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EFFECTS OF NICOTINIC ACID ON THE CHOICE OF SUBSTRATES UTILIZED DURING FASTING*

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Abstract—Two-day fasted rats receiving nicotinic acid (0·5 m-mole/kg at 2·5-hr intervals) showed the previously described depression of plasma free fatty acids (FFA) and also an elevation of plasma urea and an accelerated urinary excretion of urea. It was estimated that, during the 2-6-hr interval of nicotinic acid treatment, animals produced urea at three times the control rate and obtained nearly half their energy requirements from endogenous protein. During 6·5 hr of treatment, the total fatty acids of the kidneys and liver decreased, respectively, by 16 and 40 per cent. Data indicate that endogenous protein and fatty acids outside of adipose tissues become major energy sources when plasma FFA and blood glucose are reduced, respectively, by nicotinic acid and fasting.

NICOTINIC acid has been widely used as a clinical drug for lowering blood lipids and as an experimental tool for investigating metabolic control mechanisms. A basic effect of nicotinic acid is to retard the release of free fatty acids (FFA) into the blood and to inhibit the lipolytic effect of norepinephrine, fasting, and diabetes.[1–6] The result is a reduction in plasma FFA and a retardation of FFA uptake by the liver. Carlson and co-workers believe that the latter effect is responsible for the desired reduction in plasma concentrations of triglyceride and cholesterol.[1, 7, 8]

It is obvious that an inhibition of lipolysis, as produced by nicotinic acid, must have important metabolic consequences for the whole animal. Studies with intravenously injected ^{14}C-labeled free fatty acids reveal that plasma FFA are normally the chief source of the fat burned in the body;[9] and a variety of techniques indicates that the proportion of total calories obtained from the catabolism of fats, carbohydrates, and amino acids depends largely on the relative concentrations at which they are available. It is not surprising that, while lowering plasma FFA, nicotinic acid has been reported under various conditions to increase the fraction of total calories derived from carbohydrate.[10–12] Eaton *et al.*,[13] however, have observed that, in man, the respiratory quotient does not rise promptly during nicotinic acid treatment and suggested that part of the intracellular lipids outside of adipose tissues constitute sizable reserves of substrate for energy metabolism.

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Experiments were conducted according to the "Principles of Laboratory Animal Care" of the National Society for Medical Research.

The present studies concern the metabolic effects of nicotinic acid in rats when the blood glucose and, particularly, the liver glycogen have been reduced by 2 days of fasting. A first portion of the studies was carried out in a gradient-layer calorimeter and demonstrated an elevation in respiratory quotient (R.Q.) during nicotinic acid treatment. The elevated R.Q. could, of course, be explained either by an increased rate of catabolism of endogenous protein or by a net destruction of carbohydrate stores. Therefore, the rate of urea production was estimated in nicotinic acid-treated and control rats; and the quantity of three main components of body carbohydrate was determined at specified intervals during nicotinic acid treatment. The total fatty acids in the kidneys and liver were also measured at specified times in order to spot-check the hypothesis that portions of the fat outside of the adipose tissues can be utilized as a major energy source when plasma concentrations of glucose and FFA are low.

METHODS

The animals for these studies were male Sprague–Dawley rats which had been fasted for 2 days; they weighed, on the average, about 300 g. The injection mixture of nicotinic acid used was approximately isotonic to rat plasma, containing 0·07 M NaCl and 0·1 M nicotinic acid in a 2:1 molar ratio as the sodium salt and the free acid. To test animals, 5 ml/kg of this solution (i.e. 0·5 m-mole/kg nicotinic acid) was injected i.p. and reinjected at 2·5-hr intervals until the animals were sacrificed by decapitation. To control animals, 0·9% NaCl was injected at the same volume and frequency.

Calorimetry study

A first study was performed in a calorimeter which has been described elsewhere.[14] Groups of eight animals were used in each experimental run. As soon as all eight had been injected, either with nicotinic acid or saline, each was placed in a separate compartment of a metal rack and transferred promptly to the dark interior of the calorimeter, which was maintained at 20°. After animals had spent 60 min in this new environment, measurements of oxygen consumption, CO_2 production, R.Q., and radiative and evaporative heat losses were commenced; these measurements were then recorded for 80 min and represented total values for the eight animals. Animals were then taken out, reinjected, and returned to the calorimeter; and, after a 60-min wait, data were again recorded for a second 80-min observation period. In about half the experimental runs, this procedure was finally carried out a third time. Calorimetric data were obtained during 60–140, 210–290, and 360–440 minutes after the initiation of treatment with nicotinic acid or with saline.

