Research Exam Semester 2 – 2019-2020

June 12, 2020

The questions must be answered in English. If you cannot remember a specific English term, you may use the Dutch term.

Be precise in your answers. Adding correct but irrelevant information will not increase your score. Adding incorrect information, even if it is irrelevant, will lower your score.

During the exam, you may want to consult these books (see link in Brightspace):

- Baynes & Dominiczak: Medical Biochemistry
- Campbell: Statistics at square one
- Donders: Literature Measurement errors
- Fletcher: Clinical Epidemiology
- van Oosterom en Oostendorp: Medische Fysica
- Petrie and Sabin: Medical Statistics at a Glance
- Turnpenny: Emery's Elements of Medical Genetics
- Form with statistical formula’s

Question 1
Q3: What we can learn from urine – dr. H. Pluk
(20 points)

Urinary sucrose and fructose to validate self-reported sugar intake in children and adolescents: results from the I.Family study
Intemann et al. European Journal of Nutrition volume 58, pages1247–1258 (2019)

Purpose
Excessive consumption of free sugar increases the risk for non-communicable diseases where a proper assessment of this intake is necessary to correctly estimate its association with certain diseases. Urinary sugars have been suggested as objective biomarkers for total and free sugar intake in adults but less is known about this marker in children and adolescents. Therefore, the aim of this study is to evaluate the relative validity of self-reported sugar intake using urinary sugars in children and adolescents.

Methods
The study was conducted in a sample of 228 participants aged 5–18 years of the I.Family study that investigates the determinants of food choices, lifestyle and health in European families. Total, free and intrinsic sugar intake (g/day) and sugar density (g/1000 kcal) were assessed using 24-h dietary recalls (24HDRs). At least 19 children and adolescents per center, of both sexes, agreed to recall their diet using a 24HDR and to provide a morning urine sample on the same day of the dietary recall. Urinary sucrose (USUC) and urinary fructose (UFRU) were measured in the urine samples. Correlation coefficients and regression models were used to investigate the relationship between intake of different types of sugar and urinary sugars.

Results
The Pearson correlation between usual sugar density calculated from multiple 24HDRs and the sum of USUC/Cr and UFRU/Cr (USUC/Cr + UFRU/Cr) was 0.40. Linear regression models showed statistically significant positive associations between USUC/Cr + UFRU/Cr and the intake of total and free sugar.

Conclusions
These findings support the relative validity of total and free sugar intake assessed by self-reported 24HDRs in children and adolescents.

A. The assessment of dietary intake (by self-reported 24-h dietary recalls) is especially challenging in children. Give three reasons why. **(3 points)**

The methods of the presented study were described as follows:

Laboratory methods
Collected morning urine samples from the eight I.Family study centers (located in Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain, and Sweden) were sent for analysis to the Dept. of Biochemistry of the University of Naples under standard shipping conditions (either at − 20 °C or at − 80 °C). Urinary sucrose (USUC) and urinary fructose (UFRU) concentrations were determined using an enzyme-based kit (sucrose/D-glucose/D-fructose from Boehringer Mannheim) and a Perkin Elmer Lambda Array spectrophotometer to measure the absorbance rate. All determinations were run in triplicate. Detected concentrations were in the range of 1–150 mg/L. Within this detection range, linearity of measurements was observed. Assay control solutions in the range of expected values for sucrose and fructose were provided in the enzyme-based kit. Values for this control were remarkably stable (glucose concentration: 100.6 ± 5.9 mg/L, mean ± standard deviation, coefficient of variation 5.9%).

