Splitting of milk fat

Fat is present in milk in the form of very fine balls (droplets), which are stabilized by proteins as a protective colloid. In milk fat, glycerol is esterified with short-chain fatty acids. These fats are only subject to a small degree of cleavage by pancreatic lipase in the small intestine. The vast majority is taken up directly by the enterocytes (passive penetration of the apical membrane due tolipophilia) and cleaved intracellularly. The resulting short-chain fatty acids are transported to the liver for further metabolism. Due to the fine distribution of the fat droplets, milk fat offers an ideal prerequisite for rapid hydrolysis by lipase in the test tube.

Task:

The formation of free fatty acids from milk fat by exposure to pancreatic lipase is demonstrated with the indicator phenolphthaflax (turning points 0, 8.2 and 12 (red – colourless – violet – colourless) and the effect is measured by a change in pH.

Material:

Pipettes, test tubes, water bath (37 °C), pH meter, packets of milk, 1M NaOH, lipase from Pancreas, 1 % phenolphthalein solution, 0.9 % NaCl solution

Execution:

CAVE: NaOH is a strong base. Use goggles.
CAVE: Be careful when boiling the enzyme: the liquid boils brutes and "shoots" out of the vessel. Keep the opening of the tube away from the body.

Dissolve 5 0 mg of lipase and dissolve in 10 ml of 0.9% NaCl solution (slightly soluble, shake for 10 min, then discontinue). Boil 2 ml of it in a test tube: hold the tube over a Bunsen burner using a clamp and bring the solution to a boil. Allow the solution to cool briefly.
In two test tubes, pipette the following contents:
Per tube:

- 5 ml milk (3.5% fat)
- (approx.) 2 drops of 1% phenolphthalein solution
- Approx. 50 µl 1 N NaOH

The addition of NaOH leads to a clear pink coloration by alkalization. Use a pH electrode to measure the pH value and make a note of it. Then add between 200 and 500 µl of boiled lipase solution to tube 1 - depending on the group number (see protocol sheet) - and the same amount of uncooked lipase solution in tube 2.

Record the amount added and measure the pH. Incubate both tubes at 37 °C in a water bath and observe the color change (within 10-60 min). Write down the time and measure with increasing intervals (at the beginning after approx. 30 sec, later after several minutes) the pH value.

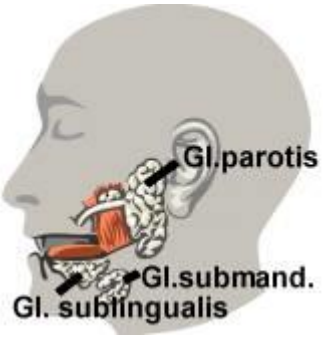
Evaluation:

Log and discuss your findings/observations and those of your colleagues! Plot the pH versus time on a graph. Compare low-fat and regular milk.

Measurement of salivary amylase

Carbohydrate digestion begins in the oral cavity. The enzyme amylase, which breaks down long-chain starch molecules into simple sugars, is present in saliva.

The amylase is secreted by the parotid gland (glandula parotis) and the submandibular salivary gland (glandula submandibularis). The former is purely serous, the latter is a mixed seromucous gland. Serous means that the saliva it produces is purely liquid (watery) without any slimy (mucous) additives. Therefore, parotid saliva is thin, slightly alkaline and rich in proteins and enzymes, especially amylase. In addition, the saliva of the parotid contains immunoglobulins, which are used for the immunological defense in the mouth.



Fibers of the glossopharyngeal nerve (Nervus IX) are responsible for the parasympathetic innervation of the parotid gland. They regulate salivation. The parotid gland is sympathetically innervated by fibers of the superior cervical ganglion.

The submandibular salivary gland is innervated by the autonomic nervous system. The parasympathetic nerve fibers originate from the upper nucleus of the salivary gland (nucleus salivatorius superior) and leave the cranial cavity with the chorda tympani, a branch of the seventh cranial nerve (nervus facialis).

Task:

Determine the amount of saliva secreted by the parotid gland.

