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THE EFFECT OF HIGH FAT DIET UPON THE PRODUCTION OF REACTIVE CARBONYLS IN HYPOXIC HEART. THE EFFECT UPON CONNECTIVE TISSUE

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ABSTRACT

The concentration of reactive lipid metabolites (malondialdehyde, formaldehyde, acetaldehyde and acetone) was assayed in rat heart reperfusates after 30 min ischemia in animals fed a high fat diet. A considerable increase in oxo-groups possessing metabolites was found in animals fed high fat diet as compared to animals fed a standard peletted diet or a carbohydrate enriched diet. This increased level of carbonyl compounds could be brought down to control level by transferring the animals to the standard peletted diet, but with some delay. After six months of feeding the experimental animals with a standard peletted diet (following 6 months period of high fat diet feeding) brings the level of carbonyl compounds down to the control level. If tail tendons from three months old rats fed standard peletted diet are incubated in the reperfusate obtained from animals fed high fat diet, their solubility after CNBr treatment is distinctly decreased. Also, it was observed, that rat tail tendons of animals kept on a high fat diet were more resistant towards CNBr solubilization compared to controls. If these tendons (apparently polymerized during the feeding experiment *in vivo*) were incubated with the 45 min reperfusate *in vitro*, a further increase in the CNBr treatment resistant core was revealed. We conclude that high fat diet feeding of laboratory rats leads to an increased production of reactive carbonyls which can be recovered in heart reperfusate and may cause an increased resistance of structural proteins (collagen) towards CNBr cleavage.

Key words: Carbonyls, Hypoxic heart, Connective tissue, Fat diet, Lipid peroxidation

INTRODUCTION

Carbonyl group possessing compounds, typically reactive carbonyl metabolites produced by peroxidation of unsaturated lipids (like malondialdehyde, formaldehyde, acetaldehyde and acetone) play an important role in *in vivo* modification of long lived proteins, particularly collagen. Carbonyl arising compounds from this peroxidation are

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considerably reactive and their involvment in non-enzymatic modification of amino acids and proteins has been the subject of several studies (1-4). Reactive aldehydic intermediates of lipid metabolism are likely to compete for similar reactive sites in biopolymers (proteins, ε-amino groups of lysine) as aldehydic sugars which are known to produce advanced glycosylation end products (AGEs) by a reaction known as nonenzymatic glycation or Maillard reaction (5-7).

In previous studies we have investigated changes in connective tissue in fully fed and food restricted animals with age (8,9). In particular, changes in UV absorbance, fluorescence, thiobarbituric test and hexosyllysine were assayed. It was well documented that animals which have experienced a long-term food restriction period were lower in these parameters. Since changes of blood glucose levels were not documented in either aged or in fasted animals (7), further studies were directed towards assaying other carbonyl bearing compounds in tissues which may compete for the glucose binding sites in long lived proteins. Using reperfusion of rat heart in a Langendorff apparatus after 30 min ischemia, we were able to demonstrate that while there was no difference in the carbonyl compounds present in reperfusates from animals of different age, the amount of released carbonyl compounds was much lower in animals kept on 50% restricted diet. If tail tendons from 3 months old rats were incubated in the reperfusate, their solubility after CNBr treatment was decreased so that the tendons resembled material obtained from old animals. Since no differences in the amino acid composition of the CNBr digestion unaccessible material were observed, it was concluded that the carbonyl containing lipid metabolites were able to create a CNBr insoluble core in connective tissue. Since food restriction regimes (see our previous communication — Deyl and Mikšík (10)) lead to a decrease of reactive carbonyls in tissues, it was the aim of the present communication to reveal whether a situation can be introduced in which tissue carbonyl level will be enhanced, by increasing the fat intake of the experimental animals.

MATERIALS AND METHODS

Animals

Four groups of male Wistar rats (10 animals per group) were used. The animals were individually haused. Five randomly selected animals were used in the experiments. The following groups of animals were assembled: (1) animals fed high fat diet for their whole life, (2) animals fed high fat diet for 5 months and then switched to a standard peletted diet for 1 month; (3) the same regime as in 2 except that the standard peletted diet refeeding was extended to two months after 5 months on a high fat diet and (4) animals kept on a high fat diet for 6 months followed by a 6 months period of standard peletted diet feeding. The high fat diet consisted of 2000 g standard peletted chow (see below), 5 g NaCl, and 1430 g lard + flax oil mixture (this mixture contained 17.5% of linolenic acid in the lipid component) and was fortified by adding 2 g/100 g of the supplementation mixture (see Table 1B).

