Effect of High Fat and High Fat-High Protein Diets on Biosynthesis of Niacin From Tryptophan in Rats

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Conversion of tryptophan to niacin in male weanling albino rats, fed high fat and high fat-high protein, was studied. High fat diet suppressed niacin biosynthesis from administered tryptophan. High protein along with high fat was found to overcome the deleterious effect of high fat on the conversion of tryptophan to niacin. Prolonged feeding of high fat decreased urinary, blood and tissue levels of niacin in rats. It is suggested that the observed effects of high fat feeding might be due to high production of ketone bodies.

It has been reported that high fat feeding to rats causes increased production of the ketone bodies, acetoacetate and β -hydroxybutyrate (1). Kotake observed pyridoxine deficiency and an increase in xanthurenic acid excretion in rats fed high fat and tryptophan (2). Acetoacetate and β -hydroxybutyrate have been reported earlier by us to suppress the biosynthesis of niacin from tryptophan in rats (3). The present experiments were undertaken to study the effect of high fat feeding on the biosynthesis of niacin from tryptophan in rats. As it has been observed that a high percentage of dietary protein overcomes niacin deficiency (4), the effect of high protein along with high fat in the diet was also studied.

MATERIALS AND METHODS

1. Experiments Performed.

Two sets of experiments were performed. In the first set male weanling albino rats weighing 40–50 g each were divided into three groups. They were fed the experimental diets deficient in niacin, the compositions of which are given in Table 1. The rats of the first group were fed the basal diet (Diet 1). Second group rats were fed a high fat diet (Diet 2) and a high fat-high protein ration (Diet 3) was fed to the animals of the third group. After feeding these diets for 5 weeks, urinary excretions of niacin and N^1 -methylniacinamide (NMN) in these rats were studied (Table 3). Tryptophan was then administered to the rats of all the three groups, orally and intraperitoneally alternately and urinary excretions of niacin and NMN were determined. The results are shown in Table 4. The urinary collec-

tions were made for successive two day periods. The urine from two rats fed the same ration was pooled for each period of collection. In all cases, collections were made from the experimental groups in each series at the same time in order to obtain data for comparable periods of all groups.

Another set of experiments was performed simultaneously to study the effect of feeding high fat and high fat-high protein to rats for prolonged periods along with niacin. Male albino rats (40–50 g body wt) were divided in three groups and fed the above mentioned diets, which were supplemented with niacin (1 mg per 100 g), for a period of 90 days. Urinary excretions of niacin and NMN were studied periodically, *viz.* after 15, 30, 45, 60, 75 and 90 days of feeding (Table 5). The animals were then killed and their blood, liver and muscle analysed for niacin. The results are shown in Table 6.

2. Diets

The niacin deficient diet (Diet 1) used in the experiment was the one described by Schweigert and Pearson (4). High fat diet (Diet 2) contained 40 % groundnut oil, while high fat-high protein diet (Diet 3) consisted of 40 % groundnut oil and 50 % casein. In each case the substitutions were made for an equal weight of sucrose. The composition of the experimental diets is given in Table 1. The animals were given food and water *ad libitum*.

TABLE 1
Composition of the Experimental Diets

Constituent	Diet 1	Diet 2	Diet 3
	<i>g/100 g of diet</i>		
Casein (vitamin free)	12	12	50
Salt mixture (Hawk-Oser)	4	4	4
Groundnut oil	4.7	40	40
Fortified shark liver oil.	0.3	0.3	0.3
L-Cystine	0.3	0.3	0.3
Sucrose	78.7	43.4	5.4

B-vitamins per 100 g of ration: thiamine 250 μ g, riboflavin 300 μ g, pyridoxine 250 μ g, calcium pantothenate 2 mg, choline chloride 100 mg, Inositol 100 mg, biotin 10 μ g, folic acid 200 μ g.

and Control groups received 1 mg per 100 g of niacin in their ration.

3. Analytical Methods

Niacin in urine and tissues was estimated by the microbiological method described by Hawk *et al.* (5). Urinary excretion of NMN was estimated by the fluorometric method of Huff and Perlzweig (6).

RESULTS AND DISCUSSION

From Table 3 it is seen that the rats fed high fat, niacin-deficient diet (Diet 2) for a period of 5 weeks excreted lower amounts of niacin and NMN in urine than the animals fed Diet 1. However, there was no decrease in urinary excretion of niacin and NMN in those fed high fat-high protein, niacin deficient diet (Diet 3). This may be due to the fact that, high amounts of tryptophan are available

TABLE 2

Effect of High Fat and High Fat-High Protein Diets on Body Weight of Rats Receiving Niacin Deficient and Supplemented Diets

Dietary regimen	Average body weight after weeks of feeding											
	Niacin deficient						Control (1 mg niacin/100 g diet)					
	0	1	2	3	4	5	0	1	2	3	4	5 weeks
	<i>g per rat</i>											
Diet 1	50	65	70	68	61	60	48	65	71	82	94	101
Diet 2	50	49	43	43	41	38	48	63	62	70	76	81
Diet 3	50	69	68	61	58	64	45	62	75	75	88	98

TABLE 3

Urinary Niacin and N¹-Methylniacinamide in Rats Fed High Fat and High Fat-High Protein Diets Deficient in Niacin

Dietary regimen	Urinary niacin	Urinary NMN
	<i>μg/rat/day</i>	<i>μg/rat/day</i>
Diet 1	8.6	26.1
Diet 2	5.5	15.3
Diet 3	8.5	28.4

The values of niacin and N¹-methylniacinamide are averages from 12 rats, 6 determinations on the pooled urine of 2 rats in each.