Material:

Salivette, balance, reflotron, centrifuge tube

Execution:

Weigh the contents of a salivette on the analytical balance and note the value. Slide it onto the exit of the parotid gland. This is on both sides at the height of the 2nd grinding teeth of the upper jaw. Remove this "cotton roll" after every minute for 5 minutes and weigh it again.
After 5 minutes: Put the piece of cotton in the container provided and place it in the centrifuge tube provided. Centrifuge at 2000 rpm for 3 minutes. Measure the flow using a pipette (hopefully you have more than 700 µl) and transfer the solution to a new centrifuge tube. Dilute 1:500 by mixing 20 µL of saliva and 9.98 mL of water in a 10 mL centrifuge tube. Apply 30 µl of the liquid to an Amylase Reflotron test strip and measure the value of the amylase (if the value was too high, you would have to dilute more and repeat this step.
CAVE: Do not dispose! The activity of the amylase is measured

Evaluation:

Create a graph showing the increase in weight over time.

Enzymatic splitting of starch

Starch is hydrolytically cleaved during digestion by α-amylases (including ptyalin) in saliva and pancreatic secretion, resulting in dextrins as end products. In contrast to α-amylases, the ß-amylases in germinating plant seeds split the starch at the end of the molecule (exoamylases), resulting in maltose residues as products. The designations α- / ß-amylases refer to the site of hydrolytic cleavage within the starch molecule and therefore have no relation to α- or ß-glycosidic bonds of individual monosaccharides!

Lugol's solution is an iodine-iodine-potassium solution. It is used to demonstrate strength; Starch molecules consist of several linked alpha-glucose molecules that attach themselves to form a helically wound chain with the elimination of water. Iodine forms a blue color complex with starch because the iodine molecules can store themselves in the "screw cavities" of the starch.

Task:

Detection of starch and determine the activity of the enzyme amylase.

Material:

Starch solution (briefly Boil 1% to dissolve), Lugol's solution (prepared iodine-iodine-potassium iodide solution (1%), distilled water, oral saliva (from previous experiment or 1% amylase solution), 2% NaCl, 0.1 N HCl, 0.1 NNaOH
Cuvettes, test tubes, Pasteur pipettes, water bath, spectrophotometer

Execution:

Starch digestion

- Prepare a batch I in cuvettes, a second batch II (with DOUBLE quantities) in test tubes
-

-	A1	A2	A3
Stärke (1 %)	0,5 ml	0,5 ml	0,5 ml
NaCl 2 %	---	---	---
HCl 0,1 N	---	0,01 ml	0,1 ml
NaOH 0,1 N	---	---	---
Aqua dest	0,5 ml	0,5 ml	0,4 ml

	B1	B2	B3
Stärke (1 %)	0,5 ml	0,5 ml	0,5 ml
NaCl 2 %	0,2 ml	0,2 ml	0,2 ml
HCl 0,1 N	---	0,01 ml	0,1 ml
NaOH 0,1 N	---	---	---
Aqua dest	0,3 ml	0,3 ml	0,2 ml

	C1	C2	C3
Stärke (1 %)	0,5 ml	0,5 ml	0,5 ml
NaCl 2 %	0,2 ml	0,2 ml	0,2 ml
HCl 0,1 N	---	---	---
NaOH 0,1 N	---	0,01 ml	0,1 ml
Aqua dest	0,3 ml	0,3 ml	0,2 ml

- Add approx. 10 µl (depending on the color intensity) of Lugol's solution to each cuvette.
- Measure the absorbance at 590 nm in the spectrophotometer. Initially calibrate to 0 with sample C3.
- Add 30 µl of amylase (i.e. oral saliva) to each tube (1-3) and vortex immediately.
- All batches are then placed in a 37°C water bath.
- Depending on the change, measure the absorbance again every minute.
  CAVE: Vortex before each measurement and wipe the outside of the cuvette.

Sugar test

- After the end of the starch digestion, add 2 ml of Fehling I and 2 ml of Fehling II. Gently heat the mixture over the Bunsen burner.

