Research Exam Semester 3 – 2019-2020

January 17, 2020

During the exam, you have access on a computer to these books:

- 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 formulas

You are allowed to use a calculator of the type Casio FX-82MS.

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.

Write your name and student number on the first page of each question!

Name:

Student Number:

Question 1
Q5: Cancer etiology and prognosis – prof. dr. B. Kiemeney
(20 points)

Metformin and the risk of renal cell carcinoma: a case-control analysis.

Abstract, European Journal of Cancer Prevention

Introduction: Metformin use has been associated previously with a decreased risk of cancer, but its association with renal cell carcinoma has not yet been investigated in observational studies. We aimed to explore the association between the use of metformin and other antidiabetic drugs and the risk of renal cell carcinoma (RCC).

Methodology: We carried out a case-control analysis in the UK-based Clinical Practice Research Datalink. We included individuals with an incident RCC between January 1995 and December 2013 younger than the age of 90 years. Six controls per case were matched on age, sex, calendar time, general practice, and number of years of active history in the Clinical Practice Research Datalink before the index date. We included BMI, smoking, alcohol consumption, hypertension, and diabetes mellitus as potential confounders in a multivariate model using logistic regression to calculate odds ratios with 95% confidence intervals, and we carried out a sensitivity analysis restricted only to diabetic cases and controls.

Results: Long-term use of metformin was not associated with an altered relative risk of RCC (≥30 prescriptions, adjusted odds ratio 1.18, 95% confidence interval 0.88-1.58), nor was use of other antidiabetic drugs. Results in the sensitivity analysis including only diabetic cases and controls were largely the same.

Conclusion: Use of metformin or other antidiabetic drugs was not associated with a materially altered risk of RCC. Further studies are warranted.

a. Take a look at the aim of the study in this abstract. What is the dependent and independent variable given their research question? (2 pts)

b. Would the authors be able to analyze sex as a risk factor for RCC using the selected 3506 cases and 21038 controls? (1 pts) Provide a reason why (or why not) (3 pts)

c. Take a look at Table 1. Explain the odds ratio of 1.13 (0.86 – 1.48) for "Metformin 1-29" in words (see the bolded line in the table). (3 pts)

Table 1: ORs for RCC for antidiabetic drugs in all cases and controls

Drugs and numbers of prescriptions	Cases [n (%)] (n=3506)	Controls [n (%)] (n=21038)	Odds Ratio (95% CI)
Metformin			
No previous use	3260 (93.0)	19 879 (94.5)	1.00 (Referent)
Any use	246 (7.0)	1159 (5.5)	1.15 (0.90 – 1.46)
1-29	**128 (3.7)**	**609 (2.9)**	**1.13 (0.86 – 1.48)**
≥ 30	118 (3.4)	550 (2.6)	1.18 (0.88 – 1.58)
Sulfonylurea			
No prior use	3320 (94.7)	20 072 (95.4)	1.00 (Referent)
Any use	186 (5.3)	966 (4.6)	0.91 (0.72 – 1.16)
Insulin			
No previous use	3431 (97.9)	20 658 (98.2)	1.00 (Referent)
Any use	75 (2.1)	380 (1.8)	0.99 (0.75 – 1.31)

d. Calculate the *Population attributable proportion (= Etiological fraction among the population)* of Metformin ≥30 and provide an interpretation for this estimate. (5 pts)

e. Would the authors be able to analyze age as an effect modifier for metformin use and RCC using the selected 3506 cases and 21038 controls? (1 pt) Provide a reason why (or why not). (2 pts)

f. The authors conclude: " Use of metformin or other antidiabetic drugs was not associated with a materially altered risk of RCC. Further studies are warranted." Provide only <u>two</u> reasons why further studies might be warranted. (3 pts)

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Question 2
Q5, Q6: Modelling physiological systems – dr. T. Oostendorp (15 points)

Modelling the pharmacokinetics of ethanol

Figure 2.1 shows the plasma concentration of ethanol recorded in a subject after drinking "ad fundum" (i.e: in one gulp) a glass of water containing 40 gr of alcohol.

Figure 2.1 Ethanol plasma concentration after consuming 40 gr of ethanol.

