SOME OBSERVATIONS ON EXPERIMENTAL MODEL FOR INDUCING DIABETES IN MICE AND RATS.

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ABSTRACT

Diabetes mellitus, or simply diabetes, is a group of metabolic diseases in which a organism has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). It is known that some dietary behaviors may increase the likelihood in which certain diseases occur both in humans and animals. The unidirectional diet may alter both the metabolism, as well as the level of some blood components (hormones, sugars, lipids etc.), which may be markers for the incidence of some morbid entities. These concepts may be applied to laboratory animals in order to induce metabolic syndromes in experimental conditions similar to the ones seen in humans. Diabetes in animals most commonly affects middle-aged and older animals and is most common in female dogs and male cats. There are two types of diabetes in animals, uncomplicated diabetes and diabetes with ketoacidosis. When diabetes occurs in young animals, it is often genetic and may occur in related animals.
The purpose of the experimental investigations was the possibilities of inducing a hyperglycemic syndrome in mice (C57Bl6 strain) and in rats (Sprague Dawley strain), a syndrome similar to that found in humans after consuming fructose-containing processed foods.Pure substances like casein, maltodextrin, sucrose, fructose, cellulose were used, in two diets: - standard diet according to AIN-93M; - experimental diet consisted in total replacement of the corn starch, of the sucrose and of the maltodextrin with a 60% fructose diet. Compared to the standard diet fed lot, the glucose tolerance was disturbed in the experimental lot after 39 days of feeding with the 60% fructose diet, and glycosuria was detected at female with the two species after 79 days, which may indicate the disturbance of the dietary metabolism, that is characteristic to the hyperglycemic syndrome.

Key words: *diabetes experimental, mice, rats*

INTRODUCTION

In the last decades the demand for sucrose, and more recently for high fructose corn syrup (HFCS) has increased in order for them to be added to juices, jams, jellies, pastry and dairy products. Therefore, if in 1970 the demand for HFCS was an insignificant 0.23 kg per person per year, in 1997

it had reached 28.4 kg (Putnam and Allshouse, 1999). HFCS is cheaper and sweeter than sucrose, it increases the palatability of the products it is included in, thus replacing sucrose in industrial food products, making possible an over-feeding in humans (Yudkin, 1967; Bray et al., 2004). Details of the different types of sweeteners tendencies in the USA are presented in Figure 1 and Figure 2 (Putnam and Allshouse, 1999).

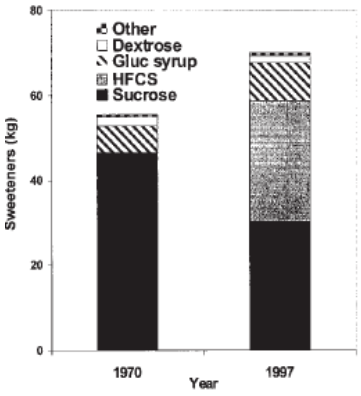

Figure 1. Annual intake per person of sucrose, fructose syrup (HFCS), glucose syrup, dextrose and other sweeteners (bee honey etc.).

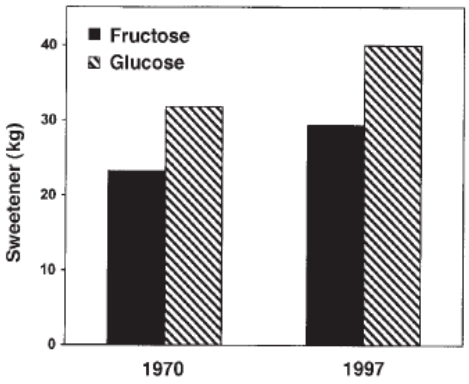

Figure 2. Annual intake per person of fructose and glucose syrup.

In the last decades numerous clinical observation and experiments have gathered that have pointed out that an increased intake of refined sugars, such as HFCS (High Fructose Corn Syrup) and sucrose (a disaccharide formed of fructose and glucose) is associated with arterial hypertension, obesity, diabetes mellitus, kidney and cardio-vascular diseases, both in humans and laboratory rodents.

