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Metabolic Interrelationships Software Application

INTERACTIVE LEARNING TOOL FOR INTERMEDIARY METABOLISM*

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Adrie J. M. Verhoeven‡§, Mathijs Doets¶, Jos M. J. Lamers‡, and Johan F. Koster‡

From the ‡Department of Biochemistry and ¶Institute of Education, Erasmus Medical Center Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

We developed and implemented the software application titled Metabolic Interrelationships as a self-learning and -teaching tool for intermediary metabolism. It is used by undergraduate medical students in an integrated organ systems-based and disease-oriented core curriculum, which started in our medical faculty in 2001. The computer program provides an interactive environment in which students learn to integrate the major metabolic pathways as well as their hormonal control mechanisms as far as they depend on nutritional status. Students can explore the time- and tissue-dependent changes in mammalian intermediary metabolism during a feeding-fasting cycle. Starting from a whole-body view of interorgan nutrient fluxes, the student can make excursions to individual organs and, from there, to increasing levels of molecular detail and to explanatory animations. The application is well received by students and staff.

Keywords: Intermediary metabolism, nutritional status, educational software, medical curriculum.

Like many medical schools, the Erasmus Medical Center Rotterdam now teaches biochemistry and molecular biology as part of an integrated organ systems-based and disease-oriented core curriculum. Since 2001, biochemistry in the first and second year is no longer taught in separate courses, but the discipline is fully integrated into several multidisciplinary thematic modules, each lasting 15–17 weeks. The teaching of "energy metabolism" has been assigned to parts of the first year module titled Disorders of the Cardiovascular, Pulmonary, and Kidney Systems and "intermediary metabolism" to the second year module titled Disorders of Nutrition, Metabolism, and Hormonal Regulation. In the first year thematic module, biochemistry teachers first briefly repeat the basics of cell biology and biochemistry. This is followed by more specific lessons on the energy requirements of (cardiac) muscle during aerobic and anaerobic performance, as needed for the understanding of functions of the cardiovascular system. The second year thematic module deals with intermediary metabolism, which is focused on whole-body energy balance in general, the storage of nutrients after food intake, the sequential mobilization of carbohydrates, fat, and protein during fasting, and the dysregulation of metabolism in *e.g.* obesity and diabetes. Teaching these subjects is limited to a three-week period in which students also have to devote attention to other subjects, such as maintenance of body composition, protein-energy malnutrition, inherited metabolic diseases, vitamin deficiencies, and drug disposal by the liver. In 2001, the medical curriculum not only changed from a discipline-based to an integrated organ systems-based curriculum but also from a teacher-centered to a student-centered self-learning formula. This change in curriculum urged teachers to reconsider the formula and the content of their teaching program.

In our long standing teaching experience, intermediary metabolism has always been a rather difficult subject for medical undergraduates. Traditionally, courses in intermediary metabolism start from the individual biochemical pathways and their regulation. Although students have done reasonably well in memorizing the individual pathways, all too often they get lost when it comes to the integration of the major pathways, *e.g.* in different nutritional states, during and after exercise, or in metabolic derangements due to various diseases. The digitalized Metabolic Pathways map and the IUBMB-Nicholson Minimaps [1] are excellent tools to study all the interconnecting pathways for the initiated, but their huge detail obscures the overall picture and is likely to frighten away the uninitiated. Moreover, medical students have little knowledge of and appreciation for molecular structures, which makes it even harder for them to see the logic behind the interconnecting pathways. In addition, students have difficulty integrating the different but interdependent roles of various tissues and organs into an overall picture of whole-body metabolism. Our opinion is that teaching of intermediary metabolism may be better served by starting immediately

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§ To whom correspondence should be addressed: Dept. of Biochemistry, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands. E-mail: a.verhoeven@erasmusmc.nl.

