Turku sugar studies XIII

Effect of the diet on certain clinico-chemical values of serum

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Mäkinen, K. K. & Scheinin, A. Turku sugar studies. XIII. Effect of the diet on certain clinico-chemical values of serum. *Acta Odont. Scand.* 33, Suppl. 70, 265—276, 1975 ; reprinted 34, 371—380, 1976.

Pooled and individual serum samples of subjects on lont-term sucrose, xylitol and fructose diets were analyzed for Ca, Mg, K, Na, inorganic phosphate, bilirubin, ascorbate, alkaline and acid phosphatase, amylase, transaminases, lactate dehydrogenase, and amino acids. Most serum samples were obtained from the last phases of the two-year dietary regimen. Significant differences between the three experimental groups were not found with regard to any of the compounds or enzymes studied. Almost significant differences were observed for amylase which was lower in the xylitol and the fructose groups than in the sucrose group, all these values being within the normal range. The results indicate that xylitol and fructose do not induce significant changes in liver function tests, nor in serum level of electrolytes, ascorbate or serum enzymes when their oral administration takes place in the same scale as that of sucrose.

Key-words: Sucrose; fructose; xylitol; serum; enzymes; dietetics

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Dietary xylitol has been reported to evoke changes in blood chemistry in laboratory animals. Thus, rats given xylitol 2 g per kg body weight have higher bilirubin and alkaline phosphatase levels than control animals (*Heraud*, 1973). On the other hand, xylitol consumption has been reported to decrease the plasma calcium and magnesium concentration (*Igarashi et al.*, 1973). A strict xylitol diet has also been found to decrease the activity of amylase in whole saliva (*Mäkinen & Scheinin*, 1975 b), whereas the formation of salivary lactoperoxidase was simultaneously increased manyfold when compared to the consumption of a sucrose diet (*Mäkinen, Tenovuo & Scheinin*, 1975). These observations suggest that various dietary sugars may evoke specific chemical responses in physiological fluids.

Based on the above findings, it was found necessary to perform additional studies on serum samples of the subjects involved in the present studies. This paper provides information about the analyses of bilirubin, alkaline and acid phosphatases, transaminases, lactate dehydrogenase, amylase, Mg, and Ca. Additionally, certain serum enzymes and the following serum compounds were analyzed: amino acids, ascorbic acid, sodium, potassium, and inorganic phosphorus. Isoelectric focusing of serum phosphatases and proteins was simultaneously carried out.

The effects of chronic xylitol, sucrose and fructose diets on lipid and carbohydrate metabolism have been described separately (*Huttunen, Mäkinen & Scheinin*, 1975). These observations dealt with serum concentrations of lactate, pyruvate,

glucose, cholesterol, and urate during the two-year trial.

MATERIAL AND METHODS

1. *Experimental design*

The study was carried out mainly in order to evaluate differences in the caries increment rate as affected by almost complete substitution of sucrose (S) by fructose (F) and xylitol (X) during a period of 2 years. Most of the studies of this paper were performed on serum samples obtained at the end of the trial. The number of subjects participating was 30—33 in the S-group, 33—38 in the F-group, and 47—50 in the X-group. The exact numbers depended on the dietary phases involved (*Mäkinen & Scheinin*, 1975 a, b). In spite of the heterogeneity of the groups in regard to age and sex all chemical values are reported here shown as three comparable groups. The values of the subjects were naturally also inspected individually. In detail, the experiments carried out were as follows:

a. *Sodium, potassium, magnesium, calcium* and *inorganic phosphorus* were analyzed at the 22-month phase.
b. *Ascorbic acid* was assayed at the 13.5-month phase.
c. *Bilirubin.* Direct, indirect, and total bilirubin were determined with the 18.5-month samples.
d. *Amylase* was analyzed at the 22-month phase.
e. *Alkaline phosphatase* was determined with the 18.5- and 22-month samples. These were also subjected to isoelectric focusing to study the focusing pattern of the enzymes involved.
f. *Acid phosphatase.* This assay was carried out with the 22-month samples using isoelectric focusing.
g. *Transaminases.* Serum alanine aminotransferase and aspartate aminotransferase were determined at the 5.5- and 22-month phases in non-fasting subjects, and at the 10.5- and 18.5-month phases in fasting subjects.
h. *Lactate dehydrogenase.* Serum lactate dehydrogenase was assayed as above.
i. *Amino acids.* Serum amino acid composition was determined with samples obtained at the 13.5-, 18.5- and 22-month phases of the trial.

