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A PHYSIOLOGICAL BIOKINECT MODEL FOR THE [7(N)-^{3}H]-CHOLESTEROL DOSIMETRY

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ABSTRACT

Cardiovascular diseases (CVD) are a major source of deaths worldwide according to WHO (World Health Organization). It is well-known that the change of the level of plasma lipoproteins, which are responsible for the cholesterol transport in the bloodstream, is a main cause of these diseases. For this reason, to know the biokinetic parameters of plasma lipoproteins and quantifies them is important to correct and deepen the understanding of associated diseases. The main objective of this work is to provide a biokinetic model in order to estimate the radiometric dose, due to the intake of [7(N)-^{3}H] –Cholesterol in physiological issues, in metabolic studies. The internal dosimetry is important to know the biological effects of radiation. The model was based on SCHWARTZ et al (2004), using parameters for the plasmatic lipoproteins and ICRP 30 (1979) gastrointestinal tract; the dose in the compartments were calculated using the MIRD methodology and the compartimental analysis by Matlab® software. The coefficients were estimated for an adult phantom with a body mass of 73.3 kg.

1. INTRODUCTION

The dyslipidemia, alteration in the level of lipids or currents lipoproteins, is induced for changes in the production, catabolism or excretion, as consequence of genetic, environmental factors, inappropriate diet and/or sedentary life style [RABELO, 2001]. According to the National Program of Education on Cholesterol, hypercholesterolemia, particularly increased LDL (low density lipoprotein), is the main predictor of CVD (Cardiovascular Diseases). This fact is due to LDL particle, which contains 70% of cholesterol, in the bloodstream and is the main target of medical intervention [The Third Report of the National Cholesterol Education Program (NCEP), 2001]. The increase of HDL (high density lipoprotein) in the serum levels decreases the relative risk of CVD. The mechanism for this protective effect is the property of the HDL to make the reverse cholesterol transport or removing it from the cells and transporting to the liver for excretion. The HDL, also, prevents oxidation and aggregation of LDL particles in the arterial wall (atheroma), reducing the atherogenic potential of lipoproteins [GIULLUM, 2000].

In the literature, there are works related to cholesterol employed as radioactive tracers, related to the blood and the high incidence of cardiovascular diseases. Then, it should be treated as a crucial component of cells and a precursor of steroid hormones and bile acid membranes [LEHNINGER et al., 2006]. Studies with artificial lipid emulsion labeled with radioactive isotopes, ^{3}H (tritium) and ^{14}C (carbon-14), are alternatives for labeling the cholesterol in biokinetic studies of physiology [LEES et al., 1983; LEES et al., 1985;].

The knowledge of dosimetric aspects for the employment of tracers was not fundamentally estimated, but indirectly, making use of the allometry theory as described by Maranhão and co-workers [MARANHÃO et al., 1996]. Basically, they used the ALI limits (annual limit of intake). For ^{3}H and ^{14}C, the estimates made are relatively precarious due to the fact that ALI parameter is dependent on the chemical form from the marked product and it is not known, specifically, for the ^{3}H-Cholesterol and ^{14}C-Cholesterol parameters.

Radiotracers, as ^{3}H-Cholesterol, are often used for metabolic studies [WOOD, 1967], being a very sensitive method for detecting substances in small quantities. Generally, a tracer must have three main properties: (1) to have the same phenomenological properties of the labeled substance, (2) to be added to the labeled molecule in a quantity that does not change its normal physiological behavior, and (3) to be easily detected as a separate entity [VICINI, 2008]. However, public concern with the biological effects of radiation is high, making studies with any form of radiation to be submitted to rigorous evaluations in ethic committees.

