PBPK modeling of tocopherol transport and absorption in rats

Dwaipayan Mukherjee, Sastry S. Isukapalli, and Panos G. Georgopoulos

Computational Chemodynamics Laboratory
http://ccl.rutgers.edu
Exposure Measurement and Assessment Division
Environmental and Occupational Health Sciences Institute
170 Frelinghuysen Road, Piscataway, NJ 08854

EOHSI, the Environmental and Occupational Health Sciences Institute, is a joint Institute of UMDNJ – Robert Wood Johnson Medical School and Rutgers, The State University of New Jersey

1. Introduction

Vitamin E was discovered by Evans and Bishop more than 75 years ago as a lipid-soluble substance in lettuce and wheat, necessary for the prevention of fetal death. Initially considered only as a factor responsible for reproduction, however, its importance in the animal body has been later found to be much more diverse. It is found to act as an important anti-oxidant, preventing and slowing down many degenerative processes in the body. It has been found to be important in the prevention of geriatric syndromes like Alzheimer and Parkinson's disease. The action of this substance in the animal brain has been a topic of recent research.

Vitamin E is composed of 2 main classes of compounds, tocopherols and tocoentrinols. Vitamin E occurs in nature in eight different forms: α, β, γ, and δ tocopherols and tocoentrinols[1]. Out of these, α and γ tocopherols are most abundant in nature and are predominantly present in natural food substances as compared to the other tocopherols and tocoentrinols[1]. The term ''tocopherol'' is used after the Greek words ''tokos'' (childbirth), ''phero'' (to bring forth), and ''ol'' (alcohol) [2]. Natural food substances contain mainly α and γ tocopherols in varying amount. However, it is α-tocopherol which is found to have the maximum biological effect regarding metabolism and storage in tissues[1]. This work focuses only on the behavior of α-tocopherol in the physiological system of the mouse. So all mention of tocopherol in this report may be assumed to mean α-tocopherol only.

2. Modeling

The study of tocopherol in physiological systems has been confined to experimental animal studies and time-dependant studies of fate of dietary tocopherol in humans. The earliest study of tocopherol transport by lipoproteins was carried out by Lewis, Quaife and Page in 1954[3]. Precise measurements in response to tocopherol intake were carried out by McCormick, Cornwell, and Brown in 1960[4]. Later a number of studies[5,6] have used deuterated tocopherol to study the entire process of tocopherol absorption and distribution. Much recently, researchers have tried to elucidate individual processes which take part in the overall uptake of tocopherol. Tocopherol transport pathways across the enterocyte of the small intestine[7] and in the hepatocytes of the liver[8] have been investigated. Organism level Physiologically-based Pharmacokinetic (PBPK) models have been used very frequently for risk estimation and studying fate of uptake events. This modeling approach considers the various tissues as compartments and models transport of a chemical to and from these compartments and also metabolism and generation in the compartments. The absence of such PBPK modeling efforts for tocopherol is indeed striking. The role of tocopherol as a therapeutic over and above its normal dietary needs is a relevant question now and PBPK modeling can bridge the gap between insights from experiments and clinical tests and dose-effectivity analysis.

The PBPK model attempted here follows the basic structure of the model used by Isukapalli *et al.*[9] in analyzing chloroform exposure. However, many changes have been brought about to the basic model regarding the modes of intake, the tissues involved and

the mechanisms of absorption. We have considered 2 modes of intake – oral ingestion (both in solid form as well as suspension) and intra-venous injection. Generally a PBPK model considers the tissues as well-mixed compartments with substance interchange between them and the body fluid described by partition coefficients and the metabolism within tissues governed by rate equations. We have also followed the same procedure here. The transport, absorption and metabolism of tocopherol have been widely studied by in-vivo animal and human experiments. However, many aspects of the behavior of tocopherol within the body are still not clear. The present modeling follows the results of the experimental research till date. This modeling being a study of the rat physiological system, studies on rats have been used wherever available. However, human values have also been taken with suitable justification. The basic mechanism of tocopherol transport, absorption and metabolism does not differ in different mammals. A whole body PBPK model necessarily requires many approximations and assumptions and they have been made wherever unavoidable. Such approximations and future possible refinements of the model have been presented under discussions. A brief account of the process of in-vivo transport, absorption and metabolism of tocopherol as well as the values and methods for estimating them in the PBPK model have been presented below.

2.1 In-vivo tocopherol distribution

2.1.1 Tissues involved in the absorption and metabolism of tocopherol

Tocopherol is transported by both the lymphatic as well as the vascular circulatory system and is distributed all over the physiological system. However, experiments in rats have shown (Bjorneboe *et al.*[10]) that some tissues preferentially take up and store tocopherol over the others and hence we will concentrate on only those tissues to make our model simpler. The tissues with their percentage of tocopherol content are presented below in Table 1.

Table 1. Amounts of tocopherol absorbed in various tissues. (Bjorneboe et al.[10])

Organ	Tocopherol (nmol/organ)	Percentage
Liver	425	28.5
Muscle	622	41.7
Adipose	319	21.4
Adrenal	12	0.8
Kidney	58	3.9
Spleen	30	2
Heart	9.3	0.6
Lung	9.3	0.6
Testis	4.6	0.3
Cerebrum	2.3	0.2
Total	1491.5	100

For the modeling we consider only the tissues which have higher than 1% of the total tocopherol. We also consider brain, despite it having lesser percentage as it has a high value of tissue:blood partition coefficient (Table 2) and gut because it has an important role in the incorporation of tocopherol into lipid chylomicrons. All other tissues (viz. Adrenal, Heart, Testis) are lumped as Additional tissues (AT). Consequently, the entire physiological system is divided into the following 8 tissue compartments and 3 body fluids: Liver, Kidney, Brain, Muscle, Spleen, Fat, Gut, Additional Tissues (AT), Blood, Bile and Lymph.

