Certified translation from the Czech language

Division of Clinical Physiology, Centre for Research on Diabetes, Metabolism and Nutrition, 2nd Department of Internal Medicine, 3rd Faculty of Medicine and University Hospital Královské Vinohrady, Prague. Chief doctor: Dr. Jan Gojda, tel.: 26716 3031

Project title: Determination of glycaemic and insulin index of nutrition of MANA drink

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Introduction

Physiological minimum

Living organisms require a supply of energy nutrients in order to ensure their survival. These nutrients include sugars, fats and proteins. Of all the nutrients, the most tightly regulated is the sugar metabolism, especially with regard to the fact that under normal conditions the nervous system is dependent exclusively upon the use of glucose (dextrose, the simplest hexose monosaccharide). A whole range of hormones play a role in the glucose metabolism on the level of the entire organism. In practical terms, the most important hormone is insulin. Insulin is released from the pancreas, and in the target tissues (skeletal muscle, fat tissue and liver) enables the entry of glucose into the cells. In principle it is released after food is consumed, and its task is to remove ingested glucose from circulation and clear it into the cells. Insulin is an anabolic hormone, which means that it supports storage processes in the cells. It is precisely from this that its metabolic effects are derived, by which it supplies the nutrients necessary for tissue growth. Between meals, at times of fasting, the insulin level is low and the organism breaks down stored nutrients, from which it sustains cellular processes. In the past, this manner of hormonal control of cycles of eating and fasting enabled our predecessors to survive periods of long-term insufficiency of nutrients, alternated with relatively brief cycles of plenty. In other words, storage of nutrients in times of surplus and their utilisation in times of insufficiency.

Glycaemic and insulinaemic index

The glycaemic and insulinaemic index of a food represents its capacity to increase the level of glucose (glycaemia) and the level of insulin (insulinaemia) in the blood. In simplified terms, these parameters are set in such a manner that the response of the level of glucose/insulin in the blood at the time is compared between the tested food and a referential food, usually pure glucose. The indexes are expressed in percentages of the response of the referential food (glucose has an index equal to 100%). Consumption and absorption of glucose leads to a transitional increase in its levels in circulation, before it is displaced into the cells through the effect of insulin. Insulin is released precisely on this impulse, namely the increase in the level of glucose. For each food we can therefore determine the extent to which its consumption leads to an increase in glycaemia (glycaemic index), and the extent to which it triggers the release of insulin (insulinaemic index). Because glucose is the simplest sugar, its absorption is very rapid, and the majority of other foods have a glycaemic index lower than 100. The insulinaemic index mostly correlates with the glycaemic index (corresponding rise of glycaemia and insulinaemia).

The glycacemic index (GI) indicates the speed at which the carbohydrates contained in the tested food are absorbed in comparison with glucose, in layman's terms how “fast” the carbohydrates are. It has been repeatedly demonstrated that foods with high GI are linked with the advance of obesity. By contrast, interventions which reduce GI in diet by changing the choice of foodstuffs lead to an improvement in metabolic condition and to slimming (Juanola-Falgarona et al. 2014; McMillan-Price and Brand-Miller 2006).

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The insulinaemic index (II) indicates the extent to which the given food increases the release of insulin in comparison with glucose or a referential food. It is known that glucose is a significant stimulus for the release of insulin, but it is known that protein-rich foods, despite the fact that they do not increase glycaemia following consumption, also support the release of insulin. This phenomenon is evidently linked to the need for anabolic signalling by insulin for the formation of complex macro-compounds from the ingested proteins. By contrast with GI, there are few population studies which compare the relationship of the II to the maintenance or increase of body weight and the risk of advance of obesity or diabetes. Isolated studies have been conducted which associate the intake of foods with a high II with the risk of advance of diabetes (Mirmiran et al. 2015). However, to date it is not possible to state with certainty the impact of a diet with an isolated high II.

Methodology

The glycaemic and insulinaemic indexes (GII) were stipulated according to the established methodology (FAO/WHO Carbohydrates in human nutrition. Report of a Joint FAO/WHO Expert Consultation (Wolever et al. 1991; Holt et al. 1997). The details of the protocol are below.

Subjects. The study included ten healthy, non-obese volunteers (7 men, 3 women; average age 25.5 ± 0.9 years, average body weight 75 ± 5 kg). The protocol, approved by the ethical commission of the 3rd Faculty of Medicine, was in accordance with the Helsinki Declaration, and was implemented in accordance with the rules of good clinical practice. Each participant signed an informed consent before the commencement of the study. All the tests were conducted at an accredited centre (Division of Clinical Physiology, 2nd Department of Internal Medicine, University Hospital Královské Vinohrady, Šrobárova 50, Prague 10).