Hence, the need for guidance on the safe use of radiation led, in 1928, to the formation of the International Commission on Radiological Protection (ICRP), which since then has coordinated its development worldwide, spreading radiation protection guidelines for accidental exposure, and the occupational exposure of patients to ionizing radiation [VICINI, 2008]. The Nuclear Medicine Society established, in 1968, a standard methodology for internal doses analysis, developing a committee called MIRD (Medical Internal Radiation Dose), which provided a broad framework for evaluating the absorbed dose in the organs, tissues, compartments and individual cells from the internally deposited radionuclides [BOLCH et al., 2009].

The MIRD methodology defines that bodies which concentrate the activity are called source organs; tissues or organs that undergo the action from the radiation sources of organs are called target organs. The same body may be simultaneously organ source and target organ itself [STABIN et al., 1999]. In MIRD protocol, to know the biokinetic parameters used, the variation of the concentration of radionuclide in function of time in the organ source is estimated. Initially, the biokinetic distribution is inferred from information gathered from animal studies. Thus, the study tries to extrapolate the biokinetic parameters obtained from animal experiments to humans, using the theory of Allometry. If the experimental results inspire confidence, it is liberated for experiments to be conducted directly in humans [SIEGEL et al., 1999].

In its publication number 30, the ICRP defines biokinetic models for diverse radioisotopes, comprising the respiratory tract and the gastrointestinal tract [ICRP 30, 1979]. Since then, the development of more complex models, which simulate the biokinetic physiology and molecules biochemistry, was stimulated with the computational advance [DISTEFANO, 1984]. However, the specific radiotracers related to dosimetric data used in these models are few in the literature: for example, ^{3}H-cholesterol as a tracer, despite being widely used in terms of dosimetric aspects, is poorly described in the literature [WOOD, 1967].

2. METODOLOGY

In the present study, the physiological biokinetic model was developed using parameters of the biokinetic described by Schwartz [Schwartz et al., 2004] that involves lipoproteins. Also, the gastrointestinal model described in the ICRP [ICRP 30, 1979] was added to the final model proposed in this work, in order to represent the cholesterol ingestion. To make a connection between the lipoprotein physiology and the gastrointestinal model, one more compartment, named chylomicron, was added in the development of the proposed model, which is presented in Fig.1.

