

## ORIGINAL ARTICLE

# Effects of oral L-carnitine, L-lysine administration and exercise on body composition and histological and biochemical parameters in pigeons

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## Summary

The purpose of this study was to examine whether L-carnitine and its precursor L-lysine could have any beneficial effect in racing pigeons, and if so, whether this effect is influenced by the extent of exercise (short-distance flight: 135 km vs. long-distance flight: 580 km). Birds were divided into seven groups of animals. Group 1: negative control, no flight, no treatment, Group 2: positive control, placebo treatment before the short-distance flight, Group 3: 200 mg/day L-carnitine treatment before the short-distance flight, Group 4: 400 mg/day L-lysine treatment before the short-distance flight, Group 5: positive control, placebo treatment before the long-distance flight, Group 6: 200 mg/day L-carnitine treatment before the long-distance flight, Group 7: 400 mg/day L-lysine treatment before the long-distance flight. L-carnitine, L-lysine and distilled water (placebo) were orally administered (tube feeding) for 7 days before flight. Just after returning home, blood samples were collected and analyzed for glucose, fructosamine, cholesterol, triglycerides and thiobarbituric acid reactive substances. Pigeons were euthanized using carbon dioxide as an inhalation agent, and the whole body was subjected to proximate analysis. The status at arrival was referred to as a basis for comparison. Sex did not affect the measured parameters. As a result of the L-carnitine and L-lysine administrations, the body fat mobilization was higher during the 580 km flight, whereas no changes were noted during the 135 km flight. The main changes in the measured blood parameters were caused by the extent of exercise. This experiment considered the extent of exercise as a factor potentially modulating L-carnitine supplementation effects. In conclusion, flight distance affected several parameters but the supplements of L-carnitine and L-lysine were not effective in the tested situations.

## Introduction

L-carnitine was discovered in 1905 as a component of beef muscle tissue, hence the name: Latin *carnis* means flesh or meat. The chemical structure was firmly established in 1927, when Tomita and Sendju

discovered the  $\beta$ -position of the OH group. The naturally occurring L-form was defined as 'physiological carnitine'. It is a free amino acid (L-3-hydroxy-4-N,N,N-trimethylaminobutyrate) which has been found to improve fat metabolism in different organs and tissues. L-carnitine is involved in

numerous metabolic processes and its effects result particularly from its significance for fat metabolism. The main property in this respect is the carnitine-mediated transport of long-chain fatty acids through the inner mitochondrial membrane (Fritz and Marquis, 1965). Long-chain fatty acids are activated outside the mitochondria by forming acyl-coenzyme A (acyl-CoA), to which the inner mitochondrial membrane is impermeable. Long-chain fatty acids therefore require a carrier to be transported to the site of  $\beta$ -oxidation. L-carnitine performs this shuttle function by replacing the CoA outside the mitochondrion and transporting the fatty acid into the mitochondrial matrix as acyl-L-carnitine. Acyl-CoA is then regenerated inside the mitochondrial matrix and conveyed to the site of  $\beta$ -oxidation. Thus, energy production from long-chain fatty acids is directly dependent on L-carnitine.

L-carnitine occurs naturally in most feedstuffs in varying amounts. For instance, animal protein sources and dairy products are rich in it (Blum and Baumgartner, 1997), but vegetable and animal fats do not contain any L-carnitine. Endogenous synthesis of L-carnitine occurs predominantly in the liver and kidney from L-lysine (Borum and Fisher, 1983). This biosynthesis, together with the uptake of L-carnitine from feedstuffs and complete feed is sufficient to cover normal requirements. This is not the case, however, when relating to stress, higher performance and sustained exertion (racing and sport animals). L-carnitine supplementation may be of major prophylactic and/or therapeutic significance in intensive animal production. Dietary L-carnitine supplementation has been widely used in nutrition of different animal species, such as turkey, chicken (Barker and Sell, 1984; Rabie and Szilágyi, 1988; Deng et al., 2006), pigeon (Janssens et al., 1998, 2000a,b), swine and dogs (Gross et al., 1988), foals (Hausenblasz et al., 1996) and rabbits (Seccombe et al., 1987).