Biochemical studies

Subsequent studies were carried out with rats maintained in separate cages at room temperature (23–26°).

The rate of urea production was estimated during the 2–6-hr interval after the start of the injection schedule with nicotinic acid or saline. Half the animals were sacrificed at 2 hr; the other half were transferred to metabolic cages at that time

and sacrificed 4 hr later. The urea in urine and plasma samples were determined by measuring the CO_2 evolved in Barcroft-Warburg manometers during incubation with urease (from Sigma) in acetate buffer, pH 5·0. The urea produced in the animals was assumed to equal the quantity excreted into urine plus that accumulating in the body. The quantity accumulating in the body during this 4-hr period was estimated from the plasma concentrations of urea observed at 2 and 6 hr and was calculated on the assumption that urea was distributed in 700 ml body water/kg having the same urea concentration as had the plasma. In estimating urea excretion, the residual urine in the bladder was taken carefully into account. All animals were handled 110 min after the start of the injection schedule (their tails were washed with cool water) in an attempt to induce micturition. Urine was rinsed from the bladders of animals sacrificed at both 2 and 6 hr by repeated injection and withdrawal of acetate buffer. The urea recovered from the bladders at 2 hr provided an estimate of the quantity of previously excreted urea which was retained within the remaining animals at the start of the 4-hr urine collection period. The quantity of urea excreted over the 4-hr period was calculated to equal the quantity discharged on the metabolic cages plus that retained at 6 hr in the bladder minus that initially present in the bladder.

In other studies, blood glucose,[15] lactate,[16] and plasma free fatty acids[17] were determined by standard methods. Total fatty acids were also measured in kidneys and livers. Prior to analysis, the livers were quickly removed, freed of extraneous tissue, rinsed in 0·9% NaCl, blotted, and weighed. The kidneys were dissected free from the capsule and ureter, and part of the pelvis was removed. The organs were homogenized in at least 20 ml chloroform:methanol (a 2:1 v/v mixture) by means of the revolving blades of a VirTis tissue grinder. The procedures for saponifying the total lipids, extracting the fatty acids, and titrating them were as previously described.[18] For the kidney analyses, however, extra care was required to keep the lipids suspended in alcoholic KOH during saponification. Liver triglycerides were also determined by Van Handel's alternate method in which silicic acid was used to remove phospholipids from the lipid extract.[19]

In one study, glycogen was measured in the whole-animal carcass. In this procedure, animals were first anesthetized by an intravenous injection of sodium pentobarbital, the skin and subcutaneous fat were promptly removed, and the remaining carcass was fragmented in a Waring blender for 5 min in 1200 ml 5% (w/v) trichloroacetic acid (TCA). An aliquot (A_1) of this homogenate was twice extracted by homogenization in 5 vol, of 5% TCA. Since quantitative extraction of glycogen in TCA at room temperature is extremely difficult to achieve,[20] the speed and duration of homogenization were carefully controlled. The last two homogenizations were performed in a VirTis tissue grinder having a rated maximum velocity of 40,000 rpm. For these operations, the grinder was operated at a near maximal speed for 2 min each. In several instances, the last homogenization in 5% TCA was carried out in a ground-glass homogenizer at 90–95°. This procedure, which Kemp *et al.* have shown to yield almost quantitative extraction of glycogen,[21] was found to increase the glycogen yield by about 20–25 per cent. In all cases the homogenates of A_1 were combined. The glycogen from an aliquot of this pooled homogenate was precipitated with ethanol and measured colorimetrically with anthrone reagent by the method of Carrol *et al.*[22]

RESULTS

Table 1 summarizes the data on respiratory quotient and oxygen consumption rate (Q_{O_2}). At each of the three observation periods, the R.Q. of nicotinic acid-treated animals was elevated to a rather uniform extent above the levels observed in controls.

