf(1, '\t\t ka = %8.5f ', param(1))
fprintf(1, '\t\t k2 = %8.5f ', param(2))
fprintf(1, '\t\t k5 = %8.5f\n', param(3))

```

```
% Solve the system with the estimated parameters
tv = linspace(min(t), max(t));
Yfit = solve_system(param, tv);

figure(1)
plot(t, y(:,1), 'co', 'Linewidth', 1.5, 'Markersize', 10)
hold on
plot(t, y(:,2), 'o', 'Color', [0.9 0.6 0.1], 'Linewidth', 1.5, '
    Markersize', 10)
plot(tv, Yfit(:,1), 'b', 'Linewidth', 1.5)
plot(tv, Yfit(:,2), 'r', 'Linewidth', 1.5)
xlabel('time (days)', 'Fontsize', 16)
ylabel('TSH (mU/L) - FT4 (pmol/L)', 'Fontsize', 16)
legend('TSH measurements', 'FT4 measurements', 'TSH solution', 'FT4
    solution', 'Fontsize', 16)
title('Solution after parameter estimation', 'Fontsize', 20)
set(gca,'box','off')
```

B

Results

Validation of the set-point theory with the Nijmegen dataset

	$TSH_{post\text{-}op}$	$FT4_{post\text{-}op}$	$TSH_{pre\text{-}op}$	$FT4_{pre\text{-}op}$	$FT4_{expected}$	Validated
P01	1.17	21.40	1.17	13.30	17.12	No
P02	2.00	19.40	2.00	13.18	15.52	No
P03	1.21	17.08	1.21	10.21	13.67	No
P05	2.04	25.19	2.04	16.33	20.15	No
P06	0.94	19.95	0.94	15.82	15.96	Yes
P07	0.72	23.80	0.72	16.23	19.04	No
P08	0.78	14.84	0.78	10.68	11.85	Yes
P09	3.68	23.33	3.68	9.47	18.66	No
P11	1.28	22.11	1.28	11.27	17.69	No
P12	0.49	17.97	0.49	13.69	14.37	Yes
P13	0.69	15.27	0.69	11.82	12.21	Yes
P14	2.27	11.77	2.27	9.50	9.41	Yes
P16	2.49	16.93	2.49	11.78	13.55	No
P17	0.99	18.23	0.99	13.77	14.59	Yes
P20	2.70	16.35	2.70	14.01	13.08	Yes

VALIDATION OF THE SET-POINT THEORY WITH THE ERASMUS DATASET

	$TSH_{post\text{-}op}$	$FT4_{post\text{-}op}$	$TSH_{pre\text{-}op}$	$FT4_{pre\text{-}op}$	$FT4_{expected}$	Validated
001.005	2.14	21.90	2.14	14.04	17.52	No
001.006	1.97	20.64	1.97	15.69	16.52	Yes
001.017	1.90	19.76	1.90	12.18	15.81	No
001.019	1.13	19.70	1.13	14.65	15.76	Yes
001.025	1.72	22.78	1.72	17.09	18.22	Yes
001.027	0.27	21.29	0.27	17.73	17.03	Yes
001.032	0.64	23.61	0.64	19.44	18.89	Yes
001.033	1.17	19.34	1.17	15.62	15.48	Yes
001.034	1.71	24.59	1.71	18.0	19.67	Yes