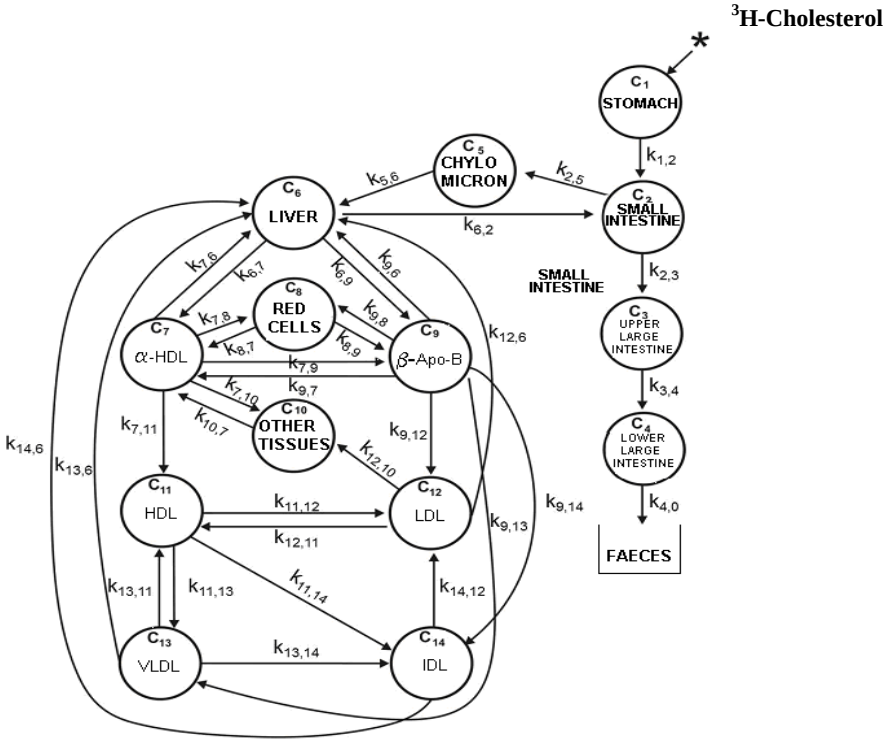

Figure 1: Compartmental Model Proposed: The input of the analysis (^{3}H-Cholesterol) in the system is represented by an asterisk. The compartments represented by circles, can be a physical place where a substance lives or its chemical state. The kinetic parameters $k_{i,j}$, represented by arrows, are the constant fractions of the content transfers of the C_i compartment that transfers to the Cj compartment (adapted to SCHWARTZ et al, 2004; ICRP 30, 1979).

As shown in the model described in Fig. 1, the flux parameters were inserted for the ^{3}H-Cholesterol in each compartment and a constant was obtained between one compartment and the other (ki,j). The transfer constants of the ^{3}H-Cholesterol in the model were calculated using the (k) values and the Matlab® software. The (k) values presented in Tables 1 and 2 show that ki,j values are the transfer time of the cholesterol labeled with tritium, in each compartment.

Table 1: The parameters used from the model of Schwartz et al, 2004.

	Pool (Cholesterol)	Flux	k	$k_{i,j}$	
Compartments	**(µmol/70 kg)**	**(µmol/min/70 kg)**	**i,j**	**(minutes^{-1})**	**(seconds^{-1})**
Liver - α-HDL	4882	32.4	6.7	0.0066	0.000111
Liver - β-apoB	4882	15.4	6.9	0.0031	5.26E-05
α-HDL - Liver	841	33.6	7.6	0.039	0.000666
α-HDL – Red Cells	841	15	7.8	0.018	0.000297
α-HDL - β-ApoB	841	50.9	7.9	0.06	0.001009
α-HDL – Other Tissues	841	6.8	7.10	0.0081	0.000135
α-HDL – HDL	841	2.5	7.11	0.003	4.95E-05
Red Cells - α-HDL	6687	15	8.7	0.0022	3.74E-05
Red Cells - β-ApoB	6687	4	8.9	0.0006	9.97E-06
β-ApoB - Liver	2838	11.2	9.6	0.004	6.58E-05
β-ApoB - α-HDL	2838	54.2	9.7	0.02	0.000318
β-ApoB – Red Cells	2838	4	9.8	0.0014	2.35E-05
β-ApoB - LDL	2838	0.6	9.12	0.000211	3.52E-06
β-ApoB - VLDL	2838	0.14	9.13	4.93E-05	8.22E-07
β-ApoB - IDL	2838	0.06	9.14	2.11E-05	3.52E-07
Other Tissues - α-HDL	24191	7.2	10.7	0.0003	4.96E-06
HDL - LDL	2750	7.2	11.12	0.0026	4.36E-05
HDL - VLDL	2750	2.8	11.13	0.001	1.7E-05
HDL - IDL	2750	0.7	11.14	0.0002	4.24E-06
LDL - Liver	6335	1.44	12.6	0.0002	3.79E-06
LDL – Other Tissues	6335	0.39	12.10	6.16E-05	1.03E-06
LDL - HDL	6335	6.8	12.11	0.0011	1.79E-05
VLDL - Liver	290	0.8	13.6	0.0027	4.6E-05
VLDL - HDL	290	1.4	13.11	0.0048	8.05E-05
VLDL - IDL	290	0.7	13.14	0.0024	4.02E-05
IDL - Liver	306	0.7	14.6	0.0023	3.81E-05
IDL - LDL	306	0.8	14.12	0.0026	4.36E-05

Table 2: The parameters used from the ICRP 30, 1979 with the Chylomicron compartment.

	k	$k_{i,j}$	
Compartments	**i,j**	**Hour^{-1}**	**Seconds^{-1}**
Stomach-Small Intestine	1.2	1	0.000278
Small Intestine-Upper Large Intestine	2.3	3.47E-05	2.42E-05
Small Intestine-Chylomicron	2.5	0.076923	2.14E-05
Upper Large Intestine-Lower Large Intestine	3.4	0.041667	1.16E-05
Lower Large Intestin-Excretion	4.0	3.47E-05	2.416E-05
Chylomicron-Liver	5.6	3.024	0.00084
Liver-Small Intestine	6.2	0.01033	2.87E-06

The dose was calculated using the MIRD methodology that makes use of the energy deposited in the organ and tissues [STABIN et al., 1999] and the Matlab® software to perform the calculations.

