Cholesterol is an essential structural and functional element in the human body. In biological membranes, cholesterol determines their physical properties by being involved in the stabilization of lipid rafts and membrane proteins (1, 25, 26). It is needed for cell-to-cell communications, intracellular transport, nerve conduction, and the response to endocrine signaling (4, 7, 23). Cholesterol acts also as an important precursor in the synthesis of steroid hormones (estrogen, progesterone, and testosterone), vitamin D₃, and cholic acid (2). Consequently, it takes part in the stress response, maintaining the body’s salt and water levels as well as in the regulation of calcium and phosphorus metabolism (3, 8, 17, 27).

The chemical structure of cholesterol was described in 1888 by Friedrich Reinitzer (19), and a single cholesterol molecule is formed by 27 carbon atoms, 46 hydrogen atoms, and 1 oxygen atom grouped in 3 regions: a hydrocarbon tail, a ring structure region with 4 hydrocarbon rings, and a hydroxyl group (1, 19). Due to the polar hydroxyl group, cholesterol is insoluble in water, and, thus, it does not travel well by itself in the bloodstream; instead, it is transported in the form of lipoproteins, particles containing lipids and proteins allowing polar fats and cholesterol to move through the water inside and outside cells (3). These lipoproteins are classified into five main types depending on their density and include low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs), commonly referred to as “bad” and “good” cholesterol, respectively.

Maintaining physiologically healthy level of cholesterol (cholesterol homeostasis) is a complex processes involving multiple factors (10). The metabolism of lipoproteins plays a central role here since lipoproteins mediate the transport of cholesterol to and from tissues (6). Other factors include the intake of dietary cholesterol (~2–3 g/day), regulated cholesterol de novo synthesis (~1 g/day), and controlled cholesterol usage, recycling, and excretion (1, 21). On the other hand, abnormalities in cholesterol serum levels lead to health problems. In particular, prevalent in Western countries are cardiovascular problems associated with high blood LDL levels and lowered HDL levels. In fact, a high blood cholesterol level has been identified as a classic coronary risk factor. It is suspected to cause one-third of coronary heart diseases, which cause up to 2.6 million deaths each year (according to the World Health Organization). Leading a healthy lifestyle focused on a low-cholesterol high-fiber diet and physical activity is advocated to prevent elevated blood cholesterol, whereas statins (cholesterol de novo synthesis inhibitors) are the most widely prescribed class of drugs for lowering high blood cholesterol (12, 22). Although much is already known about cholesterol circulatory transport and de novo synthesis, the exact mechanism responsible for cholesterol homeostasis and its disturbance remain not fully understood and even ambiguous (11, 24).

Obtaining detailed knowledge of human cholesterol homeostasis and the development of cardiovascular diseases is limited, due to ethical restrictions, to research centered on animal feeding studies, epidemiological surveys, or clinical trials (16, 18). The majority of controlled studies can be performed on animals, but species-to-species variability in the plasma cholesterol response to dietary cholesterol as well as the distinctly different plasma lipoprotein profiles of most animal models make the clinical applicability of the obtained findings complicated. Mathematical modeling of cholesterol homeostasis can help in gaining further understanding of the problem and facilitate the selection of the optimal preventive measures and treatment methods. To our knowledge, not much has been done in this area. A few attempts to model cholesterol transport were made in the 1970s, along with studies assessing the long-term turnover of plasma cholesterol examined by injecting intravenously isotopically labeled cholesterol (13). The previous work included models of lipoprotein dynamics, e.g., a model of the fluid dynamics of lipid accumulation on the arterial walls (14) and chemical kinetics of LDL oxidation (5), as well as a model of lipoprotein metabolism (2). Building on that, we have previously developed a two-compartment mathematical model
to investigate cholesterol circulatory transport and de novo synthesis in the liver and showed its applicability to investigate the processes associated with high blood cholesterol diagnosis and treatment (15). As students often have problems with understanding the key players and their relationship in maintaining cholesterol homeostasis, we prepared a step-by-step algorithm to use the model and prepared three interactive exercises to facilitate students’ learning experiences. Here, we demonstrate the teaching aspects of our model for the complex mechanisms of cholesterol homeostasis aimed toward undergraduate medical students or those in related fields.

METHODS

Two-Compartment Mathematical Model of Cholesterol Homeostasis

Details on the development of the model can be found in Ref. 15. Briefly, we have reviewed cholesterol homeostasis-related publications and gathered textbook knowledge to outline the key pathways of human cholesterol circulatory transport and de novo synthesis (Fig. 1). We distinguished the following six main stages.