The serum samples were obtained from non-fasted persons at approximately 2—3 p.m. or from the same persons in the morning after 14 h fasting (8 a.m.). The blood samples of approximately 40 ml (5 ml at the 13.5-month phase) were centrifuged within 20 min in cold (+ 4°C) for 10 min at 10000 x *g*. The serum was withdrawn and stored at — 20°C until used, usually within three months after the collection. Ascorbate, bilirubin and the phosphatases were analyzed using fresh samples. Details about the experimental groups, sugar consumption, and other pertinent information were given earlier (*Mäkinen & Scheinin*, 1975 a, b).

2. *Chemical analyses*

Sodium, potassium, magnesium, and calcium were determined with atomic absorption spectrophotometry using Perkin-Elmer Atomic Absorption Spectrophotometer Model 303. Potassium was determined with an Osram discharge lamp and all other metals with hollow cathode lamps. The assays were based on the instructions provided in the Perkin-Elmer Manual (Analytical Methods for Atomic

Absorption Spectrophotometry, January, 1964). Inorganic phosphorus was assayed according to the *Lowry* and *Lopez* (1946) method.

Serum ascorbate was assayed according to *Ordell* (1952) on 1.0 ml samples obtained between 8 and 9 p.m. from test persons who were allowed to consume prior to the blood collection normal foodstuffs containing the sugars involved (in the form of breakfast).

The determination of bilirubin (conjugated, free, and total) was based on direct measurement of the molar absorptivity of diluted serum at 455 nm (*White, Haidar & Reinhold*, 1958). The method is not reliable for low bilirubin concentrations, but can be applied by altering the dilution ratio enough to get reliable readings. The accuracy of this method was limited to approximately 0.5 μmol/l. The assays were performed 18 hours after the collection of the samples. The centrifuged sera were kept this time at 0°C. To avoid possible errors due to turbidity, a correction factor was introduced (*Fog*, 1958 a, b).

3. *Enzyme assays*

Amylase was assayed by the method of *Henry & Chiamori* (1960). Alkaline and acid phosphatase were assayed as suggested by *Bessey, Lowry & Brock* (1946) using *p*-nitrophenyl phosphate as substrate. The reaction mixtures consisted of 0.3 ml of 0.05 M glycinate buffer, pH 10.5 (or 0.05 M β,β-dimethylglutarate buffer, pH 5.0), of 0.2 ml of water, and of 0.1 ml of diluted serum (1 : 9, diluted with water). The above buffers contained $MgCl_4$ (0.0005 moles per litre) and *p*-nitrophenyl phosphate (0.055 moles per litre; sodium salt).

When the phosphatase activity was determined in the fractions resulting from isoelectric focusing, 0.1 ml of a $ZnCl_4$ solution (61.2 mg/100 ml) was added to abolish the inhibitory effect of the Ampholine ampholytes simultaneously present. In these cases only 0.1 ml of water (instead of 0.2 ml) was added to the reaction mixtures. The transaminases were determined for GPT (*Reitman & Frankel*, 1957) and GOT (*Babson & Phillips*, 1965; *Doumas & Biggs*, 1969). Lactate dehydrogenase was assayed according to *Wroblewski & La Due* (1955).