Evaluation:

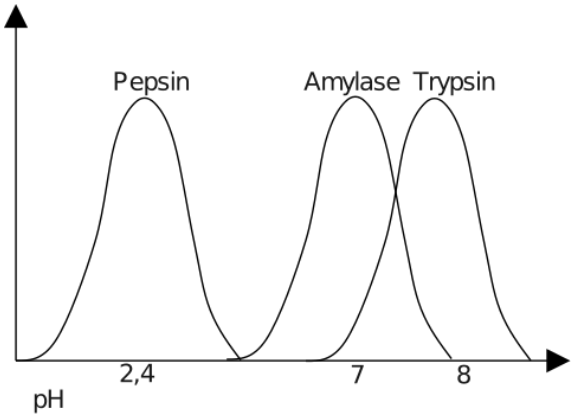
Explain the visible result and plot the absorbance of the samples against time in a graph. Determine the gradient of the straight line and use a calibration curve to calculate the starch reduction per hour. Compare the strength values with the observation from the Fehling sample.

Protein digestion

During digestion, proteins are enzymatically split by proteases into (di-, tri-)peptides and amino acids. The specific cleavage takes place with hydrolysis of the peptide bond according to the following principle:

$$R_1\text{-}CO\text{-}NH\text{-}R_2 + H_2O \rightarrow R_1\text{-}COOH + H_2N\text{-}R_2$$

Depending on the structure-specific hydrolysis, proteases are differentiated into endopeptidases and exopeptidases. Endoenzymes are primarily secreted in the stomach and upper small intestine (why?). They break bonds within a molecule, resulting in multiple fragments. Endopeptidases include pepsin, trypsin, chymotrypsin, elastase. Pepsin is a phosphoprotein with a length of 327 amino acids. Pepsinogen is formed in the main cells of the stomach as an inactive precursor of pepsin. Under the action of hydrochloric acid in the gastric juice, pepsinogen is converted into the active protease pepsin by autoproteolysis.
Trypsin is a hydrolase secreted by the exocrine pancreas that can cleave proteins as a serine protease. Trypsin is secreted in the form of a proenzyme as trypsinogen and activated in the duodenum by the enzyme enteropeptidase or by proteolysis by already activated trypsin molecules.
Trypsin has its optimum effect at a pH of 7 to 8 and, as an endopeptidase, mainly cleaves for basic amino acids.



Task:

Measurement of pepsin and trypsin activity and determination of the pH optimum of both enzymes.
Material:
Hard-boiled egg, pepsin and trypsin solution (100 mg/mL in 0.9% NaCl), hydrochloric acid (0.1N), NaOH solution (0.0001N), Bradford's reagent (optional: Biuret's reagent (0, 2M Na-K-Tartrate, 20mM CuSO4 in 0.2M NaOH), Folin-900 Biuret Reagent -Phenol Reagent), Aqua dest.
Safety goggles, test tubes or centrifuge tubes, pipettes, water bath

Execution:

Preparation:

- Have the lecturer assign you to a group (A, B or C).
- Then pipette the specified reagents into a test tube according to the scheme below (don't forget to label!).
- To start, withdraw 100 µl of the mixture. Place them in labelled Eppendorf tubes (“pre”) and put them in the refrigerator at 4°C.
- Determine the pH of your solutions and write them down.

Pipettierschema:

Gruppe A, D

	A1	A2	A3	A4	A5	A6	A7
Pepsin	---	---	---	---	---	---	---
Trypsin	---	---	---	---	---	---	---
0,1 N HCl	---	1 ml	100 µl	10 µl	---	---	---
10^{-4} N NaOH	---	---	---	---	1 ml	100 µl	10 µl
0,9 % NaCl	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl
Aqua dest.	1,3 ml	0,3 ml	1,2 ml	1,3 ml	0,3 ml	1,2 ml	1,3 ml

Gruppe B, E

	B1	B2	B3	B4	B5	B6
Pepsin	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl
Trypsin	---	---	---	---	---	---
0,1 N HCl	---	1 ml	100 µl	10 µl	---	---
10^{-4} N NaOH	---	---	---	---	10 µl	100 µl
Aqua dest.	1,3 ml	300 µl	1,2 ml	1,3 ml	1,3 ml	1,3 ml

Gruppe C, F

	C1	C2	C3	C4	C5	C6
Pepsin	---	---	---	---	---	---
Trypsin	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl
0,1 N HCl	---	1 ml	100 µl	10 µl	---	---
10^{-4} N NaOH	---	---	---	---	10 µl	100 µl
Aqua dest.	1,3 ml	300 µl	1,2 ml	1,3 ml	1,3 ml	1,3 ml

Reaction start:

- Cut off a small piece of egg (white) - approx. 250 mg.
- Weigh it with the analytical balance and note the value.
- Add the protein piece to your sample tube.
- All test tubes are then placed in a water bath at 37°C for SEVERAL hours. Note the start time.