Feed of pigeons is generally based on grains of various cereals, legumes or oil seeds. The natural L-carnitine content of the seeds varies between 5 and 15 mg/kg feed (Blum and Baumgartner, 1997). Pigeons absorb L-carnitine exclusively from plant-based feed. For flight, fatty acids are the most important source of energy for providing the wing muscles with ATP (Blum and Baumgartner, 1997). It can be supposed that L-carnitine supplementation has a positive effect on fat metabolism during flight. The purpose of this study was to examine whether the oral provision of L-carnitine and its precursor L-lysine have any beneficial effect in racing pigeons,

and if so, whether this effect is related to the extent of exercise (flying distance).

## Materials and methods

Two consecutive experiments were carried out by adult (2–4 year) carrier pigeons (*Columbia livia domestica*). In the first experiment the flying distance (as an extent of exercise) was 130 km (short-distance flight), whereas in the second experiment 580 km (long-distance flight). In the experiments, birds were divided into seven groups of animals. Experimental groups were as follows.

Group 1: F $\emptyset$ T $\emptyset$  = no flight–no treatment, negative control,  $n = 6$  (three males/three females);

Group 2: FST $\emptyset$  = Flight: short distance–no treatment, positive control, placebo treatment,  $n = 6$  (three males/three females);

Group 3: FSTC = Flight: short distance–treatment: L-carnitine, (Lonza, Basel, Switzerland), 200 mg/day,  $n = 6$  (three males/three females);

Group 4: FSTL = Flight: short distance–treatment: L-lysine, (REANAL, Budapest, Hungary), 400 mg/day,  $n = 6$  (three males/three females);

Group 5: FLT $\emptyset$  = Flight, long distance–no treatment, positive control, placebo treatment,  $n = 8$  (three males/five females);

Group 6: FLTC = Flight: long distance–treatment: L-carnitine (Lonza), 200 mg/day,  $n = 8$  (four males/four females);

Group 7: FLTL = Flight: long distance–treatment: L-lysine (REANAL), 400 mg/day,  $n = 8$  (four males/four females).

L-carnitine, L-lysine and placebo treatment (distilled water) were orally administered (tube feeding) during 7 day before flight. As according to the literature, in humans, 30–50% of total  $\epsilon$ -N-trimethyllysine is converted to carnitine (Rebouche et al., 1989), in this experiment a double dose of L-lysine compared with L-carnitine was used. Animals were fed *ad libitum* with whole grains and seeds (Table 1). Liquid vitamin (Jolovit<sup>®</sup>, Phylaxia Pharma, Budapest, Hungary) and trace mineral supplements (Blitzform<sup>®</sup>, Dr. Hesse Tierpharma, Germany) were also available in drinking water. Pigeons were kept together so that the actual feed intake could not be measured individually. Total intake from the different seeds was measured daily and the average consumption of the seed mixture was calculated according to this data (Table 1). Grains fed were subjected to proximate analysis (Herlich, 1990). The apparent metabolizable energy content (MJ/kg) of the grain mixture was

**Table 1** Proportion of the different seeds in the daily ration according to the pigeons' selection and its nutrient content

|   |                               |
|---|-------------------------------|
| Sunflower seed ( <i>Helianthus annuus</i> ) | 25%                           |
| Corn ( <i>Zea mays</i> )                    | 15%                           |
| Wheat ( <i>Triticum aestivum</i> )          | 15%                           |
| Pea ( <i>Pisum sativum</i> )                | 10%                           |
| Millet ( <i>Panicum miliaceum</i> )         | 10%                           |
| Mineral mix†                                | 1 ml/1 l of<br>drinking water |
| Limestone grits                             | <i>ad lib</i>                 |
| Composition from table values‡,§            |                               |
| Crude Protein (%)                           | 15.57                         |
| Metabolizable energy (MJ/kg)                | 16.22                         |
| Ether extract (%)                           | 14.60                         |
| Crude fibre (%)                             | 7.03                          |
| Sorghum ( <i>Sorghum vulgare</i> )          | 7%                            |
| Barley ( <i>Hordeum sativum</i> )           | 5%                            |
| Wetch ( <i>Vicia sativa</i> )               | 5%                            |
| Hempseed ( <i>Cannabis sativa</i> )         | 5%                            |
| Rice ( <i>Oryza sativa</i> )                | 3%                            |
| Vitamin mix*                                | 1 ml/1 l of<br>drinking water |
| Calculated daily intake                     |                               |
| Feed  | 37.5 g/bird                   |
| Crude protein                               | 5.8 g/bird                    |
| Metabolizable energy                        | 0.6 MJ/bird                   |
| Ether extract                               | 5.5 g/bird                    |
| Crude fibre                                 | 2.6 g/bird                    |