We will use a two-compartment model to study the clearing of ethanol from the body.

a. Based on figure 2.1, what will these compartments represent? (2 pts)

Compartment 1:

Compartment 2:

In linear kinetics, the amount of ethanol converted per hour by the liver, $\Psi(/)$, is given by

$\Psi(/) = 1 \quad 3(/)$

With constant 1 the clearance and $3(/)$ the plasma ethanol concentration.

b. Explain how you can conclude from figure 2.1 that the conversion of ethanol by the liver cannot be described by linear kinetics. (3 pt)

The proper way to describe ethanol conversion by the liver is by Michaelis-Menten kinetics:

$$\Psi(/) = \frac{V_{\max}}{K_{\mathrm{m}} + c(t)}\, 3(/)$$

With $4_{\max}$ the maximum conversion rate and 8_{m} the concentration at which $\Psi(/) = \_^{\&}{}_{+}\, 4_{\max}$.

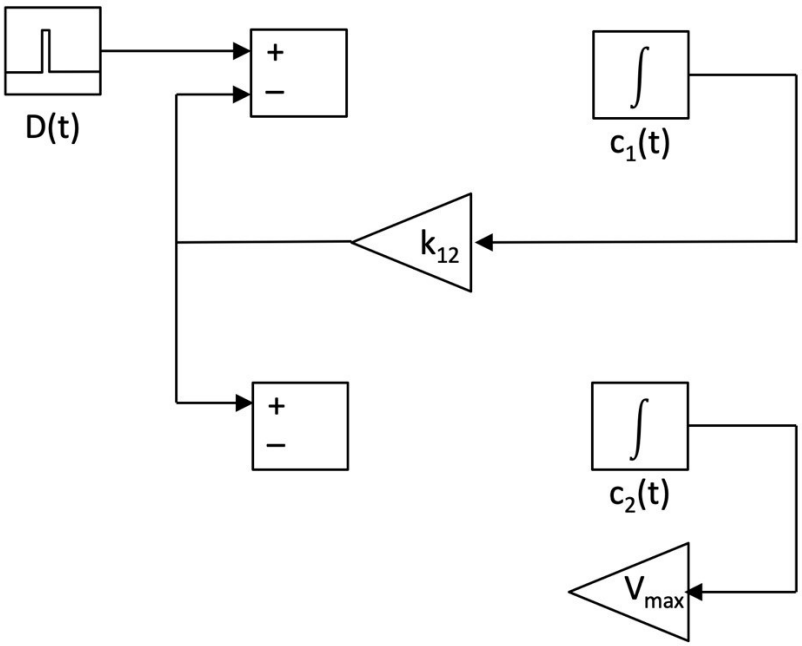
c. Show that the system equation for the second compartment is

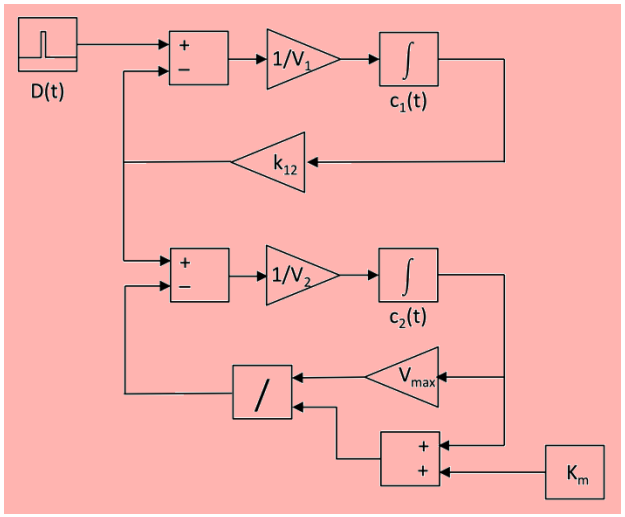
$$\frac{d}{dt}\ 4_{+}3_{+}(/) = 1_{\&+}3_{\&}(/) - \frac{4_{\max}}{8_{\mathrm{m}} + 3_{+}(/)}\ 3_{+}(/)$$

With 4_{+} the volume of compartment 2, $1_{\&+}$ the clearance from compartment 1 to compartment 2, and $3_{\&}$ and 3_{+} the concentrations in compartment 1 and 2 respectively. (4 pt)

Below an incomplete diagram is shown of the Simulink implementation of the twocompartment model for the clearing of ethanol from the body. For compartment 1 linear kinetics can be assumed. $\mathrm{B}(/)$ is the dose velocity.

d. Complete the diagram (6 pt).