2.1.2 Intake mode: Ingestion

Tocopherol being a highly lipophilic compound is highly insoluble in the aqueous medium of the gut and hence is absorbed along with the fats present in the diet. Absorption and metabolism of tocopherol is highly related to lipid metabolism. Fats are absorbed from the small intestine into the lymphatic system of the body before reaching the liver where they are metabolized and discharged into the blood circulation as one of LDL, HDL or VLDL. A schematic diagram of the process is shown in the above figure. Fat is broken down into small globules in the stomach and after entering the small intestine is mixed with the bile salts from the gall bladder and pancreatic lipase from the pancreas. Lipase breaks fats into monoglycerides and fatty acids, which together with the bile salts form molecular aggregates known as mixed micelles (Traber *et al.*[11]). These micelles are able to dissolve other lipophilic compounds like tocopherol and carry tocopherol to the intestinal membrane where they are absorbed by the villi. However many researchers have found disparities in the extent of tocopherol absorption as given by analysis of the lymph and that given by the analysis of feces (Friedrich W.[12]), suggesting the presence of another pathway of tocopherol absorption independent of the lymph. The suggested pathway is through the hepatic portal vein (MacMahon *et al.*[13]). The complete distribution pathway of vitamin E from the intestine to the tissues, in conjunction with lipids is represented schematically in the following figure.

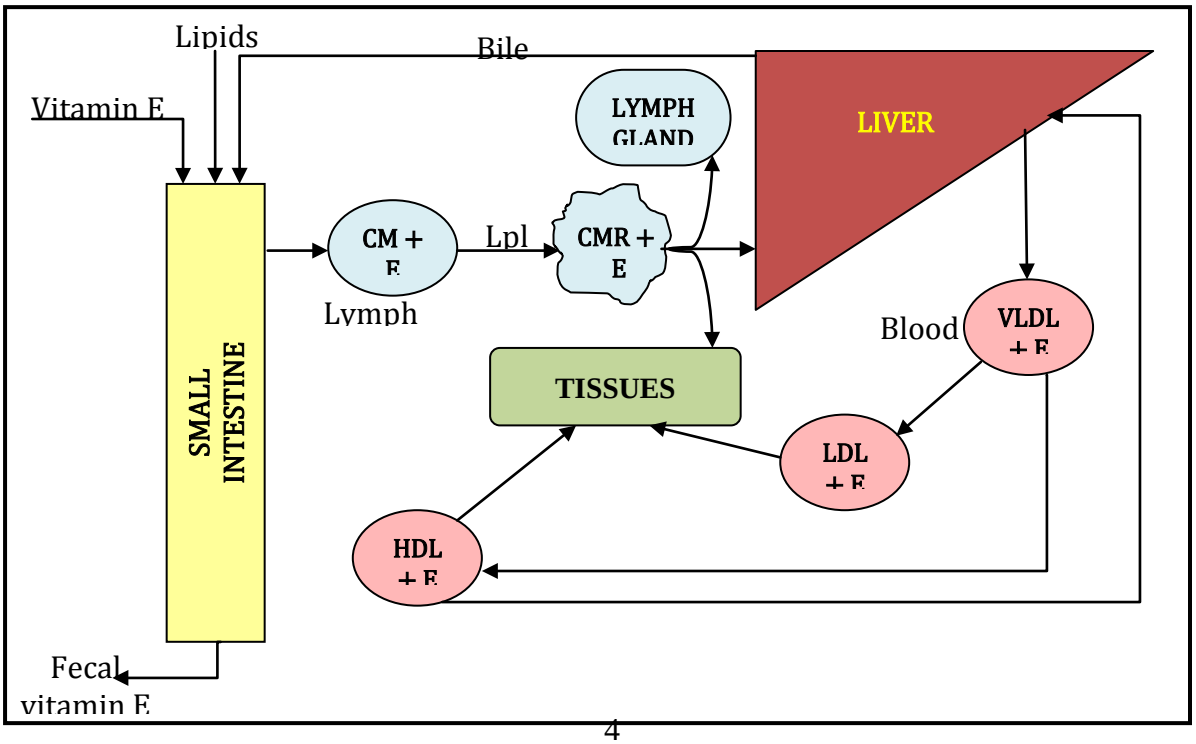


Fig.1. Transport and absorption of tocopherol along with lipid for the purpose of PBPK modeling, the schematic diagram of the entire process may be represented as shown below:

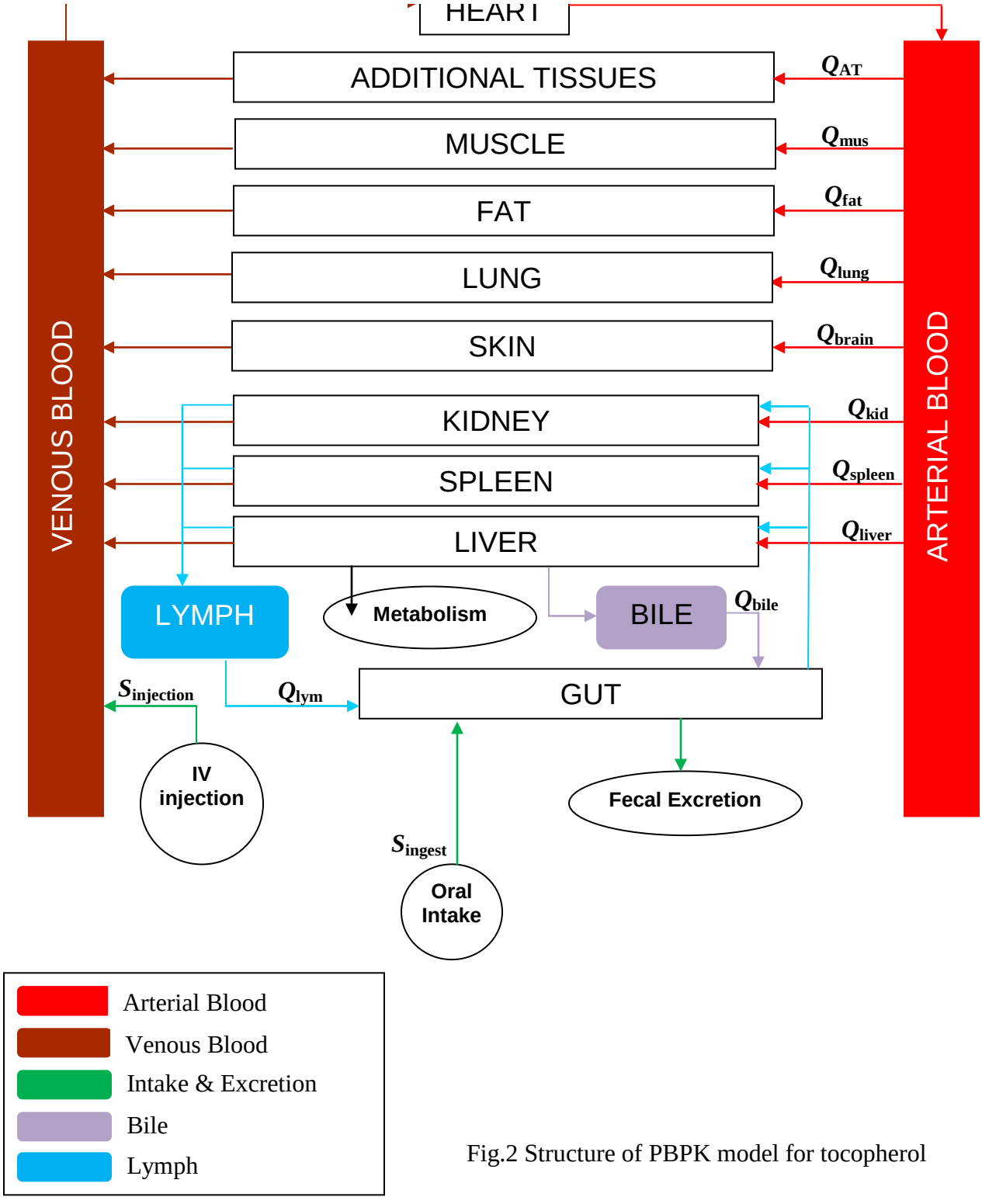


Fig.2 Structure of PBPK model for tocopherol

2.1.3 Small Intestine

After ingestion, some time is required for the food to get masticated in the alimentary canal and then emulsification in the small intestine. For tocopherol, this is a physical process which happens at a certain rate. The time lag for an ingested food bolus to reach the intestine in human has been estimated by Clifford et al.[14] to be 0.08 day, i.e. 1.92 hours. In rats this time lag is supposed to e smaller. Experiments in rats fed with radioactive tocopherol show a time lag of about 1 hour (Bjorneboe et al.[10]) before tocopherol reaches the lymphatic system. So we take the time lag to be 1 hour. Lipid digestion and absorption takes some time and the absorption of tocopherol is limited by this process and the time lag is due to this. This time lag is introduced "mechanically" by adding 60 minutes to the time of oral intake. In the small intestine, lipids are emulsified with bile and pancreatic lipase and micelle formation takes place in which tocopherol is taken up. The rate constant for this process has been estimated by Clifford et al.[14] (Table 3). After this, the micelles or chylomicrons are absorbed by the enterocytes on the lumen of the small intestine. This was initially thought to be a process of passive diffusion (Traber et al., MacMahon et al.). But recently Reboul et al.[7] showed that the scavenger receptor SRB-1 is involved in tocopherol absorption in the intestine. Reboul et al. showed this process to follow Michaelis-Menten type kinetics and is dependent on micellar tocopherol concentration in the intestine. The Michaelis-Menten type constants for this process are tabulated in Table 3. In our modeling, we have coupled the 2 processes of uptake into chylomicrons and uptake of chylomicrons as one process with the slower rate. The uptake rate of chylomicrons by the intestinal enterocytes is slower and the entire process may be thought to be proceeding at the slower rate. Tocopherol not absorbed in the intestine is excreted with feces, which has been estimated to be 30% by MacMahon & Neale[15] and also confirmed later by Clifford et al.[14]. There is some time-lag in the colon before a substance reaches the feces. This rate constant has also been estimated by Clifford et al. and is tabulated in Table 3.