Tests. A total of three tests were conducted within the framework of the protocol (the minimum interval between the tests was 7 days), in random order. Two tests were conducted with a referential food (glucose, Glucopur 50g, Naturamyl a.s., Czech Republic) and one test with the tested food (MANA drink, Mark 3, in a dose equivalent to 50g of carbohydrates, the composition of the preparation is shown in fig. 1) in an equivalent volume of 467 ml. The respondents were examined on an empty stomach (12 hours), a peripheral venous cannula was applied. After a relaxation interval a basal blood sample was taken from the patients (time 0 minutes), after which the food in question was consumed, and subsequently a venous blood sample was taken at regular intervals (15, 30, 45, 60, 90, 120 minutes).

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Fig. 1. Composition of preparation MANA drink, Mark 3, Haeven Labs, s.r.o., Prague, Czech Republic

NUTRITION INFORMATION / NÄHRWERTE / NUTRIČNÍ HODNOTY

Serving Size 1 bottle MANA / Portionsgröße pro Flasche / Velikost porce v láhvi (330 ml / 400 kcal)
Servings Per Bottle / Portionszahl pro Flasche / Počet porcí v láhvi: 1

[MARK 3]

Average nutritional values / Durchschnittliche Nährwerte / Průměrné nutriční údaje		100 ml	1 serving / 1 portion / 1 porce	RI %* 1 serving / RM %* 1 portion / RHP %* 1 porce
Energetic content / Brennwert / Energetická hodnota	kJ/kcal	508 / 121	1675 / 400	20 %
Energetic content from fats / Brennwert der Fette / Energetická hodnota z tuků	kJ/kcal	226 / 54	754 / 180	9 %
Fats / Fette / Tuky (g)		6	20	28,6 %
of which saturates / davon gesättigte Fettsäuren / z toho nasycené (g)		0,6	2	10 %
of which monounsaturated / davon einfach ungesättigte Fettsäuren / z toho mononenasycené (g)		3,5	11,6	-
of which polyunsaturated fat / davon mehrfach ungesättigte Fettsäuren / z toho polynenasycené (g)		1,6	5,2	-
of which Omega-3 Fatty acid / davon Omega-3- Fettsäuren / z toho omega-3 mastné kyseliny (g)		0,4	1,4	-
DHA (Docosahexaenoic acid) / (Docosahexaensäure) / (Dokosahexaenová kyselina) (mg)		55	182	-
EPA (Eicosapentaenoic acid) / (Eicosapentaensäure) / (Eikosapentaenová kyselina) (mg)		30	100	-
ALA (Alpha-Linolenic acid) / (Alpha-Linolensäure) / (Alfa-Linolenová kyselina) (mg)		307	1014	-
Carbohydrates / Kohlenhydrate / Sacharidy (g)		10,7	35,5	13,7 %
of which sugar / davon Zucker / z toho cukry (g)		2,5	8,4	9 %
Fibre / Ballaststoffe / Vláknina (g)		1,2	3,8	15 %
of which soluble fiber / davon lösliche Ballaststoffe / z toho rozpustná (g)		0,4	1,4	-
of which insoluble fiber / davon unlösliche Ballaststoffe / z toho nerozpustná (g)		0,7	2,4	-
Proteins / Eiweiß / Bílkoviny (g)		6,3	20,8	42 %
Salt / Salz / Sůl (g)		0,3	1	17 %

	100ml	1 serving	RI %*
Vitamin A (mcg)	48,5	160	20 %
Vitamin B1 (mg)	0,06	0,2	20 %
Vitamin B2 (mg)	0,1	0,3	20 %
Vitamin B3 (mg)	1	3,2	20 %
Vitamin B5 (mg)	0,4	1,2	20 %
Vitamin B6 (mg)	0,1	0,3	20 %
Vitamin B7 (mcg)	3	10	20 %
Vitamin B9 (mcg)	13,3	44	20 %
Vitamin B12 (mcg)	0,3	1	50 %
Vitamin C (mg)	4,8	16	20 %
Vitamin D2 (mcg)	0,4	1,3	25 %
Vitamin E (mg)	0,8	2,5	21 %
Vitamin K1 (mcg)	4,5	15	20 %
Vitamin K2 type 7 / Vitamin K2 typ 7 (mcg)	15,5	51	-
Potassium / Kalium / Draslík (K) (mg)	121	400	20 %
Iodine / Jod (I) (mcg)	9	30	20 %
Magnesium / Hořčík (Mg) (mg)	22,7	75	20 %
Calcium / Vápník (Ca) (mg)	48,5	160	20 %
Iron / Eisen / Železo (Fe) (mg)	0,9	3	20 %
Zinc / Zink / Zinek (Zn) (mg)	0,6	2	20 %
Manganese / Mangan (Mn) (mg)	0,1	0,4	20 %
Copper / Kupfer / Měď (Cu) (mg)	0,1	0,3	32 %
Selenium / Selen / (Se) (mcg)	3,3	11	20 %
Chromium / Chrom / (Cr) (mcg)	3,6	12	33 %

