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ORIGINAL CONTRIBUTION

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Estimation of available energy of dietary fibres by indirect calorimetry in rats

Summary *Background* Knowledge of energetic availability of dietary fibres is important for human nutrition. But up to now results are often different and depend on the methods used. Estimation of metabolisable energy of dietary fibres (mainly by balance technique) is a time-consuming procedure and needs special technical effort. *Aim of the study* Validation of the experimental design for short-term studies by using indirect calorimetry with feeding below maintenance requirement to evaluate the energetic availability of dietary fibres and their influence on absorption velocity of carbohydrates (CHO). *Methods* Energy expenditure and CHO oxidation (including short-chain fatty acids as fermentation products) were estimated in Wistar rats over 23 h after being fed a basal diet for the first day (300 KJ/kg$^{0.75}$, 20 % protein, 3 % fat, 77 % CHO) followed by supplementation with either microcrystalline cellulose, the soluble rye fibre arabinoxylan, apple pectin, amylomaize starch (with 48 % of resistant starch) or gelatinised wheat starch (200 KJ/kg$^{0.75}$ each) as control for the following days. Energetic availability was determined by comparing the increase of CHO oxidation after addition of gelatinised wheat starch with that of the dietary fibres tested. *Results* In comparison to wheat starch (100 %), the following energetic availability of the dietary fibres was found: microcrystalline cellulose 14 %, arabinoxylan 33%, pectin 39 %, amylomaize starch 62 %. The time-course of CHO oxidation indicated that microcrystalline cellulose enhances, whereas the soluble rye fibre slows down the velocity of CHO absorption due to the different consistency of the intestinal contents modified by the kind and properties of the used dietary fibres. After intake of arabinoxylan or pectin, CHO oxidation remained at a higher level during the experimental period elucidating an increased activity of fermentation to short-chain fatty acids. *Conclusions* Short-term experiments in rats using indirect calorimetry are a suitable method for comparative estimation of the energetic availability of dietary fibres. Results are partly in agreement with values estimated by long-term *in vivo* methods.

Key words Dietary fibre – cellulose – resistant starch – pectin – arabinoxylan – available energy – indirect calorimetry – rats

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Introduction

Among the diverse direct and indirect physiological effects of dietary fibres, two aspects of their physiological characterisation are important for human dietetics:

1) Estimation of the energetic availability of different non-nutritive carbohydrates due to their microbiological degradation and transformation to short-chain fatty acids.
2) Characterisation of the influence of dietary fibres on the time-course of carbohydrate (CHO) absorption (aiming at the dietary treatment of type 2 diabetes).

Balance methods in animals as well as in human beings are mainly used to evaluate to what extent the energy of dietary fibres is available for the organism. These exper-

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iments are time consuming and require great technical and analytical effort. The time-course of blood glucose levels is the main method to evaluate the influence of dietary fibres on the velocity of glucose absorption from the intestinal tract, but a high variability limits its significance [1].

There is only sparse information about indirect calorimetry in human beings to study the influence of poorly digested carbohydrates as maltitol, lactulose, resistant starch, cellulose, pectin or raw potato starch on energy expenditure, respiratory quotient and metabolic fuel oxidation [2–5]. But these experiments were not focused on the estimation of energy availability and the tested compounds were administered alone or together with glucose.

The purpose of this study is to present an approach for the rapid estimation of the available energy from different types of dietary fibres in combination with a well balanced diet in rats as well as their influence on postprandial CHO oxidation by using indirect calorimetry.

Materials and methods

Dietary fibres

The acute effect of the following CHO (dietary fibres) was evaluated in combination with a well balanced semisynthetic diet on energy metabolism.

Microcrystalline cellulose

The microcrystalline cellulose preparation "Heweten 20" was obtained from Rettenmaier u. Söhne, Ellwangen, Germany.