4. *Isoelectric focusing*

The isoelectric focusing of 5.0 ml serum samples was performed with the LKB Isoelectric Focusing System (column 8101) according to the instructions of the supplier (LKB Produkter, Bromma, Sweden). Details of the methods have been described earlier (*Mäkinen & Mäkinen*, 1972). The pH gradient was determined with a 39030 Beckman Combination Electrode at + 25°C.

5. *Amino acid analyses*

Serum amino acid analyses were carried out with the Beckman Unichrom Amino Acid Analyzer according to the standard procedure (described in the Beckman Manual).

RESULTS

1. *Sodium, potassium, magnesium, calcium, and inorganic phosphorus*

Fig. 1 shows the concentration of the metals and Fig. 2 that of inorganic phosphorus in serum. There were practically no differences between the sugar groups.

2. *Ascorbate*

The ascorbate determinations were performed with the S- and X-groups at the

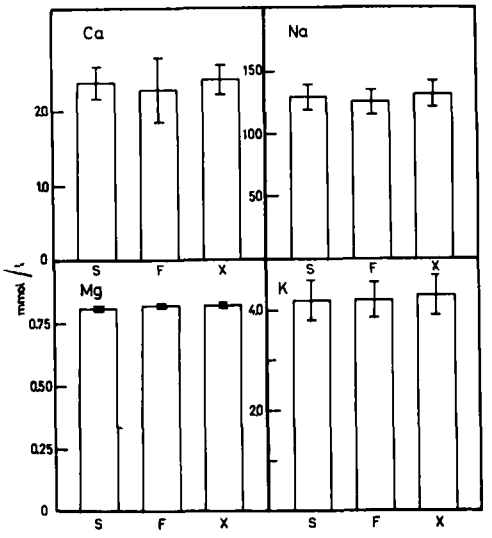

Fig. 1. The concentration of sodium, potassium, calcium, and magnesium in serum. 22-month phase (non-fasting state).

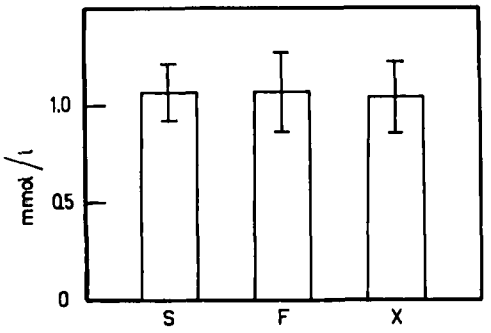

Fig. 2. The concentration of inorganic phosphorus in serum 22-month phase (non-fasting state).

13.5-month phase of the trial. The means and standard deviations are shown in Fig. 3. There was no significant difference between the groups.

3. *Bilirubin*

The bilirubin assays were performed on serum obtained at the 18.5-month phase of the trial. All subjects red normal bilirubin values at the dietary phase indicated. In addition to the bilirubin

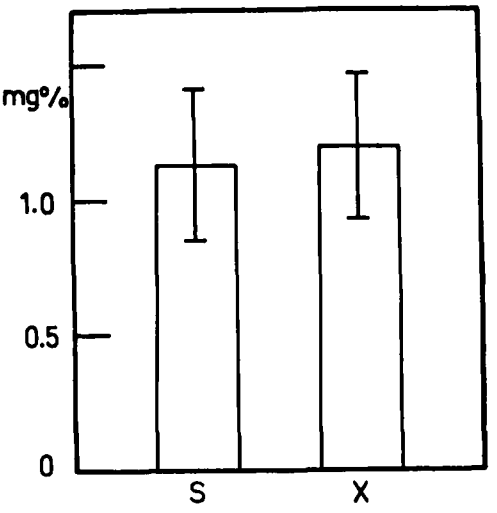

Fig. 3. The concentration of ascorbate in serum. 13.5-month phase (non-fasting state).

Table I. *The concentration of bilirubin in serum (18.5-month phase, fasting state)*

Sugar	Bilirubin (μmol per l)			
	Total		Conjugated (direct)	
	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Sucrose	8.0	4.0	1.0	0.5
Fructose	7.5	4.0	1.0	0.5
Xylitol	8.0	3.5	1.0	0.5

assays, all blood samples during the course of the trial were inspected visually immediately after collection of blood. Except for occasional turbidity in postprandial samples the color was normal in all samples throughout the study.