D(t)
+
–
$c_1(t)$
k_{12}
+
–
$c_2(t)$
V_{max}


D(t)
+
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$1/V_1$
$c_1(t)$
k_{12}
+
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$1/V_2$
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V_{max}
/
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+
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Question 3
Q5: Molecular Cancer Research – dr. P. Groenen
(15 points)

1. Evaluation of Western Blot setup

NRAS is frequently mutated in melanoma. It activates the kinase cascade BRAF-MEK-ERK. A student in the lab performs a Western blot to determine the amount of phosphorylated ERK (pERK). The following antibodies are used: an antibody directed against phosporylated ERK and an antibody against tubulin (major component of the cytoskeleton). The following samples are used; a melanoma sample with a NRAS activating Q61L mutation, a melanoma sample that has no NRAS mutation and a melanoma sample that has the BRAF V600E mutation.

a. Please sketch the Western blot (similarly to figure 3.1) that shows the activation status of the pathway in the melanoma. Moreover, indicate what the controls are, and why these are used. (4 pt)

2. Evaluation of Western Blot analysis & Signal Transduction

In the figure below, Western blots of five different tumor cell types are depicted that all carry a different BRAF fusion protein.These fusions lead to constitutive phosphorylation of BRAF. In this experiment, these tumor cells were left untreated (most left lane) or were treated with the mTOR kinase inhibitor Everolimus (one dose, 10µM) alone or in combination with the MEK kinase inhibitor Trametinib in different concentrations (0.1, 1 or 10 nM).

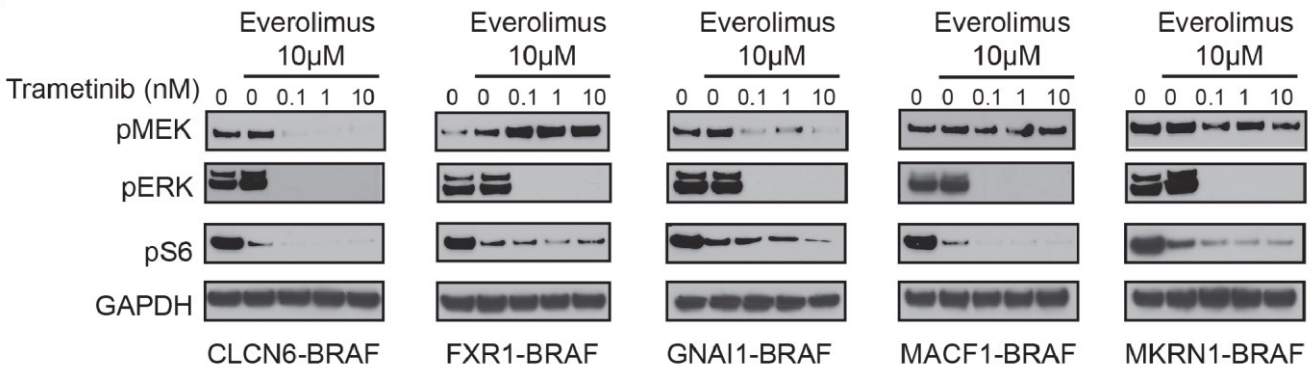

Figure 3.1. Western blot analysis of the indicated proteins in five different tumor cell types (CLCN6-BRAF, FXR1-BRAF, GNAI1-BRAF, MACF1-BRAF and MKRN1-BRAF) and five different treatment conditions

b. Phosphorylated S6 (pS6) is a kinase that is directly activated by the mTOR kinase. Is the inhibition of the mTOR kinase with Everolimus sufficient to completely inhibit the phosphorylation of S6 (pS6) in these five cell types? Explain in your answer, which blots/lanes you have evaluated to answer this question. (2 pt)

c. What is the additional effect of the combination treatment with Everolimus and Trametinib on the inhibition of the phosphorylation of S6 (pS6)? Please describe the findings in all 5 cell types. (4pt)