Arabinoxylan

The soluble rye fibre arabinoxylan was prepared according to the procedure of Fengler et al.1988 [6] modified by Täufel et al. [7]: 250 g bran of rye (variety Clou) were heated in a reflux condenser with 700 ml of 80 % ethanol. The residue was extracted with 1000 ml and after that with 500 ml of water for 30 min at 30 °C. After filtration and concentration at 40 °C, pancreas α-amylase and trypsin were added to depolymerise starch and protein (6 h at 40 °C). The solution was filtered (Celite 445) and dialysed. The dialysate was heated (30 min at 100 °C). Further enrichment of soluble arabinoxylan was reached by precipitation of the dissolved dialysate with 96 % ethanol (80 % saturation). The coagulate was resolved in water, again filtered and lyophilised.

The composition of the arabinoxylan preparation used was pentoses 81.3 %, total hexoses 10.6 %, starch 0.54 %, protein (N × 5.76) 4.0 %, water 3.8 % and ash 0.6 %.

Pectin

High-esterified apple pectin was obtained from Pektinwerk Werder, Germany. It was purified by extraction with acidic 65 % ethanol. The purified pectin had the following parameters: galacturonan 58 %, degree of esterification 61.4 %, intrinsic viscosity [η] 445 g/ml galacturonan. The galacturonan content was determined by the *meta*-hydroxybiphenyl method [8]. Methyl ester groups were analysed by the chromotropic acid method [9]. The intrinsic viscosity [η] which is related empirically to the molecular weight by the Mark-Houwink relation [10] was determined in 0.155 M NaCl at 25.0 °C and pH 6.0 using a Ubbelohde viscosimeter.

In the pectin preparation used approximately 25 % of galactose, arabinose, xylose and rhamnose residues were present in the form of nondigestible polysaccharides. These occur partly in the rhamnogalacturonan fractions of the pectic substances or accompany polysaccharides of pectin in the cell wall.

Amylomaize starch

High-amylose corn starch "Hylon VII" (National Starch and Chemical Company, Neustadt, Germany) with an amylose content of 70 % and with 45.7 % of type III resistant starch was used. The total starch content was determined after alkaline solution by using the enzymatic starch UV assay (Boehringer, Mannheim, Germany). Resistant starch was analysed by the *in vitro* method of Englyst et al. [11].

Wheat starch

Wheat starch from Cerestar (Zülpich, Germany) in a gelatinised form was used as a reference for well digestible carbohydrates.

Energetic value

Energetic values of all components were estimated using combustion calorimetry.

Indirect calorimetry

Apparatus

Energy expenditure and metabolic fuel oxidation were estimated from oxygen consumption, carbon dioxide production and urinary nitrogen excretion over 23 hours in open circuit metabolic cages (Simax, Czech Republic). The oxygen concentration in the air was measured paramagnetically with a 'Permolyt 2' analyser and the carbon dioxide concentration by infrared absorption with an 'Infralyt 4' analyser (Junkalor, Dessau, Ger-

many). Air and catalytically burned ethanol were used for calibration purposes. All electrical signals from six different channels (four from animals, two from calibration) were monitored and received by a 'unit 575/AMM 2' (Keithley Instruments GmbH, Munich, Germany) and a personal computer (PC-AT 386 SX) [12].

Calculation

Energy expenditure and CHO oxidation (as anhydroglycose units) were calculated according to Brower [13], based on caloric equivalents of mean body protein, lipids and anhydroglucose units (including the oxidised short-chain fatty acids, mainly acetate with a RQ of 1.0; the small error due to the lower RQ of propionate and butyrate was neglected in this comparative study) for animal calorimetric experiment and related to the metabolic body size ($kg^{0.75}$).

Animals

Male Wistar rats (Schoe-Wist, Versuchstierzucht Schönwalde, Germany) with an average body weight of 300 g were housed individually prior to the experiments in wire-bottomed cages in a room with controlled humidity and temperature (23 °C) and a fixed 12 hour light-dark cycle (07^{00} to 19^{00} light). The animals received a pellet diet (Altromin, Lage/Westfalia, Germany) and drinking water *ad libitum*.