Two types of controls were used. The first control group (10 animals) was fed standard peletted diet (see Table 1 for composition). The other control group (10 animals) was fed a diet consisting of 2000 g standard peletted chow, 5 g NaCl and 1430 g starch.

In other words while the high fat diet contained 40 % fat by weight, the second control diet contained an equal proportion of carbohydrate. In order to compensate for the addition of carbohydrate, also this diet was added 2 g/100 g of the supplemetation mixture. When comparing the composition of resulting diets with the nutrient requirements of rats (see Nutrient Requirements od Laboratory Animals, Natl. Acad. Sci. US, 1978) all the key components were present in a considerably higher amount than required for pregnant laboratory rats in all dietary regimes used.

TABLE 1

Composition of the Standard Peletted Diet

A) Basic Component

Compound	g/100 g
Wheat scrap	60.0
Dried milk*	13.0
Casein	13.0
Pollard	8.5
Supplementation mixture	5.0
Soybean oil	0.5

* Contained 28% fat in dry weight

B) Composition of the Supplementation Mixture

Component	Amount
Calcium carbonate	84.43 g
Calcium phosphate	220.7 g
Sodium chloride	150.0 g
Iron(II) sulfate	31.95 g
Copper(II) sulfate	0.392 g
Zinc(II) sulfate	0.44 g
Manganum(II) carbonate	1.88 g
Kalium iodide	0.026 g
Thiamin (B_1)	240 mg
Riboflavin (B_2)	160 mg
Pyridoxin (B_6)	80 mg
Cobalamin (B_{12})	0.6 mg
Tocopherol acetate (E)	2000 mg
Folic acid	120 mg
Inositol	2 000 mg
Choline hydrochloride	20 000 mg
Calcium pantothenate	160 mg
Retinol	100 000 int. unit
Calciferol (D_2 + D_3)	240 000 int. unit
Yeast	400 g
Talc, obduction components and antioxidants	ad 1 000 g

Both control diets (standard peletted and carbohydrate enriched) and fat enriched diets were available *ad libitum* and the animals had a free access to water. Animals of body mass 200 g (42 ± 3 days old) were taken into the experiment. There were no significant differences in the growth curves.

Isolated heart preparation

The procedure used followed that published by Otani *et al.* (11). The rats were anesthesized by intraperitoneal injection of pentobarbital (80 mg/kg body mass) and hearts were removed and mounted on a non-recirculating Langendorff perfusion apparatus as rapidly as possible. Retrograde perfusion was done at a 100 cm H_2O pressure ($9.8 \cdot 10^3$ Pa) with oxygenated, normothermic Krebs-Henseleit bicarbonate buffer containing 3% of serum albumin (Fluka, Buchs, Switzerland) declared as low in fatty acid content. First the heart preparation was allowed to equilibrate for 10 min at 37°C with nonrecirculating Krebs-Henseleit bicarbonate buffer. The retrograde aortic flow was then terminated and the hearts were made ischaemic for 30 min in physiological saline (pH 7.4) at 37°C. Then reperfusion was started with the Krebs-Henseleit solution (pH 7.34) for 60 min at normothermia. Samples of the perfusate were collected prior to ischemia, after ischemia and during reperfusion and assayed for lipid metabolic products.

Derivatization of metabolites in perfusate

The analytical procedure used followed that, published by Cordis *et al.* (12).

Samples taken during the individual stages of perfusion were derivatized with 2,4-dinitrophenylhydrazine (DNPH). A 310 mg aliqout of DNPH was dissolved in 100 ml 0.2 M HCl and 0.1 ml of this DNPH reagent (3.13 μmol) was added to 1.5 ml of the perfusate in a 20 ml screw capped PTFE vial. After adding 0.5 ml of water the test tubes were Vortex mixed followed by the addition of 10 ml of n-pentane. The test tubes were shaken for 30 min and the derivatization reaction was allowed to proceed at room temperature. The organic phase was removed and the aqueous phase was reextracted with the same amount of n-pentane; both extracts were combined and taken to dryness under a steam of nitrogen at 30°C. The dry residue was redissolved in 200 μl of acetonitrile and after filtration directly used (0.2 μm Nylon-66 membrane filter, Rainim, Woburn, MA, USA) for chromatography.