After the end of the reaction:

- After the reaction time (SEVERAL hours to overnight), remove the piece of egg and place it on filter paper to dry for approx. 30 minutes.

• Weigh the piece of egg and record the value.
• Centrifuge the sample at maximum speed for 5 min ("post") and then withdraw another 100 µl ("post").
• Detect the amount of protein in the sample at the beginning (pre) and at the end (post) using a Bradford protein test:
o Make a serial dilution of BSA (4, 2, 1, 0.5, 0.25 mg/mL)
o For each sample (A1-C6) and BSA solution, add 1 ml Bradford reagent to a cuvette
o Pipette (per sample A1-C6 and BSA standard) 20 µl protein solution (once "pre", once "post") into the cuvette.
o Let samples ≥ 10 minutes at R.T. incubate
o Measurement at 595 nm in the spectrophotometer (pre and post)

Evaluation:

Calculate the theoretical pH in solutions A1-C6. Determine the change in protein concentration and weight between pre and post. Draw a graph showing changes in protease activity versus pH. Pay attention to possible reaction time differences. Discuss the results of groups A-C.

Urine test strips

A urine test strip is a semi-quantitative rapid test for urine analysis. The detection of various components in the urine allows conclusions to be drawn about various diseases. There are different types of urine test strips with one to eleven test parameters. Common parameters are pH, leukocytes, nitrite, protein, glucose, ketone bodies, urobilinogen, bilirubin, blood and hemoglobin. The corresponding ingredients can also be estimated in terms of their concentration using a color comparison scale. Urine test strips only require a small amount of urine and are a quick and inexpensive test method, but they only serve as a rough guide

Task:

Determine the parameters of the Combur-9 test of your urine.

Material:

Test strips: Combur-9 or EasyScreen 10SL

Execution:

Hold a test strip in midstream urine for 1 second. Wipe off the side edges of the test stick and excess urine drops on a clean swab/non-woven paper. Compare the color of the test strip with the color pattern on the test strip vial after 30-60 seconds.

Evaluation:

Create descriptive statistics and compare the measured values (especially the pH value) in relation to the eating habits with a t-test.

Determination of heart rate and performance index

The important circulatory parameters include blood pressure and pulse rate. The latter is the result of the rhythmic activity of the heart and changes when the organism is under stress. The measurement of the pulse rate thus enables the determination of the heart rate under different conditions and an assessment of the training status (fitness) of the healthy subject. The resting pulse should be reached again no later than 3 minutes after the effort. Performance indices can be calculated with measurements at multiple points in time. The performance index (LI) according to James Ruffier has undergone various modifications. The pulse frequencies are determined at three points in time:

P0 = heart rate at rest
P1 = pulse rate immediately after exercise (30 squats in < 45 s)
P2 = pulse rate exactly 1 min after the end of the second measurement (P1)

The performance index according to Ruffier is to be determined from these three values:

$$LIR = (P0 + P1 + P2 - 200)/10$$

A variant of this was provided by Dickson:

$$LID = ((P1-70)+2*(P2-P0))/10$$

Rating scales are used to assess the training status of a subject. The dependency on age, gender, weight and other parameters is not fully taken into account, so that the performance can only be roughly estimated using the following scale:

Ruffier:

LIR = <0	=	very good adjustment to effort
LIR = 0 to <5.0	=	good adjustment to effort
LIR = 5.0 to <10.0	=	normal adjustment
LIR = 10.0 to <15.0	=	insufficient fit
LIR = ≥15	=	low adjustment, med. Rating recommended

Dickson:

LID = <0	=	excellent performance
LID = 0 to < 2.0	=	very good performance
LID = 2.0 to < 4.0	=	good performance
LID = 4.0 to < 6.0	=	normal performance
LID = 6.0 to < 8.0	=	poor performance
LID = 8.0 to < 10.0	=	very poor performance
LID ≥ 10	=	poor exercise adaptation

Task:

Determining the performance indices according to Ruffier and Dickson.