Diet and feeding schedule

After a 14 hour fasting period, the animals were transferred at 8 a. m. into metabolic cages from glass (Simax, Czech Republic) and received 300 $kJ/kg^{0.75}$ of a basal diet (20 % casein, 3 % sunflower oil, 72 % wheat starch, 1 % vitamin-mix and 4 % minerals, w/w), totally gelatinised by cooking with the threefold amount of water. Oxygen consumption, carbon dioxide production and urinary nitrogen excretion were estimated during the following 23 hours. After 2 days on the pellet diet, the procedure was repeated using the same rats with the addition of 200 $kJ/kg^{0.75}$ of the dietary fibres (first trial: cellulose, soluble dietary fibre from rye; second trial: cellulose, pectin, amylomaize starch). Due to this experimental design each rat served as its own control. To avoid structural changes by heat, the tested dietary fibres were mixed with the basal diet after cooking. Each trial was concluded by feeding the basal diet with the addition of 200 $kJ/kg^{0.75}$ of totally gelatinised wheat starch.

Evaluation of energetic availability of dietary fibres

Energetic availability of the tested dietary fibres was defined as the ratio of CHO oxidation increase over basal diet after addition of dietary fibre and the increase after addition of the same amount of gelatinised wheat starch (based on the estimated energy content).

Statistical analysis

Data in tables and figures are expressed as mean ± SEM. Analyses of variance (one-way ANOVA) followed by Tukey-Kramer test was used to compare the different diets. The levels of significance are given in the tables.

Ethical considerations

The animal experiment was approved by the Ministry of Nutrition, Agriculture and Forestry of the Federal State Brandenburg, Germany.

Results

Energy expenditure, respiratory quotient (RQ) and carbohydrate oxidation over 24 h after intake of a basal diet and after addition of microcrystalline cellulose or soluble rye fibre arabinoxylan in comparison to addition of gelatinised wheat starch are given in Table 1. Due to an energy intake below maintenance requirement, energy expenditure is not dependent on energy intake. However, RQ and total CHO oxidation increase significantly with the amount of available carbohydrates released from the added dietary fibres and wheat starch, respectively. In Table 2, the results of the second experiment after addition of cellulose, pectin and amylomaize starch are summarised. As in the first trial, energy expenditure is independent of the given diet and higher than the energy intake, an advantage for comparing the influence of the added dietary fibres on carbohydrate oxidation. By comparing the increase of CHO oxidation of the added cellulose and the soluble dietary fibre from rye with that of the gelatinised wheat starch, an energetic availability of 13.6 and 33.4% can be calculated. As outlined by the increase on RQ corresponding with an equivalent increase of CHO oxidation, the energetic availability of pectin and amlyomaize starch was calculated to be 38.9 and 61.9%, respectively. As in the first trial, 15.4 % of the energy of microcrystalline cellulose was available for the organism, emphasising good reproducibility of the presented method, likewise outlined by the low coefficient of variation of the carbohydrate oxidation after the basal diet (10.79 ± 0.39 and 10.85 ± 0.52 $g/kg^{0.75}$, Tables 1 and 2, respectively).

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Tab. 1 Energy expenditure, respiratory quotient (RQ) and carbohydrate oxidation in rats after feeding a basal diet and after addition of soluble rye fibre, microcrystalline cellulose or wheat starch (mean ± SEM; n = 7)

	Energy expenditure ($kJ/kg^{0.75}$ x d)	RQ	Carbohydrate oxidation[1] ($g/kg^{0.75}$ x d)	Energetic availability in comparison to wheat starch[2]
Basal diet (BD) (300 $kJ/kg^{0.75}$ x d)	622.3 ± 3.9	0.801 ± 0.001	10.79 ± 0.39	
BD + cellulose (200 $kJ/kg^{0.75}$ x d)	593.6 ± 7.1	0.814 ± 0.005	11.90 ± 0.67	13.6
BD + rye fibre (200 $kJ/kg^{0.75}$ x d)	644.3 ± 6.6	0.823 ± 0.005	13.52 ± 0.53*	33.4
BD + wheat starch (200 $kJ/kg^{0.75}$ x d)	584.9 ± 8.8	0.877 ± 0.004	18.97 ± 0.66*	100

[1] Glucose units including metabolites formed from the dietary fibre (mainly acetate with a RQ of 1.0);
[2] see Materials and methods: evaluation of energetic availability of dietary fibres; level of significance: * $p < 0.05$ (Tukey difference: 2.258) compared with basal diet.