TABLE 1. EFFECT OF NICOTINIC ACID ON RESPIRATORY QUOTIENT AND RATE OF OXYGEN CONSUMPTION

Treatment	Time after start of injection schedule								
	60–140 min			210–290 min			360–440 min		
	N*	R.Q.	Q_{O_2}†	N	R.Q.	Q_{O_2}	N	R.Q.	Q_{O_2}
Nicotinic acid	18	0·795	912	18	0·768	912	8	0·760	948
Saline	14	0·755	960	14	0·736	930	7	0·726	924
Difference		0·040	−48		0·032	−18		0·034	24
S.E. of difference		0·0068	24		0·0068	24		0·0098	35

* N refers to the number of experimental runs in the calorimeter, each made with eight rats.
† Q_{O_2} is expressed as ml O_2 (converted to 0° C and 760 mm Hg)/kg/hr.

The effects on Q_{O_2} were somewhat complex. Saline-treated controls were consistent in showing a slightly higher Q_{O_2} during the first observation period than during the subsequent two periods. The Q_{O_2} of the nicotinic acid-treated group was essentially the same at all three intervals and differed from that of controls ($P < 0.05$) only during the first exposure to the calorimeter.

TABLE 2. EFFECT OF NICOTINIC ACID ON UREA PRODUCTION

Urea values	Nicotinic acid-treated group		Saline-injected controls	
	Mean	S.E.	Mean	S.E.
4-Hr urine sample (+ bladder contents) (m-mole/kg body wt.)	9·01	0·40	4·40	0·24
Initial bladder contents (m-mole/kg body wt.)	0·80	0·20	0·81	0·26
Urea excreted per 4 hr	8·21	0·45	3·59	0·34
Final plasma concentration (μmole/ml)*	10·36	0·61	4·72	0·19
Initial plasma concentration (μmole/ml)*	6·63	0·38	4·58	0·15
Difference (μmole/ml)	3·73	0·72	0·14	0·24
Estimated urea production in 4 hr (m-mole/kg)	10·62		3·69	

* Initial determinations of urea washed from the bladder and of blood plasma concentrations were made 2 hr after a first injection of saline or nicotinic acid. The "final" sample of blood plasma was obtained 4 hr later, after two additional injections of saline or nicotinic acid. At each of these two times, eleven test animals and eleven controls were sacrificed.

Table 2 shows that, during the 2–6-hr interval after the onset of the prescribed injection schedule, nicotinic acid-treated animals excreted urea at more than twice the rate of controls. In the test group, plasma urea concentration was elevated at 2 hr and was further elevated at 6 hr to more than twice the control level. It was

estimated that the nicotinic acid-treated animals produced urea at nearly three times the rate of the control animals.

Table 3 summarizes data on the concentration of various carbohydrate components during nicotinic acid treatment. No change in body composition with respect to carbohydrates was observed. It is concluded that, during nicotinic acid treatment, the quantity of carbohydrate synthesized from amino acids was similar to the quantity oxidized to CO_2 and water.

TABLE 3. POSSIBLE CHANGES IN COMPONENTS OF BODY CARBOHYDRATE AFTER NICOTINIC ACID

	Starting condition	150 min	240 min	300 min	270 min	450 min
Carcass glycogen (mg/kg body wt.)	1023 (9)*	982 (9)		1036 (9)		1004 (9)
S.E.	50·8	70·9		85·7		78·4
Blood glucose (mg/ml)	0·62 (6)		0·61 (6)	0·56 (8)	0·59 (6)	0·61 (8)
S.E.	0·016		0·035	0·018	0·014	0·013
Blood lactate	0·079 (9)		0·078 (9)	0·103 (8)	0·093 (9)	0·104 (8)
S.E.	0·0099		0·0056	0·0068	0·0110	0·0087
Estimated carbohydrate pool (mg/kg)†	1264			1267		1260

* In parentheses are the number of animals on which the indicated determination was made.
† Estimates of the carbohydrate pool are based on the assumption that lactate and glucose are distributed, respectively, in total body water (700 ml/kg) and in a smaller pool of 300 ml/kg at the same concentrations observed in blood.