2.1.4 Lymphatic system

Tocopherol, after absorption in the small intestine enterocytes, is taken up by the lymphatic system. Normally ingested food substances after absorption in the intestine, is taken up in the blood circulation of the portal vein, which carries it to the liver for metabolism. However, lipids and lipophilic substances are taken up in the lymphatic system. The extent of the absorption of a compound in the lymphatic system is indicated by its lipophilicity, which may be measured by its octanol-water partition coefficient (Jandacek et al.[16]). It has been observed that a $\log_{10}P$ of at least 5 causes the substance to enter the lymphatic system (Jandacek et al.). This work also documents some lipophilic organometallic compounds and their partition coefficients. The corresponding value for tocopherol has been calculated to be around 12.2 (Cooper et al.[17]). So we may assume that due to its high lipophilicity, tocopherol is mostly absorbed in the lymphatic system and its portal vein absorption may be safely neglected. Bjorneboe et al. also did not find any tocopherol in portal blood. Lymph is basically a fluid which alternates between the intercellular fluid and the plasma of blood. In blood, lipophilic compounds are carried in

the plasma and so in this modeling we do not create any distinction between blood and lymph. Table 2 shows the final percentage of tocopherol absorbed in the various tissues. We neglect the tissues which have less than 1% of the tocopherol. The amount of tocopherol taken up by these tissues is governed by their tissue:plasma partition coefficients (Table 2).

Table 2. Flow rates of various body fluids (for a 250 gm rat)

Body fluid	Flow rate	Source
Blood	74 mL/min	Davies & Morris[23]
Bile	22.5 mL/day	Davies & Morris[23]

2.1.5 Intake mode: Intravenous injection

Tocopherol when intravenously injected, directly enters the blood stream and follows the same course of events as orally ingested tocopherol after its release in the blood circulation. In this case, it bypasses the lymphatic system and reaches the organs faster. It however takes some time to bind to the lipoproteins LDL, HDL and VLDL in the blood plasma but that time lag has been neglected.

2.1.6 Metabolism and excretion

Metabolism of α-tocopherol in the animal body is very limited. Less than 1% of the ingested α-tocopherol is excreted in the urine. The most important route of excretion is fecal elimination. About 70% of the tocopherol ingested is absorbed, the rest being excreted with feces (MacMahon et al.[13]). Absorbed α–tocopherol is mostly stored in the tissues[11]. However MacMahon et al. found the presence of some polar compounds formed from tocopherol metabolism. The major metabolic pathways of tocopherol have been compiled by Chow et al.[2]. It was thought that the only metabolic product formed from tocopherol was tocopheryl quinone[19]. But later CEHC (carboxyethyl-hydroxychromans) were found to be the major metabolites from tocopherol compounds. Clifford et al.[14] estimated the tocopherol metabolism taking place in the liver and estimated fractional rate constants for changes occurring between different pools of tocopherol in-vivo. We have averaged the rate constants for liver for the 2 different pools of tocopherol and approximate the process by a single rate constant (in Table 3.). In this modeling, we do not follow the fate of the metabolic products of tocopherol, which are excreted by the kidney through urine. However, the liver also undertakes two other important processes. It metabolizes some of the tocopherol (see next section) and releases some tocopherol into bile[20] which recirculates to the gut. Bjorneboe et al.[20] calculated that about 1.92% of tocopherol goes into bile per day which has been assumed to be negligible.

Table 3. Rate constants in tocopherol absorption and metabolism

Process	Location	Rate constants	Source
Absorption in chylomicrons	Small Intestine	*k = 0.841 per day*	Clifford et al., 2006[14]
Absorption of chylomicrons	GI Lumen	V_{max} *= 0.5 nmol/min,* K_m *= 84.3 µM*	Reboul et al., 2006[7]
Metabolism of tocopherol	Liver	*k = 1.0 per day*	Clifford et al., 2006[14]
Release into bile	Liver	*k = 0.0192 per day*	Clifford et al., 2006[14]
Transport into feces	Colon	*k = 0.785 per day*	Clifford et al., 2006[14]

2.1.7 Tissue:Blood partition coefficients

The tissue:blood partition coefficients are calculated for the various tissues from the algorithm developed by Poulin & Krishnan[21]. The algorithm uses the lipid and water contents of the tissues, the values for which were taken from the same paper, except those of spleen which were taken from the work of Christie and Noble[22].

Table 4. Tissue:Blood partition coefficients

Tissue/Organ	Tissue : Blood partition coefficient
Liver	9.378
Brain	12.954
Muscle	7.485
Kidney	7.784
Lung	1.13
Spleen	2.569
Adipose	158.327
Gut	∞
Additional tissues	0

2.1.8 Tissue volumes and blood flow

The volumes of the various tissues have been estimated by various workers. Most of the values in the table below are taken from Davies and Morris[23], the rest being from the work of Wu et al.[24]. Davies and Morris present all data for a 250 gm rat. Since most of the data are from that work, we have stuck to the 250 gm rat. The data from the other

work is however for a smaller rat (139 gm) and so the values have been scaled according to the volume ratios among tissues which are uniform for a species irrespective of size.

Table 5. Tissue volumes and percentage of cardiac flow.

Tissue	Volume (ml)	Percentage of cardiac flow
Liver	19.6	18.65
Brain	1.2	1.76
Kidney	3.7	12.43
Spleen	1.3	0.85
Lung	2.1	100.0
Fat	10.0	0.54
Gut	11.3	10.14
Muscle	245.0	10.14
Blood	13.5	-
Additional Tissues[a]	70.0	100.0

[a] Calculated from Wu et al.[18], adding up eosophagus, heart, large intestine, skeleton, skin & stomach.

2.1.9 Summary

The tissues and processes described above have been summarized in Fig.3 below.