The amounts of free, conjugated, and total bilirubin were normal in all cases (Table I). There was no tendency in the X-group to show higher values than the two other groups. The amount of free (indirect) bilirubin varied approximately from 3.0 to 6.5 μmol/l in each sugar group.

4. *Amylase*

Serum amylase activity was determined at the 22-month phase of the trial (Fig. 4). The activity was highest in the S-group and lowest in the F-group. The differences between the S- and F-groups ($p = 0.03$), and the S- and X-groups ($p = 0.035$) were found almost significant.

5. *Alkaline and acid phosphatase*

The results of the serum alkaline phosphatase determinations of individual samples are shown in Fig. 4. No significant differences between the experimental groups were found.

The isoelectric focusing patterns of the serum alkaline phosphatase activity were similar in all groups. The same was true for acid phosphatase (Fig. 5). The results were similar at both the 18.5- and 22-month phases.

6. *Transaminases*

Serum transaminase determinations were carried out twice on non-fasting and twice on fasting subjects. The results (Table II) indicate that there were no significant differences between the groups. In a number of cases the serum transaminase activity was practically nil. These values were not included in the calculations of Table II. The total number of these zero values in the four assay periods was as follows: alanine aminotransferase, sucrose (7); fructose (12); xylitol (10); aspartate aminotransferase, sucrose (3); fructose (1); xylitol (0).

7. *Lactate dehydrogenase*

Table II indicates that serum lactate dehydrogenase values were almost similar in all sugar groups and at all examinations.

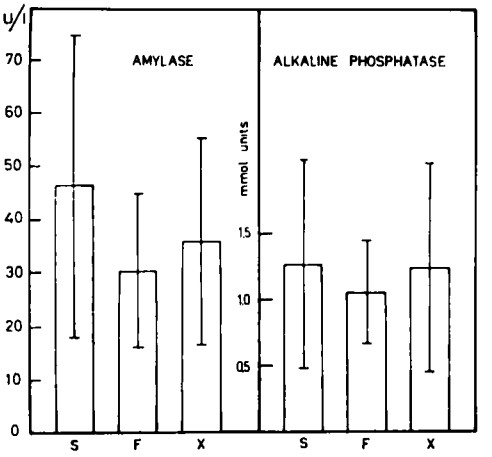

Fig. 4. The activity of amylase and alkaline phosphatase in serum. 22-month phase (non-fasting state).

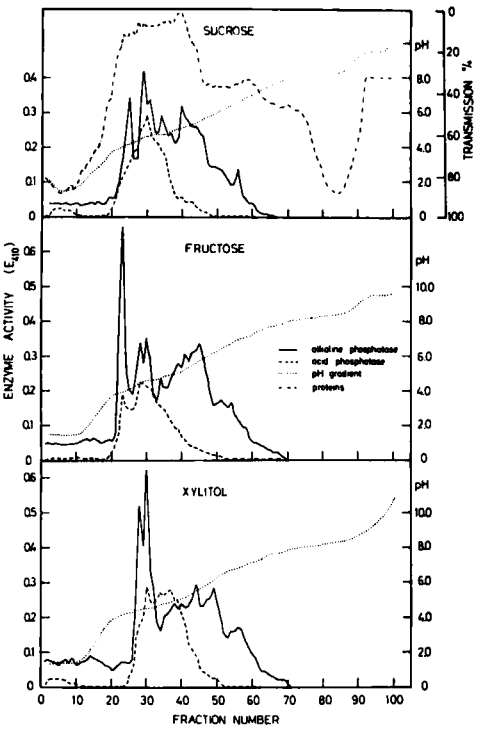

Fig. 5. Isoelectric focusing of pooled serum samples (5 ml) representing the three sugar groups. pH gradient 3.5—10. The per cent transmittance scale (for proteins) stands for all part figures. 22-month phase (non-fasting state).