TABLE 4. CHANGES IN BLOOD AND LIVER AFTER NICOTINIC ACID

	Starting condition*			240 min*			270 min*		
	Mean	S.E.	N	Mean	S.E.	N	Mean	S.E.	N
Plasma FFA (μmole/ml)	0·82	0·107	7	0·12	0·010	9	0·14	0·024	9
Liver size (g wet wt./kg body wt.)	25·7	0·63	9	23·8	0·41	9	22·4	0·62	9
Liver total fatty acids (m-mole/kg body wt.)	3·98	0·200	9	2·79	0·123	9	2·38	0·087	9
Liver triglyceride (m-mole/kg body wt.)	1·074	0·0144	9	0·267	0·0030	9	0·108	0·0015	9

* Animals representing the "starting condition" were sacrificed about 9:00 a.m. before receiving nicotinic acid. Other animals received nicotinic acid at 2·5-hr intervals and were sacrificed at 4 or 6·5 hr after the start of treatment.

Table 4 indicates that, during nicotinic acid treatment, plasma FFA were reduced by 80 per cent and that livers showed a net loss of 40 per cent of their total fatty acids over a 6·5-hr period. Most of the depletion occurred during the first 4 hr. Of this depletion, more than half could be accounted for in the triglyceride fraction, which decreased by 70 per cent during the first 4 hr and by 90 per cent in the full 6·5-hr period.

The kidneys, as organs which do not secrete fat and can presumably lose fatty acids only by oxidizing them, were also studied. As Table 5 indicates, the kidneys showed a 16 per cent decrease in their total fatty acids over a 6·5-hr period. The reduction in kidney total fatty acids during the 4–6·5-hr period of treatment was apparently real ($P < 0.05$) and similar in quantity to that observed during the first

TABLE 5. EFFECTS OF NICOTINIC ACID ON KIDNEYS

	Duration of nicotinic acid treatment			Saline control*	S.E.M.D.†
	0 min	240 min	270 min		
Number of animals	10	10	10	10	
Organ (wet) wt (g/kg body wt)	7·4	7·6	7·4	7·4	
Total fatty acid (μmole/g kidney)	92·8	83·8	77·0	94·2	2·77
Total fatty acid (μmole) per kg body wt	687	639	580	697	27·6

* Saline controls were sacrificed 6·5 hr after the first of three injections with 0·9% NaCl.
† The standard error of the difference between any two means (D.F. = 36).

4 hr of treatment. The effect of nicotinic acid on the total fatty acids of the kidneys was smaller than that observed in the livers.

DISCUSSION

A key effect of nicotinic acid appears to be to increase the fraction of total calories derived from the oxidation of carbohydrate.[11–13] Free fatty acids have been shown, *in vitro*, to have a glucose-sparing action on the perfused rat heart[23, 24] and on skeletal muscle;[25] and Carlson *et al.*[12] consider that the reduction of plasma FFA by nicotinic acid releases tissues from this inhibition on their glucose catabolism. Nicotinic acid has been shown to bring about an increased utilization of carbohydrate in briefly fasted animals and presumably had this effect in the severely fasted rats in the present study.

It is tempting to compare the action of nicotinic acid on urea production with that of phlorhizin. Nicotinic acid would be considered to speed the loss of carbohydrate through oxidation, while phlorhizin, acting rather specifically on the kidneys,[26] brings about a marked loss of glucose in the urine. Apparently, endogenous protein is utilized for glucogenesis at an accelerated rate when carbohydrate has been depleted by fasting and is being lost or utilized at an increased rate. The phlorhizinized rat, after 2 days without food, has been reported to excrete urea at a quite similar rate to that at which the fasted nicotinic acid-treated rats produced urea in the present study.[27] However, this analogy should not be carried too far. Although the phlorhizinized rat, in degrading endogenous protein, uses the glucogenic amino acid chiefly for glucose synthesis, it remains to be shown whether the fasted nicotinic acid-treated animal does likewise.

In the present study, body carbohydrate was shown not to accumulate in the body during nicotinic acid treatment. Therefore, the endogenous protein which was degraded sufficiently to yield urea may be considered to have been entirely catabolized, though in part by an indirect route involving glucose synthesis. The quantity of

total calories obtained ultimately from protein catabolism can be estimated from the urea data.