Chromatography of 2,4-dinitrophenylhydrazones

Filtered samples (25 μl) were loaded in a 3 μm Beckman Ultrasphere C_{18} column 7.5 cm x 4.6 mm I.D. in a Spectra-Physics Chromatograph (San Jose, CA, USA). Injection of samples was done by the split stream technigue; a Bondapak C_{18} Guard Pak column was mounted into the separation system. Detection was done by using a Waters model 490 multiwavelength detector (Millipore, Milford, MA, USA) at 307, 325 and 356 mm. Elution was isocratic with acetonitrile-water-acetic acid (40:60:0.1, v/v/v) at a flow rate of 1 ml/min (typical running time less than 20 min). After each run the column was washed with acetonitrile containing 0.1% (v/v) of acetic acid. Dinitrophenylhydrazone standards were prepared in the laboratory by reacting 50 ml 2,4-dinitrophenylhydrazine solution (310 mg/100 ml 2M HCl) with a 2-5 molar excess of formaldehyde (FDA), acetaldehyde (ADA), malondialdehyde (MDA) and acetone. The hydrazone precipitate was filtered off, dried and recrystallized from methanol. Standard solutions of 2,4-dinitrophenylhydrazones containing 50 ng/μl were used for spiking purpose. Calibration was linear in the range 10 pmol - 6.25 nmol of each standard. The identity of the peaks was confirmed by comparing the retention times with those of authentic standards. Spiking of the mixture with appropriate standards was done as well. Accuracy of the method was checked by standard addition

technique. Within run and inter run variations were 1 and 5% and were the same as reported by Cordis *et al.* (12).

In vitro reaction of tail tendons with lipid metabolites

Intact rat tendon fibres were incubated with the 45 min perfusate (in order to get as much of the reactive carbonyls in the perfusate as possible) *in vitro*. Fibres were washed overnight at 10°C in 5 mM sodium phosphate buffer containing 0.9% NaCl, pH 7.4 and then incubated with the 45 min perfusate which was made 3 mM with respect to sodium azide at 37°C for 24 days.

Solubilization of tendons by CNBr

About 10 mg wet weight of tissue was treated with 1 ml of 70% formic acid containing 100 mg of CNBr at 30°C for 6 h, and dried in vacuum. After addition of 10 mM acetic acid with intensive mixing, centrifugation in an Eppendorf microcentrifuge yielded the CNBr-soluble and -insoluble fractions. These fractions were lyophilized and weighed.

Statistical calculations were based on the Student's t-test.

RESULTS AND DISCUSSION

As demonstrated in Table 2 after a 30 minutes of ischemia heart perfusates contain lipid metabolites possessing reactive carbonyls, namely MDA, FDA, ADA and acetone. The increase in concentration of these metabolites in high fat diet fed animals is quite considerable. When control animals are compared with those staying on high fat diet, an increase in MDA, FDA, ADA and acetone levels — 63% ($P<0.01$), 34% ($P<0.10$), 56% ($P<0.10$) and 42% ($P<0.001$), respectively (for 15 min perfusion time) — was observed in the experimental groups. In a subsequent set of experiments we have tested to what extent the increased level of reactive carbonyls in high fat diet fed animals could be brought back to control level by transferring the animals to standard peletted diet feeding. It was demonstrated that in animals kept for five months on a high fat diet, the subsequent period of one month standard pelletted diet feeding decreases the levels of reactive aldehydes in the reperfusate (both after 15 and 45 min reperfusion), close, but not quite to the control level. A prolonged period (6 months) of refeeding with normal pelletted diet brings the concentration of reactive carbonyls in the reperfusates (both 45 and 15 min reperfusion) back to control level. This indicates that in animals transferred back to standard pelletted diet after some period of high fat diet feeding, lowering of the concentration reactive carbonyls in heart reperfusates occurs with some inertia. This may be ascribed, e.g. to fat deposits in the investigated tissue.