These harmful effects of fructose excess on health may be attributed to the way fructose is metabolized. The assimilation of monosaccharides, of glucose, galactose and fructose as well of some pentose respectively, implies two mechanisms: *active transport against gradient* and *facilitated transport (passive)*. The greatest absorption capacity is observed in the duodenum and superior jejunum, progressively decreasing in the inferior jejunum and ileum.

Glucose and *galactose* are actively transported thru the microvillous epithelial cell by a transporting protein called SGLT-1. This SGLT-1 protein uses the energy of the Na^+ gradient in the active transport of glucose and galactose. SGLT-1 transports two Na^+ and one molecule of either

glucose or galactose that compete in crossing the brush border of the small intestinal mucosa. The potential difference of Na^+ ions is regulated by the Na^+/K^+/ATP-ase dependent pump that is present in the bazo-lateral membrane of the intestinal epithelium cells.

Glucose and galactose leave the enterocytes by facilitated (passive) transport in the bazo-lateral membrane. The transporting protein responsible with the efflux of glucose and galactose is GLUT-2, this protein being present in the liver, the kidneys and the pancreatic cells as well.

The transport of fructose is facilitated (passive) by a transporter protein named GLUT-5, which is *specific* for fructose and is not inhibated by glucose, galactose or other sugars.

Fructose leaves the enterocytes by crossing the bazo-lateral membrane of the intestinal epithelial cells with the help of the same GLUT-2 transporter used by glucose and galactose.

A first advantage of fructose is the lack of competition in intestinal absorbtion, the transporter protein GLUT-5 being *specific* for fructose and not being inhibited by glucose or galactose. After absorbtion from the gastrointestinal tract, the fructose is transported by the portal circulation to the liver, in which case it will enter the hepatocytes with the help of GLUT-5 transporter – independent of insulin, and is quickly metabolized (Smith Jr et al., 1953; Sato et al., 1996).

In hepatocytes, the fructose is phosphorilated in the presence of ATP, forming fructose-1-phosphate, in a reaction catalyzed by fructosekinase. The resulting fructose-1-phosphate is cleaved by aldolase B in glyceraldehyde and dihydroxyaceton phosphate. Both metabolites may be converted to glyceraldehyde-3-phosphate. Thus, the fructose molecule is metabolized in two phosphate trioses that avoid the main pathway controlling the glycolysis, that being 6-phosphofructokinase, phosphofructokinase is activated by AMP and AMP-cyclic and is inhibited by ATP, representing the direct link between the cell's energetic state and glycolysis (Elliott et al., 2002).

As well, phosphofructokinase, a hepatic enzyme that regulates glycolysis in hepatic cells, acts negatively on the glucose anabolism, while fructose may bypass this control mechanism and be metabolized in glycerol-3-phosphate and acetil-coenzime A. These two intermediary metabolites are then used as substrate for glyceride synthesis, contributing to very low density lipoproteins (VLDL) production (Basciano et al., 2005; Qu et al., 2007).

More, because of the lack of expression of the transporter protein GLUT5 in β-pancreatic cells, fructose, unlike glucose, does not directly stimulate the

insulin secretion (Sato et al., 1996). High quantities of fructose may rapidly stimulate lipogenesis and tryglicerides accumulation in liver, that will consequently reduce the sensibility to insulin and the hepatic resistance to insulin and to glucose intolerance (Basciano et al., 2005).

MATERIAL AND METHOD

The ingredients used to make the purified diets were pure substances, acquired from firms from country and from Germany, that had Quality Certificates and conformity declarations in order to attest both their quality, as well as their purity. Thus: Casein (Kuk Romania SRL); methionine (Nutristar Romania); corn starch, fructose and maltodextrin (Brenntag SRL); sucrose (Lemark SRL); cellulose (J. Rettenmaier & Sohne GMBH); soy oil (S.C. Ultex SA); vitamin-mineral premix (Nutristar Romania); food die Brilliant blue G (Sigma-Aldrich).
The two types of diet were made from the pure substances mentioned above, the first type of diet was a standard one according to AIN-93M (Table 1), and the second experimental diet was imagined for the study, of 60% fructose (Table 2), in which the corn starch, sucrose and maltodextrin have been fully replaced with fructose.