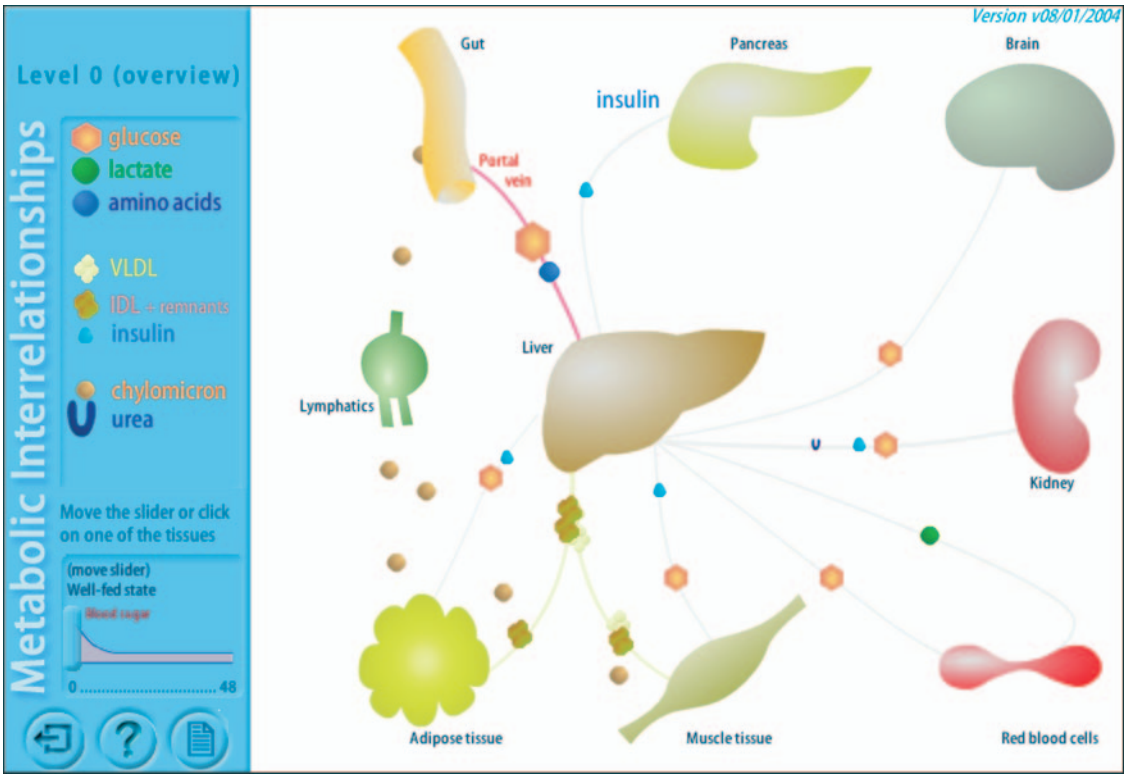


FIG. 1. **Opening screen of the computer program Metabolic Interrelationships.** The *main screen* shows the different organs. *Moving symbols* depict fluxes of the nutrient involved. The *left panel* gives, from *top* to *bottom*, an indication of the level of detail, a *legend* explaining the different symbols, and a *slider* with which the student can change the elapsed time since the last meal. At the *left corner bottom*, three icons indicate an *exit button*, a *help button*, and a *text button* that opens an explanatory text box corresponding to the main panel.

from the perspective of the whole body before introducing the biochemical details of the various pathways and specifying differences between organs [2].

To meet the needs of an integrated organ systems-based and student-centered curriculum (self-learning by exploration), we decided to develop a multimedia application as a tool for self-learning the interrelations between nutritional status and intermediary metabolism for medical undergraduate students. This software describes the sequential changes that occur in the metabolism of carbohydrates, fats, and protein following ingestion of a mixed meal and subsequent prolonged fasting. Starting from a whole-body perspective, overall nutrient fluxes and outlines of biochemical pathways are described for different organs. At any time point and for every organ, the student can zoom in to increasing levels of biochemical detail or to explanatory animations of processes that are difficult to understand from the printed page, *e.g.* glycogen (de)-branching or the carnitine shuttling of acyl-CoAs over the mitochondrial membrane.

DESCRIPTION OF THE SOFTWARE

Metabolic Interrelationships is a multimedia application written in Macromedia Director. The program can be run as a stand-alone Windows executable or it can be started from a web browser, provided that the Macromedia Shockwave player is installed. To enable use of the program in computer classrooms, sounds have been omitted; instead, boxes with explanatory texts light up at appropriate times and mouse movements.