Material:

Stopwatch

Execution:

While sitting still, determine the radial pulse on the forearm (P0) by palpation for 15 s. Do 30 squats in 45 seconds or less. Stop and record the time. Measure the radial pulse (P1) again immediately after the squat. Repeat the measurement after one minute (P2), then every 30 s until the pulse rate normalizes or for at least 4 min.

Evaluation:

Display the heart rate curve in graphic form and calculate the individual performance indices. Compare the two indices using a paired t-test. Discuss your results!

Determination of carbohydrates in food

Sugars are found in many foods and serve as a source of energy. With the help of the Fehling test, some sugars can be detected. The deep blue solution of the Cu(II) complex is reduced by the aldehyde group of the glucose. A red-brown to orange-colored precipitate of Cu2O forms. The detection is positive for monosaccharides and for the disaccharides fructose and lactose. Since both latent carbonyl functions are blocked in sucrose, the test for cane sugar is negative.
The detection also works well with strongly colored liquids such as cola. Solids must first be dissolved in water or a suspension prepared and then decanted.

Material:

Chemicals

- Fehling I and II
- Glucose, fructose, sucrose
- Sample material
- Water

Materials

- Test tubes
- Bunsen burner
- Spatula
- Wooden clip
- Pipettes

Task:

Determination of sugar in food.
CAVE: Take a sugary and sugar-free food with you.

Execution:

- Pipette 0.5 ml of 0.1% carbohydrate solution into an Eppi and add 1 drop of Lugol's solution to each one. Check the color development of the carbohydrate solutions in response to the addition. The different sugar solutions are used as controls.
- Place 2 mL each of the carbohydrate solutions and food sample in a test tube. Add 2 ml of Fehling I and 2 ml of Fehling II solution. Carefully heat the mixture over the Bunsen burner (CAVE: delayed boiling)

Evaluation:

Observe the discoloration and interpret the result.

Solutions:

Fehling's solution I

- 7 g $CuSO_4$ in 100 ml H_2O.

Fehling's solution II

- 35 g KNa tartrate and 10 g NaOH in 100 ml H_2O.

Renal function - determination of creatinine clearance

The kidneys cleanse the body of metabolites and foreign substances (e.g. drugs). Three mechanisms are responsible for this:

1. Glomerular filtration
2. Tubular reabsorption
3. Tubular secretion

The physiological unit of the kidney, the nephron, begins with filtration (in the glomeruli). If a substance is then neither reabsorbed nor additionally secreted, this substance can be used to determine the "glomerular filtration rate" (GFR) to determine kidney functionality. This is given as clearance (C), which is defined as the plasma volume (Vplasma) that is completely freed from substance X per unit of time (t):

$$C_X = \frac{[X]_{Urin}}{[X]_{Plasma}} \times V_t$$

With Vt = urinary time volume in ml/min
Clearance is referred to as exogenous when a substance foreign to the body is administered (e.g. insulin). If one uses a substance formed by the body itself, one speaks of an endogenous measurement. For example, you can use creatinine, an end product of muscle metabolism.
This results in the clearance formula:

$$C_{Kreatinin}[\frac{\frac{ml}{min}}{1{,}73m^2}] = \frac{[Kreatinin]_{Urin} \times Vol_{Urin} \times 1{,}73}{[Kreatinin]_{Plasma} \times t \times KO}$$

t = collection time in min.
KO = body surface
1.73 = standard body surface of a 75 kg person in m2, the reference ranges are based on this value

Reference range for adults, age-related (Jaffé reaction) – specification of $\bar{x} \pm s$ (taken from L.Thomas: Labor und Diagnose)

Jahre	♀	♂
20-29	91 ± 19	117 ± 23
30-39	96 ± 25	98 ± 39
40-49	76 ± 26	98 ± 22
50-59	74 ± 24	88 ± 21
60-69	60 ± 15	76 ± 22
70-79	49 ± 12	64 ± 15
80-79	41 ± 14	45 ± 15

