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Influence of changes in diet on the dynamics of $^{13}C/^{12}C$-ratio in selected urinary steroids. Part I: Study design, $^{13}C/^{12}C$-ratio of applied foodstuffs and effect on anthropometrical data

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1. Introduction

Ingested food provides the subunits for all body compounds such as carbohydrate, fat and protein, thereby also determining carbon isotope ratios of steroids[1], expressed in terms of $\delta\,^{13}C$ in per mille (‰) against a standard VPDB : $\delta\,^{13}C = [(r_{sample}/r_{VPDB}) - 1] \times 1000$
Well known are differing carbon isotope ratios of endogenous steroids in people of diverse continents or changes in fossil tooth enamel [2] which can be attributed to differing nutrition. The motivation of the study was to examine effects of altered nutritional content in ^{13}C on $^{13}C/^{12}C$-ratios of endogenous steroids. Resulting data was analyzed to answer two complex problems:

1. As every metabolic steps incorporates a fractionation, there could be different kinetics in incorporating ^{12}C versus ^{13}C into endogenous steroids classified as precursors or as potential steroids of abuse. This could lead to positive IRMS-results.
2. From the velocity and onset of changes in steroid $\delta\,^{13}C_{VPDB}$ ‰ -values we might conclude on size and kinetics of the steroid pool and their precursors as well as components that serve as a resource for de-novo-biosynthesis.

Abbreviations: BMI: Body-Mass-Index, CAM: Crassulacean-Acid-Metabolism, EA: elemental analysis, PEP: Phosphoenolpyruvate, RuBisCo: Ribulose-1,5-bisphosphatcarboxylase

2. Study design: Diet habits, protocol and assumptions

Ingested food serves as the precursor for steroid biosynthesis, assuming that the steroid precursor pool in the body is appropriately small and enough time is allowed [1]. To investigate this effect, seven healthy young male and female persons, including two vegetarians, collected urine samples for two weeks as the basis for obtaining baseline reference values. These persons lived then for a period of four weeks on a ^{13}C-enriched diet before returning to a diet with a $^{13}C/^{12}C$ ratios regarded typical for western Europe. The normal diet consisted mainly of C_3-plant-origin and the ^{13}C-enriched diet consisted of more than 80% of C_4-plants with no cholesterol allowed, thus limiting steroid biosynthesis to de-novo-biosynthesis. Two average characteristics of participating test persons ensured a fast turnover of a comparatively small precursor pool, namely a low weight to height ratio (68,5 kg/179 cm resulting in a BMI of 20,9) and a fast metabolic rate of 2850 kcal/day (including 700 kcal/person x day for sport activities). Nutrition and anthropometric data were registered and interpreted daily (Table 1), as reduction of adipose tissue, loss of weight or altered body composition was not desired. Degraded body compounds could serve as components for de-novo steroid biosynthesis.

Table 1: food and weight protocol for each test person

date/ time	nutrition (exact declaration [g], composition: fast food, preparation, % fat)	drinks [ml]	training/action illness	weight/ % body fat

3. C_4-plants and CAM-plants compared to C_4-plants: $\delta^{13}C_{VPDB}$ and metabolism

Less transpiration and no photorespiration gives C_4-plant-metabolism an advantage in special climatic conditions. As a consequence of a high CO_2-affinity of C_4-plants PEP-Carboxylase - compared to C_3-plants carbon-assimilating enzyme RuBisCo - less fractionation is evident and $^{13}C/^{12}C$-ratios resemble values of air [3, 4](Table 2).

Table 2: distribution and $\delta\,^{13}C_{VPDB}$ of plants

	Distribution	$\delta\,^{13}C_{VPDB}$
C_3-plants	dominant in cool climate	-27 ‰
C_4-plants	evolution in warm/arrid climate	-13 ‰
CAM-plants	extremely dry environments	-13 ‰ - -27 ‰

The carbon dioxide fixation proceeds in two steps. There is spatial separation between mesophyll and bundlesheath cells in C_4-plants and temporal separation between night and day in CAM-plants. The first step (Figure 1) involves fixation into oxaloacetic acid, the second step supplies the fixed carbon dioxide via RuBisCo to the Calvin cycle, adding it to Ribulose-1,5-bisphosphate and generating two molecules of phosphoglycerate [4] .