\*Jolovit® (Phylaxia Pharmat) provided the following amounts per litre of vitamin mix: Vitamin A, 14 000 000 IU; cholecalciferol, 1 400 000 IU; vitamin E, 10 500 IU; Na panthothenate, 6550 mg; menadione, 175 mg; choline Cl, 140 000 mg; thiamine, 1400 mg; Vitamin B<sub>2</sub>, 2100 mg; Vitamin B<sub>6</sub>, 1400 mg; Vitamin B<sub>12</sub>, 14 mg; nicotinic acid, 14 000 mg; d-biotin, 35 mg; folic acid, 175 mg.

†Blitzform® (Dr. Hesse Tierpharma) provided the following in mg/5 ml of mineral mix: K, 450; I, 250; Fe, 45; Mo, 0.04; Co, 0.063.

‡National Research Council (1994).

§Mackrott (1992).

calculated with the formula for poultry of the European Union:

$$0.155 \text{ CP} + 0.343 \text{ CF} + 0.167 \text{ St} + 0.130 \text{ Su},$$

where CP is crude protein g/kg, CF is crude fat g/kg, St is starch g/kg and Su is sugars g/kg (Larhier and Leclerq, 1992). The pigeons were transported to the releasing site (1 day before the race) and were given corn and water exclusively. The study was conducted on a sunny, hot mid-summer day (ambient temperature: 30 °C).

Before transporting to the releasing site and after returning home, pigeons were weighed individually. Just after returning, home blood samples from the brachial veins of the left wing were collected and analysed. The blood plasma and red blood cells were

separated by centrifugation at  $4000 \times g$  for 5 min, red blood cell haemolysate was made with nine-fold volume of redistilled water. Plasma and red blood cell haemolysate was frozen at  $-20$  °C until analysed. The following parameters were determined: whole blood glucose, serum fructosamine, plasma cholesterol (CHOL), plasma triglyceride (TG) and thiobarbituric acid reactive substances (TBARS) from blood plasma. The analysis of glucose was completed by D'Cont Cabrio reflection photometer (Boehringer, Mannheim, Germany) by using test strip of C-Test. Serum fructosamine was determined according to Oppel *et al.* (2000). Cholesterol was measured according to Allain *et al.* (1974), TG according to Werner *et al.* (1981), using commercial kits (REANAL). Thiobarbituric acid reactive substances content of blood plasma was determined by using 2-thiobarbituric acid reaction (Placer *et al.*, 1966).

After taking the blood samples, pigeons were euthanized using carbon dioxide as an inhalation agent. After measurement of the heart and liver weight, samples were taken from the following tissues: heart, liver, kidneys, breast muscle and lungs. Until the histological examination, samples were fixed in a buffered 10% (v/v) formaldehyde solution. The histological examination was carried out by freezing method and by use of paraffin sections. Sections were stained by haematoxylin-eosin. For detection of lipids the Fat Red stain was used and for the polysaccharides, the periodate-Schiff reaction was used. Subsequently, the whole body (including the feathers) as well as grains fed were subjected to proximate analysis (Herlich, 1990).

The status of arrival was referred to as a basis for comparison between the measured biological parameters of pigeons. At the same time, pigeons of Group 1 (negative control) were sampled equally to the others.

Four pigeons did not return home from the long-distance flight (two males from the Group 5 and two males from the Group 6), so finally, parameters of 44 birds were available for evaluation.

Analysis of variance was made by single trait general linear model, where fixed effects were treatment (groups) and sex.

$$P_{i,j} = \mu + \text{treatment}_i + \text{sex}_j + \text{error}_{i,j}$$

where  $P_{i,j}$  = parameters evaluated,  $\mu$  = overall mean of the parameter,  $\text{treatment}_i$  = fixed effect of treatment (1–4),  $\text{sex}_j$  = fixed effect of sex (male/female),  $\text{error}_{i,j}$  = residual effect specific for each observation.

All data preparation and processing were carried out by the use of STATISTICA6 (StatSoft, 2003) program package. Within the results, we give the

LSM and SEM by treatments as well as the significance of effects and between-effects. Later on, Duncan test was carried out as a *post hoc* comparison of means.