As shown in Table 2, perfusates (routinely 5 ml) contained 32-52 nmol/ml of oxo-functionalities per ml (for 45 min perfusion time). Consequently 160-260 nmol were available for protein modification (controls and animals kept on high fat diet for one year). If we assume that the 10 mg of tendom taken for incubation contain 2-3 mg of pure collagen type I, i.e. 3.10^{-8} mol α-chains (i.e. 30 nmol) there is a 5.3 to 8.7 molar excess of the oxo-functionalities in the system. In a tissue (tendom) it is likely that not all the collagen molecules will be easily accessible to react with oxo-moiety; consequently the local excess of oxo-functionalities to the available free amino groups would be possibly higher.

Fig. 1 shows the time course of progressive insolubilization of the tendom samples upon incubation with control perfusate (standard peletted diet or starch enriched diet), perfusate obtained with animals kept for 6 months on the high fat diet, upon incubation

TABLE 2

Concentrations of Lipid Metabolites (MDA – malondialdehyde, FDA – formaldehyde, ADA – acetaldehyde) in rat heart perfusates after 30 min ischemia. Reperfusion 15 and 45 min (n=5; nmol/ml perfusate). Mean ± SD

Regime	Age	MDA-DNPH	FDA-DNPH	ADA-DNPH	Acetone-DNPH
Perfusion time: 15 min					
Controls	6 months	0.09 ± 0.02	3.17 ± 0.96	4.02 ± 2.03	19.57 ± 2.77
	1 year	0.08 ± 0.02	3.15 ± 0.83	3.93 ± 1.90	20.01 ± 2.55
Carbohydrate enriched diet	6 months	0.10 ± 0.02	2.98 ± 0.97	3.96 ± 2.00	20.01 ± 2.87
	1 year	0.10 ± 0.02	3.05 ± 1.03	3.90 ± 2.05	19.75 ± 2.89
High fat diet	6 months	0.12 ± 0.02*	3.98 ± 0.76	5.23 ± 0.96	26.21 ± 1.99*◊
	1 years	0.13 ± 0.02*◊	4.21 ± 0.63	6.15 ± 1.12	28.32 ± 2.12*◊
High fat diet 5 months, 1 month withdrawal	6 months	0.12 ± 0.02*	3.66 ± 0.76	3.69 ± 1.03°	23.02 ± 1.19*°
High fat diet 5 months, 2 months withdrawal	7 months	0.12 ± 0.02*	3.25 ± 0.78	4.55 ± 0.87	20.15 ± 0.87°†
High fat diet 6 months, 6 months withdrawal	1 year	0.08 ± 0.04°	3.15 ± 0.69°	4.00 ± 0.96°	19.20 ± 1.25°†

Perfusion time: 45 min

Controls	6 months	0.15 ± 0.04	5.87 ± 1.97	5.83 ± 1.97	20.68 ± 2.03
	1 year	0.15 ± 0.04	5.98 ± 2.03	5.63 ± 1.45	20.40 ± 1.99
Carbohydrate enriched diet	6 months	0.18 ± 0.06	6.02 ± 1.35	5.78 ± 1.65	21.42 ± 1.96
	1 year	0.20 ± 0.04	5.94 ± 2.00	5.82 ± 1.43	20.93 ± 2.04
High fat diet	6 months	0.28 ± 0.02*◊	7.46 ± 1.79	7.92 ± 2.01	32.15 ± 2.86*◊
	1 year	0.26 ± 0.02*◊	7.22 ± 2.01	7.87 ± 1.97	36.20 ± 2.35*◊
High fat diet 5 months, 1 month withdrawal	6 months	0.23 ± 0.04*°	6.88 ± 2.12	6.78 ± 1.72	28.00 ± 1.79*◊°
High fat diet 5 months, 2 months withdrawal	7 months	0.20 ± 0.02*°	6.02 ± 1.68	6.23 ± 1.70	24.00 ± 2.21*°†
High fat diet 5 months, 7 months withdrawal	1 year	0.14 ± 0.02◊°†‡	5.76 ± 1.79	5.75 ± 1.99	19.22 ± 2.17°†‡
High fat diet 6 months, 6 months withdrawal	1 year	0.13 ± 0.06°†‡	5.89 ± 2.03	5.87 ± 2.12	21.02 ± 2.53°†

Statistical differences (*P*<0.05) from bottom upwards: comparison with controls —*, carbohydrate enriched — ◊, high fat diet — °, high fat diet 5 months, 1 month withdrawal — †, high fat diet 5 months, 2 month withdrawal —‡. In all regimes the same age categories (6 months or 1 year) were compared, only in case of 7 months (high fat diet 5 months, 2 months withdrawal) old animals the data were compared with the 6 months old groups.

