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Effects of dietary fat and nitrite on plasma and corporal density

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SUMMARY. Two groups of nine weanling male rats each were fed different diets for 60 days. Group A (control) was fed a full casein diet containing 17% protein. The group B received the same diet plus nitrite, fried bacon and proline. Diet B induced increased body weight gain and increased plasma l-lactic acid and cholesterol levels, as well as a decrease in plasma selenium. We suggest that the adverses effects of diet B are related to peroxidation, with an increased nutritional need for selenium.
Key words: nitric, selenium, needs, lactic acid, cholesterol

RESUMO. Efeitos da gordura e nitrito da dieta na densidade corporal. Dois grupos de nove ratos desmamados foram alimentados com diferentes dietas por 60 dias. O grupo A (controle) recebeu dieta completa com 17% de proteina (caseina). O grupo B, recebeu a mesma dieta adicionada de nitrito, bacon frito e prolina. A dieta B provocou: aumento do ganho de peso; aumento dos niveis plasmáticos de ácido láctico e colesterol, com redução do selênio. Sugere-se que os efeitos adversos da dieta B estejam relacionados à peroxidação, como aumento das necessidades nutricionais de selênio.
Palabras Chave: nitrito, selênio, ácido láctico, colesterol.

INTRODUCTION

Nitrite is present in most foods as a natural component or as an additive. Extensive evidence suggests that nitrite in the body is a precursor of N-nitrosamines and N-nitrosamides which exert a carcinogenic action through nucleic acid methylation. The effects are enhanced by the presence of some amino acids such as proline, and by the ingestion of fried bacon. (1)

The nitrosamine content in consumer products, such as bacon, were significantly reduced in the last years, but it still present. (2) Friying the nitrite-cured bacon (3) results in the formation of some carcinogenic volatile N-nitrosoproline (NPro) which is excreted by the urine. However Wagner and Tannenbaum (4) found no differences of urine NPro among groups consuming lunches of hot dogs containing proline plus nitrite, or, nitrite-free, suggesting that the nitrosation was insignificant compared to endogenously produced nitrite.

The bioavailability of nutrients (BA) reflects their final utilization in the body through the process of digestion, intestinal absorption, competition and interaction, utilization by the tissues, and excretion. Thus, BA depends not only on the nutrient content of ingested food but also on the entire system of food processing and on the physiological conditions of the organism, i.e., it depends on both extrinsic and intrinsic factors. Under special conditions, the needs for some nutrients can be modified. (5,6,7)

The present experimental study was undertaken to determine whether the presence of nitrite and bacon in diet produce some impairment in the plasmatic selenium and cholesterol levels.

MATERIAL AND METHODS

Animals: Two randomised groups of weanling male wistar rats having an average initial weight of 112.7± 5.95g were fed different diets for 60 days. The animals (9 per group) were maintained in individual cages.

Diets: The basic diet (group A, control)l consisted of 200g/Kg casein completed with all nutrients. Group B received the basic diet plus 24 mg/Kg sodium nitrite, 100g/Kg bacon and 10mg proline (Diet NB).

Rats were fed *ad libitum* for 60 days and Food Intake was measured. At the end of the experimental period six animals/ group were anesthetized with ether, decapitated and bled.

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Plasma cholesterol, selenium and L-lactic were determined. The composition of the diets is given in Table 1.

TABLE 1
COMPOSITION OF DIETS. DATA ARE REPORTED PER Kg.

Diet	A Control	B NB
Casein (g)	200	200
Nitrite (mg)	—	24
Bacon* (g)	—	100
Proline (mg)	—	10
Minerals (g)**	40	40
Vitamins (g)**	50	50
Oil (g)	80	—
Starch(+)	<1.000	<1.000
Protein %(++)	17.6	17.9
Fat %(++)	8	8.20
Selenium (ug/g)(++)	0.105	0.105

* Fried Bacon in 10ml oil
+ Starch- to complete 1 Kg
** Minerals, vitamins (8)
++ Determined in diets
Protein - Kjeldahl method (N x 6,25) (8)
Fat- Extraction of 2g of the diet with chloroform/metanol (2/1)
Selenium - As described in Methods.

Determinations

Dietary protein: Dietary protein was measured by the classical Kjeldahl (9), method (N x 6.25).

Lactic acid. Lactic acid was determined by oxidation in the presence of NAD^+ and L-lactate dehydrogenase (L-LDH) to pyruvate, which is trapped in the presence of L-glutamate and glutamate-pyruvate transaminasse (GPT):

$$\text{L-lactate} + NAD^+ \xrightarrow{\text{L-LDH}} \text{pyruvate} + NADH + H^+$$

The resulting NADH is measured by absorbance at 340 nm and absorbance is compared to a reference (Boehringer Mannheim Reagents)(10).
Selenium. Selenium was measured by the fluorimetric method, modified (11)*.