3. Evaluation of Western Blot analysis & Hypoxia

Small cell lung cancer (SCLC), the most aggressive type of lung cancer. Treatment is still very suboptimal and the investigators want to exploit the altered tumor metabolism. Increased glycolysis provides a rapid way to generate ATP but also leads to raised lactic acid levels, leading to intra-tumoral acidosis. To counterbalance lactate accumulation, cancer cells use monocarboxylate transporters MCT1 and MCT4 for intracellular uptake of lactate. Inhibition of MCTs has been proposed to selectively target highly glycolytic cancer cells. Here, investigators studied AZD3965, an MCT1 specific inhibitor. They also investigated the effects of hypoxia. Two cell lines were studied (DMS114 and DMS79).

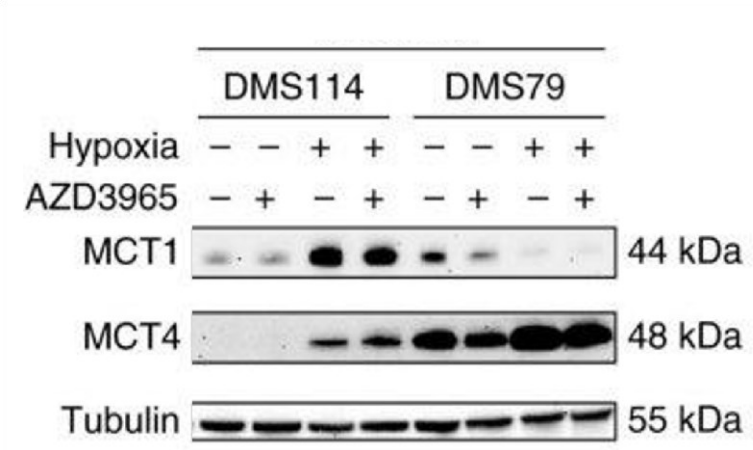

Figure 3.2. Protein expression analyses of MCT1, MCT4 and tubulin in DMS114 and DMS79 *cell lines treated with the MCT1 inhibitor AZD3965 in either a normoxic or a hypoxic environment.*

d. Is the capacity for lactate accumulatin successfully inhibited by the treatment of AZD3965 under hypoxic conditions? (5pt)

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Question 4
Q6: Meta-analysis and more – dr. J. in ’t Hout
(15 points)

Meta-analysis of the effect of vitamin C in ICU patients

Observational studies on Vitamin C demonstrated that critical illness is associated with low levels of vitamin C, but randomized clinical trials (RCTs) of vitamin C, alone or in combination with other antioxidants, have yielded contradicting results. A meta-analysis was conducted, based on RCTs comparing vitamin C with control (placebo or none), in intensive care unit (ICU) patients.

Overall mortality was the primary outcome. The results of one of the subgroup analyses is displayed in Figure 4.1 on the next page.

a. What is the name of this plot? (1p)

b. Explain what is represented by the individual horizontal lines (including length of the lines) with the squares per study, the vertical line, and the summarizing diamonds. (5p)