First, exogenous cholesterol enters the gastrointestinal tract during meals with foods containing animal cholesterol-rich products (Fig. 1, pathway 1), and, upon reaching the small intestine, it is absorbed into the lymphatic system in the form of chylomicrons. These gradually release fatty acids (energy source) and form remnants, which enter the bloodstream and are taken up by the liver via specific receptors (pathway 2).

Second, bile circulates between the intestine and liver (pathways 3 and 4). Bile released from the liver contains up to 8% cholesterol, whereas bile returning to the liver via the portal vein contains an additional amount of cholesterol derived from membranes of dead cells of the intestinal epithelium (up to 500 mg cholesterol/day). An additional factor linking bile and cholesterol is cholic acid, which constitutes >60% of bile and is partially synthesized in the liver from dead enterocytes. The amount of cholesterol in the second compartment is dependent on the amount of cholesterol derived from membranes of dead cells (Fig. 1, pathway 5). The synthesis rate depends on the amount of cholesterol already present in the liver: in principle, the lower amount of cholesterol, the higher the de novo synthesis rate and vice versa.

Fourth, cholesterol is released from the liver into the bloodstream in the form of very-low-density lipoproteins, which are transformed to intermediate-density lipoproteins (pathway 6) and subsequently to LDLs (pathways 7 and 8). LDLs are captured by specific cellular receptors (pathway 8). LDLs are commonly referred to as bad cholesterol as the development of arteriosclerosis (thickening and hardening of the walls of arteries, hindering blood flow) has been linked with the occurrence of oxidized forms of LDLs. Oxidized cholesterol is accumulated in macrophages (pathway 9), which are gradually converted into foam cells building up atherosclerotic lesions.

Fifth, the reverse cholesterol transport, back from the peripheral tissues to the liver, is based on apolipoprotein A1, which is synthesized in the liver. With the help of macrophages, apolipoprotein A1 is enriched in redundant cholesterol in the body. Lipoproteins involved include pre-β-1-HDLs, pre-β-2-HDLs, HDL3s, and HDL2s.

Finally, cholesteryl ester transfer protein facilitates the exchange of cholesterol ester between the lipoproteins (pathway 10). These six stages were translated into a two-compartment model, where the first compartment represents blood flowing through the liver and the second compartment reflects blood in the peripheral tissues (Fig. 2). The model is described by a set of two ordinary differential equations expressing the rate of changes of the cholesterol mass in the two compartments, respectively (Eqs. 1 and 2).

\[
\frac{dm_1}{dt} = k - m_1 + k_2m_2 - k_1m_1 + m_{in} - m_{out} \quad (1)
\]

\[
\frac{dm_2}{dt} = -k_2m_2 + k_1m_1 - m_{in} + m_{diet} \quad (2)
\]

The amount of cholesterol in the first compartment is dependent on the rate of exchange with the first compartment, respectively (Eqs. 1 and 2).

Fig. 1. Overview of the current knowledge of main biochemical processes of cholesterol homeostasis. C, dietary cholesterol intake; CM, chylomicrons; CR, cholesterol remnants; FFA, free fatty acids; APOA1, apolipoprotein A1; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein; CETP, cholesteryl ester transfer protein; oxidized cholesterol; LCAT, lecithin-cholesterol acyltransferase; PLTP, phospholipid transfer protein; ABCA1, ATP-binding cassette subfamily A member 1.
Values of model parameters

Table 1. Values of model parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Model Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_1$</td>
<td>Cholesterol mass in the blood flowing through the liver (compartment I)</td>
<td>2,400 mg (15)</td>
</tr>
<tr>
<td>$m_2$</td>
<td>Cholesterol mass in the peripheral blood (compartment II)</td>
<td>8,600 mg (9)</td>
</tr>
<tr>
<td>$V_1$</td>
<td>Blood volume in the liver (compartment I)</td>
<td>12 dl (2)</td>
</tr>
<tr>
<td>$V_2$</td>
<td>Peripheral blood volume (compartment II)</td>
<td>43 dl (2)</td>
</tr>
<tr>
<td>$k$</td>
<td>Rate of de novo cholesterol synthesis</td>
<td>1,500 mg²/min (3)</td>
</tr>
<tr>
<td>$k_{12}$</td>
<td>Rate of cholesterol transport from the liver to blood (compartment I to II)</td>
<td>1.0 min⁻¹ (15)</td>
</tr>
<tr>
<td>$k_{21}$</td>
<td>Rate of cholesterol transport from blood to the liver (compartment II to I)</td>
<td>3.58 min⁻¹ (15)</td>
</tr>
<tr>
<td>$m_{out}$</td>
<td>Cholesterol taken up from the intestine to the liver</td>
<td>1 mg/min (20, 21)</td>
</tr>
<tr>
<td>$m_{out}$</td>
<td>Cholesterol used as a precursor of bile acids and forming part (~8%) of bile</td>
<td>1.4 mg/min (15)</td>
</tr>
<tr>
<td>$m_{diet}$</td>
<td>Exogenous cholesterol obtained through diet</td>
<td>0 ± 1.1 mg/min (20, 21)</td>
</tr>
<tr>
<td>$m_{tot}$</td>
<td>Cholesterol taken up by peripheral tissues and muscles</td>
<td>0.234 mg/min (20, 21)</td>
</tr>
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Table 2. Content and objective of the three interactive student tasks for cholesterol homeostasis