3. RESULTS AND DISCUSSION

The k values obtained for the model proposed in Fig.1, using the Matlab® software, generated the curves of the tritium activity per time, for each compartment. To calculate the dose of tritium labeled cholesterol, an activity of 1MBq was used. For better visualization, the abscissas of tritium activity curves per time are plotted in logarithmic scale. These curves represent the transference time of the tritium labeled cholesterol at each compartment and they are presented in Figs. 2, 3 and 4. The curves are selected according to the type of the structures; Fig. 2 presents the curves of gastrointestinal tract compartments, while Fig. 3 shows the curves that represent the lipoprotein compartments. The curves of the compartments of liver, red cells and the other tissues are shown in Fig.4.

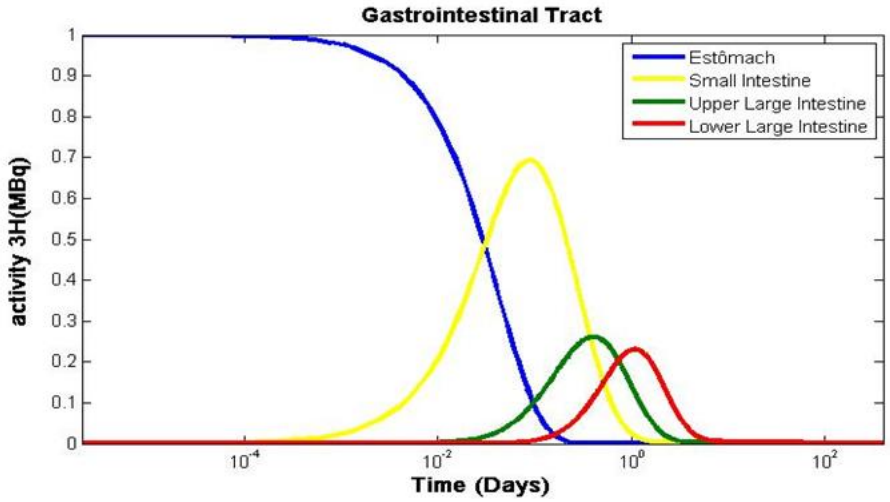

Figure 2: The transference of the ^{3}H-Cholesterol in the gastrointestinal tract.