Table 1.

The composition of the diet made of purified ingredients AIN-93M

Index	Ingredients	Quantity (gr.)
1	Casein	140
2	DL- methionine	3
3	Corn starch	465
4	Maltodextrine	155
5	Sucrose	100
6	Cellulose	50
7	Soy oil	40
8	Vitamin-mineral premix	45
9	Choline	2

After the individual dosing of the substances, the samples were mixed for 15 minutes, while distilled water was added (about 1 litre/10 kg mix), in order to ensure the needed consistency to pellet the product. The pelleting was done with the help of a Alvan Blanch granulator from the Institute of Cellular Biology and Pathology “Nicolae Simionescu”. After pelleting, the two diets were dehydrated by depositing them for 48 hours in a

thermostated room at 37^0C. The excess fructose diet is kept in spaces lacked in humidity, because fructose is highly hygroscopic, and the pellets become sticky if kept in humid conditions.

Table 2.

The composition of the diet made with fructose constituted of purified ingredients

Index	Ingredients	Quantity (gr.)
1.	Casein	210
2	DL- methionine	3
3	Fructose	600
4	Cellulose	79.85
5	Palm oil	50
6	Vitamin-mineral premix	55
7	Bitartrate choline	2
8	Food coloring	0,15

The lab animals used came from Animaleria SPF "Stațiunea Băneasa" of Cantacuzino Institute, using: - Mice from strain C57Bl6 6 weeks old, males and females equally, with roughly the same body weight (Table 3).

Table 3.

Groups of mice strain C57Bl6 used in the experiment

Sex Group / ID-animal		Group 1 witness weight	Group 2 fructose weight
♂	N-normal-uncut ears	18.7gr	19.0 gr
	UD-right ear	16.9 gr	18.4 gr
	US-left ear	16.7 gr	19.0 gr
	AU- both ears	17.0 gr	18.2 gr
	2UD – twice the right ear	18.1 gr	16.5 gr
♀	N-normal-uncut ears	15.2 gr	14.4 gr
	UD-right ear	14.1 gr	13.8 gr
	US-left ear	15.2 gr	13.9 gr
	AU- both ears	13.8 gr	14.3 gr
	2UD – twice the right ear	13.8 gr	13.1 gr

Rats from strain Sprague Dawley (SD) 6 weeks old, males and females equally, with roughly the same body weight (Table 4). The identification of animals was possible with the help of some cuts made to the ear pavilion, as seen in Tables 3 and 4. First, the animals were habituated in the accommodation rooms for experimental animals, in regard with the microclimate, hygene and feeding norms needed for these protocols (Curcă, 2004).

Table 4.

Groups of rats strain Sprague Dawley (SD) used in the experiment

Sex Group / ID-animal		Group 1 witness weight	Group 2 fructose weight
♂	N-normal-uncut ears	160.5 gr	152.8 gr
	UD-right ear	116.6 gr	183.2 gr
	US-left ear	152.9 gr	126.2 gr
	AU- both ears	152.5 gr	131.1 gr
	2UD – twice the right ear	166.5 gr	185.8 gr
♀	N-normal-uncut ears	139.0 gr	125.1 gr
	UD-right ear	141.1 gr	137.2 gr
	US-left ear	154.0 gr	145.2 gr
	AU- both ears	142.9 gr	136.2 gr
	2UD – twice the right ear	142.2 gr	165.7 gr

The experimental room has a microclimate controlled by the conditioning system, ensuring the optimum of temperature and humidity (t^0C 18-22; UR 55-65%). The acclimatization period was 7 days, meanwhile the animals were accommodated 5 individuals of the same sex in one per cage. During that period, the transition from the natural diet (of natural ingredients: cereals, proteic shrots of soy and sun-flower) to the standard purified one administered *ad libitum*. The composition of this diet is mentioned above (Table 1).