<table>
<thead>
<tr>
<th>Task</th>
<th>Description</th>
<th>Teaching Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monitoring blood cholesterol levels</td>
<td>Students select a few model parameters, e.g., rates of cholesterol exchange between compartments, modify their values (e.g., increasing/decreasing by 10%), and monitor and analyze the corresponding changes in blood cholesterol levels</td>
<td>Familiarity with physiological processes behind cholesterol homeostasis as captured by the model; understanding the relationship between changes in model parameters and blood cholesterol levels</td>
</tr>
<tr>
<td>High blood cholesterol treatment</td>
<td>Students simulate the increase of the blood cholesterol levels due to impaired cholesterol uptake by tissues for 2 mo (reducing the value of ( m_{\text{in}} ) by 85%) and monitor high blood cholesterol level treatment with statins over a month, modeled by lowering values of ( k_{\text{de novo}} ) cholesterol synthesis; after a month, students stop the treatment and observe blood cholesterol levels for a week</td>
<td>Familiarity with applying the model to simulate possible real-case clinical scenarios</td>
</tr>
<tr>
<td>Mastering cholesterol homeostasis</td>
<td>Students implement their own ways of disturbing blood cholesterol levels and treatment strategies</td>
<td>Mastering the usage of the model; deepening and strengthening cholesterol homeostasis knowledge</td>
</tr>
</tbody>
</table>

**Interactive Tasks**

**Interactive task 1: monitoring blood cholesterol levels over a range of physiological conditions.** After familiarizing themselves with running the simulations, students are asked to generate steady-state solutions using the default values of parameters as shown and assuming no dietary cholesterol intake, that is, \( m_{\text{diet}} = 0 \). The proper solution yields blood cholesterol values (\( C_2 \)) oscillating (due to numeric solutions) around 200 mg/dl. Cholesterol concentration values instead of mass are used to be able to compare the results with standard laboratory practices and cholesterol levels guidelines (e.g., \( C_2 = m_2/V_2 \), where \( V_2 \) is the peripheral blood volume). Now, students are asked to experiment with the values of the model parameters and observe the changes in blood cholesterol levels.

For instance, responses to 10% or 20% increase and decrease changes in the model parameters \( m_{\text{out}} \) and \( k \) can be observed (Fig. 4, A and B). Students can compare effects of the different parameters values on blood cholesterol levels and discuss which pathway disturbances have the largest influence on cholesterol homeostasis.

**Interactive task 2: treating high blood cholesterol levels with de novo cholesterol inhibitors.** In the second task, students simulate an elevated blood cholesterol level and its treatment as well as weekly discontinuation of the treatment. Overall, students make three iterations through the algorithm, solving the differential equations for the three cases (raising the blood cholesterol level, treatment, and treatment discontinuation; Fig. 4C).

High blood cholesterol levels can be caused, for instance, by a diet rich in animal products or by less prevalent genetic disorders affecting the LDL receptor in tissues (familial hypercholesterolemia). Given our model, students can simulate such blood cholesterol level increases by reducing values of \( m_{\text{out}} \), the parameter responsible for cholesterol uptake by peripheral tissues and muscles. Assuming 2-mo of disturbed cholesterol uptake, students can observe an increase of \( C_2 \) up to 275 mg/dl. A full set of parameters for this run includes the following: initial values of \( m_1 = 2,400, \ m_2 = 8,600, \ k_{12} = 3.5, \ k_{21} = 1, \ k = 1,500, \ m_{\text{in}} = 1, \ m_{\text{out}} = 1.4, \ m_{\text{tis}} = 0.0351, \) and \( m_{\text{diet}} = 0 \) and the 2-mo interval expressed in minutes (\( t_0 = 0, t_1 = 86,400 \)).