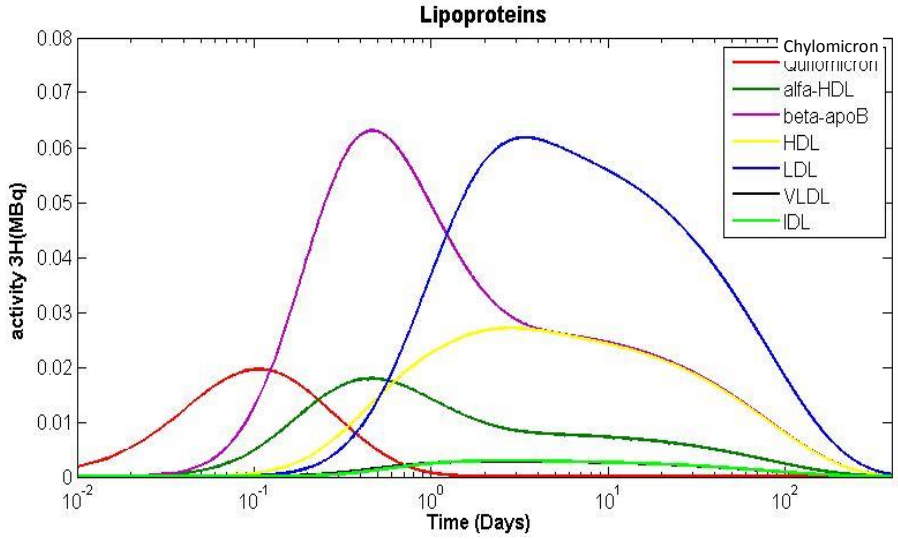

Figure 3: The transference of the ^{3}H-Cholesterol in the lipoprotein compartments.

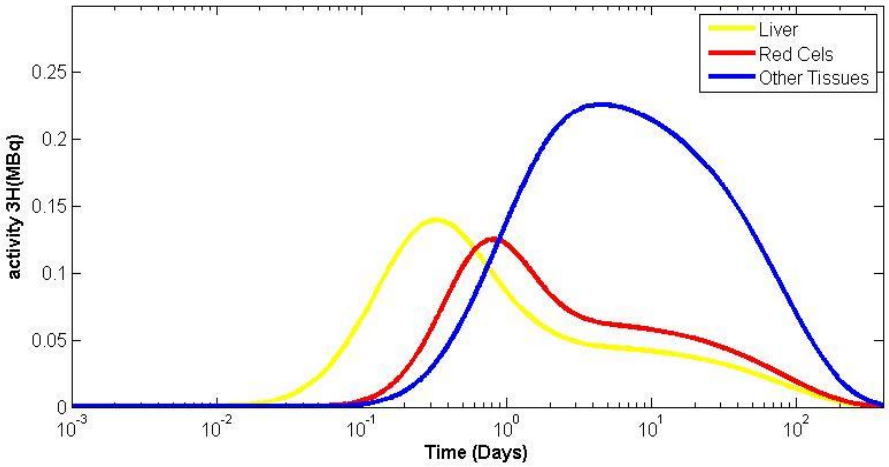

Figure 4: The transference of the ^{3}H-Cholesterol in the liver, red cells and other tissues compartments.

As it can be observed from Fig. 2, the curve of the compartment that represents the stomach shows a different behaviour from other gastrointestinal compartments. The stomach compartment receives the tritium labelled cholesterol directly, in its maximum activity and, then, presents a decrease as the time passes. On the other hand, the other gastrointestinal compartments start from zero, reach a peak representing the maximum activity of cholesterol, which was 1 MBq and, thereafter, transferring cholesterol to the next compartment. In Figs. 3 and 4, the maximum values of the activities are consistent with the profile of the curve, for a better view. These curves were used to calculate the dose using the parameter that was called Area Under the Curve (AUC). This parameter gives the values of the total disintegrations of tritium atoms (^{3}H) in each compartment. The same way, the total energy that was distributed between the compartments was calculated.

MIRD methodology, which considers the energy deposited in the organs and tissues, was used to perform the calculation of the dose with the values of the organs and tissues mass that are described in the ICRP 106 [ICRP 106, 2008] for an adult man of 73.3kg. To perform the calculation of the dose, it was used the deposition energy in joule (J) of the ^{3}H-Cholesterol, divided by the mass (kg) of each compartment. The dose obtained was in J/kg that is equivalent to Gray (Gy), according to equation 1:

$$D(Gy) = \frac{\text{Energy of } 3\text{H in the Compartments (J)}}{\text{Mass of the compartment (kg)}} \quad (1)$$

The doses obtained from the model for an adult of 70 kg are described in Table 3.

Table 3. The Absorbed Dose in the compartments of the model for an intake of 1 MBq of ^{3}H-Cholesterol.