The program is based on the description of nutrient fluxes between eight different organs (gut, liver, muscle, brain, adipose, kidney, erythrocytes, and pancreas) during one feeding-starvation cycle, as described in the integrative chapter on metabolic interrelationships in Devlin [3] written by Harris and Crabb [4]. The program opens with a view of the eight organs in the well fed state immediately after ingestion of a mixed meal and entry of the digestion products into the circulation (Fig. 1). By moving a slider, the student can gradually change the elapsed time since the last meal from 0 to 48 h. This will gradually change the view from the well fed state to successively, the postabsorptive, the early fasting, and the prolonged fasting state. Fluxes of nutrients between different organs through the circulation are indicated by the continuous flow of symbols from one organ to the other. Changes in flux magnitudes are indicated by an increase or decrease of symbol sizes. At any time during the feeding-starvation cycle, a simple mouse click at each organ will zoom into the metabolic processes that occur within this organ (Fig. 2). At each sublevel, the student can again change the elapsed time since the last meal and examine the changes in metabolic routes within this particular organ. Changes in flux magnitude and direction of the metabolic routes are indicated by the appearance or fading of arrows between key intermediate metabolites. Simultaneously, the changes in plasma concentrations of the pancreatic hormones insulin and glucagon and their main targets in each organ, are indicated. Magnifying glass icons overlaying branch points and control sites within the metabolic routes indicate that

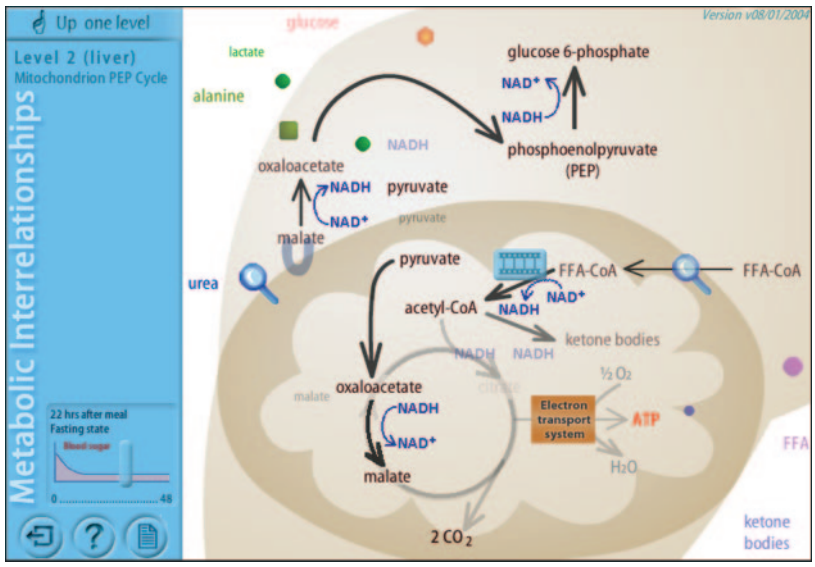


FIG. 2. **When liver glycogen is exhausted, the liver produces glucose from gluconeogenesis and ketone bodies from adipocyte-derived fatty acids.** This is a *screen shot* representing the situation in the liver at 22 h after the last meal (see *slider* at the *left bottom corner*) after two consecutive mouse clicks on the liver in the opening screen (see *level indicator* at the *left top corner*). Processes that occur in the mitochondria are clearly discriminated from those in the cytosol. The two *magnifying glass icons* indicate a deeper level of biochemical detail underneath, which can be opened by clicking there. By clicking on the *"up one level" field* in the *left upper corner*, one can go to the previous screen. By clicking on the *filmstrip icon*, an animation of β-oxidation can be viewed.