Table II. *The activity of serum alanine aminotransferase and aspartate aminotransferase (both in U/l), and lactate dehydrogenase (LDH; U/l)*

Enzyme		5.5 Months (Non-fasting state)			10.5 Months (Fasting state)			18.5 Months (Fasting state)			22 Months (Non-fasting state)		
		S	F	X	S	F	X	S	F	X	S	F	X
Alanine amino-transferase	$\bar{x}$	8.2	9.2	9.4	4.8	5.3	6.6	7.6	6.3	5.8	5.5	5.4	5.2
	S.D.	7.48	6.68	7.11	3.65	4.52	4.64	6.69	4.45	3.75	2.95	3.31	2.73
Aspartate amino-transferase	$\bar{x}$	8.1	8.5	9.8	6.7	7.6	7.9	8.2	7.9	8.5	8.5	8.2	8.7
	S.D.	4.14	4.98	4.81	1.74	2.09	2.25	1.95	2.09	1.76	2.39	2.33	2.45
LDH	$\bar{x}$	194	186	203	265	223	249	248	229	201	237	242	243
	S.D.	45.95	46.21	56.27	65.65	70.70	61.32	72.28	81.46	67.92	61.07	51.86	56.18

Table III. *The concentration of amino acids of pooled serum samples collected at the phases indicated. The values are given in μmoles/1000 ml serum. At the 13.5-month phase fructose group samples were not analyzed*

Amino acid	13.5 Months (Non-fasting state) Sucrose	Xylitol	22 Months (Non-fasting state) Sucrose	Fructose	Xylitol
Taurine	38	47	50	45	46
Urea	1405	1848	1057	1101	1064
Hydroxyproline	*	*	*	*	*
Aspartic acid	*	*	25	25	28
Threonine	122	121	94	98	86
Serine	131	155	129	115	88
Asparagine + Glutamine	352	230	358	321	200
Proline	186	186	216	220	152
Glutamic acid	143	171	150	131	202
Citrulline	30	27	19	17	15
Glycine	183	348	262	232	250
Alanine	379	384	391	351	331
α-Amino-n-butyric acid	15	16	19	17	15
Valine	206	219	232	212	202
Cystine	*	*	*	*	*
Methionine	18	26	20	17	13
Isoleucine	63	71	75	63	62
Leucine	118	131	149	128	124
Tyrosine	47	63	63	70	54
Phenylalanine	61	67	73	90	65
Ornithine	56	73	100	77	82
Ammonia	327	317	258	298	316
Lysine	170	185	109	157	124
Histidine	78	84	74	83	84
Arginine	171	172	94	88	100

* Traces

Table IV. *The concentration of neutral and acidic amino acids of serum samples of nine subjects who consumed high amounts of either sucrose, fructose or xylitol. The samples (non-fasting state) were obtained 22 months after the start of the trial. The values are given in μmoles/1000 ml of serum*

Amino acid	Sucrose				Fructose				Xylitol			
	No 1 (♀)	16 (♂)	86 (♂)	Mean	No 25(♀)	19 (♀)	15 (♂)	Mean	No 124(♀)	29 (♀)	47 (♂)	Mean
Taurine	55	57	47	53	32	112	67	70	60	41	55	52
Urea	1189	1631	776	1199	1551	2078	1243	1624	750	1370	1110	1077
Aspartic acid	*	*	*	*	*	*	*	*	*	*	*	*
Threonine	92	143	112	116	98	98	102	99	95	133	131	120
Serine	111	132	92	112	93	118	136	116	122	164	120	135
Glutamic acid	92	99	128	106	47	90	62	66	138	100	156	131
Proline	148	274	185	202	200	235	200	193	115	180	188	161
Glycine	209	199	222	210	202	294	262	253	228	465	239	311
Alanine	273	465	355	364	302	407	446	385	238	235	315	263
Cystine	*	*	*		*	15	15		*	*	*	
Valine	154	270	190	205	151	241	187	193	200	202	219	207
Methionine	15	30	16	20	21	20	21	21	17	24	28	23
Isoleucine	51	100	53	68	49	67	48	55	72	76	77	75
Leucine	95	189	102	129	92	141	95	109	123	118	151	131
Tyrosine	56	95	59	70	60	66	45	57	46	60	85	64
Glutamine Asparagine	304	545	238	362	575	499	442	505	260	318	207	262
Phenyl-alanine	48	81	63	64	84	69	60	71	67	59	76	
Sugar consumption (kg per 2 years)	79	123	71		92	92	126		68	46**	76	