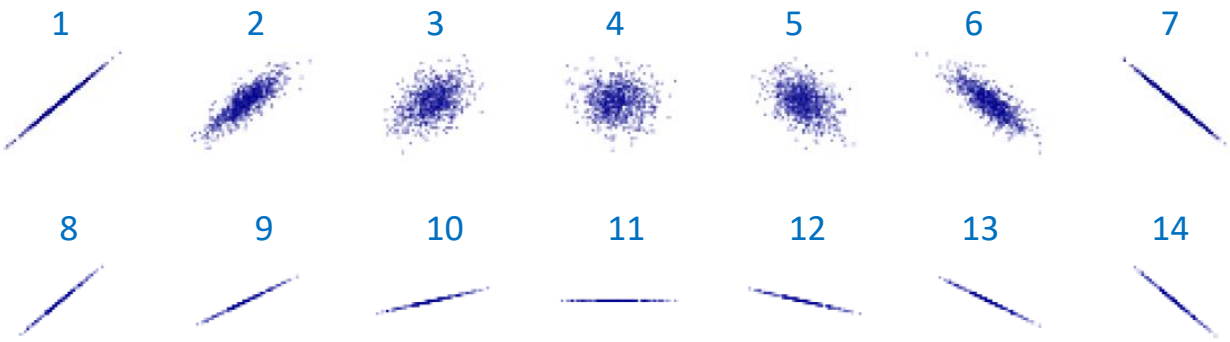
Analyses
Urinary concentrations of sucrose and fructose for all participants were expressed as mg/L of urine and also as mg/g of creatinine (USUC/Cr, UFRU/Cr). The (mean/usual) sugar density was calculated as (mean/usual) total sugar intake (in gram) per 1000 kcal of (mean/usual) total energy intake.

B. Identify five critical steps in the experimental setup (as presented in the abstract and methods section above); explain shortly why these are critical steps to get valid results in this experiment. **(10 points)**

C. Explain why urinary concentrations of sucrose and fructose were expressed as mg/L of urine and also as mg/g of creatinine (USUC/Cr, UFRU/Cr). **(3 points)**

The researchers state the following result: “The Pearson correlation between usual sugar density calculated from multiple 24HDRs and the sum of USUC/Cr and UFRU/Cr (USUC/Cr + UFRU/Cr) was 0.40. Linear regression models showed statistically significant positive associations between USUC/Cr + UFRU/Cr and the intake of total and free sugar.”

D. Sketch a graph that represents this Pearson correlation. Select the best fitting representation of this correlation from the examples below (circle the graph you choose, or mention the number of the representation (1 to 14)) and complete the graph by naming the x- and y-axis. If you cannot sketch explain in words what you would see in the intended graph and name the axes. **(4 points)**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Question 2
Q4: Modelling Epidemic Outbreaks – dr. T. Oostendorp
(15 points)

The standard model that is used to predict the number of infectious people in an epidemic is the SIR model. In this model, the differential equations for the number of susceptible people $S(t)$ and infectious people $I(t)$ are

$$\frac{d}{dt}S(t) = -\frac{R_0}{D}I(t)\,\frac{S(t)}{N}$$

$$\frac{d}{dt}I(t) = \frac{R_0}{D}I(t)\,\frac{S(t)}{N} - \frac{I(t)}{D}$$

with

N	population size
R_0	basic reproductive number
D	average duration of infectiveness in days

A. Explain why the minus term in the differential equation of $I(t)$ is $-I(t)/D$. (4 pts)

B. *Note: there are two versions of question B; the first one is for students that did the module Modelling Epidemic Outbreaks in 2020 and learn R, the second one is for those that did it in the years before and learned Simulink.*

Version 1 (students in 2020; R):

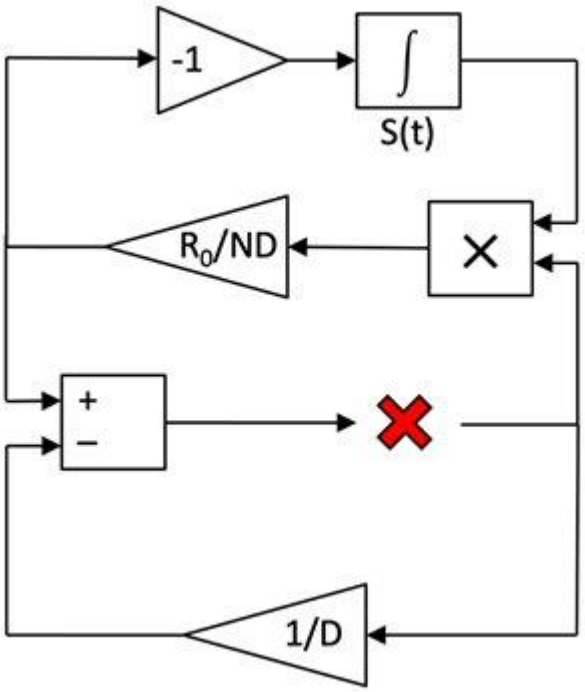
Below is a skeleton for a program in R that implements the SIR-model. Replace the three dotted lines by the code needed in order to make the script compute the number of infected people. (5 pts)

```
simDuration <- 100
deltaT      <- 1
nSteps      <- simDuration/deltaT
N  <- 100
R0 <- 2
D  <- 7
S  <- numeric(nSteps)
I  <- numeric(nSteps)

T[1] <- 0
I[1] <- 0
```

```
S[1] <- N-I[1]


for (i in 1:Steps)
{
        ...
        newResistants <- I[i]/D * deltaT
        T[i+1] <- T[i] + deltaT
        ...
        ...
}
plot(T, I, type='l', xlab='t (days)', ylab='I(t)')
```