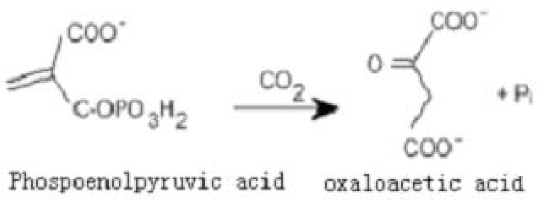

Figure 1: fixation of carbon dioxide into oxalacetate

4. Results

4.1. δ $^{13}C_{VPDB}$ of food used in the ^{13}C-depleted part of the study (4 weeks)

$^{13}C/^{12}C$-analysis of 100 food products with C-IRMS restricted the food content that could be used in the 4-week-diet to millet, corn, sugar cane, amaranth, pineapple and their products (Table 3). Favoured for the diet were those ingredients with a $\delta^{13}C_{VPDB}$ of -10 up to –13, highlighted in the table are those products that contain additional C_3-ingredients and have a lower $\delta^{13}C_{VPDB}$. Whole meals were also analysed and consist of 80 % or more of C_4-origin. Of special interest is the egg substitute which was not dispensable to make food more edible, $\delta^{13}C_{VPDB}$ of meat if the cattle is fed from corn and $\delta^{13}C_{VPDB}$ of tuna, which feeds on phytoplankton with a high ^{13}C-content. Tuna was incorporated in small amounts into the diet, but meat with desired $^{13}C/^{12}C$-ratios could not be bought in any quantity.

Table 3: δ $^{13}C_{VPDB}$ of food, median of 3 analyses as analysed by EA-IRMS

ingredient	product	δ^{13}C VPDB
amaranth	popcorn	**-12,58**
corn	powergel	**-11,60**
corn	bread	**-13,50**
corn	polenta	**-11,17**
corn	lolly	**-11,73**
corn	cornflakes	**-12,04**
corn	noodles	**-12,46**
corn	flour	**-11,46**
corn	germ oil	**-15,64**
corn	starch	**-12,46**
corn	corncob	**-11,67**
corn	in the can	**-12,08**
corn div.sorts	tortilla chips	**-16,82**
millet	flakes	**-13,40**
millet	porridge	**-16,35**
millet	honey pops	**-17,55**
millet	whole grain	**-12,73**
pineapple	fruit	**-13,64**
pineapple	dried	**-13,02**
pineapple	slices	**-11,97**
pineapple	pieces	**-12,64**
sugar cane	sugar	**-12,88**
sugar cane	whole sugar	**-10,49**
sugar cane	jelly sugar	**-11,73**
sugar cane	rum 40 %	**-11,33**
sugar cane	alcohol 40 %	**-11,65**
sugar cane	div. marmalade	**-12,92**
sugar cane	pear jelly	**-13,57**
meat	young steer	**-16,97**
egg substitute	no cholesterin	**-20,66**
tuna	filet no oil	**-16,28**

whole meals	additional ingredient	δ^{13}C VPDB
corn cake	raisin	**-14,63**
millet pilaw	ginger, carrot, onion, raisir	**-14,22**
corn noodles	paprika, wild onion, onion	**-14,30**
corn pancake	brokkoli, carrot	**-19,75**
corn chowder	potato	**-18,08**
corn, millet cake	recipe	**-14,91**
sugar cane jelly	pineapple	**-13,32**
millet hirsotto	mushroom	**-13,69**
corn soup	tomato, onion	**-17,92**
corn bread	basilikum, parmesan (20g)	**-13,81**
amar.corn cookies	raisin, soda	**-13,75**
corn dumplings	tomato sauce, cheese, car	**-14,28**
corn dumplings	mushroom, cheese, zucch	**-16,69**
corn noodles	tomato, parsley	**-12,21**
millet roulade	cabbage, brokkoli, tomato	**-17,75**
corn dumplings	pure	**-12,59**
corn, millet bread	candida	**-11,63**
millet casserole	carrot, onion, brokkoli	**-15,81**
millet boats	zucchini	**-17,33**
corn pizza	tomato, paprika, wild onior	**-13,65**
corn dumplings	mushroom, cheese, zucch	**-16,69**
tuna sauce	cornstarch, weeds, tomatc	**-16,55**
corn pizza	tomato, paprika, cheese	**-19,18**
corn pizza	tomato, mushroom	**-17,18**
corn cake	corn, sugar cane, canela	**-14,31**