* Traces

** The consumption is given for one year, the subject having been transferred from the F-group.

8. *Amino acid analyses*

Two types of experiments were carried out:

a. Analyses on three serum pools.

b. Analyses on individual serum samples of subjects who consumed exceptionally high amounts of either sucrose, fructose or xylitol.

The amounts of sugar considered high were those amounting to the annual sugar consumption in Finland per capita (approximately 45 kg in 1973), or exceeding this value. From each of the three sugar groups three such representative subjects were studied. In case *b* only neutral and acidic amino acids were determined.

The amino acid analyses performed on serum pools are shown in Table III. The results of the 13.5- and 22-month tests are presented, because the 18.5-month results were similar to the 22-month results. There were practically no differences between the sugar groups. The 13.5-month analysis showed a difference between sucrose and xylitol groups in glycine levels. This was, however, not detected at the 22-month phase. The

Table V. *Hemoglobin, leucocytes and sedimentation rate of red blood cells*

Parameter		5.5 Months (Non-fasting state)			10.5 Months (Fasting state)			18.5 Months (Fasting state)			22 Months (Non-fasting state)		
		S	F	X	S	F	X	S	F	X	S	F	X
Hemoglobin (g/l)	$\bar{x}$	133	131	134	139	135	141	140	136	138	134	133	133
	S.D.	15.82	13.70	14.82	14.54	12.55	13.93	13.76	14.77	16.25	15.11	11.55	13.35
Leucocytes	$\bar{x}$	5888	5821	5731	5573	5341	5443	5711	4943	5440	5974	5719	5882
(x 10^6 liter^{-1})	S.D.	1512	1661	1697	1415	1740	1162	2013	1776	1895	1594	1389	1474
Sedimentation	$\bar{x}$	8	7	9	7	7	8	6	8	10	8	11	12
(mm/hr)	S.D.	7.77	5.88	7.08	5.12	5.95	6.51	4.38	5.93	8.39	6.91	9.09	9.49

results obtained with nine subjects, consuming particularly high amounts of sugars, are shown in Table IV. All values were within the range of normal amino acid composition of human serum.

9. *Hematological values*

Table V shows the results of the quantitative hematological studies. There were no significant differences between the groups.

10. *Values of pregnant subjects*

Table VI shows the chemical serum values of pregnant subjects. All pregnancies, deliveries and infants were normal. Due to the restricted size of this fraction of the material the findings were not analyzed for differences between sugar groups, and between pregnant and non-pregnant subjects.

11. *General health*

During the two-year dietary regimen and at its termination no changes in the general health conditions were observed. Transient osmotic diarrhea, observed in a number of the xylitol subjects in the beginning of the trial, has been separately described (*Mäkinen & Scheinin*, 1975 a).

DISCUSSION

The purpose of the blood determination reported in this paper and in the paper of *Huttunen, Mäkinen & Scheinin* (1975) was to provide reliable data on the safety of chronic fructose and xylitol diets for human subjects. The paper dealing with carbohydrate and lipid metabolism (*Huttunen, Mäkinen & Scheinin*, 1975) indicated that the three sugar groups did

not differ in any detectable way in regard to parameters studied, although several subjects consumed high amount of fructose and xylitol during the trial. This observation is in agreement with earlier experience on the effects of oral fructose and xylitol on human metabolism (*Huttunen*, 1971; *Berg, Bickel & Matzkies*, 1973; *Schmidt, Fingerhut & Lang*, 1964; *Spitz et al.*, 1970; *Dubach, Feiner & Forgo*, 1969; *Lang*, 1971).