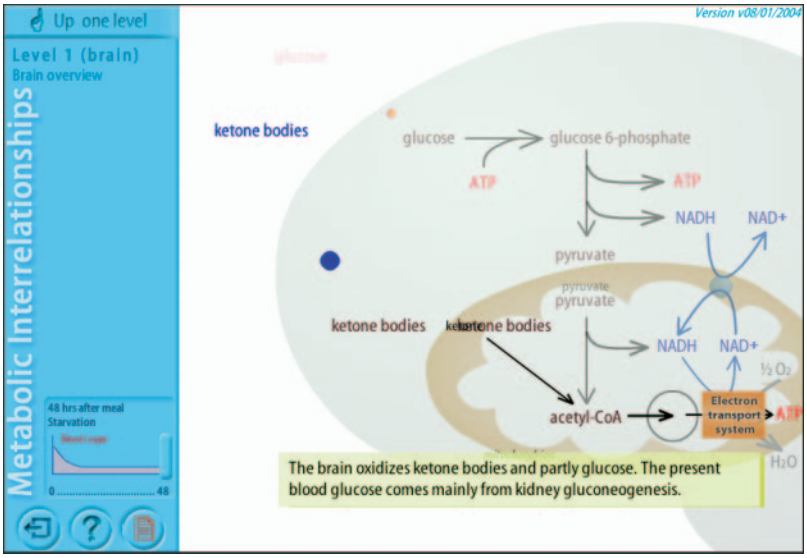
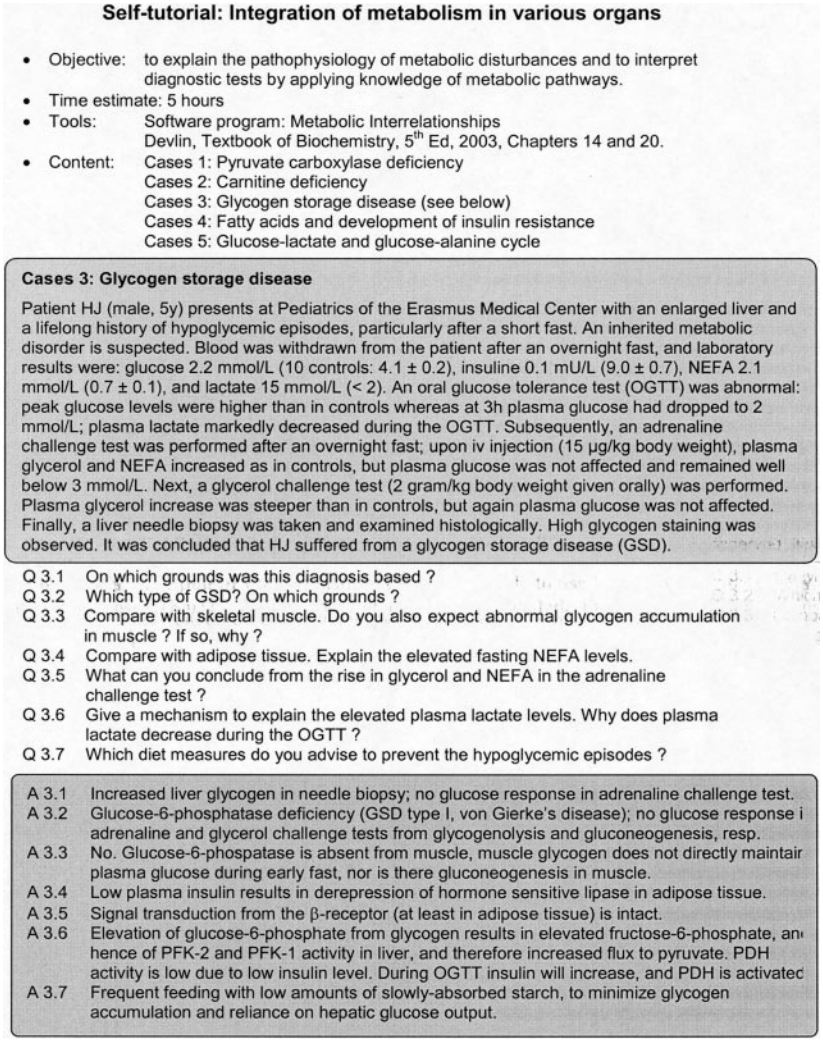


FIG. 3. **Upon prolonged fasting, the brain is able to use ketone bodies for energy generation.** This is a *screen shot* representing the situation in the brain at 48 h after the last meal (see *slider* at the *left bottom corner*) after one mouse click on the brain in the opening screen (see *level indicator* at the *left top corner*). By moving the mouse cursor over the text icon in the *bottom corner* of the *left panel*, an explanatory text box is projected on *top* of the image. This *window* is the highest level of detail currently offered for the brain.

a window with a higher level of biochemical detail is present underneath, which may be entered on a mouse click. Similarily, a filmstrip icon indicates the presence of explanatory animations underneath (Fig. 2). For the liver, four consecutive upward levels of biochemical detail have been built in. For the other tissues, fewer or even no upward levels have been designed yet. Throughout the program, appropriate short explanatory text boxes are made available by the movement of the mouse cursor over a text icon (Fig. 3).