ENG | *Reference intake of an average adult (8 400 kJ / 2 000 kcal). Percentage daily values are based on 2000 kilocalories diet plan. It is possible that your personal diet plan requires higher or lower energetic intake.

DE | *Referenzmenge der Tageszufuhr für einen durchschnittlichen Erwachsenen (8400 kJ / 2000 kcal). Die prozentualen täglichen Empfehlungen basieren auf einem Ernährungsplan mit 2000 kcal. Ihre Richtwerte können, abhängig von Ihrem Plan, höher oder niedriger liegen.

CZ | *Referenční hodnota příjmu u průměrné dospělé osoby (8400 kJ / 2 000 kcal). Procentuální denní doporučení jsou založena na dietním plánu 2 000 kilokalorií. Je možné, že vaše osobní hodnoty jsou vyšší nebo nižší, záleží na vašem plánu.

RI acc. to EFSA / GDA nach EFSA / RHP dle EFSA	2 000 kcal	2 500 kcal
Fat / Fette / Tuky	70g	87,5g
Saturated Fat / gesättigte Fettsäuren / Nasycené tuky	20g	25g
Sodium / Natrium / Sodík	2 400 mg	3 000mg
Potassium / Kalium / Draslík	2 000 mg	2 500mg
Carbohydrate / Kohlenhydrate / Sacharidy	260g	325g
Protein / Eiweiß / Bílkoviny	50g	62,5g
Dietary Fiber / Ballaststoffe / Vláknina	25g	31,2g

Delicious. Time-Saving. Balanced food for everybody. / Köstliche. Effiziente. Ausgewogene Mahlzeit für jeden Mensch. / Lahodné. Rychlé. Vyvážené jídlo pro každého.

#DrinkMana

Analyses. Immediately after the sample was taken, the blood was centrifuged and aliquots of plasma were frozen at a temperature of -80°C. All the samples were analysed together. The plasmatic concentration of glucose was measured using hexokinase reaction (Konelab Glucose analyzer, Thermo Fisher Scientific, Oy., Finland), and the plasmatic concentration of insulin was measured using chemilumiscence enzyme immunoassay (Immulite 2000, Siemens A.G., Germany). The analyses from plasma were conducted by a certified laboratory (Institute of Laboratory Diagnostics, University Hospital Královské Vinohrady).

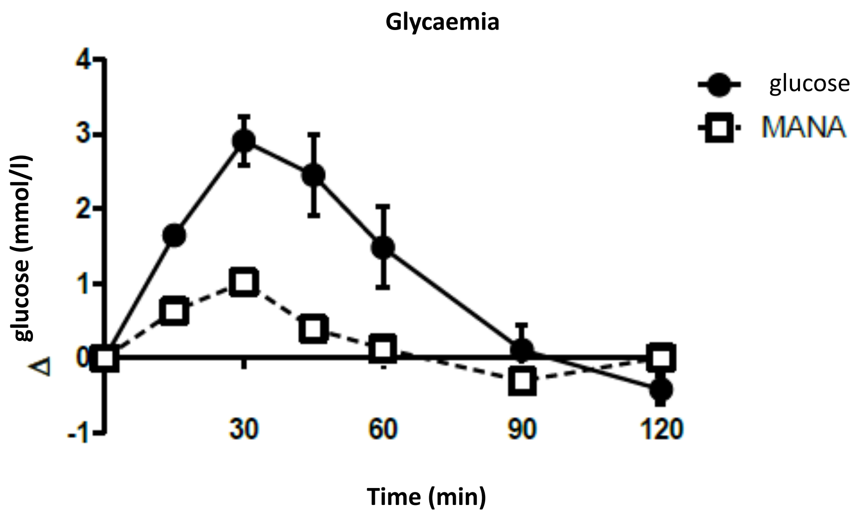
Calculation and statistics. The glycaemic response was calculated from the measured glycaemias over a period of 120 minutes as an incremental area under the curve (iAUC) for the individual tests, calculated with the aid of a trapezoidal model. The insulinaemic response was calculated from the measured insulinaemias, adjusted to 1000kJ of ingested energy (~ 60g glucose and 189.4 ml MANA drink), as an incremental area under the curve (iAUC) for the individual tests, calculated with the aid of a trapezoidal model. The individual values beneath the initial level were excluded.