Blood samples were collected for dosing glycemia, both in the glucose tolerance test (GTT), as in the control dosing, which were made by penetrating the lateral vein of the tail, found at the limit of the anterior third and the middle third of the tail, practicing a small incision with a scalpel blade, collecting the blood drop directly on the reactive strip.

Glycemia was measured with ACCU CHEK ACTIVE Glucometer (Roche diagnostics). Prior to sampling, the animals were dieted, with no hydric diet, for 16-18 hours. The glucose tolerance test or *the orally provoked hyperglycemia* is a functional test that explores the reaction of the organism to the glucidic overload. Thus, after 16-18 hours of alimentary diet the basal glycemia (T0) is determined, after which glucose solution 20% is administered as gavage in dose of 1 gram per 1 kg live weight. Then the glycemia is measured after 15, 30, 60 and 120 minutes after the gavage, revealing information about the capacity of insulin releasing or the peripheric resistance to insulin (http://EMPReSS). If there are altered values for basal glycemia or after feeding fructose, this test can be redone of it may

infirm diabetes mellitus diagnostic. In the glucose tolerance test gavage needles 18G for rats and 22G for mice were used.
The urine analysis was done for the animals that presented increased values of the glycemia, these being tested for glycosuria with the help of CYBOW strips (DFI Co, Ltd). The urine was directly sampled on the reactive area of the strip.Animals were weighted weekly during the experiment with the help of the SARTORIUS-type scale for animals.

RESULTS AND DISCUSSION

The ingredients of the purified diet that ensure the nutrients needed are presented in Table 5, resulting in the quantity of components, as well as the chemical parameters that were estimated and realized.

Table 5.

The purified diet that ensures the upkeep necessary, AIN-93M

No.	**Ingredients:**	**Quantity** (gr)	**Estimated analytical parameters**	
			Estimated	Realized
			Nutrient Quantity%	Nutrient Quantity%
1	Casein	140	Protein 14,02%	Protein 14,0%
2	DL- methionine	3	Sugars 68,0%	Sugars 68,0%
3	Corn starch	465	Lipids 4,1%	Lipids 4,1%
4	Maltodextrin	155		
5	Sucrose	100		
6	Cellulose	50		
7	Soy oil	40		
8	Vitamin-mineral premix	45		
9	Betartrate choline	2		

This diet was administered to the animals of both groups during the habituation period for the first week and then only to the witness group for the whole testing period. The purified diet with fructose was administered to the animals in the second group during the testing period (Table 6). The diet and the water were administered *ad libitum.* At the end of the habituation period, which lasted a week, the animals were evaluated from the glucose tolerance point of view. The glycemia values resulted were considered reference values for the normal animals before the diet alteration. The results of the glucose tolerance test are given in real values in the next tables (Table 7 for mice and Table 8 for rats). From the data analysis in Table 7 and the respective diagrams it can be observed the uniformity of reaction to

the glucose tolerance test in mice C57Bl6 of both sexes, befor administerng the fructose diet and to the rapid decrease of the glycemia after T15.

Table 6.

The purified experimental diet with fructose 60%.

No.	**Ingredients:**	**Quantity** (gr)	**Estimated analytical parameters**	
			Estimated	Realized
1	Casein	210	Nutrient Quantity%	Nutrient Quantity%
2	DL-methionine	3	Protein 18.5%	Protein 18.5%
3	Fructose	600	Sugars 60.0 %	Sugars 60.04 %
4	Cellulose	79.85	Lipids 5.0%	Lipids 5.05%
5	Palm oil	50		
6	Vitamin-mineral premix	55		
7	Bitartrate choline	2		
8	Food coloring	0.15		

It can be noticed that the glycemia value of the entire male group in T0 with 108,8 mg/dl blood average and the standard average 5,59. The results of the glucose tolerance test done on SD rats after the habituation period was over are presented in Table 8. After analysing the data found in table 8, as well as the respective diagrams, it can be observed the uniformity of reactions between sexes to the glucose tolerance test and the continous glycemia between T15-T30 minutes.

Also, there is a slower response to the glucose load in rats as opposed to mice. After determing the glucose tolerance test after 39 days of feeding with the experimental diet in mice, the data were written in table 9.