The program is developed as an add-on application so that modules and animations can easily be added afterward. In fact, additions and improvements are made on a yearly basis. Currently, we are working on the incorporation of plasma lipoprotein metabolism and the elaboration of amino acid metabolism. We have also planned to include a self-assessment module with a limited number of multiple choice questions and model answers. However, it is clearly not our intention nor within our competence to present complete maps of interconnecting metabolic pathways. For this, the users are referred to the eloquent IUBMB-Nicholson Mini-maps, which are readily accessible on the Internet [5]. Also, we have not included molecular structures of any of the metabolites, because these are well presented in textbooks and on the Internet.

We have chosen to enable the students to gradually, instead of stepwise, change the metabolic condition from the well fed to the prolonged fasting state. In this way, the user gets a better feeling about the time frames that may be involved, and it stresses the fact that changes in metabolism occur gradually over time rather than abruptly. However, this forced us to decide at which time after a mixed meal liver glycogenolysis or gluconeogenesis starts or when the brain starts to consume ketone bodies, etc. By nature, these time points cannot be given exactly, because this will differ between individuals and may depend on other conditions, such as meal size, body composition, or physical activity. We have arbitrarily set the time frame for gluconeogenesis to fall again and for the brain to start oxidation of ketone bodies well within 48 h after the last meal. Further quantitative changes in whole-body metabolism will continue thereafter. Full adaptation will be attained only after about three weeks of total starvation [6], a rather extreme condition.



Self-tutorial: Integration of metabolism in various organs

- Objective: to explain the pathophysiology of metabolic disturbances and to interpret diagnostic tests by applying knowledge of metabolic pathways.
- Time estimate: 5 hours
- Tools: Software program: Metabolic Interrelationships
  Devlin, Textbook of Biochemistry, 5th Ed, 2003, Chapters 14 and 20.
- Content: Cases 1: Pyruvate carboxylase deficiency
  Cases 2: Carnitine deficiency
  Cases 3: Glycogen storage disease (see below)
  Cases 4: Fatty acids and development of insulin resistance
  Cases 5: Glucose-lactate and glucose-alanine cycle

Cases 3: Glycogen storage disease

Patient HJ (male, 5y) presents at Pediatrics of the Erasmus Medical Center with an enlarged liver and a lifelong history of hypoglycemic episodes, particularly after a short fast. An inherited metabolic disorder is suspected. Blood was withdrawn from the patient after an overnight fast, and laboratory results were: glucose 2.2 mmol/L (10 controls: 4.1 ± 0.2), insuline 0.1 mU/L (9.0 ± 0.7), NEFA 2.1 mmol/L (0.7 ± 0.1), and lactate 15 mmol/L (< 2). An oral glucose tolerance test (OGTT) was abnormal: peak glucose levels were higher than in controls whereas at 3h plasma glucose had dropped to 2 mmol/L; plasma lactate markedly decreased during the OGTT. Subsequently, an adrenaline challenge test was performed after an overnight fast; upon iv injection (15 µg/kg body weight), plasma glycerol and NEFA increased as in controls, but plasma glucose was not affected and remained well below 3 mmol/L. Next, a glycerol challenge test (2 gram/kg body weight given orally) was performed. Plasma glycerol increase was steeper than in controls, but again plasma glucose was not affected. Finally, a liver needle biopsy was taken and examined histologically. High glycogen staining was observed. It was concluded that HJ suffered from a glycogen storage disease (GSD).