Intraindividual variability of glycaemic/insulinaemic response in the two referential tests did not differ significantly (two way ANOVA for interaction time v test glycaemia, $p = 0.99$; insulinaemia $p = 0.99$), and as a result was evaluated for the calculation as the average ± SEM from two measurements. The glycaemic/insulinaemic index was calculated as a percentage proportion between the average iAUC of glycaemia/insulinaemic for Glucopur and MANA drink ($iAUC_{MANA}$ / $iAUC_{GLU}$ x 100). The results are expressed as average ± SEM, statistical significance is evaluated as $p < 0.05$. The software GraphPad Prism 5.03, GraphPad Software Inc., USA, was used for all statistical operations.

Results

The kinetics of the plasmatic concentrations of glucose and insulin are illustrated in fig. 2-5 in relative and absolute changes. The glycaemic response for the MANA drink is substantially and statistically significantly lower in comparison with glucose ($p < 0.0001$ for interaction time v test, two-way ANOVA). The insulinaemic response in the case of the MANA drink is substantially and statistically significantly lower in comparison with glucose ($p < 0.0001$ for interaction time v test, two-way ANOVA).

Fig. 2. Glycaemic response



The graph illustrates the average incremental values of glycaemia over time. The data is displayed as average ± SEM; $p < 0.0001$ for interaction time v test, two-way ANOVA.

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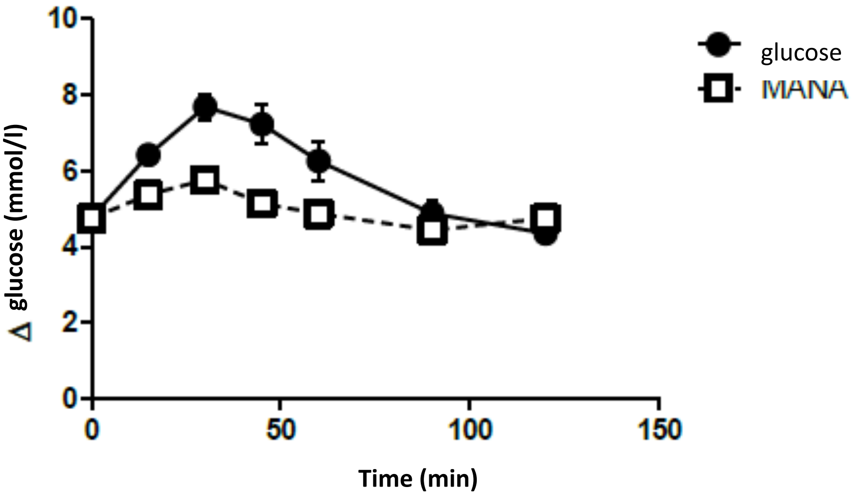
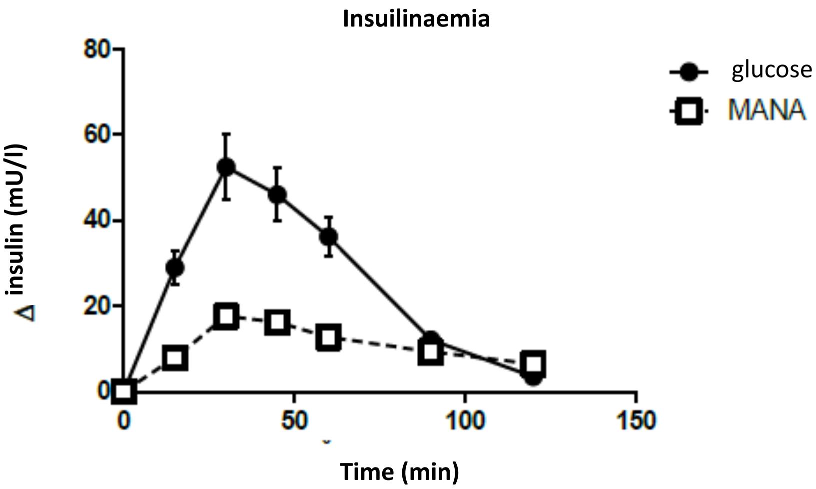


The graph illustrates the average incremental values of glycaemia over time. The data is displayed as average ± SEM; *p* < 0.0001 for interaction time v test, two-way ANOVA.