Version 2

Below is an unfinished Simulink diagram that implements the SIR model. What type of block should be inserted at the red cross, and what name should be given to that block? (5 pts)



Let's assume that in 2025, the covid-25 pandemic will develop. Social distancing will again be enforced in order to bring down the value of R_0 for covid-25. The RIVM (Dutch Institute for Public Health and environment) states that the number of infectious people will decrease if that value can be kept below 1.

C. Explain why the number of infectious people will decrease when $R_0 < 1$. **(3 pts)**

The plot in figure 2.1 shows the result of the SIR model for corona, where at day 40 the value of R_0 changed instantaneously from 2 to 1.3.

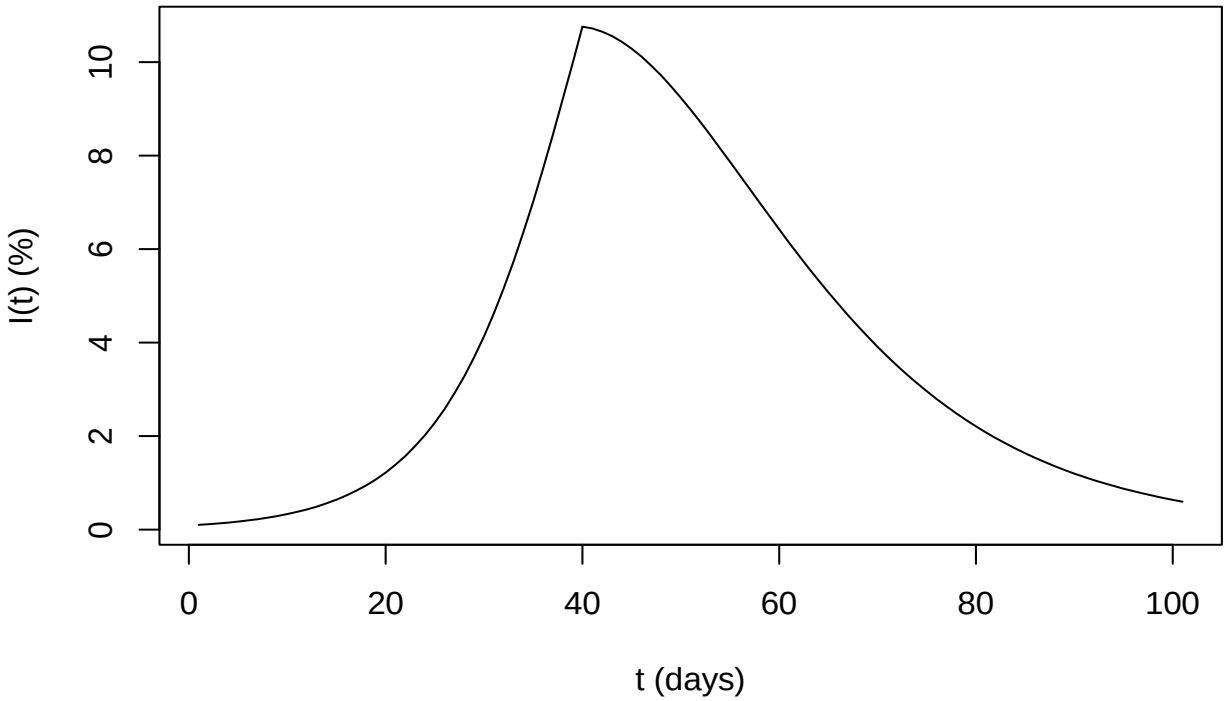


Figure 2.1 Predicted number of infectious people if R_0 changes from 2 to 1.3 on day 40.