Q 3.1 On which grounds was this diagnosis based ?
Q 3.2 Which type of GSD? On which grounds ?
Q 3.3 Compare with skeletal muscle. Do you also expect abnormal glycogen accumulation in muscle ? If so, why ?
Q 3.4 Compare with adipose tissue. Explain the elevated fasting NEFA levels.
Q 3.5 What can you conclude from the rise in glycerol and NEFA in the adrenaline challenge test ?
Q 3.6 Give a mechanism to explain the elevated plasma lactate levels. Why does plasma lactate decrease during the OGTT ?
Q 3.7 Which diet measures do you advise to prevent the hypoglycemic episodes ?

A 3.1 Increased liver glycogen in needle biopsy; no glucose response in adrenaline challenge test.
A 3.2 Glucose-6-phosphatase deficiency (GSD type I, von Gierke's disease); no glucose response i adrenaline and glycerol challenge tests from glycogenolysis and gluconeogenesis, resp.
A 3.3 No. Glucose-6-phospatase is absent from muscle, muscle glycogen does not directly maintair plasma glucose during early fast, nor is there gluconeogenesis in muscle.
A 3.4 Low plasma insulin results in derepression of hormone sensitive lipase in adipose tissue.
A 3.5 Signal transduction from the β-receptor (at least in adipose tissue) is intact.
A 3.6 Elevation of glucose-6-phosphate from glycogen results in elevated fructose-6-phosphate, an hence of PFK-2 and PFK-1 activity in liver, and therefore increased flux to pyruvate. PDH activity is low due to low insulin level. During OGTT insulin will increase, and PDH is activated
A 3.7 Frequent feeding with low amounts of slowly-absorbed starch, to minimize glycogen accumulation and reliance on hepatic glucose output.

FIG. 4. **Part of a self-tutorial that is based on the use of the software program Metabolic Interrelationships.** This self-tutorial is scheduled at the end of the three-week subtheme on Intermediary Metabolism. Only one of the five cases in this self-tutorial is given in detail here. Shorthand answers are not supplied together with the tutorial, but are made available to the students via the intranet.

EMBEDDING OF THE PROGRAM IN THE CURRICULUM

For teaching intermediary metabolism to second year medical students, we have included several written tutorials in the syllabus that comes with the thematic module titled Disorders of Nutrition, Metabolism, and Hormonal Regulation. These tutorials use Devlin [3] as the accompanying textbook on biochemistry. The program Metabolic Interrelationships is made available to the students via the university intranet and as a CD-ROM accompanying the syllabus. Use of the software program is self-explanatory.

After an introductory lecture on the "storage" and "production" mode of metabolism [6], the students are initiated with the program in a teacher-assisted computer practical. Their assignment is to determine the routes of exogenous glucose, amino acids, and fatty acids from the intestine to their final destination and storage forms. Subsequently, they are asked to determine the sources and fates of the endogenous glucose, amino acids, and fatty acids after a 24-h fast. At this stage, the metabolic pathways are examined without much biochemical detail. During the remainder of the three weeks, students acquire knowledge on the interconnecting biochemical pathways and details of the mechanisms of their regulation via three lectures on metabolic interconversions during starvation, lipoprotein metabolism, and metabolic diseases, successively. They deal with the five phases of glucose homeostasis, the various metabolic effects of alcohol [7], the principles of regulation of metabolic pathways, and the role of the endocrine pancreas in corresponding teacher-assisted assignments lasting 2 h each. Simultaneously, they have non-assisted tutorials on fuel utilization in athletes, the ultimate hunger strikers in Northern Ireland during the 1970s [8], diet and lipoproteins, regulation and counter-regulation, energy expenditure during extreme endurance exercise of Antarctic travelers [9], and the vitamin deficiencies accompanying alcoholism. For these self-study tutorials, which take the students ~3 h each, use of the software program is advised and indicated in the text. In a final self-study tutorial on inherited metabolic diseases, students have to work with the software program to explain the metabolic consequences of *e.g.* pyruvate carboxylase and carnitine deficiency and of glycogen type I storage disease. As an example, a part of this self-tutorial is given in Fig. 4. During the three weeks, model answers to the self-tutorials are made gradually available to the students for feedback and self-correction. Assessment follows two weeks later as part of an integrated exam in which multiple choice and open-ended questions on intermediary metabolism are alternated with questions on the other subjects taught during these weeks. In this exam, 21 of 56 questions (37.5%) concern intermediary metabolism, in agreement with the relative study load of this discipline during