Second, students model the treatment of the elevated blood cholesterol level with statins, a class of drugs used to lower cholesterol levels by inhibiting the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase, which plays a central role in the production of cholesterol in the liver. This can be directly simulated by lowering the values of \( k \) de novo cholesterol synthesis, e.g., by 30%, and by administering treatment over a month. Students change the parameter values to \( m_1 = 3,379, \ m_2 = 11,828 \) (as obtained at the end of the 2-mo increasing blood cholesterol period), \( k_{12} = 3.5, \ k_{21} = 1, k = 1,050, m_{\text{in}} = 1, m_{\text{out}} = 1.4, m_{\text{tis}} = 0.0351, \) and \( m_{\text{diet}} = 0 \) as well as the 2-mo interval expressed in minutes (\( t_0 = 86,400, t_1 = 129,600 \)). Finally, to observe the changes in blood cholesterol during a weekly discontinua-
tion of the treatment, students change the parameters back to the first set, adjust the initial values of cholesterol mass as obtained at the end of the 1-mo treatment ($m_1 = 2,645$ and $m_2 = 9,258$), and set a weekly time interval ($t_0 = 129,600$, $t_1 = 139,680$). The changes of blood cholesterol over the three periods (2-mo blood cholesterol increase, 1-mo treatment, and 1-wk treatment discontinuation) can be shown on one graph (Fig. 4

Interactive task 3: further disturbing and treating cholesterol homeostasis. The model offers ample possibilities for observing changes in blood and liver cholesterol levels under a wide range of physiological conditions, simulated by varying values of model parameters. To ensure students have an active understanding of the modeled cholesterol homeostasis processes, the third task requires students to find their own way of cholesterol homeostasis disturbances and countermeasure strategies. For instance, students can simulate other treatments used for lowering high blood cholesterol. In addition to statins, common treatment includes drugs that bind bile to lower the amount of cholesterol returned to the liver via the portal vein and/or changing diet to the one rich in high-fiber products that increase intestinal peristalsis, reducing intestinal cholesterol absorption (both modeled by reducing values of $m_{in}$). Another example of improving unbalanced cholesterol homeostasis is by increasing levels of good cholesterol (HDLs) and lowering levels of bad cholesterol (LDLs). Drugs inhibiting cholesteryl ester transfer protein activity, involved in transferring cholesterol esters between different lipoprotein fractions, are currently under clinical trials, and their effect can be modeled by a simultaneous reduction of $k_{21}$ values and an increase of $k_{12}$ values.

DISCUSSION

Maintaining healthy levels of blood cholesterolhas been established as a key to prevent the development of cardiovascular diseases. Nowadays, basic blood tests measure blood cholesterol levels, and it is a common knowledge that preventing high blood cholesterol levels should be centered on healthy and active lifestyles. Despite that, we found that students struggle with learning about cholesterol homeostasis, most likely due to the fact it is an intensely regulated process involving many factors, both at the molecular and tissue level (10, 21). To this end, we show how a relatively simple two-compartment model of circulatory cholesterol transportation and de novo synthesis can help students to actively learn, understand, and memorize basic mechanisms behind cholesterol homeostasis.

We offered an opportunity to follow the above interactive exercises to first-year biomedical engineering students. During the 90-min computer practical, the majority of students were able to get comfortable with operating the scripts, especially the more user-friendly Matlab version, and were able to complete all three interactive tasks. Students gave positive feedback and confirmed that the hands-on experiments on cholesterol homeostasis were fun and helpful. By allowing a great flexibility in the third task, it was easy to spot and help students with difficulties. The more ambitious students recognized the
limitations of the model and were eager to discuss ways to improve it. For instance, the model in its current state cannot simulate the gallbladder pathway, in which bile and within in cholic acid, containing 8% of cholesterol, is secreted from the liver to the gallbladder for storage. This level of regulation can be interpreted in the physiological, non-abstract, context, e.g., $k_{12}$ and $k_{21}$ can be considered as LDL and HDL fractions, respectively, and their default ratio is equivalent to the recommended optimal healthy HDL-to-LDL ratio of 3.5 to 1.

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DISCLOSURES
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AUTHOR CONTRIBUTIONS
Author contributions: A.W. performed experiments; A.W. prepared figures; A.W. and O.H. edited and revised manuscript; J.B. analyzed data; J.B. interpreted results of experiments; O.H. drafted manuscript; K.K. conception and design of research; K.K. approved final version of manuscript.

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