Table 7

The results of the glucose tolerance test (Gl) in mice strain C57Bl6 that will be fed with experimental diet

Sex Group ID-animal		Live weight gr	ml. sol. Gl 20% gavage	T0 Gl mg/dl	T15 Gl mg/dl	T30 Gl mg/dl	T60 Gl mg/dl	T120 Gl mg/dl
♂	N	17.6	0.088	114	250	231	188	107
	UD	17.1	0.0855	105	211	188	147	107
	US	17.8	0.089	113	171	201	144	123
	AU	18	0.09	111	233	194	162	165
	2UD	16.5	0.0825	101	237	235	186	147
Average		**17.4**	**0.087**	**108.8**	**220.4**	**209.8**	**165.4**	**129.8**
d.s.		0.60	0.00	5.59	30.98	21.72	20.88	25.60
♀	N	14	0.07	107	228	122	170	96
	UD	13.3	0.0665	118	150	209	111	101
	US	14.2	0.071	97	266	158	138	84
	AU	13.7	0.0685	127	252	221	142	116
	2UD	13.2	0.066	139	168	192	155	111
Average		**13.68**	**0.0684**	**117.6**	**212.8**	**180.4**	**143.2**	**101.6**
d.s.		0.43	0.00	16.46	51.35	40.35	21.92	12.62

After 39 days of feeding the mice C57Bl6 with fructose diet the folowing observations can be made, by analising the diagrams above:

- the uniformity of reaction between the individuals from the same group as well as between sexes dissappears;
- the average value of the basal glycemia both in males and females decreases. This decrease of the average basal glycemia in both sexes may be the consequence of the alimentary intake of glucose with decompensation of glycogenolysis and gluconeogenesis;
- the basal glycemia values in male mice have a wider distribution with a standard derivation of 35,27 compared with the moment T0 of the test when the animals ate the standard diet and the basal glycemia values were concentrated around the average, the standard deviation being 5,59;
- a remakable important aspect in both sexes is the tardiv hypoglicemia and the decrease of the glicemia to T120 under the basal glicemic value T0. This situation may be the consequence of exhausting the hepatic glycogen reserves; - the male mouse identified US is remarked by a flat curve that may be the expresion of hyperinsulinemia, characteristic to the incipient stages of hyperglicemic syndromes.
- the male mouse identified 2UD is remaked because of the slow response, with no return to the initial value, not even after 120 minutes after the oral

administration of glucose solution, indicating insulin resistance. In the female group, the female identified UD is noticed due to the peak in T15, when glycemia reached 411 mg/dl, indicating a weak secretion of preformed insulin. After determining the glucose tolerance test after 39 days of feeding rats SD with experimental diet, the data presented in table 10 were obtained. After analyzing the data from the table, it can be observed that the male rat identified "N" died in the first day of the experiment, after his weight dropped from 180.2 gr to 147.3 gr. Following the tendency of weight evolution in the male "N" it was observed a slight increase of the weight after 7 days of fructose diet intake, then a constant decrease. As well, in this rat in the 11th day of basal glycemia control test, his 85 mg/dl glycemia dropped under the average of the group of 100 mg/dl blood.

Table 8

The glucose tolerance test (Gl) done on rats strain Sprague Dawley (SD) that are fed with the experimental diet

Sex Group ID-animal		Live weight gr	ml. sol. Gl 20 % gavage	T0 Gl mg/dl	T15 Gl mg/dl	T30 Gl mg/dl	T60 Gl mg/dl	T120 Gl mg/dl
♂	N	180.2	0.901	110	225	213	159	127
	UD	215.3	1.0765	105	160	200	147	107
	US	150.8	0.754	134	237	192	183	130
	AU	156.5	0.7825	114	167	236	195	113
	2UD	223.3	1.1165	108	185	203	168	122
Average		**185.22**	**0.9261**	**114.2**	**194.8**	**208.8**	**170.4**	**119.8**
d.s.		33.13	0.17	11.54	34.5	16.9	19.0	9.63
♀	N	142.2	0.711	98	199	204	166	112
	UD	158.4	0.792	107	179	197	129	109
	US	159.5	0.7975	92	188	204	126	110
	AU	151.5	0.7575	102	206	181	126	109
	2UD	192	0.96	99	231	241	193	115
Average		**160.72**	**0.8036**	**99.6**	**200.6**	**205.4**	**148**	**111**
d.s.		18.80	0.09	5.50	19.88	22.01	30.32	2.55