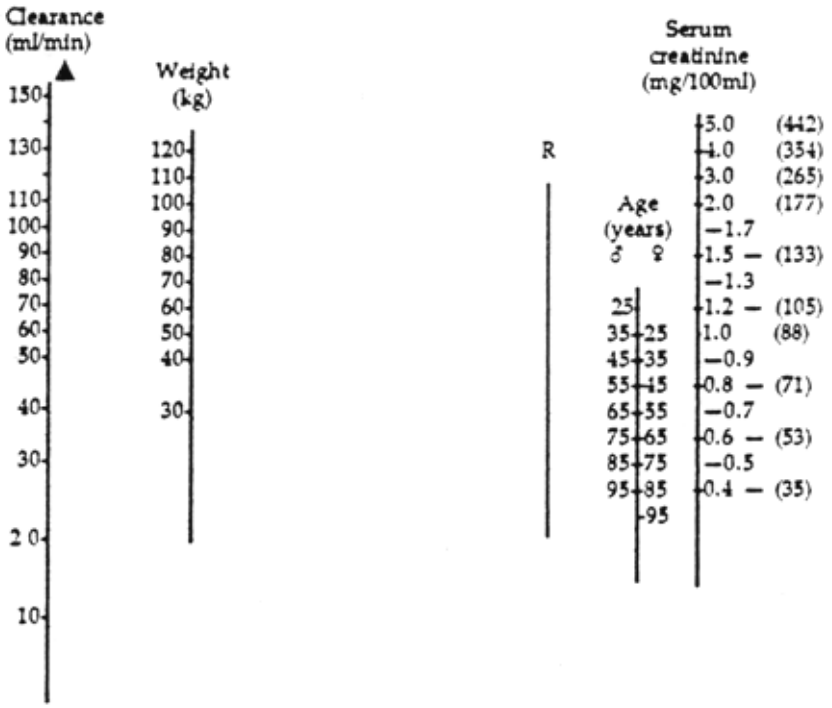


Fig. 1: Nomogram for estimating creatinine clearance

Task:

Determine your kidney's creatinine clearance.

Material:

Measuring vessels (for urine), pipette, reflotron with lancing device and creatinine test strips.

Execution:

CAVE: Wear gloves when working with blood

a. Determining the blood creatinine concentration

As in the "Blood" part of the exercise, stab yourself in the sanitized ring finder. Using a capillary, collect 30 µl of blood and apply it to a Creatinine Reflotron test strip. Insert the test strip into the device and read the value after approx. 2 minutes.

b. Determination of the urinary minute volume

(It would be best to collect all urine from 24 hours)

Note the time of the last bladder emptying before the internship (t1). During the internship, collect the urine (at least 2 hours) and ensure that you drink enough fluids. After the last bladder emptying, the time is also noted (t2) and the urine volume is determined (measurement in a measuring cylinder).

c. Determination of urine creatinine concentration

Dilute the urine sample

• If the urinary minute volume is <1.0 ml/min, dilute 1:40
• If the urinary minute volume is >1.0 ml/min, dilute 1:20

Then 30 µl are pipetted onto a creatinine Reflotron test strip and measured (see above). The urine creatinine concentration is obtained by multiplying by the dilution value.