Fig. 3. Insulinaemic response



The graph illustrates the average incremental values of the insulinaemic response per 1000kJ of energy. The data is displayed as average ± SEM; *p* < 0.0001 for interaction time v test, two-way ANOVA.

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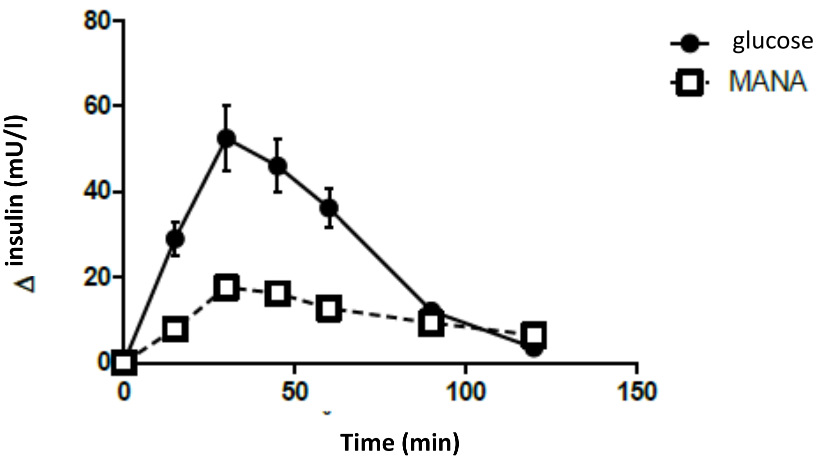

The graph illustrates the average incremental values of the insulinaemic response per 1000kJ of energy. The data is displayed as average ± SEM; $p < 0.0001$ for interaction time v test, two-way ANOVA.

Table 1. Areas under the curve and glycaemic/insulinaemic index of MANA drink

Characteristics	GLUCOSE	MANA DRINK	INDEX	*p*-value
120 min iAUC glucose (mmol/l x min)	151.1 ± 97	44.4 ± 45	29 ± 16	0.0056
120 min iAUC insulin (mU/l x min)	3140 ± 1001	1287 ± 546	41 ± 9	< 0.0001

10 subjects were administered 50 g of glucose or 467 ml of MANA drink (50 g of carbohydrates). iAUC was calculated with the aid of a trapezoidal model. The glycaemic/insulinaemic index is expressed as ($iAUC_{MANA}$ / $iAUC_{GLU}$ x 100). The glycaemic index is calculated from the measured values, the insulinaemic index from the values adjusted to 1000kJ of ingested energy. The values are stated as the average ± standard deviation. The stated *p*-value for the unpaired Student t-test is $iAUC_{MANA}$ v $iAUC_{GLU}$.

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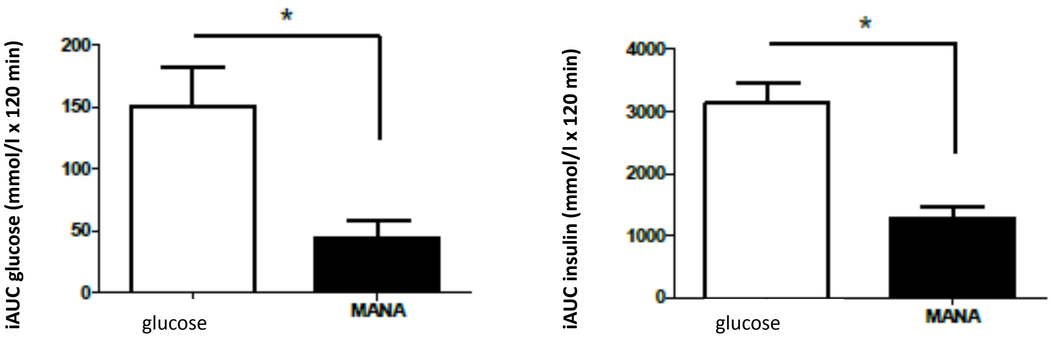
Fig. 4. Comparison of iAUC of glucose and insulin.