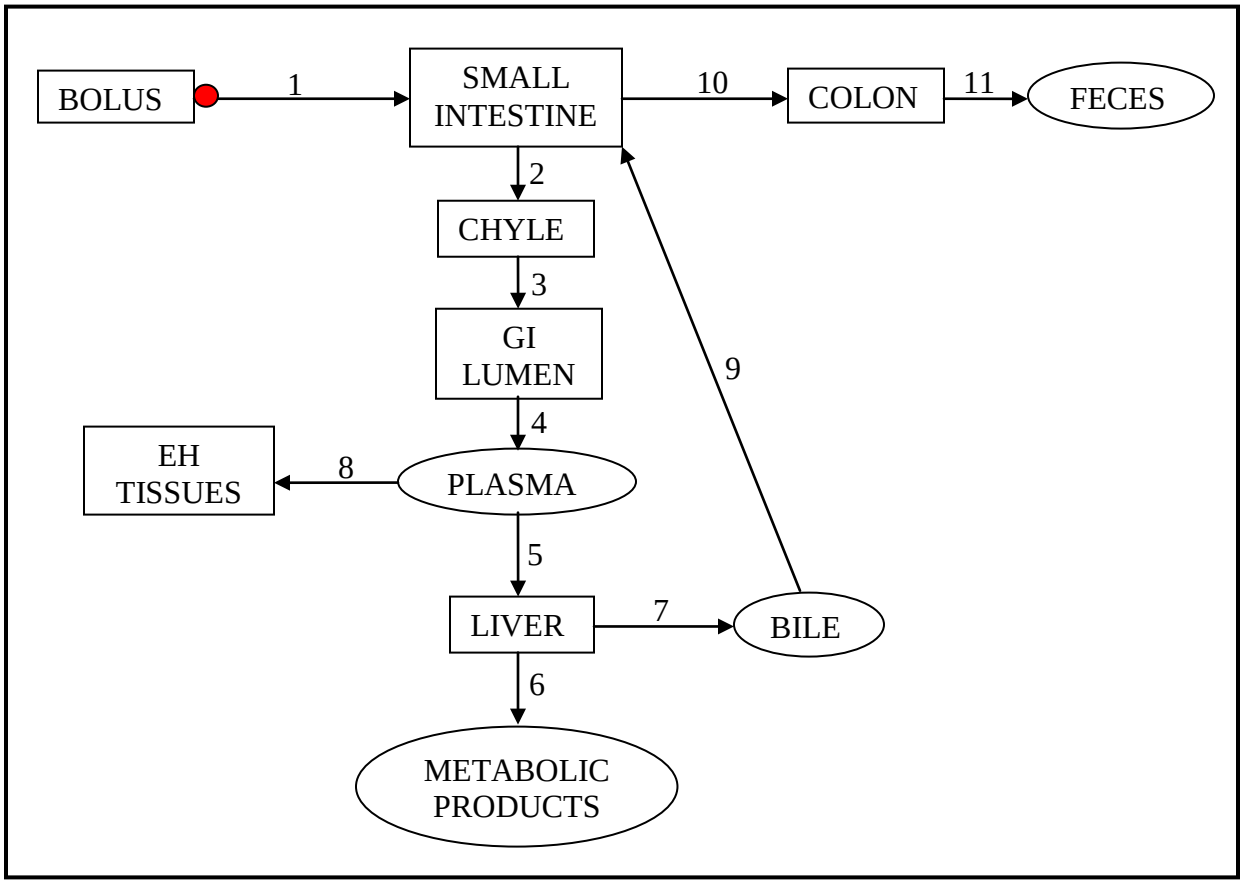


Fig. 3. Schematic diagram of tocopherol distribution and metabolism in the physiological system along with the tissues and body fluids involved. (Only the fluids, tissues and processes related to tocopherol are shown. Additional time lags are lumped at points shown with red circles.)

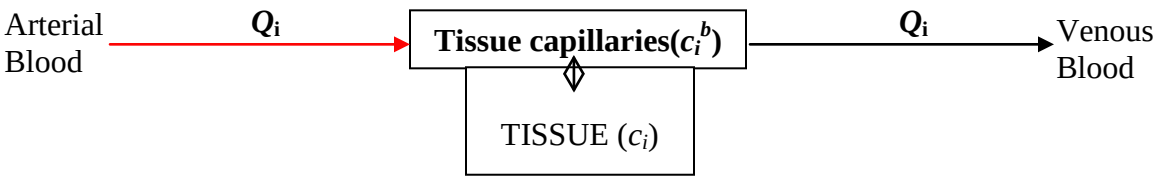
Brief description of the processes in Fig. 3

1. Time delay after oral intake for movement of food bolus to small intestine. (See section on small intestine) This delay has been "mechanically" added.
2. Rate of formation of chylomicrons in small intestine. Volumetric rate constant, *k* in Table 3. Rate, $R\ (mmol/d) = k\ (d^{-1}).V(ml).c(mmol/ml)$
3. Rate of absorption of chylomicrons by the GI Lumen. Rate constant estimated from the Michaelis-Menten constants in Table 3 assuming first order uptake. By a comparison of their rates it is seen that between the processes described here and the last one, this takes place slower. So this rate is taken to be the overall rate of the process.
4. Rate of release into blood plasma is assumed to be instantaneous and so the overall rate is the rate described in the last point.
5. Rate of uptake by liver is governed by the tissue : plasma partition coefficient (Table 4).
6. Rate of metabolism of tocopherol by liver is given by the first order rate constant give in Table 3.
7. Rate of tocopherol release into bile is negligible as discussed before.
8. Rate of uptake by extra-hepatic tissues is governed by the tissue : plasma partition coefficient summarized in Table 4.
9. Rate of tocopherol transport in bile is not important as tocopherol release in bile has been neglected.
10. As discussed earlier, 30% of the ingested tocopherol goes into the colon instantaneously.
11. Rate of excretion in feces is governed by the rate constant given in Table 3.