Compartments	µGy
Stomach	2.07
Small Intestine	2.94
Upper Large Intestine	16.52
Lower Larger intestine	46.80
Chylomicrons	0.017
Liver	11.81
α-HDL	0.73
Red Cells	5.82
β-ApoB	2.48
Other Tissues	2.13
HDL	2.36
LDL	0.53
VLDL	0.25
IDL	0.26

In Table 3, the dose obtained is represented in micro Gray (µGy), only for a better presentation of the dose that was very low. In Table 3, it can be observed that among all compartments and, more specifically for the gastrointestinal tract, the major dose received was in the lower large intestine, of 46.8 µGy. This part of the intestine received, approximately, 50% of the cholesterol from the small intestine and the upper large intestine; it, also, received the cholesterol in the form of biliary acids from the liver to excretion.

Besides the organs of the gastrointestinal tract, a high dose was found in the liver with 11.81 µGy. The high flow rate of cholesterol found in this compartment is due to liver production of lipoprotein, which has the function of transporting cholesterol into the bloodstream and bringing the remaining cholesterol back to the liver.

To compare the level of the doses founded in the model and to demonstrate that the doses presented in Table 3 are very low, we can highlight some values of doses that are usually used in specific diagnostics, making use of different types of radiation such as X-rays and radiopharmaceuticals. According to the Health Physics Society (United Kingdom, 2010), exams that use X-rays, such as endoscopy of the upper digestive tract, the maximum dose allowed is 1500 µGy/exam and for a computed tomography of abdomen, the dose of 10000 µGy/exam is applied to the patients. These doses are many times higher than the major dose from the radiotracer 3H-Cholesterol that was 46.8 µGy. For diagnosis that makes use of radiopharmaceuticals such as ^{67}Ga (gallium), used for the diagnosis of tumors, the dose is 11000 µGy and this dose is accepted by the medical community. These factors justify the use

of radiotracers like e^{3}H-Cholesterol. It should be emphasized the importance of these tracers in physiological studies and for the kinetics of lipoproteins. It helps in the comprehension of pathology associated with cardiovascular diseases, due to alterations of the levels of lipoproteins in the blood.

Another important comparison is for the use of stable isotopes, which for ethics committees are safer than radioactive isotopes. However, as demonstrated in this study, the dose for patients subjected to the use of ^{3}H-cholesterol as a radioactive tracer is very low, which justifies the use of the tracer. Moreover, the method is much less complex compared to the use of stable isotope. To analyze the tritium labeled cholesterol, it is only necessary one aliquot of blood plasma, whose activity is measured with liquid scintillator [REDGRAVE et al., 2001]. With stable isotopes, the analyses is more complex, needing equipment of analyzes that are more specific, such as like mass spectrometer with gas chromatography or mass spectrometer coupled with a nuclear magnetic resonance device [DICK C. et al., 2004]. Thus, expensive and laborious equipment is required for the preparation and manipulation of samples. The labeling form for both, the stable and the radioactive isotopes, have the same difficulty; the labeling of the cholesterol occurs in the steroid nucleon of the molecule, which is formed only by carbon and hydrogen, whereas for stable isotopes there are the examples of ^{13}C and ^{2}H (deuterium). Thus, as demonstrated in this study, taking into account the low doses calculated in the model and the measurement methodology compared to the use of stable isotopes, the use of 3H-cholesterol as a radioactive tracer is justified.

4. CONCLUSIONS

The compartmental model proposed is adequate to predict the doses to be received by each organ of the model, in patients submitted to physiological and biokinetic studies with ^{3}H-cholesterol. The highest dose obtained from the ^{3}H-Cholesterol was quantified in the lower large intestine as 46.8 μGy. The results presented in this paper contribute, as subsidy, for metabolic studies using ^{3}H-Cholesterol, based on human physiology, for the calculation of doses. The dose received by the patient during diagnostic X-ray procedures and radiopharmaceuticals in nuclear medicine are often higher than that received from ^{3}H-Cholesterol use. The proposed model constitutes a decision tool for ethics committees in order to approve experimental protocols to use ^{3}H-Cholesterol.

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