4.2. Relation in nutrients and effect on anthropometric data

The food chosing protocol was interpreted using a computer assisted analysis for relationships with the various nutrients: during the 4-week-diet the carbohydrate content was higher and less fat and protein was consumed. While this looked like an ideal diet (Figure 2, Table 4), after 2 weeks, half the volunteers felt sick or minor infections were observed. The protein content and quality as well as the limited vitamin and mineral intake could be responsible for these observations [5].

Table 4: mean macronutrient content in different phases of the study

	carbohydrate	fat	protein
baseline	50 %	30 – 37 %	14 %
C_4-diet	60 – 65 %	25 %	10 %

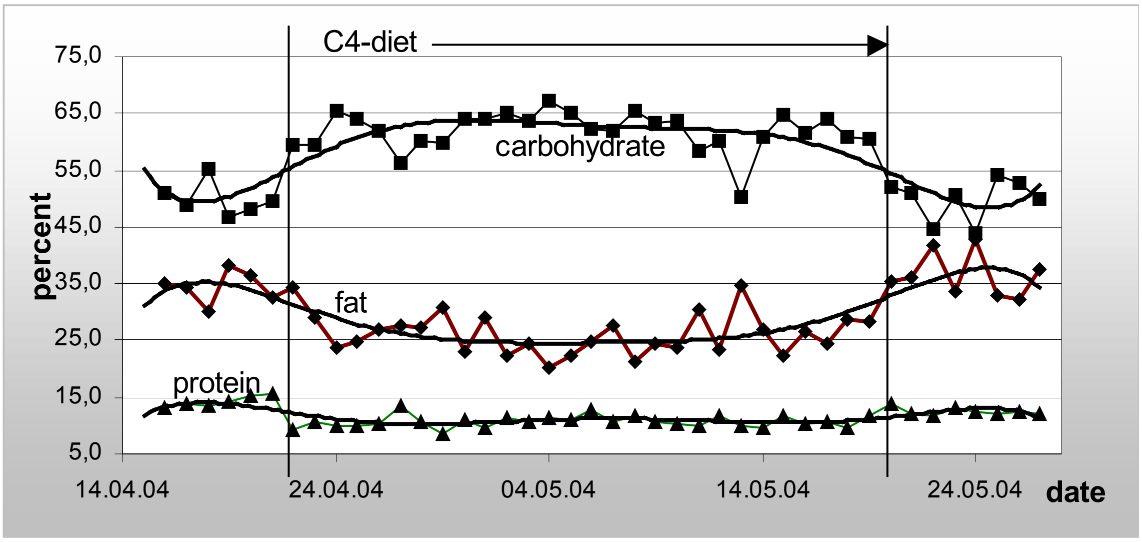

Figure 2: Relation in nutrients during the three phases of the study

Total maximum weight loss during the ^{13}C-depleted diet was attributed to a negative caloric sum of 6000 kcal/person equivalent to nearly 1 kg of fat tissue (Figure 3, Figure 4). Regarding the total mean energy intake of 120400 kcal/person in 7 weeks the negative energy balance and weight loss can be neglected. The high overall energy expenditure up to 4000 kcal/person ensures that ingested food will serve as the resource for steroid biosynthesis. The day by day energy balance is positive if intake is higher than calculated expenditure and vice versa. Day by day energy expenditure is cumulated to the energy expenditure sumcount. Model assumptions regarding energy uptake and expenditure calculations and resultant sumcount for every test individual (not shown) as well as means of test persons coincide well with the anthropometric data body weight and % body fat (Figure 3, Figure 4). Thus mathematical calculations and adoptions of models for energy uptake, expenditure, daily requirements of persons [5] and the adoption and transfer of food protocol concerning amount and quality of food seem to be sufficiently exact. Since weight and body fat correlate for the mean values of the volunteers as well as for individual changes (Figure 4), body weight loss can be attributed to diminution of fat tissue.