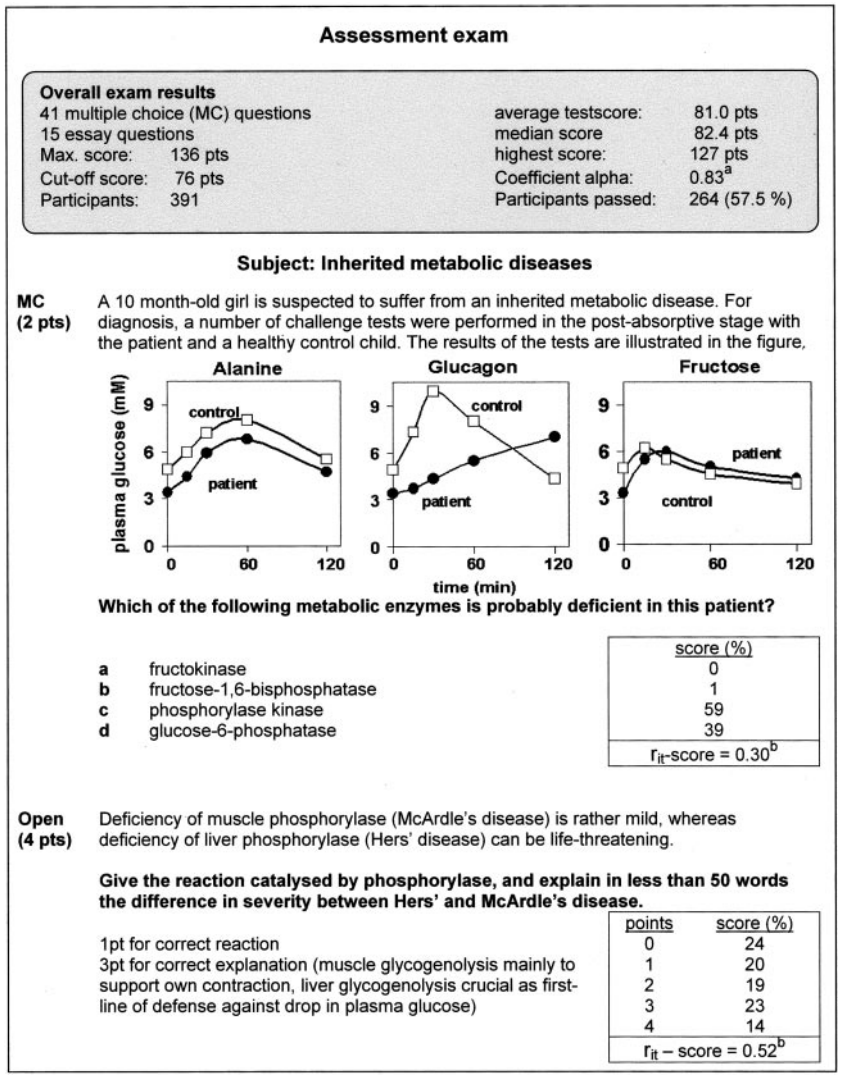


FIG. 5. **Performance of example exam questions on intermediary metabolism.** The assessment exam was held among second year medical students during Spring 2005. Overall exam results and two sample questions related to the self-tutorial in Fig. 4 are given. *a*, coefficient α is a reliability index for the exam and is a measure for the internal consistency of the exam. α ranges from 0 to 1 and should be >0.75. *b*, the r_{it}-score is the linear correlation coefficient between the item *versus* the total test score for all participants and is a measure for the discrimination power of this item. The r_{it}-score ranges from -1.0 to $+1.0$ and should preferably be >0.25.

the assessed period. Two sample items from the assessment held among second year medical students in Spring 2005 are given in Fig. 5. Note that the metabolism items are production-type rather than recall questions.