By correlating this evolution of the body weight with the basal glycemic value in the 11th day and the aspect of the liver during the necropsy (yellow clay colored with rounded edges), the perturbation of the metabolism due to the lack of food intake of glucose may be deduced.

Table 9

The glucose tolerance test (Gl) done in the 39th day of feeding the mice strain C57Bl6 with experimental diet

Sex Group / ID-animal		Live weight gr	ml. sol. Gl 20 % gavage	T0 Gl mg/dl	T15 Gl mg/dl	T30 Gl mg/dl	T60 Gl mg/dl	T120 Gl mg/dl
♂	N	14.6	0.073	104	179	117	67	63
	UD	15.4	0.077	124	214	129	69	48
	US	13.1	0.0655	45	114	75	62	48
	AU	14.6	0.073	46	166	87	57	37
	2UD	13.8	0.069	90	278	273	221	111
Average		**14.3**	**0.0715**	**81.8**	**190.2**	**136.2**	**95.2**	**61.4**
d.s.		0.88	0.00	35.27	60.81	79.53	70.48	29.23
♀	N	9.8	0.049	78	275	146	78	60
	UD	10.4	0.052	64	411	174	85	59
	US	9.4	0.047	60	183	172	121	60
	AU	12.5	0.0625	67	177	169	155	66
	2UD	10.7	0.0535	56	174	155	108	53
Average		**10.56**	**0.0528**	**65**	**244**	**163.2**	**109.4**	**59.6**
d.s.		1.20	0.01	8.37	102.4	12.15	30.81	4.62

Table 10

The results of the glucose tolerance test (Gl) done in the 39th day of feeding the rats SD with experimental diet

Sex Group ID-animal		Live weight gr	ml. sol. Gl20 % gavat	T0 Gl mg/dl	T15 Gl mg/dl	T30 Gl mg/dl	T60 Gl mg/dl	T120Gl mg/dl
♂	N	147.3	-	-	-	-	-	-
	UD	214.9	1.0745	87	182	197	178	155
	US	138.1	0.6905	66	172	159	127	29
	AU	167.7	0.8385	168	170	189	161	145
	2UD	233.3	1.1665	138	182	184	130	116
Average		**180.26**	**0.9425**	**114.75**	**176.5**	**182.25**	**149**	**111.2**
d.s.		37.51	0.22	46.63	6.40	16.40	24.70	57.27
♀	N	123.3	0.6165	105	143	268	156	91
	UD	133	0.665	97	185	238	230	121
	US	145.5	0.7275	244	272	272	243	180
	AU	148.9	0.7445	119	153	194	157	144
	2UD	212.8	1.064	151	177	230	195	120
Average		**180.26**	**0.9013**	**143.2**	**186**	**240.4**	**196.2**	**131.2**
d.s.		41.94	0.21	60.00	51.03	31.73	40.27	33.13

By analysing the diagrams of the glucose tolerance test done after 39 days of feeding with fructose diet of the rats SD the following observations occur:

- the basal glicemia increases only in some individuals, both males and females. Fron the male group the individuals identified with "AU" and "2UD" are noticed due to the basal glicemia that increased with 47.3% and 21% respectively to the group average. From the female group the female "US" is noticed, having the basal glicemia increase by with 70.3%;
- there is no more uniformity of reaction both between individuals of the same groups as well as between different sexes. The T0-T15 interval represents the oral glucose asimilation phase, meanwhile the preformed insulin is consumed. Thus, in the male rat group the stabilization of the average value of glicemia to 141.2 mg/dl, with the standard deviation 6.40 noticed at T15 is observed, demonstrating the presence of preformed insulin. 15 minutes after oral administering of glucose the newly sinthesised insulin secretion begins, marked by the increase of standard deviation, indicating the individual capacity of synthesis and captation of glucose. The curves made by glicemia in T30, are ascendant both in males as in females,showing the existance of an insufficient secretion of a peripheric resistance to insulin.