D. Explain why the number of infectious people decreases after 40 days, even though at that moment R_0>1. (3 pts)

Question 3

Q4: Population research: Associations and causal relations – dr. F. de Vegt (20 points)

Use '*Elnaz Daneshzad et al - Association between a **low-carbohydrate diet** and sleep status, depression, anxiety, and stress score – abstract and tables*.'

A. What is the research question in the study of Daneshzad et al?
   In addition, specify the determinant, the outcome and the study population (3 pts).

B. The study design was a cross-sectional study. Describe the set-up of a cross-sectional study and mention one advantage and one disadvantage of this study design (3 pts).

C. Instead of a cross-sectional study, researchers could have designed a randomised controlled trial (RCT) to investigate the same research question. How does a RCT look like for this research question? (3 pts)

D. See Table 4 in the abstracts and tables. In total 188 subjects suffer from poor sleep. If per quartile of LCD score the numbers of patients were given, which test must be used to determine whether the percentage 'poor sleepers' differs among the quartiles? (1 pt)

E. In Table 4 four outcome parameters are analysed with binary logistic regression. The statistical section mentioned '…*and the significance value set as P < 0.05'*.
   Which methodological problems did the authors <u>not</u> deal with? (3 pts)

F. What is the interpretation of the result 0.44 (0.19; 0.98) mentioned in Table 4? (3 pts)

G. Explain why 'duration of diabetes' might be a confounder in the research of Daneshzad et al. (2 pts)

H. What has been done in the research of Daneshzad et al. to control for confounders? (2 pts)

Question 4
Q4: T- and B- cells in the lab – dr. E. Blaney Davidson
(20 points)

Amanda is researching T-cells in the spleen. She wants to visualize the T-cell receptor (TCR) using immunohistochemistry (IHC). Amanda made formalin fixed sections from human spleen on glass and stained these for the T-cell receptor TCR, but found no staining.

A. Name three experimental factors related to the IHC protocol that could have caused the lack of staining. (3 pts)

B. After having figured out what went wrong in 1a, Amanda repeats the immunohistochemistry for TCR of the spleen. But while incubating the 3,3′-Diaminobenzidine on her sections, she is interrupted by an important phone call and forgets about her staining for a while. When she finishes her phone call and gets back she is doubtful about her staining. Can she still objectively use her immunohistochemically stained sections to interpret the amount of TCR in the spleen? Explain why she can or cannot. (3pts)

A patient recovered of Covid-19 displays antibodies to COVID-19 in the circulation. An indirect ELISA is a suitable method to measure antibody levels in the circulation. An indirect ELISA means that you have a combination of a detecting antibodies and a labeled conjugate.
The following materials are available in the lab: standard ELISA plates, blocking buffers, washing buffers, substrate for horse radish peroxidase (HRP) and an ELISA plate reader.
The following antibodies, plasma and antigens are available in the lab:

- Avidin-HRP conjugate
- Guinea pig(Ig) anti-rat Ig
- Biotinylated Hamster(Ig) anti-rat Ig
- Rabbit(Ig) anti-human Ig
- Rat (Ig) anti-human Ig
- Biotinylated Rat(Ig) anti-rabbit Ig
- COVID-19 spike protein solution
- Plasma of a patient who recovered from Covid-19.

C. Write down the ELISA procedure to determine the level of anti-COVID-19 antibodies in blood of a recovered patient in a step-wise manner. In total there are 6 steps. Only indicate the sequence of steps regarding the application of antigen and antibodies that are required in this indirect ELISA (so blocking and washing steps do not need to be indicated).(4 pts)

D. What is the reason to include a blocking step?

In order to determine whether a change in cytokine concentration has influenced the composition of lymphocyte subpopulations in blood, you like to use flow cytometry to investigate the four main lymphocyte populations (Helper T cells, Cytotoxic T cells, B cells and NK cells). You use a blood sample for your analysis.

E. Which antibody combinations should be used in flow cytometry to determine each population of lymphocytes in the blood separately? Provide an answer for the four main lymphocyte populations. (4 points)

F. How should you perform the flow cytometry analysis to evaluate correctly the lymphocyte populations? Describe the different plots by mentioning their axes in the correct rank order, starting with forward scatter/ side scatter. Describe which cell population(s) can be selected in each plot by using this rank order. (5 points)