2.2 Model structure

2.2.1 Balance of tocopherol around a general tissue compartment

Let *i* denote the general index, which takes up different values for the 10 tissue compartments. (Fat -1, Muscle – 2, Brain – 3, Liver – 4, Gut – 5, Kidney – 6, Spleen – 7 and Additional Tissues - 8)



The model is developed on the assumption of flow-limited distribution. The transport of tocopherol to the different tissues is limited by the blood flow to the tissues. Tocopherol concentration in the tissue reaches a fast equilibrium with the tissue capillaries and is related to the capillary concentration by the partition coefficient.

Balance of tocopherol around the i[th] tissue compartment gives:

$$V_i \frac{dc_i}{dt} = Q_i \left(c_{ar} - c_i^b \right) + R_i ,$$

where c_i = conc. of tocopherol in the tissue
c_{ar} = conc. of tocopherol in the arterial blood
c_i^b = conc. of tocopherol in the tissue-blood capillaries
R_i = rate of metabolism of tocopherol within the tissue
Q_i = blood flow in and out of the tissue
V_i = volume of the tissue (Table 5)

There are 2 processes in the biotransformation of tocopherol which are important in this study. One is the packing of tocopherol into the lipid chylomicrons which happens in the enterocyte of the small intestine. The second important process is the metabolism of tocopherol in the liver. The rates of these 2 processes have been estimated from a study by Clifford et al.. (See the sections on small intestine and metabolism.) So R_i is zero for all i except i = 4, 5.

The concentrations of tocopherol in the tissue and the capillaries are related by the tissue:blood partition coefficients as: $P_i = \frac{c_i}{c_i^b}$

Venous blood takes up tocopherol from each compartment and so has an average concentration: $c_{ven} = \frac{1}{Q_{ven}} \sum_i Q_i . c_i^b$

3. Simulation results

Three different case studies have been studied using the model described above to simulate the results. The case studies considered are:

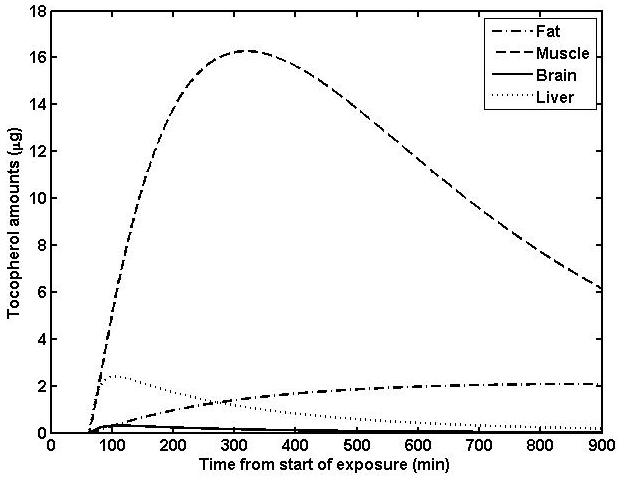
- **Only oral ingestion of tocopherol in solid form.**
- **Only intra-venous injection of solubilized tocopherol**
- **Both oral ingestion as well as injection with a time gap**

Only oral ingestion
20 µg of solid tocopherol is ingested. The dosage must be given to the rats mixed with normal balanced diet.

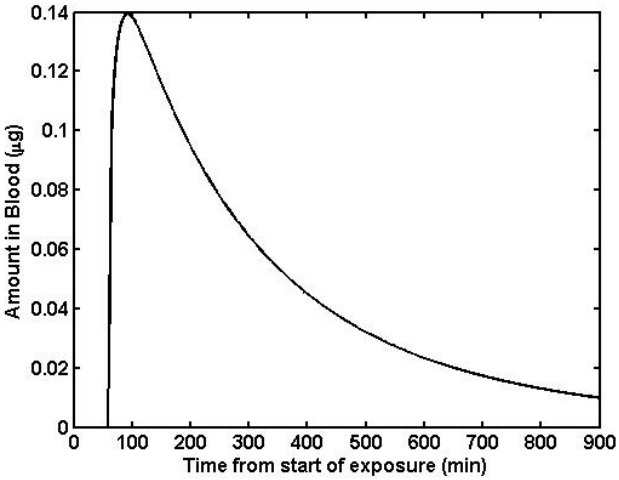
Only intra-venous injection
10 µg of tocopherol is injected at a time point of 1 hour.

Both oral ingestion and intra-venous injection

Oral ingestion in the beginning is followed by an injection after 4 hours. Respective amounts are the same as above.