This study was approved by the Animal Use and Care Administrative Advisory Committee of the Hungarian Scientific Veterinary Chamber and complies with European Union directives regarding the use of experimental animals (CECAE, 1992).

## Results

Histological examination showed an active hyperaemia in the lung and dilatation of bronchial tubes of pigeons participating in the long-distance race (Group 5: FLTØ, 6: FLTC and 7: FLTL). Accumulation of brownish black substances in the cytoplasm of macrophages could be observed. Focal, lymphohistiocytoid infiltration in the interstitium of lung, liver and kidneys and the occurrence of the chondroid islet were also detected.

Body weight loss during long-distance flight ranged between 70.5 and 84.9 g whereas weight loss during short-distance flight was significantly ( $p < 0.001$ ) less and ranged between 30.7 and 37.2 g (Table 2). In this respect, neither L-carnitine nor L-lysine effects were present. The absolute and rela-

tive weights of the examined organs (Table 3) were affected by the extent of exercise but not by the L-carnitine and L-lysine administration.

Body composition of pigeons can be seen in Table 4. Independent from the flying distance, dry matter (DM) content of birds which did not fly (Group 1: FØTØ) was significantly ( $p < 0.001$ ) higher than that of the raced ones at arrival. In this respect neither L-carnitine nor L-lysine effects could be observed. With reference to the CP and ash contents of pigeons' body, there are no significant differences between the groups. It can be seen that the body fat (ether extract, EE) content of pigeons which did not fly (Group 1: FØTØ) was significantly ( $p < 0.001$ ) higher than that of the other groups which did fly. Regarding these parameters, the main differences can be seen between pigeons that were raced and pigeons which did not fly. In pigeons subjected to long-distance flight, EE content of groups that had L-carnitine (Group 6: FLTC) and L-lysine (Group 7: FLTL) supplementation was very similar and they were significantly ( $p < 0.001$ ) lower than the EE content of the raced (positive) control (Group 5: FLTØ). This effect could not be observed in pigeons subjected to short-distance flight. The amounts of mobilized body fat significantly ( $p < 0.001$ ) increased because of the enhancement of the extent of exercise.

**Table 2** Body weight of pigeons before and after flight

| Treatment                | n   | Body weight (BW) |      |                |      |                    |      |                      |     |
|--------------------------|-----|------------------|------|----------------|------|--------------------|------|----------------------|-----|
|                          |     | Initial (g)      |      | At arrival (g) |      | Change (g)         |      | Relative change* (%) |     |
|                          |     | LSM              | SEM  | LSM            | SEM  | LSM                | SEM  | LSM                  | SEM |
| FØTØ                     | 6   | 460.0            | 15.5 | –              | –    | –                  | –    | –                    | –   |
| FSTØ                     | 6   | 485.0            | 20.6 | 454.3          | 22.9 | –30.7 <sup>a</sup> | 12.3 | –6.4 <sup>a</sup>    | 2.5 |
| FSTC                     | 6   | 462.5            | 14.6 | 431.5          | 16.2 | –31.0 <sup>a</sup> | 8.7  | –6.7 <sup>a</sup>    | 1.8 |
| FSTL                     | 8   | 449.6            | 16.3 | 412.4          | 18.1 | –37.2 <sup>a</sup> | 9.7  | –8.4 <sup>a</sup>    | 2.0 |
| FLTØ                     | 6   | 490.0            | 14.6 | 419.5          | 16.2 | –70.5 <sup>b</sup> | 8.7  | –14.3 <sup>b</sup>   | 1.8 |
| FLTC                     | 6   | 512.0            | 19.6 | 427.1          | 21.7 | –84.9 <sup>b</sup> | 11.7 | –16.4 <sup>b</sup>   | 2.4 |
| FLTL                     | 6   | 518.0            | 12.6 | 443.5          | 14.0 | –75.3 <sup>b</sup> | 7.5  | –14.6 <sup>b</sup>   | 1.5 |
| P <sub>(Treatment)</sub> |     | 0.118            |      | 0.640          |      | <0.001             |      | 0.003                |     |
| Sex                      |     |                  |      |                |      |                    |      |                      |     |
| Male                     | 16† | 485.2            | 10.1 | 438.6          | 12.2 | –56.7              | 6.6  | –11.3                | 1.3 |
| Female                   | 22† | 479.9            | 7.3  | 424.2          | 8.8  | –53.2              | 4.7  | –11.0                | 1.0 |
| P <sub>(Sex)</sub>       |     | 0.669            |      | 0.348          |      | 0.669              |      | 0.845                |     |