10 subjects were administered 50 g of glucose or 467 ml of MANA drink (50 g of carbohydrates). iAUC was calculated with the aid of a trapezoidal model. The iAUC of glycaemia is calculated from the measured values, insulinaemia index from the values adjusted to 1000kJ of ingested energy. The values are stated as the average ± standard deviation. The stated *p*-value for the unpaired Student t-test is $iAUC_{MANA}$ v $iAUC_{GLU}$.

Comments

Within the framework of the protocol, the glycaemic and insulinaemic response of the tested food (MANA drink, Mark 3) and the referential food (glucose) were tested on 10 subjects in an equivalent carbohydrate dose (50 g). The data was used to determine the glycaemic index (GI) and insulinaemic index (II). Data adjusted to 1000kJ of energy was used for determining the II, because the release of insulin is triggered by the intake of any nutrients, not only glucose. As a result, the use of unadjusted values would overestimate the II (Holt et al. 1997). The data was subsequently used to determine the glycaemic and insulinaemic index.

The glycaemic index of MANA drink is 29%, the insulinaemic index 41%. In both parameters it is possible to include the preparation among foods with a low score. For comparison, the GI and II of selected regular foods are presented in table 2.

Table 2. Values of GI and II of selected foods. Processed according to the methodologies corresponding with the currently used methodology (Wolever et al. 1991; Foster-Powell et al. 2002; Holt et al. 1997).

Food	**Glycaemic index** (50g of glucose)	**Insulinaemic index** (1000kJ of energy)
MANA	29	41
Carbohydrate-rich foods		
Oatmeal	42	40
Muesli	66	46
Cornflakes	76	81
Egg pasta (spaghetti)	44	40
Wholemeal pasta (spaghetti)	37	40
French fries	75	74
White rice	64	79

Wholemeal bread	69	96
White bread	100	100
Boiled potatoes	70	121
Protein-rich foods		
Eggs	0	31
Hard cheese	0	45
Fish	0	59
Beef	0	51
Lentils	30	58
Fruit		
Apples	38	59
Oranges	42	60
Bananas	58	81
Watermelon	72	82

According to the present state of knowledge, it appears that goods with a high GI and II are metabolically unfavourable, their ingestion is linked with a high risk of obesity and the onset of type 2 diabetes. This is due to the fact that if we ingest a quantity of carbohydrates that exceeds our current requirement for their immediate utilisation (oxidation), their excess is used for the formation of fats, which are subsequently stored especially in fat tissue. For this reason, long-term population strategies exist in order to reduce the GI of foods (McMillan-Price and Brand-Miller 2006). The high potential of foods to increase glycaemia is usually linked also with a higher release of insulin, and as a result GI and II mostly correlate. Long-term high insulinaemias are linked with a risk of the development not only of diabetes, but also for example cancers. As a result, today the general nutritional trend is towards a "low insulin" diet, i.e. one based on foods with a low GI and II. Similarly, these foods are suitable for people suffering from excess weight or obesity. This is doubly the case for patients with diabetes, who need to monitor the quantity of carbohydrates in the ingested food in order to prevent an increase in their blood sugar level (glycaemia). It has been repeatedly demonstrated that a diet with a low GI/II and reduced content of carbohydrates helps control diabetes and reduces the risk of development of complications in connection with diabetes. This is reflected also in the recommendations of Czech, European and American diabetes societies. In the light of these facts, the MANA drink in its composition appears to be a suitable preparation which can be quite easily used to replace regular foods without burdening the organism with a higher risk of the advance of obesity and diabetes.

On the other hand, it is clear that under certain circumstances, the intake of "fast carbs" is desirable. This concerns situations in which the organism needs to cover suddenly increased demands for energy. This is the case especially during physical exertion. During anaerobic exertion, muscles primarily burn glucose and certain amino-acids. This is similarly the case also during highly intensive interval training. By contrast, during aerobic exertion with low intensity, it is fats in particular that are used. From this perspective, the MANA drink in its properties is suitable especially for longer lasting aerobic activity or periods of reconvalescence, which is partially thanks to the low content and very slow absorption of carbohydrates from the drink, and partially due to the relatively high content of fats. However, it is necessary to note that foods with a high II are necessary for the growth of muscle tissue, because the release of insulin leads to anabolism.

Conclusion

The MANA drink preparation is characterised by a relatively low glycaemic and insulinaemic response and a relatively low carbohydrate content. According to these parameters, it fits within the current concept of a healthy diet, and can be used to replace regular foods. It would be especially advantageous to use the drink on patients with diabetes within the framework of complex dietary treatment.

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