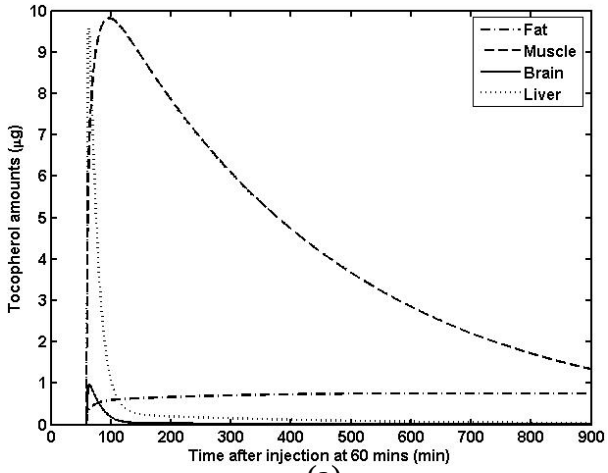


(a)

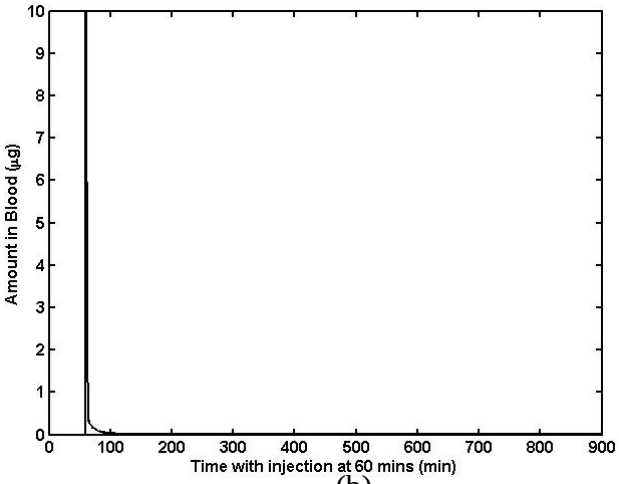


(b)

Fig.3.1. Cumulative amounts of tocopherol in (a) different tissues and (b) blood after oral ingestion

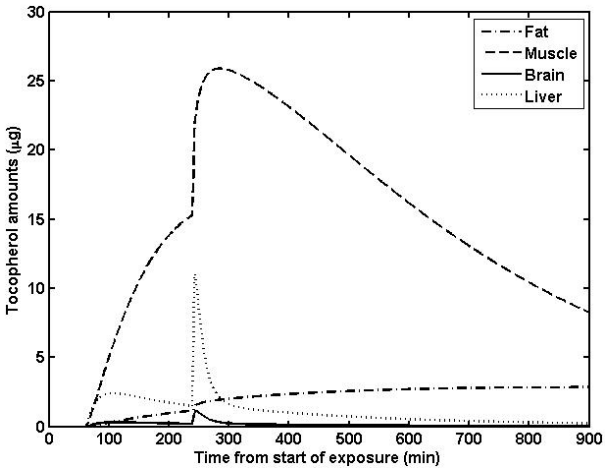


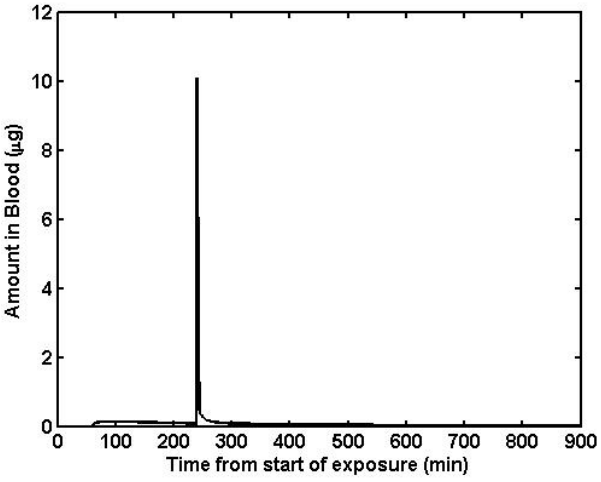
(a)



(b)

Fig.3.2. Cumulative amounts of tocopherol in (a) different tissues and (b) blood after intra-venous injection.





(a) (b)

Fig.3.3. Cumulative amounts of tocopherol in (a) different tissues and (b) blood after intra-venous injection.

4. Discussions

The oral ingestion case scenario shows the time lag of 1 hour which is the time required for the food bolus to reach the intestine after actual ingestion. Fig.3.1(a) shows the comparative analysis of tocopherol amounts in various organs. The amounts absorbed by various organs depend on their respective lipid content and so we have maximum tocopherol absorption in the adipose tissue. All tissues are known to maintain a constant concentration of tocopherol and the results are found to simulate that. Fig.3.1(b) shows the tocopherol content in blood demonstrating a sharp rise due to ingestion after which it reaches constancy. Intra-venous injection which is administered at a time-point of 1 hour shows no such time-lag as it is directly available to the blood stream. Consequently, tocopherol uptake by the tissues from the blood stream is also instantaneous. The case of both ingestion and injection also show very predictable results.

5. Future work

Figure 1 shows the actual pathway of tocopherol transport and absorption. As shown in section 2.1.4, tocopherol is a highly lipophilic substance as described by its high value of octanol-water partition coefficient. Consequently, it is expected to be absorbed predominantly within the lymphatic system. Its release in the blood stream occurs only from the liver. In this analysis, the role of the lymphatic system has been neglected. It may be possible that including the lymphatic system may only serve to bring a separate time-lag in the entire dynamics but not change the dynamics of tocopherol distribution. However, its effect should be studied, nevertheless.