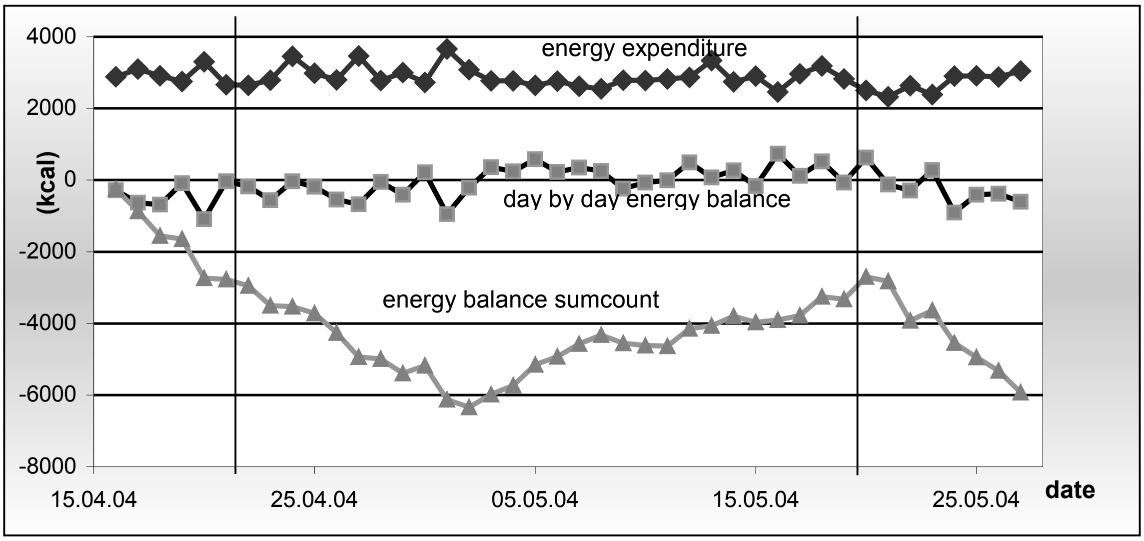

Figure 3: Effect on anthropometric data: energy

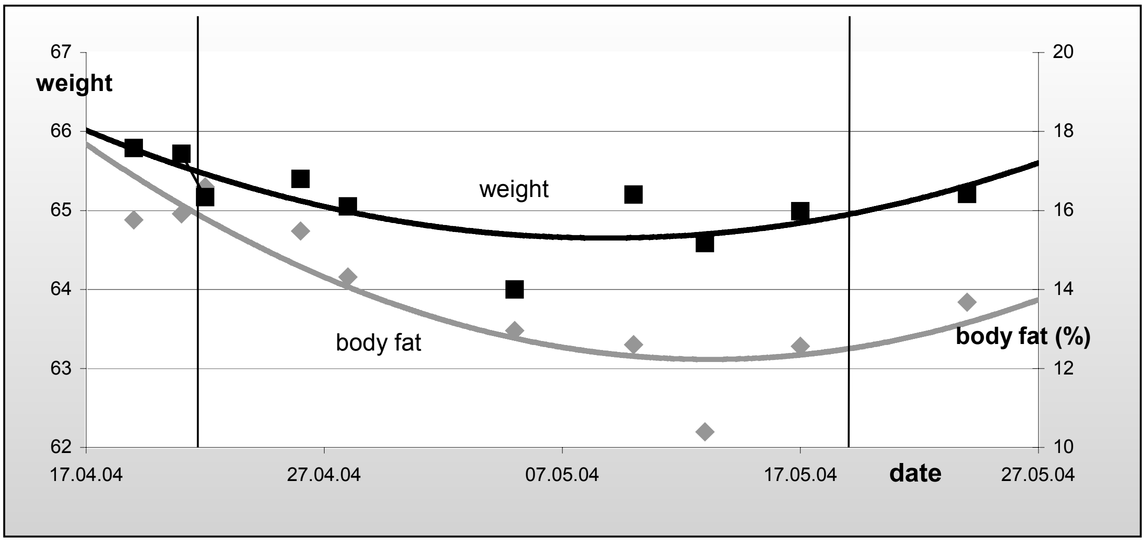

Figure 4: Anthropometric data: weight and body fat

5. Literature

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