* Laboratoire de Controle Nestlé. Selenium fluorometrie. Dosage du selenium par fluorimetric. Ciclobond I (Betacyclodextrin). 1990 (Personal information).

Plasma cholesterol (Ch) was measured using the Signos Diagnostic-cholesterol Enzymatic Determination method (12).
Plasma protein was measured by the biuret reaction (13).
Lipids in diet measured by the weight of the dry extract of chloroform/metanol (2/1).
Body density was measured by immersion in water. Three rats/group. Determined by: body weight/difference of the water volume before and after immersion of each animal.

RESULTS

The average variation in body weight and food intake is shown in Table 2.

TABLE 2
THE AVERAGE VARIATION OF BODY WEIGHT (BWV), FOOD INTAKE (FI), BODY DENSITY (D) OVER A PERIOD OF 60 DAYS FOR THE CONTROL, AND NITRITE BACON DIETS

Group	BWV (g)	FI (g/d)	D(g/ml)
A-Control	43.96 (7.57)^{a*}	24.41 (1.02)^{a}	1.32 (0.02)^{a}
B-NB	62.38 (7.00)^{b}	24.59 (0.87)^{a}	1.19 (0.02)^{b}

* mean (±sd): different letters -p<0.01 (in column)

The NB diet enhanced final body weight without changing significantly the food intake. The lowest body density value was obtained for the NB diet group (group B), suggesting an increase in fatty mass.

Plasma L-lactic acid, cholesterol, protein and selenium values are given in Table 3.

The NB diet caused an increase in plasma L-lactic acid and cholesterol.

TABLE 3
PLASMATIC L-LACTIC ACID, SELENIUM, PROTEIN AND CHOLESTEROL OF ANIMALS FED THE CONTROL AND NITRITE + BACON DIETS.

Group	L-Lactic Acid^{+} (mmol/l)	Selenium^{+} (umol/l)	Protein^{++} (g/dl)	Cholesterol^{++} (mg/dl)
A-control	0.43(0.02)^{a*}	1.14 (0.01)^{a}	80.3 (4.59)^{a}	297.14 (28.86)^{a}
B-NB	1.11 (0.02)^{b}	0.30 (0.01)^{a}	69.6 (4.59)^{b}	342.85 (32.31)^{b}

* mean ± sd: different letters -p<0.01 (in column)
+ n=6-1 ml blood of two animals selected by draw, mixed- Three mixtures were measured, representing 6 animals/group, in three «pools» (in order to economize reagents)
++n=9 -determined in 9 animals/group

DISCUSSION

The addition of nitrite, bacon and proline to the diet was studied because nitrite has been suggested to form N-nitroso compounds in the organism which are components that probably lead to increased levels of free radicals of peroxidations. Selenium is a component of the structure of glutathione peroxidase (GSHPx)(14) and is therefore essential as a metal cofactor for the activity of the antioxidation enzyme. The scavenger effect of GSHPx probably occurs when the peroxides enter the cell cytosol by reducing the fatty acid hydroperoxides(15). The addition of nitrite to the diet containing fried bacon and proline increased total body weight and probably also fat mass. Considering that the composition of the diets were not sufficiently different to explain the changes in the body weight variations, these were probably due to the different metabolic pathways.

Diet B led to increased plasma L-lactic acid, a reduction of total plasma protein, and increase in total plasma cholesterol as well as a reduction in plasma selenium. Heim et al (16) reported an increase in L-lactate levels from 0.63±0.14 to 1.31±0.1 mmol/l in tumor patients due to a blockade of metabolism at the pyruvate-lactate level.

We suggest that the NB diet provoked impaired carbohydrate metabolism, with a consequent increase in L-lactic acid even with enhancement of the body weight.

By our previous results the diet content of selenium from 0,04 up to 0,105 mcg Se/Kg is adequate for weanling rats (17). These results suggest that the presencee of nitrite-bacon led to an increase in selenium utilization perhaps due to the different fatty components of the diet, or because that more selenium may have been necessary for the scavenger action at the cell level, perhaps against peroxidation.

These data are difficult to interpret at the present time but deserve consideration. It appeared that when a diet containing nitrite fried bacon and proline is administered to rats the needs for selenium are enhanced.

This conclusion of this study is that the addition of nitrite, proline and fried bacon to the diet of rats impaired the l-lactic, protein, cholesterol and selenium plasmatic levels.

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