Tab. 2 Energy expenditure, respiratory quotient (RQ) and carbohydrate oxidation over 24 h after intake of basal diet and after addition of microcrystalline cellulose, pectin or amylomaize starch (mean ± SEM; n = 8)

Diet	Energy expenditure ($kJ/kg^{0.75}$ x d)	RQ	Carbohydrate oxidation[1] ($g/kg^{0.75}$ x d)	Energetic availability in comparison to wheat starch[2]
Basal diet (BD) (300 $kJ/kg^{0.75}$ x day)	613.3 ± 19.3	0.804 ± 0.003	10.85 ± 0.52	
BD + cellulose (200 $kJ/kg^{0.75}$ x day)	584.2 ± 10.2	0.824 ± 0.002	12.86 ± 0.18	15.4
BD + pectin (200 $kJ/kg^{0.75}$ x day)	618.0 ± 13.0	0.845 ± 0.005	15.93 ± 0.62*	38.9
BD + amylomaize starch (200 $kJ/kg^{0.75}$ x day)	607.5 ± 15.3	0.870 ± 0.005	18.93 ± 0.64*	61.9
BD + wheat starch (200 $kJ/kg^{0.75}$ x day)	620.4 ± 11.3	0.909 ± 0.005	23.91 ± 0.29*	100

[1] See Table 1;
[2] see Materials and methods: evaluation of energetic availability of dietary fibres; level of significance: * $p < 0.05$ (Tukey difference: 2.054) compared with basal diet.

Tab. 3 Available energy of dietary fibres measured with different methods (mainly with long-term balance technique in rats) in comparison to the described short-term calorimetric method

Dietary Fibre	Method	Available energy (%) Literature	Own results
Cellulose			13.6; 15.4*
Solka-floc cellulose	Dietary energy balance in rats (28 d); faecal analysis	0 [3]	
Microcrystalline cellulose	Cellulose balance with rats (14 d); faecal analysis	8.8 [20]	
Ball-milled cellulose		12.2 [20]	
Acid swollen cellulose		20.3 [20]	
Solka-floc cellulose	Dietary energy balance in rats (28 d); faecal analysis	10 [29]	
Solka-floc cellulose	Dietary energy and dietary fibre balance in rats (21 d)	1.7 [24]	
Bacterial cellulose	C^{14}-labelled balance in rats (14 d)	20.4 [21]	
Arabinoxylans (AX)			33.4
Rye bread with different AX	Degradation in large intestine of pigs	73 [18]	
Resistant starch			61.9*
Resistant starch	Calculation using fermentation stoichiometry	53 [30]	
Raw potato starch (54 % RS type II)	Postprandial carbohydrate oxidation in humans; indirect calorimetry	20 [5]	
Pectin			38.9*
Apple pectin	Dietary faecal balance in rats (14 d); faecal analysis	67 (24)	

* 2nd trial

In Figs. 1 and 2, the time-course of carbohydrate oxidation is plotted after ingestion of the tested fibre containing diets representing the velocity not only of glucose influx into circulation but also of dietary fibre degradation by intestinal flora to short-chain fatty acids and its absorption. The arabinoxylan-rich soluble fraction of rye fibre as well as highly esterified pectin from apple leads to a long-lasting higher CHO oxidation rate during the late phase of digestion. Beyond that, rye dietary fibre leads also in the initial phase of digestion to