TABLE 4

Effect of Tryptophan on Urinary Excretion of Niacin and N¹-Methylniacinamide of Rats Fed High Fat and High Fat-High Protein Diets Deficient in Niacin

Dietary regimen	Urinary niacin and N ¹ -methylniacinamide			
	Tryptophan fed ^a		Tryptophan injected ^b	
	Niacin	NMN	Niacin	NMN
	<i>μg/rat/day</i>			
Diet 1	58	688	47	591
Diet 2	37	428	29	401
Diet 3	54	669	44	604

The values of niacin and N¹-Methylniacinamide are averages from 8 rats, 4 determinations on the pooled urine of 2 rats in each

^a L-Tryptophan, 1 % in the diets.

^b DL-Tryptophan, 2.5 ml of 2 % NaHCO₃ solution, containing 10 mg of tryptophan per ml, twice daily.

TABLE 5

Urinary Niacin of Rats Fed High Fat and High Fat-High Protein Diets Supplemented with Niacin (Values are mean ± SE)

Diet	Urinary niacin after days of feeding						
	0	15	30	45	60	75	90
	<i>μg/rat/day</i>						
Diet 1	20.4 ± 2.1	21.0 ± 1.7	22.2 ± 2.3	28.6 ± 1.8	31.0 ± 2.4	35.5 ± 3.3	34.3 ± 1.7
Diet 2	22.1 ± 1.8	17.3 ± 0.8	14.4 ± 2.8	13.8 ± 2.0	14.1 ± 1.6	13.9 ± 2.1	14.9 ± 1.2
Diet 3	19.8 ± 1.3	18.7 ± 1.0	19.0 ± 0.7	27.3 ± 2.2	32.4 ± 2.2	33.1 ± 1.5	34.7 ± 2.6

TABLE 6
Blood and Tissue Niacin of Rats Fed High Fat and High Fat-High Protein Diets Supplemented with Niacin for 90 Days

Diet	No. of animals	Niacin level		
		Liver	Hamstring muscle	Blood
		$\mu\text{g/g}$	$\mu\text{g/g}$	$\text{mg}/100\text{ ml}$
Diet 1	10	178 (154-198)	84 (74-93)	1.38 (1.29-1.53)
Diet 2	6	106 (92-118)	67 (59-71)	0.84 (0.72-0.95)
Diet 3	7	181 (158-202)	91 (74-100)	1.40 (1.31-1.64)

Values in the parentheses give the range.

when 50 % casein is given to the animals. Schweigert and Pearson (4) found that 25 % casein in the diet almost completely overcame the deficiency of niacin. They attributed this to the availability of more tryptophan. We also found that the rats fed Diet 2 lost weight to a greater extent than those given Diets 1 and 3 (Table 2).

When tryptophan was administered orally or intraperitoneally, there was an increase in the excretion of niacin and NMN (Table 3). The increase in excretion of these substances was comparatively less in the rats fed Diet 2 than in those fed Diets 1 and 3 (Table 4), thus indicating a suppression of niacin synthesis from administered tryptophan in those fed high fat diet alone.

Table 5 shows urinary excretion of niacin after 15, 30, 45, 60, 75 and 90 day feeding of the rats fed with Diets 1, 2 and 3 supplemented with niacin. It is seen that the rats fed Diet 2 excreted lower amounts of niacin than those fed Diets 1 and 3. There was not much difference in excretions of the animals fed Diets 1 and 3. When the animals were killed after 90 day feeding and their liver, muscle and blood analysed for niacin, the animals fed Diet 2 (high fat) were found to contain less niacin in their blood and tissues than those kept on Diet 1 or 3 (basal or high fat-high protein).

The results of our experiments indicate that a high level of fat in the diet has a deleterious effect on the conversion of tryptophan to niacin in rats. Kotake fed rats simultaneously with a large amount of a fatty acid (Na salt) and tryptophan and found high xanthurenic acid excretion in urine (7). He attributed this to the inhibition of the enzyme related to the formation of pyridoxal phosphate from pyridoxine. The deleterious effect of high fat diet on the growth of rats fed a riboflavin deficient diet has been observed by Mannering *et al.* (8). They suggested that high fat diet might alter the intestinal synthesis of riboflavin. It is known that the conversion of tryptophan to niacin is dependent on the presence of other water-soluble vitamins such as thiamine, riboflavin and pyridoxine (9). Chakrabarti and Nath (10) have shown that acetoacetate and β -hydroxybutyrate, which are produced in large quantities in high fat feeding (1) cause considerable decrease in urine and blood levels of riboflavin in rabbits. Devyagina (11) reported that a four week treatment of atherosclerotic patients with niacin activated the anticlotting blood system and Nath and Brahmarkar (12) found that administration of acetoacetate to rabbits for 8 weeks caused hastening of blood coagulation. They suggested that the fats, causing accumulation of ketone bodies in blood (13), might be shortening the coagulation time due to accumulation of acetoacetate, thus resulting in atherosclerosis and thrombosis. Our present study seems to suggest that the observed

deleterious effects of high fat feeding on niacin biosynthesis are related to the high production of ketone bodies, which have been shown to be responsible for many deleterious effects on carbohydrate and fat metabolism reported earlier from this laboratory and confirmed by others (14, 15).

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