d. Determination of the body surface

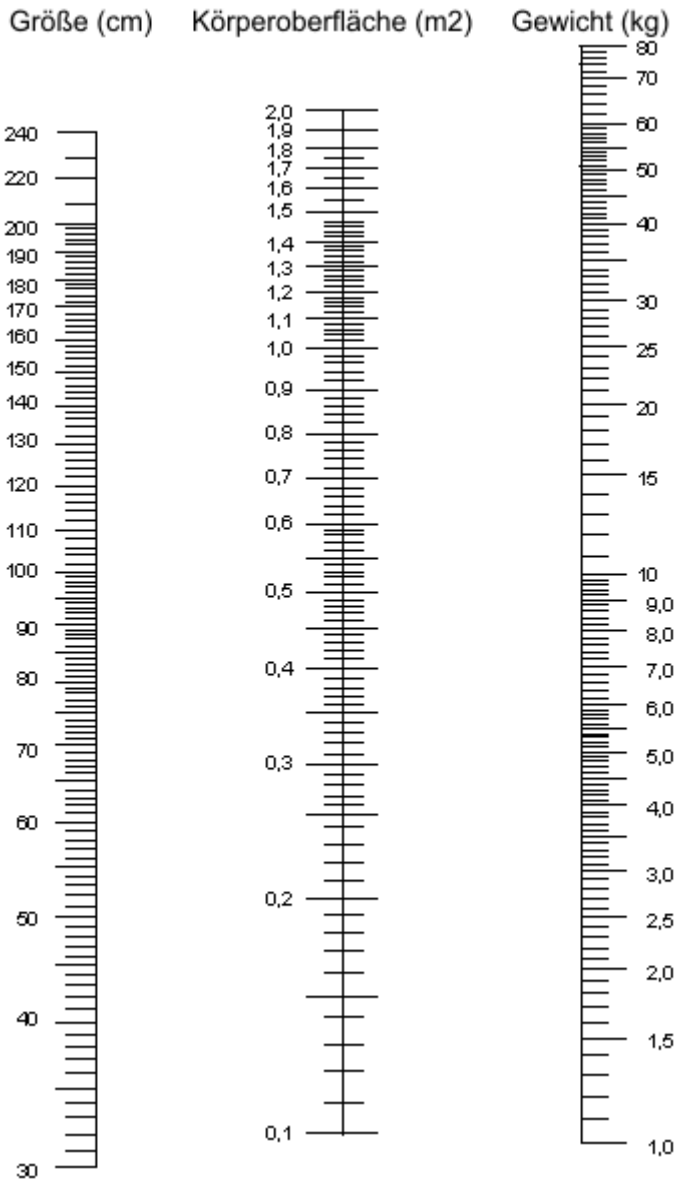


Fig. 2: Nomogram for determining body surface area from height and weight:

Evaluation:

Calculate the participants' creatinine clearance and discuss this in comparison to the normal values (e.g. with a z-test)

Glucose tolerance test

The oral glucose tolerance test (OGTT or sugar load test) is used to detect impaired glucose utilization and for early diagnosis of diabetes mellitus. It is contraindicated in manifest diabetes mellitus.
The supplied glucose initially leads to a short-term increase in the blood glucose concentration. Immediately thereafter, insulin secretion is stimulated with a subsequent drop in value. In patients with reduced insulin secretion or insulin resistance, the drop in blood glucose concentration is delayed. The 120-minute blood sugar level is higher than in healthy people.

The test is carried out in the morning (ten hours) on an empty stomach. In order to obtain a meaningful result, the subject should have consumed more than 150 g of carbohydrates per day on the three previous days. In addition, there must be no febrile illness, and test subjects should be three days apart before and after menstruation.

The supplied glucose initially leads to a short-term increase in the blood glucose concentration. Immediately thereafter, insulin secretion is stimulated with a subsequent drop in value. In patients with reduced insulin secretion or insulin resistance, the drop in blood glucose concentration is delayed. The 120-minute blood sugar level is higher than in healthy people. (according to Wikipedia)

Task:

Determine the breakdown of glucose in your blood

Material:

Blood lancets, blood glucose meter, glucose, water

Execution:

Measure the fasting glucose value by applying a drop of capillary blood from your fingertip to the test strip. Then drink a glucose solution (75 g glucose in 250-300 ml water) within 5 minutes.
Measure the glucose value again after 120 minutes (you can of course also determine the value after 10, 20, 30, 60, 90 and 120 minutes - depending on your courage! ;-) and plot the values in a time graph). You can also vary the trial (within a group, with one person drinking glucose, another drinking sucrose, etc.)

Evaluation:

Determine the absolute values and difference. Create a corresponding graph for a time measurement.
The following reference values apply:

Measurement	Normal value	Impaired Glc tolerance	Diabetes mellitus
Fasting	< 100 mg/dl	100-110 mg/dl	>110 mg/dl
120 min	< 140 mg/dl	140-200 mg/dl	>200 mg/dl