EVALUATION

Metabolic Interrelationships has been in use by second year medical classes since 2002. Although the software application has not been rigorously evaluated professionally, it is well received by both students and staff. This is evident from the feedback in the form of questionnaires and remarks at staff/student evaluation meetings. Table I gives the results of an electronic survey held in March 2005 among ~400 students at the end of the three-week course. The response rate (12%) is low but similar to that of many other surveys held during the year. The performance of the current version of the software program is favorably rated, and the level of complexity appears to be well suited. Students specifically appreciate the ease with which one can switch from general overview to molecular detail and the ability to change the time scale themselves. Nevertheless, students still consider intermediary metabolism a difficult subject to study. Because the application was introduced simultaneously with a change in the medical curriculum from a teacher-centered, discipline-based curriculum to a student-centered, integrated systems-based curriculum, it is difficult for us to determine whether the introduction of this software application has improved students' appreciation of intermediary metabolism or their performance in the in-course assessments. From the 391 students participating in the Spring 2005 exam, 246 (57.5%) passed. The difficulty index, defined as the ratio of average score over maximal score/question, of the 21 metabolism items in this exam was 0.55 ± 0.18 (mean $\pm$ S.D.) compared with 0.60 ± 0.21 for the entire exam. This suggests that the metabolism items are only slightly more difficult than the questions from the other disciplines.

One criticism of the software application is that the many moving objects on the screen seem to distract the user (Table I). This complaint is also heard from some older teaching staff members who may not be as accustomed to fast moving animations as today's students are. We feel that, when examining each pathway individually, the objects moving between these processes make it easier to understand the overall picture. Another criticism is that a certain amount of biochemical knowledge is prerequisite to fruitfully work with the program. Students also requested that we include enzyme names, extend the explanatory texts on the type of regulation (allosteric, (de)-

TABLE I
Student evaluation of the software program Metabolic Interrelationships

Number of responders	47 (12%)
How many hours did you work with the software program?	5.0 ± 2.3[a]
What is the level of complexity of the software program?	
Too easy	1 (2%)
Just right	38 (81%)
Too difficult	8 (17%)
Rate the following statements about the software program on a scale from 1 (totally disagree) to 5 (completely agree):	
Easy to use	4.02 ± 0.87[a]
Supplies sufficient additional information	3.51 ± 0.80[a]
Improved my understanding of intermediary metabolism	4.11 ± 0.70[a]
Name strong features of the software program[b]:	
Zoom function from overview to detail (17×)	
Link various pathways in several organs (12×)	
Interactive time-scale (feeding-fasting) (9×)	
Attractive, user-friendly, impressive (11×)	
Clear, illuminating (10×)	
Name weak features and points for improvement[b]:	
Data missing/please expand program at:	
explanatory texts at points of regulation (6×)	
early refed state (5×)	
enzyme names (4×)	
legends/search function (4×)	
Symbols move too fast, too nervous (5×)	
Got lost in the multiple zooming levels (5×)	

[a] Mean ± SD.
[b] Given are only those features mentioned more than three times in this survey. Students could name more than one feature.

phosphorylation, induction) at control points, and expand the program with the early refed state after a period of starvation. The software program can be improved and extended according to these suggestions. However, one should keep in mind that this application is not intended to replace textbooks. On the contrary, it should be applied in combination with standard biochemical texts, such as Devlin [3], as an alternative approach to exploring the principles of mammalian intermediary metabolism. Clearly, these days most students like to play with educational software rather than sit and read "dull, lifeless, and boring" texts [10].

CONCLUSIONS

We have developed a stand-alone software application in Macromedia Director for medical students, which enables them to learn by themselves the principles of whole-body intermediary metabolism in an interactive, enjoyable way. The need for such a program came in 2001 with the change in the medical curriculum of the Erasmus Medical Center Rotterdam, not only from a discipline-based to an integrated systems-based curriculum but also from a teacher-centered to a student-centered self-learning formula. The computer program focuses on the sequential changes that occur in the metabolism of nutrients following ingestion of a mixed meal and subsequent fast. From a display of whole-body nutrient flow, students can zoom in on selected organs and, from there, to increasing levels of biochemical detail. It is intended for use alongside a biochemical text, *e.g.* Devlin [3]. Although written for medical students, the software may also prove to be an interesting teaching tool for other disciplines, such as animal physiology, nutrition, and physical education. The application is written as an add-on program, such that it can easily be extended with additional biochemical pathways and molecular detail. The application is accessible for evaluation at www2.eur.nl/fgg/ow/coo/bioch/#english.

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