\*Relative change: BW change expressed in percent of the initial BW, Group 1: FØTØ = no flight–no treatment; Group 2: FSTØ = Flight: short distance–no treatment; Group 3: FSTC = Flight: short distance–treatment: L-carnitine; Group 4: FSTL = Flight: short distance–treatment: L-lysine; Group 5: FLTØ = Flight: long distance–no treatment; Group 6: FLTC = Flight: long distance–treatment: L-carnitine; Group 7: FLTL = Flight: long distance–treatment: L-lysine.

†When calculated data regarding the BW at arrival, BW change and relative change of BW, number of animals was less by 3 (both in males and females) than in case of the other parameters because the members of Group 1 did not fly. Remark: pigeons could drink water just the minute they arrived which might increase their arrival body weight thus also the values of 'change' and 'relative change'.

<sup>ab</sup>Means bearing different superscripts in the same column differ significantly.

**Table 3** Absolute and relative weight of liver and heart

| Treatment                | n  | Liver weight |     |                   |      | Heart weight      |      |                   |      |
|--------------------------|----|--------------|-----|-------------------|------|-------------------|------|-------------------|------|
|                          |    | Absolute (g) |     | Relative* (g)     |      | Absolute (g)      |      | Relative* (%)     |      |
|                          |    | LSM          | SEM | LSM               | SEM  | LSM               | SEM  | LSM               | SEM  |
| F0T0                     | 6  | 7.8          | 0.7 | 1.65 <sup>a</sup> | 0.14 | 5.05 <sup>a</sup> | 0.29 | 1.07 <sup>a</sup> | 0.05 |
| FST0                     | 6  | 10.1         | 0.9 | 2.11 <sup>b</sup> | 0.17 | 6.49 <sup>c</sup> | 0.36 | 1.36 <sup>b</sup> | 0.07 |
| FSTC                     | 6  | 9.0          | 0.7 | 1.94 <sup>b</sup> | 0.14 | 6.29 <sup>c</sup> | 0.29 | 1.37 <sup>b</sup> | 0.05 |
| FSTL                     | 8  | 9.6          | 0.8 | 2.11 <sup>b</sup> | 0.15 | 6.74 <sup>c</sup> | 0.32 | 1.48 <sup>b</sup> | 0.06 |
| FLT0                     | 6  | 9.7          | 0.7 | 1.97 <sup>b</sup> | 0.14 | 5.70 <sup>b</sup> | 0.29 | 1.17 <sup>a</sup> | 0.05 |
| FLTC                     | 6  | 8.0          | 0.8 | 1.61 <sup>a</sup> | 0.14 | 5.66 <sup>b</sup> | 0.30 | 1.13 <sup>a</sup> | 0.06 |
| FLTL                     | 6  | 7.8          | 0.6 | 1.50 <sup>a</sup> | 0.12 | 5.69 <sup>b</sup> | 0.25 | 1.10 <sup>a</sup> | 0.05 |
| P <sub>(Treatment)</sub> |    | 0.178        |     | 0.010             |      | 0.006             |      | <0.001            |      |
| Sex                      |    |              |     |                   |      |                   |      |                   |      |
| Male                     | 19 | 8.4          | 0.5 | 1.73 <sup>a</sup> | 0.09 | 5.95              | 0.18 | 1.23              | 0.03 |
| Female                   | 25 | 9.4          | 0.4 | 1.96 <sup>b</sup> | 0.07 | 5.94              | 0.14 | 1.26              | 0.03 |
| P <sub>(Sex)</sub>       |    | 0.104        |     | 0.044             |      | 0.979             |      | 0.786             |      |

\*Relative liver/heart weight: liver/heart weight expressed in percent of the initial BW, Group 1: F0T0 = no flight–no treatment; Group 2: FST0 = Flight: short distance–no treatment; Group 3: FSTC = Flight: short distance–treatment: L-carnitine; Group 4: FSTL = Flight: short distance–treatment: L-lysine; Group 5: FLT0 = Flight: long distance–no treatment; Group 6: FLTC = Flight: long distance–treatment: L-carnitine; Group 7: FLTL = Flight: long distance–treatment: L-lysine. Remark: pigeons could drink water just the minute they arrived which might increase their arrival body weight thus also the values of the relative liver/heart weight.