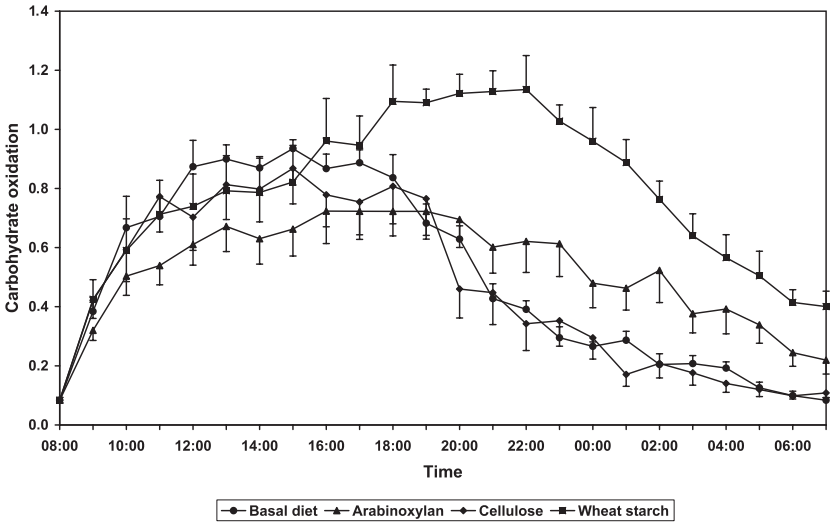

Fig. 1 Postprandial carbohydrate oxidation (g glucose units/kg$^{0.75}$ × h) in rats after intake of a basal diet (300 kJ/kg$^{0.75}$ × d; 20 % protein, 3 % fat, 77 % carbohydrate) and after addition of arabinoxylan, microcrystalline cellulose and wheat starch, respectively (200 kJ/$^{0.75}$ × d in each case) (mean ± SEM; n = 7). Levels of significance based as the areas under the curves are the same as given in Table 1.

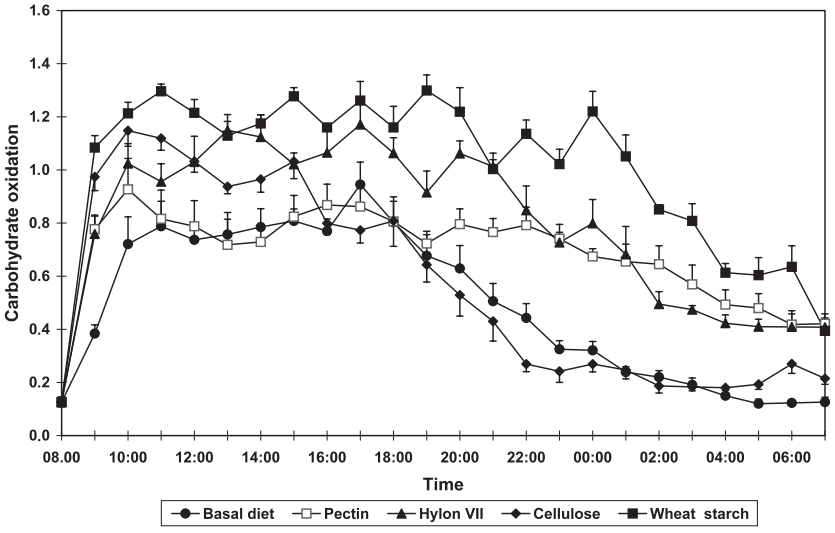

Fig. 2 Postprandial carbohydrate oxidation (g glucose units/kg$^{0.75}$ × h) in rats after intake of a basal diet (300 kJ/kg$^{0.75}$ × d; 20 % protein, 3 % fat, 77 % carbohydrate) and after addition of pectin, amylomaize starch (Hylon VII), microcrystalline cellulose and wheat starch, respectively (200 kJ/$^{0.75}$ × d in each case) (mean ± SEM; n = 8). Levels of significance based as the areas under the curves are the same as given in Table 2.

a retarded increase of CHO oxidation. However, addition of microcrystalline cellulose to the diet tends to enhance the carbohydrate oxidation in the early postprandial phase. This effect may also be caused by the different consistency of the intestinal contents modified by the concentration, kind and properties of the dietary fibres used (for instance soluble/insoluble, neutral/acidic or viscous/low-molecular).

Discussion

Depending on their structure and their functional properties, dietary fibres are important constituents of human nutrition due to their influence on the utilisation of macro- as well as micronutrients. Furthermore, evaluation to what extent these carbohydrates can contribute to whole energy body metabolism in humans as well as in animals is of interest in present nutritional science. Therefore, *in vitro* methods (e. g. fermentation procedures by using faecal flora) as well as *in vivo* methods (mainly long-term growing and balance studies in laboratory animals) were used to characterise the energetic utilisation of dietary fibres [3, 14–25].