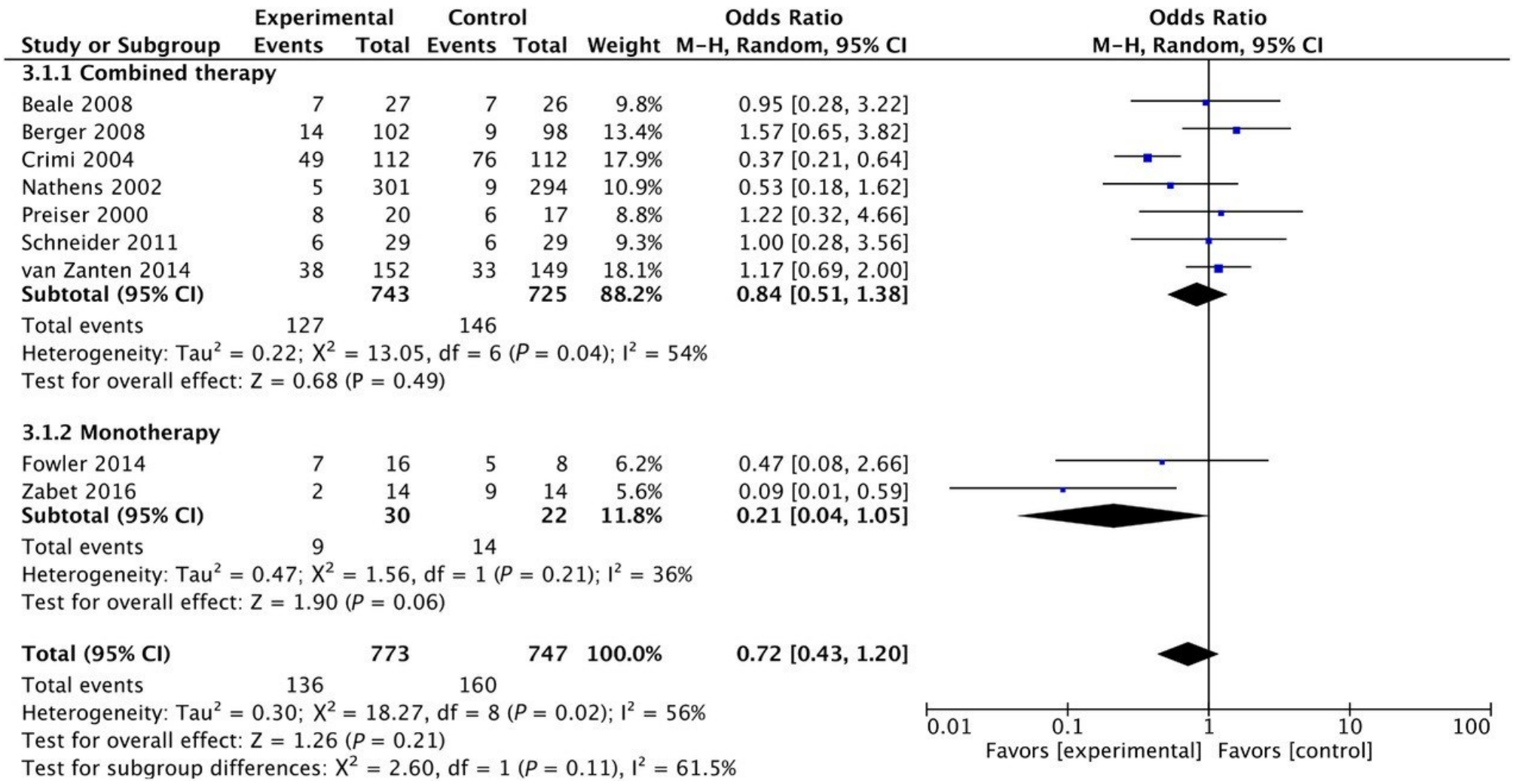

Figure 4.1 Plot of mortality in ICU patients in relation to vitamin C treatment

c. Why is it visually attractive to present the x-axis on log scale? (2p)

d. Suppose the subgroup analysis comparing the effect of combined therapy vs monotherapy in Figure 4.1 would have been statistically significant. Mention two reasons why you should be careful with the interpretation of this result. (4p)

e. Consider a large study, taken from a meta-analysis with zero between-study heterogeneity. Is this study relatively more important (for the pooled result) in a fixedeffect or in a random-effects meta-analysis? Explain your answer (3p).

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Question 5

Q6: Measuring and modelling reflexes – dr. E. Tanck & dr. T. Oostendorp (20 points)

Statics

A young woman (60 kg) is doing static exercises to strengthen the m. triceps (see figure 5.1 where you see two positions of the static exercise). A physical therapist is interested to know how high the contact force in the elbow joint is and how high the force in the tendon of the m. triceps is when the arm of the woman is at (about) 90 degrees and the rope is at (about) 45 degrees (figure 5.1 on the very left).

Known data: You may assume that masses of body parts and all distances, angles and attachment points for ligaments, tendons and muscles are known. The force that the woman is applying to the rope is 100 N.