It can be noticed that after 39 days of feeding with fructose diet, the answer to provoked hyperglicemia in the glucose tolerance test, is much slower so that the relative plateau constant between T15 and T30 initially, transformed in the case of females in an ascendant curve. The data concerning the dynamics of the average of glicemia in mice C57Bl6 and rats SD, at the beginning of the feeding with experimental diet, then in the 11^{th}, the 39^{th} and the 79^{th} day of experimental diet, ar seen in table 11 and their respective diagrams. By analysing the charts above, it can be noticed that although the rats Sprague Dawley and mice C57Bl6 belong to the same family (*Muridae*), they have different ways of metabolic response to the fructose substitution, of any glucidic source from diet. If rats have a hiperglicemic answer to the diet change, the mice C57Bl6 have a first hipoglicemic answer. Just by analising the glicemic evolution in the first phase in mice, without correlations with other metabolic parameters, the fructose excess may be considered a beneficial nutritional factor in hiperglicemic syndromes. The correlation between these two parameters, the basal glicemia and the glucose tolerance, monitored in this study by insulin, cholesterol, tryglicerid, uric acid dosage and arterial tention determination, may create a complex and complete image of the metabolic perturbances provoked by the excess of fructose in diet.

Table 11

Synoptic table with the average of glicemia values in mice C57Bl6 and rats SD, at the beginning of the feeding with experimental diet, then in the 11th, 39th and 79th day

DATE	Mouse C57/Bl6 ♂	Mouse C57Bl6 ♀	Rat SD ♂	Rat SD ♀
12.05.2011 - **initially**	**108.8**	**117.6**	**114.2**	**99.6**
23.05.2011 – **11th day**	**89.6**	**100.2**	**100**	**97.4**
17.06.2011 – **39th day**	**81.8**	**65**	**114.7**	**143.2**
27.07.2011 – **79th day**	**126.5**	**110**	**152**	**170.6**

In the 79th day of feeding with experimental diet in mice and rats, the glicemia and glucosuria were determined, the data being written in Table 12 and table 13 respectively.

Table 12

Values of glycemia and glycosuria in mice C57Bl6 in the 79th day of experimental diet

Sex	**Group**	**ID. Animal**	**Glycemia mg/dl**	**Glycosuria mg/dl**
Male	**Fructose**	US	133	Negative
		2UD	120	Negative
	Witness	2UD	173	Negative
Female	**Fructose**	N	162	+/- 100
		UD	58	+/- 100
	Witness	AU	137	Negative

Table 13

Values of glycemia and glycosuria in rats SD in the 79th day of experimental diet

Sex	**Group**	**ID. Animal**	**Glycemia mg/dl**	**Glycosuria mg/dl**
Male	**Fructose**	AU	152	Negative
		US	114	Negative
	Witness	N	216	Negative
Female Male	**Fructose** Witness	N	216	+/- 250
		US	169	+/- 100
		AU	127	Negative
	Fructose	AU	141	Negative

After analyzing the data presented in tables 12 and 13, it may be noticed the presence of glycosuria with different intensities in females of both species, while in males of both species it hyperglycemia was installed with no glycosuria.

CONCLUSIONS

The diets made from purified ingredients allowed the selective elimination of all sources of sugars and their replacement with fructose, making possible the evaluation of its effects over the metabolism.

The quantitative alteration, at a given moment, of a nutritional factor from the diet makes the evaluation of the effect very precise. This means that there are practically unlimited possibilities of alterations that can be done in a purified diet, making them a powerful research instrument.

Fructose excess diets administered to mice from strain C57Bl6 and rats from strain Sprague Dawley lead to:

- The increase of basal glycemia and the alteration of glucose tolerance;
- Glyosuria in females of both species while the males in both species were hyperglycemic and not glycosuric.

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