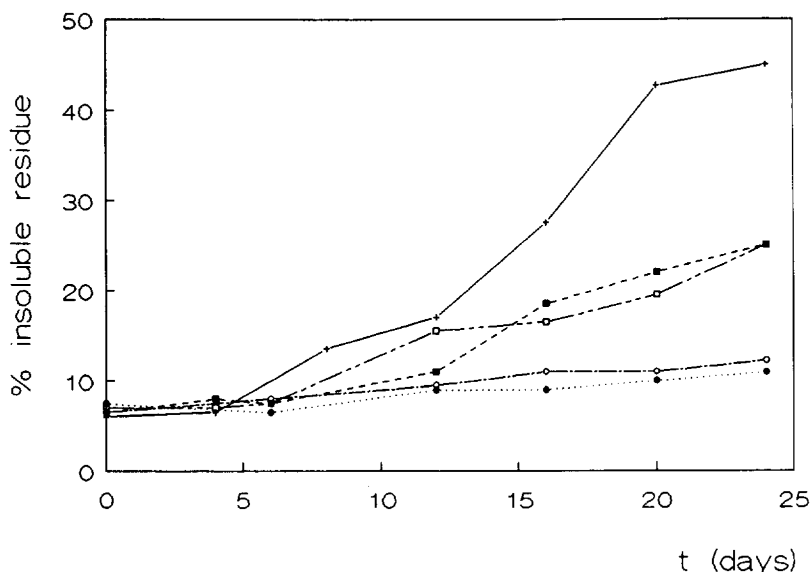

FIG. 1. Progressive insolubilization of tendon collagen upon incubation with 200 mmol/l glucose (+), acetaldehyde solution contaning 250 nmol/5 ml (■), 45 min perfusate from 6 months old animals kept on high fat diet (□), perfusate obtained from animals kept on the starch enriched diet (●) and perfusate from animals kept on the standard peletted diet (o). Data obtained by incubating tendon collagen in physiological saline did not differ from (●) and (o). Statistical differences of the percentage of the insoluble residue: glucose *vs* acetaldehyde and high fat diet $P<0.05$; acetaldehyde and high fat diet *vs* controls $P<0.05$ (evaluated after 24 days of incubation).

with 200 mM glucose and, upon incubation with a 250 nmol/5 ml acetaldehyde solution (the concentration of the oxo-groupings in the acetaldehyde solution corresponds to the concentration of the oxo-moiety in the high fat diet animals' perfusate). The resistance towards cleavage upon the incubation with high fat diet fed animals perfusate is practically the same as with the acetaldehyde solution. As expected, incubation with the glucose solution of much higher concentration results also in a much higher resistance towards CNBr cleavage.

Reactive carbonyl metabolites, particularly those containing a free aldehydic group are known to react with free amino groups of proteins; such reactions are best detected in protein structures with a long metabolic half-time. A typical representative of this protein category is collagen. However, instead of using purified collagen samples to test the ability of carbonyl containing perfusates to react with the protein, we have used rat tail tendons; these tendons are known to contain mainly collagens (type I and III), however they exhibit a definite biological structure and experiments carried out with them are closer to the physiological conditions in the organism. The presumptive counterparts for the reactive carbonyl containing metabolites, free amino groups, are much more readily accessible in a solubilized protein in comparison to a protein imbedded in a tissue. Application of such a model situation has, however, to respect (i) that a part of the protein in the tissue (rat tail tendon) may be already reacted (cross-linked) with reactive metabolites or otherwise and (ii) only a part of the total free amino groups maybe accessible to the reaction with oxo compounds. Therefore we have used a standard method for solubilizing tendon collagen. This was done by CNBr cleavage which is known to split collagen type I molecule into six

TABLE 3

Changes in the Percentage of CNBr Insoluble Residue of Rat Tail Tendoms in Rats Kept on Different Feeding Regimes and after Incubation with the 45 min Perfusates (n=10). Mean ± SD