For a rough calculation of the energy contribution of different dietary fibres in a mixed diet, a total value of 4 or 8 kJ/ g is used depending on German or British version for calculation [26]. However, for different reasons it is very useful to estimate the energetic value of the single dietary fibre for the exact calculation of the total available energy content in a diet for human as well as for animal studies.

Therefore, our study aimed at the development of the experimental design to evaluate comparable values for

energetic utilisation of different dietary fibres by using indirect calorimetry in rats in short-term experiments.

Dietary fibre and resistant starch escape from hydrolysis by alimentary enzymes in the upper gastrointestinal tract. Depending on their structure and state (isolated or included in cell walls), these substances are either not, partly or more or less completely fermented by the gastrointestinal microflora.

The experimental design used was based on the following premises:

- The oxidation of CHO and their fermentation products are below maintenance requirement equivalent to the absorbed amount of these substrates.
- The time-course of CHO oxidation after an overnight fasting and quick ingestion of the offered food enables an assessment of the velocity of the absorption of carbohydrates and their fermentation products (mainly acetate with a respiratory quotient of 1.0). According to Jequier [27], the blood glucose curve runs in the postprandial phase parallel to the CHO oxidation. Of course, the portion of dietary fibres in the test diets is relatively high, but it was chosen in these short-time experiments to elucidate distinct metabolic effects after intake without adaptation.

We have chosen different types of dietary fibres to compare results of short-term calorimetric experiments with those got from *in vitro* fermentation methods and *in vivo* balance or growing studies.

Microcrystalline cellulose was taken as an almost non-fermentable fibre.

Arabinoxylans are the high molecular, major non-starch polysaccharides in rye and other cereals consisting of a linear backbone of (1→4)-β-D-xylopyranosyl units which are partly substituted with α-L-arabinofuranosyl moieties.

Pectin is a main component of cell walls in fruits and vegetables. It consists mainly of long chains of (1→4)-α-D-galacturonosyl acid units. Besides this homogalacturonan regions, there are rhamnogalacturonan regions with attached neutral saccharide units.

Resistant starch is that part of starch which can not be split and absorbed in the small intestine [28], practically acting as a dietary fibre. We used resistant starch of type III industrially prepared from high-amylose corn starch.

In Table 3, our results are compared with data from the literature measured with different methods (mainly with long-term balance technique in rats). Due to the special structures in the polysaccharide molecules (various monosaccharide residues and types of linkages), published data are different and of high variance depending on the method used. Concerning cellulose which is often used for comparison or as a "standard", there is relatively good agreement between values obtained with our short-term calorimetric method and those of the balance technique. This is due to the fact that the available energy of cellulose is very low because of the small amount of splitting of the (1→4)-β-linkages by the intestinal microflora enzymes. Our presented values for highly fermentable dietary fibres like arabinoxylan, pectin or resistant starch differ more from other data. The reason may be caused by differences in the fine structure of the polysaccharides, in purity of the fibre preparations used and the methods used (including influence of adaptation).

Whether the time course of glucose oxidation after ingestion of dietary fibre containing diets represents the velocity of dietary fibre degradation during the gastrointestinal passage and their influence on the absorption rate of digestible carbohydrates has to be proven in further absorption experiments. The presented data can only be considered as a very rough approach. First attempts to elucidate this question has also been made by Tagliabue *et al.* [5] and Ranganathan *et al.* [31] in human beings indirect calorimetry however after administration of the pure dietary fibres (cellulose, Lintner starch, resistant starch and pectin in comparison to glucose).

Of course, the presented method for estimation of energetic availability of dietary fibres does not take into consideration that there might be an influence on the estimated results due to the high concentration of ingested dietary fibre and the missing adaptation; both factors may influence the reliability of the determined values. But the presented method seems to be very useful for comparative studies of purified dietary fibres as well as other poorly digestible carbohydrates as underlined by the successful estimation of the energetic availability of alcohols and similar substances [32]. Furthermore this short-term method has to be verified by long-term balance techniques.

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