Most proteins are considered to contain 16 per cent (w/w) nitrogen and to yield during catabolism 4·32 kcal/g. The energy obtained from the catabolism of endogenous protein can be conservatively estimated on the assumption that all the protein nitrogen released in this process entered urea synthesis. The observed production of urea in nicotinic acid-treated animals (2·7 m-moles/kg/hr) would indicate that the catabolism of endogenous protein contributed 2·04 kcal/kg/hr. The observed rate of oxygen consumption indicates that the total metabolic rate of these animals was 4·3 kcal/kg/hr. It is concluded that nearly half the total energy requirement of these animals was derived from the catabolism of endogenous protein.

Plasma FFA, in spite of their reduced concentration in nicotinic acid-treated rats, appear to have been a major energy source in these animals. In fasted animals not receiving nicotinic acid, it has been previously shown that plasma FFA are the source of most of the calories utilized by the fasted animal.[9] Carlson *et al.*[12] have reported that, at least in man, nicotinic acid does not appreciably alter the fractional turnover rate or the fraction of plasma FFA which, on leaving the blood, are burned to CO_2 and water. It appears that, after nicotinic acid treatment, the rate that plasma FFA are utilized for energy metabolism is reduced by about the same extent as is the plasma FFA level. This line of reasoning would suggest that when nicotinic acid reduced plasma by 80 per cent in the present studies, plasma FFA were still contributing 10–15 per cent of the energy requirements of the treated animals.

At this point, not more than 65 per cent of the substrate utilized for total metabolism has been accounted for in fasted animals receiving nicotinic acid. Endogenous protein and plasma FFA accounted, respectively, for about 45–50 per cent and 10–15 per cent; and it has been demonstrated that there is no net destruction of carbohydrate. It appears likely that fatty acids previously deposited in tissues other than the adipose must be used to a significant extent as energy sources during nicotinic acid treatment of fasted rats. Carlson and Nye[8] have reported that nicotinic acid decreases liver triglycerides sharply. A 390-min treatment with nicotinic acid is seen in the present study to bring about the loss from the liver of a quantity of fatty acids sufficient, theoretically, to account for an additional 15 per cent of the metabolic rate. Unfortunately, the data of Table 4 should not be interpreted in this way for two reasons. First, it is known that part of the triglyceride fatty acid secreted by the liver into the blood may be extracted by adipose tissues and not be promptly used up.[28] Second, various studies indicate that part of the plasma triglycerides are first converted to plasma FFA before being catabolized;[29, 30] and the caloric contribution of plasma FFA has already been taken into account in this discussion.

The kidney was given special attention as an organ which does not secrete fat and can presumably lose fat only by catabolizing it faster than it extracts FFA or triglyceride fatty acids from the blood. The quantity of fat depleted from the kidneys in 6·5 hr of nicotinic acid treatment was small and could account for only about 1 per cent of the total animal metabolism. However, the kidneys, though having an unusually high metabolic rate for their size, represented less than 1 per cent of body weight; and the quantity of fat which was lost from the kidneys during nicotinic acid treatment was sufficient probably to account for an important fraction of the total metabolism of those organs. This finding is consistent with the hypothesis[13]

that part of the intracellular fat of various tissues constitutes an important reserve of substrate which can be utilized when other substrates are in short supply.

It has been reported that norepinephrine elevates the metabolic rate significantly and that nicotinic acid inhibits the calorigenic effect of norepinephrine but does not otherwise affect the metabolic rate.[12] These observations provide a likely explanation of the finding that, on the first exposure to the calorimeter, saline-treated rats showed a slightly elevated metabolic rate which dropped off during subsequent exposures. Although nicotinic acid-treated animals showed an apparent increase in Q_{O_2} for the last test period, this was not significant, because the variability of Q_{O_2} was greater in that group. It is clear that even when body carbohydrate stores were depleted by prolonged fasting and plasma FFA were reduced by nicotinic acid, animals were still able to utilize sufficient substrate to maintain their metabolic rate.

It is concluded that during treatment with nicotinic acid, fasted rats use endogenous protein as the ultimate source of nearly half their energy requirements and apparently utilize to an important degree esterified fatty acids previously stored outside the adipose tissues. These changes in the choice of substrate did not involve an alteration of metabolic rate.

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