Regime	Age	Insoluble residue (weight %)	
		Non incubated	Incubated
Controls	6 months	7.92 ± 2.25	12.20 ± 0.98
	1 year	$11.36 \pm 2.18^{\S}$	$18.24 \pm 1.11^{\S}$
Carbohydrate enriched diet	6 months	7.56 ± 2.00	10.88 ± 1.02
	1 year	$10.91 \pm 1.98^{\S}$	$19.32 \pm 1.56^{\S}$
High fat diet	6 months	$13.26 \pm 1.99^{*\Diamond}$	$25.27 \pm 0.95^{*\Diamond}$
	1 year	$18.32 \pm 1.93^{*\Diamond\S}$	$27.21 \pm 1.93^{*\Diamond\S}$
High fat diet 5 months, 1 month withdrawal	6 months	$13.32 \pm 1.33^{*\Diamond}$	$24.87 \pm 1.26^{*\Diamond}$
High fat diet 6 months, 6 months withdrawal	1 year	$16.24 \pm 1.26^{*\Diamond\circ\dagger}$	$25.28 \pm 0.98^{*\Diamond}$

Statistical differences ($P<0.05$) from the bottom upwards: comparison with controls — *, carbohydrate enriched — $^\Diamond$, high fat diet — $^\circ$, high fat diet 5 months, 1 month withdrawal — † and between age categories (6 months and 1 year) — §. In all regimes the same age categories (6 months or 1 year) were compared. Difference between incubated and non incubated counterparts is always highly significant.

fragments; collagen molecules which underwent a reaction with reactive carbonyls (including aldehydic sugars) are known to form polymeric structures, parts of which remain insoluble even after intensive CNBr cleavage (13). On the other hand it is also known that aging of connective tissue involves collagen polymerization. The nature of some of the arising cross-links is known, however, the complete mechanisms of age accompanied collagen polymerization keeps to be a matter of debate for the last twenty years (14-16). Indeed if we compare the percentage of tendons that remains insoluble after CNBr cleavage increased resistance towards solubilization with CNBr can be observed in high fat diet fed animals (Table 3). It may be argued that CNBr cleavage is a too rough way of solubilization and possibly other solubilization procedures like simple acid extraction or pepsin solubilization may be better suited. In our preliminary experiments acetic acid solubilization (0.5% acetic acid) gave poorely reproducible results though some tendency in progressive insolubilization with perfusate incubated samples could be traced. Using extraction with neutral salts (salt soluble collagen) was impossible to apply because of the low yield extracted by this technique even from control samples. Solubilization with pepsin would be perhaps equally applicable as CNBr cleavage provided that the activity of the enzyme preparation could be kept constant over a long period of time (half a year). Because in our hands the activity of different pepsin batches differed considerably, we preferred to use chemical cleavage with CNBr as this procedure appeared better than enzymic cleavage.

As shown in Table 3 high fat diet feeding leads to a considerable increase in the CNBr insoluble core of rat tail tendons (non-incubated tendons); if these tendons are incubated with the 45 min perfusate, a further increase in the proportion of the insoluble core was observed. It is thus feasible to conclude that upon high fat diet feeding, reactive metabolites capable of connective tissue collagen polymerization are generated to a higher extent than with controls.

It is worth mentioning that if we replaced of fat with the same percentage of starch in the animals' chow results which aren't distinguishable from those obtained with the pelleted diet only were obtained.

At least, as shown in Table 2, increased levels of MDA, ADA, FDA and acetone were found in the heart reperfusates. If the tail tendons from the same animals are incubated in the particular reperfusate, data shown in the column "incubated" in Table 3 were obtained, indicating (i) the high affinity of oxo compounds present in the reperfusate to polymerize connective tissue proteins and (ii) that in high fat diet fed animals (and in different variations of the feeding regime) further polymerization of connective tissue proteins (collagen) in rat tail tendons is still possible.

Though the simple exchange of fat and carbohydrate and then comparison with a commercial diet is certainly not a very rigorous diet design. However synthetic diets which would be much better were not available to us (due to the high costs). Certainly intake of the same calories (see comparable mass gains) results in substantially lower intakes of other nutrients when the high fat diet is fed (proteins, fibers, vitamins etc.). However the aim of this investigation was to show whether or not high fat diet feeding may lead to the accumulation of reactive metabolites (e.g. bearing an oxo group) which could alter the structure of long lived proteins. This indeed was shown to be the case. Oxo group bearing compounds are likely to react with free NH_2- groups of the protein and, consequently, make the protein structure more resistant towards both chemical and enzymic cleavage. Of these two possibilities a higher resistance towards CNBr cleavage was demonstarted.