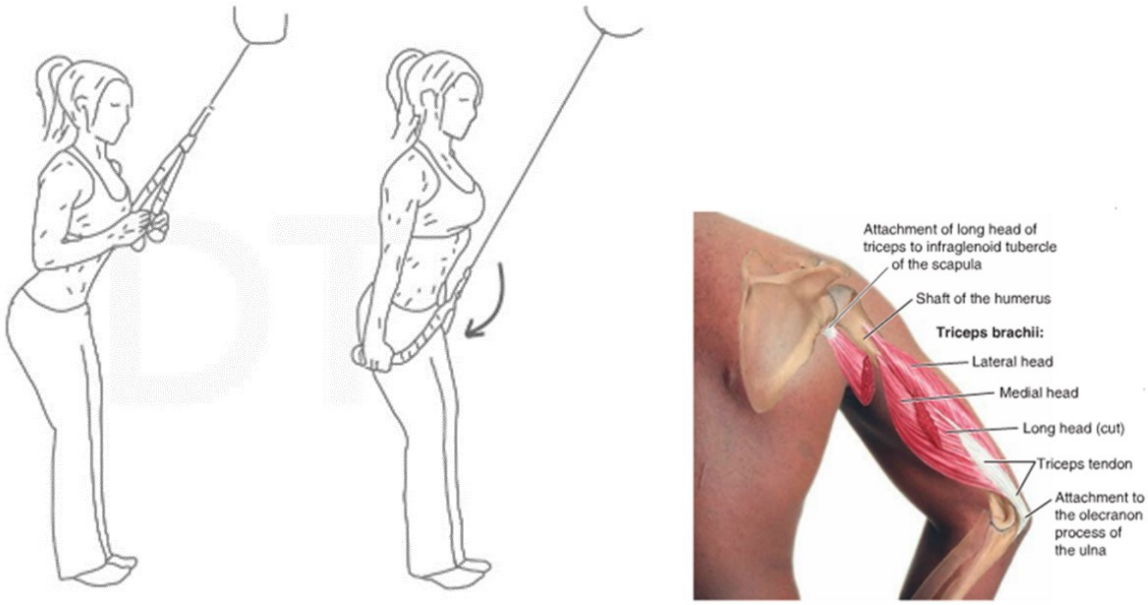


Figure 5.1 Static exercises to strengthen the m. triceps and anatomy of elbow including the attachment point of the triceps tendon (at process of the ulna).

a. On the next page, create a free body diagram (FBD) to calculate the forces in the elbow joint (contact force) and the tendon of the m. triceps. Make your diagram large enough so everything can be labeled clearly. Create a legend to specify all components of the FBD. (7 pt)

b. Give the Equilibrium equations that belong to your FBD. You do not have to calculate the forces. (4 pt)

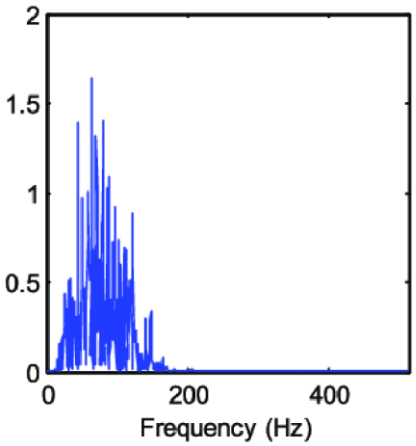
c. In case another person is doing the same exercise and the mass of the arm of that person is 4 kg higher than that of the woman, will the force in the tendon of the m. triceps be a bit higher, equal or a bit lower? Please explain. NB: the force that the person is applying to the rope is still 100 N. (4 pt)

During the exercise, the activity of the m. triceps is recorded by using surface electrodes.

d. Why is the average over time of the EMG not a good measure for the size of the EMG? (2 pt)

Figure 5.2 shows the spectrum of the EMG. In order to minimize disk space, the researcher wants to record the EMG with as low a sample rate as possible, while avoiding aliasing.

e. What sample rate should be used? Explain your answer. (3 pts)



End of the exam. Did you write your name and student number on the first page of each question?