In spite of the lack of effects of a two-year consumption of fructose and xylitol on the metabolism of man, the following points deserve attention. The serum and whole saliva (*Mäkinen & Scheinin*, 1975 b) amylase activity was highest in the S-group. The amylase assay used for whole saliva samples is, however, fairly unspecific (*Mäkinen & Scheinin*, 1975 b). It is thus more likely that the lower saliva amylase activity in the X-group was largely due to the strong reduction of dental plaque and its content of bacterial carbohydrates. It is possible that true salivary amylase was not affected or even, that it was increased. Xylitol-fed monkeys exhibited thus higher parotid and submandibular salivary amylase activity than sucrose-fed controls (*Mäkinen et al.*, 1976).

The present amylase method is, however, reliable in serum. Sucrose consumption had a stronger stimulating effect on the liberation of amylase into blood than F or X. The administration of various sugars to normal human subjects is known to result in adaptive changes in jejunal glycolytic enzymes (*Herman*, 1974; *Rosensweig*, 1974; *Greene et al.*, 1975). Related changes also appear most likely to a certain extent in serum, as indicated by the present results. It is obvious that enzymes of the whole alimentary tract are selectively affected by the chemical nature of dietary carbohydrates.

The ascorbate assays indicated that this compound was not accumulated in serum of X-consuming subjects. L-ascorbate may, however, be actively metabolized after possible formation. It has been earlier found that the incubation of L-gulonolactone in a rat kidney system did not lead to ascorbate accumulation (*Burns, Kanfer & Ashwell*, 1969).

The findings indicate that a moderate consumption of xylitol and fructose did not induce responses in the subjects, which would differ from those exhibited by the consumption of equivalent quantities of sucrose. The chemical results obtained during an oral intake of xylitol and fructose are not comparable with those obtained in studies involving parenteral administration of these sugars.

Table VI. *Chemical and hematological blood values of pregnant subjects*

Subject No.	2	7	8	8*	22	32	36
Month of pregnancy**	IV	V	IX	IX	II	VIII	VI
Sugar group	F	S	X	X	X	F	F
Sugar intake***	2.8	2.8	1.0	1.0	0.8	1.5	1.2
Na (mmol/l)		136.7		123.9	124.3		
K (mmol/l)		4.15		3.96	3.71		
Ca (mmol/l)		2.16		2.31	2.30		
Mg (mmol/l)		0.79		0.81	0.79		
P_i (mmol/l)		0.73		0.84	0.94		
Alkaline phosphatase****		1.09		3.51	0.56		
Amylase (U/l)		80.0		32.1	38.0		
IgA (g/l)*****		2.20		1.89	0.65		
IgG (g/l)		13.75		9.24	10.56		
IgM (g/l)		2.25		1.48	1.38		
Alanine aminotransferase (U/l)	16.3	5.5	19.4	9.4	3.3	4.3	7.4
Aspartate aminotransferase (U/l)	13.3	9.7	13.6	14.2	7.9	7.8	6.7
Lactate dehydrogenase (U/l)	125	288	240	250	192	144	288
Hemaglobin (g/l)	112	105	133	117	113	134	116
Leucocytes (x 10^6 $liter^{-1}$)	10260	6634	12460	6434	6834	5566	10434
Sedimentation of red blood cells (mm/hr)	41	49	41	47	20	40	19

* Second pregnancy

** Month of pregnancy at the time of blood collection

*** Average monthly use of sucrose, fructose or xylitol during pregnancy until blood collection (in kg)

**** Bessey-Lowry units

***** For the methods used, see *Mäkinen & Scheinin*, 1975b

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