CONCLUSIONS

High fat diet feeding of laboratory rats yielded a considerable increase in reactive carbonyl possessing compounds concentration in rat heart reperfusates after 30 min

ischemia. The concentration of these metabolites can be brought back to control level when high fat diet feeding is switched back to standard peletted diet; however the decrease in reactive carbonyls concentration in the reperfusates to the control level occurs with some inertia. It was demonstrated that in high fat diet fed animals there is an increase in the highly polymerized tail tendon core, which can not be solubilized by CNBr treatment; this core is to a large extent preserved even if the animals have been transferred to standard peletted diet feeding for a considerable period of time (1 - 6 months). This may be understood in such a way, that a part of the connective tissue proteins has been excluded from the normal metabolism through polymerization because of the presence reactive lipid metabolites, perhaps similar to those released from the hypoxic heart during the perfusion period.

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REFERENCES

1. Chio KS, Tappel AL. Synthesis and characterization of the fluorescent products from malondialdehyde and amino acids. Biochemistry 1969; 8:2827-2832.

2. Noberasco G, Odetti P, Boeri D, Maiello H, Adezatti L. Malondialdehyde (MDA) level in diabetic subjects. Relationship with blood glucose and glycosylated hemoglobin. Biomedicine and Pharmacotherapy 1991; 45:193-196.

3. Gómez-Sánchez A, Hermosín I, Maya I. Influence of malondialdehyde on the Maillard degradation of Amadori compounds. Carbohydr Res 1992; 229:307-322.

4. Hermosín I, Ledl F, Gómez-Sánchez A. Influence of oxidized lipids on the non-enzymic browning reaction: interaction between linolenic acid and Amadori compound. Chem Phys Lipids 1992; 63:265-270.

5. Harding JJ. Non-enzymatic posttranslational modification of proteins in vivo. Advances in Protein Chemistry 1985; 37:247-325.

6. Ledl F, Schleicher E. New aspects of the Maillard reaction in foods and in the human body. Angew Chem. Int. Ed. Engl. 1990; 29:565-594.

7. Monnier VM. Nonenzymatic glycosylation, the Maillard reaction and the aging process. J Gerontol: Biol Sci 1990; 45: B105-111.

8. Mikšík I, Stružinský R, Deyl Z. Change with age of UV absorbance of collagen and accumulation of ε-hexosyllysine in collagen from Wistar rats living on different food restriction regimes. Mech Ageing Develop 1991; 57: 163-174.

9. Mikšík I, Deyl Z. Change in the amount of ε-hexosyllysine, UV absorbance and fluorescence of collagen with age in different animal sciences. J Gerontol: Biol Sci 1991: 46: B111-116.

10. Deyl Z, Mikšík I. Age and feeding-dependent production of carbonyl compounds in hypoxic heart. The role of produced carbonyls in connective tissue modification. Mech Ageing Develop 1994; 73:47-55.

11. Otani H, Prasad MR, Jones RM, Das DK. Mechanism of membrane phospholipid degradation in ischemic-reperfused rat hearts. Am J Physiol 1989; 257:H252-258.

12. Cordis GA, Maulik N, Bagchi D, Engelman RM, Das DK. Estimation of the extent of lipid peroxidation in the ischemic and reperfused heart by monitoring lipid metabolic products with the aid of high-performance liquid chromatography. J Chromatogr 1993; 632:97-103.

13. Tanaka S, Avigad G, Eikenberry EF, Brodsky B. Isolation and partial characterization of collagen chains partially dimerized by sugar-derived cross-links. J Biol Chem 1988; 263:17650-17657.

14. Reiser KM. Nonenzymatic glycation of collagen in aging and diabetes. Proc Soc Exp Biol Med 1991; 196:17-29.

15. Reiser KM, McCormick R, Rucker RB. Enzymatic and nonenzymatic cross-linking of collagen and elastin. FASEB J 1992; 6:2439-2449.

16. Kivirikko KI. Collagens and their abnormalities in a wide spectrum of disease. Ann Medi 1993; 25:113-126.

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