Previous studies[11,2] have shown that tocopherol content in various tissues and blood is maintained at a constant level at all times. The results of modeling as presented by the plots in Figure 3, show that tocopherol amount reaches a constant level after a long time but this level is different for different amount of intake. For example the constant levels in various tissues and blood in Figure.3.1 for oral ingestion is almost double the constant levels in Figure 3.2 for injection. This is because the amount of oral ingestion (20µg) is double that of injection (10µg). To simulate that effect, we should include a feedback mechanism, which probably exists in the physiological system, which can maintain tissue homeostasis irrespective of tocopherol intake.

The values of rate constants have been taken from different works, which have been done under different conditions. Dietary conditions influence tocopherol uptake considerably. Hence the model needs to be validated against experimental results and the parameter values needs to be suitable changed to reflect the actual process.

6. References

1. Kayden H.J., and Traber M.G., Absorption, lipoprotein transport, and regulation of plasma concentration of vitamin E in humans, *Journal of Lipid Research*, 34, 1993, 343-358.
2. Chow C.K., Vitamin E, In *Handbook of Vitamins (3rd Ed.)*, Edited by Rucker R.B, and others, 2001, Marcel Dekker, New York.
3. Lewis L.A., Quaife M.L., and Page I.H., Lipoproteins of serum, carriers of tocopherol, *American Journal of Physiology*, 178, 1954, 221-222.
4. McCormick E.C., Cornwell D.G., and Brown J.B., Studies on the distribution of tocopherol in human serum lipoproteins, *Journal of Lipid Research*, 1, 1960, 221-228.
5. Ingold K.U., Burton G.W., Foster D.O., Hughes L., Lindsay D.A., and Webb A., Biokinetics of and discrimination between dietary RRR- and SRR-α-tocopherols in the male rat, *Lipids*, 22, 1987, 163-172.
6. Traber M.G., Ingold K.U., Burton G.W., and Kayden H.J., Absorption and transport of deuterium substituted 2R,4'R,8'R- α-tocopherol in human lipoproteins, *Lipids*, 23, 1988, 791-797.
7. Reboul E., Klein A., Bietrix F., and others, Scavenger receptor Class B Type I (SR-BI) is involved in Vitamin E transport across the enterocyte, *Journal of Biological Chemistry*, 281(8), 2006, 4739-4745.
8. Anwar K., Kayden H.J., and Hussain M.M., Transport of vitamin E by differentiated Caco-2 cells, *Journal of Lipid Research*, 47, 2006, 1261-1273.
9. Isukapalli S.S., Roy A., and Georgopoulos P.G., Physiologically Based Pharmacokinetic Modeling: Inhalation, Ingestion, and Dermal Absorption, In *Pharmacometrics:The Science of Quantitative Pharmacology*, Edited by Ette E.I and Williams P.J., John Wiley, 2007.
10. Bjorneboe A, Bjorneboe G.E., Bodd E., Hagen B.F., Kveseth N., and Drevon C.A., Transport and distribution of α-tocopherol in lymph, serum and liver cells in rats, *Biochimica et Biophysica Acta* 889, 1986, 310-315.
11. Traber M.A., Cohn W., and Muller D.P.R., Absorption, Transport and Delivery to Tissues, In *Vitamin E in health and disease*, Edited by Packer L., Fuchs J., 1993, Marcel Dekker, New York.
12. Friedrich W., *Vitamins*, 1988, Walter De Gruyter, New York.
13. MacMahon M, Neale G, Thompson G., Lymphatic and portal venous transport of α-tocopherol and cholesterol, *European Journal of Clinical Investigation*, 1(4), 288-294, 1971.
14. Clifford A.J., De Moura F.F., Ho C.C., Chuang J.C., Follett J., Fadel J.G., and Novotny J.A., A feasibility study quantifying in vivo human α-tocopherol metabolism, *The American Journal of Clinical Nutrition*, 84, 2006, 1430-1441.
15. MacMahon M.T., and Neale G., The Absorption of α-tocopherol in control subjects and in patients with intestinal malabsorption, *Clinical Science*, 38, 1970, 197-210.

16. Jandacek R.J., Rider T., Yang Q., Woollett L.A., and Tso P., Lymphatic and portal vein absorption of organochlorine compounds in rats, *American Journal of Gastrointestinal and Liver Physiology*, 296, G226-G234, 2009.
17. Cooper D.A, Webb R.A and Peters J.C, Evaluation of the potential for Olestra to affect the availability of dietary phytochemicals, *Journal of Nutrition*, 127(8), 1997, 1699S-1709S
18. Davies B., and Morris T., Physiological parameters in laboratory animals and humans, *Pharmaceutical Research*, 10(7). 1993, 1093-1095.
19. Drevon C.A., Absorption, transport and metabolism of vitamin E, Free Radical Research Communications, 14(4), 1990, 229-246.
20. Bjorneboe A., Bjorneboe A.G.E., and Drevon C., Serum half-life, distribution, hepatic uptake and biliary excretion of α-tocopherol in rats, Biochimica et Biophysica Acta, 921, 1987, 175-181.
21. Poulin P., and Krishnan K, A biologically-based algorithm for predicting human tissue:blood partition coefficients of organic chemicals, 14, 1995, 273-280.
22. Christie W.W and Noble R.C, The lipid composition of spleen and intestinal and popliteal lymph nodes in the sheep, Lipids, 20(6), 1985, 389-392.
23. Davies B., and Morris T., Physiological parameters in laboratory animals and humans, Pharmaceutical Research, 10(7), 1993, 1093-1095.
24. Wu L., Zhang G., Luo Q., and Liu Q, An image-based rat model for Monte Carlo organ dose calculations, Medical Physics, 35(8), 2008, 3759-3764.