<sup>abc</sup>Means bearing different superscripts in the same column differ significantly.

**Table 4** Body composition of pigeons and the amount of mobilized fat

| Treatment            | n  | Dry matter        |     | Ash               |      | Crude protein     |     | Ether extract       |      | Mobilized fat      |      |
|----------------------|----|-------------------|-----|-------------------|------|-------------------|-----|---------------------|------|--------------------|------|
|                      |    | (%)               |     | (%)               |      | (%)               |     | (%)                 |      | (g)                |      |
|                      |    | LSM               | SEM | LSM               | SEM  | LSM               | SEM | LSM                 | SEM  | LSM                | SEM  |
| F0T0                 | 6  | 46.9 <sup>a</sup> | 0.9 | 3.41              | 0.16 | 23.6              | 0.5 | 17.61 <sup>bc</sup> | 1.09 | –                  | –    |
| FST0                 | 6  | 38.7 <sup>b</sup> | 1.1 | 3.81              | 0.20 | 23.9              | 0.6 | 8.67 <sup>a</sup>   | 1.34 | 46.11 <sup>a</sup> | 0.62 |
| FSTC                 | 6  | 38.2 <sup>b</sup> | 0.9 | 3.75              | 0.16 | 24.2              | 0.5 | 8.19 <sup>a</sup>   | 1.08 | 46.10 <sup>a</sup> | 0.50 |
| FSTL                 | 8  | 38.3 <sup>b</sup> | 1.0 | 3.94              | 0.17 | 24.5              | 0.5 | 8.03 <sup>a</sup>   | 1.18 | 46.06 <sup>a</sup> | 0.54 |
| FLT0                 | 6  | 38.3 <sup>b</sup> | 0.9 | 3.57              | 0.16 | 23.6              | 0.5 | 8.12 <sup>a</sup>   | 1.08 | 52.23 <sup>a</sup> | 0.56 |
| FLTC                 | 6  | 36.4 <sup>b</sup> | 0.9 | 3.66              | 0.16 | 24.8              | 0.5 | 5.19 <sup>b</sup>   | 1.12 | 67.99 <sup>b</sup> | 0.76 |
| FLTL                 | 6  | 36.7 <sup>b</sup> | 0.8 | 3.88              | 0.14 | 25.0              | 0.4 | 4.75 <sup>b</sup>   | 0.93 | 70.16 <sup>b</sup> | 0.65 |
| P <sub>(Group)</sub> |    | <0.001            |     | 0.278             |      | 0.190             |     | <0.001              |      | <0.001             |      |
| Sex                  |    |                   |     |                   |      |                   |     |                     |      |                    |      |
| Male                 | 19 | 38.7              | 0.6 | 3.55 <sup>a</sup> | 0.10 | 24.6 <sup>a</sup> | 0.3 | 8.13                | 0.69 | 52.74              | 0.51 |
| Female               | 25 | 39.7              | 0.4 | 3.88 <sup>b</sup> | 0.80 | 23.8 <sup>b</sup> | 0.2 | 9.17                | 0.53 | 54.66              | 0.67 |
| P <sub>(Sex)</sub>   |    | 0.378             |     | 0.014             |      | 0.043             |     | 0.240               |      | 0.311              |      |

Group 1: F0T0 = no flight–no treatment; Group 2: FST0 = Flight: short distance–no treatment; Group 3: FSTC = Flight: short distance–treatment: L-carnitine; Group 4: FSTL = Flight: short distance–treatment: L-lysine; Group 5: FLT0 = Flight: long distance–no treatment; Group 6: FLTC = Flight: long distance–treatment: L-carnitine; Group 7: FLTL = Flight: long distance–treatment: L-lysine.

<sup>abc</sup>Means bearing different superscripts in the same column differ significantly.

According to the present results, no major differences were detected between the males and the females.

Results of blood analysis are summarized in Table 5. After long-distance flight as an effect of exercise, glucose had decreased significantly ( $p < 0.05$ ) when compared with the values in pigeons that were

not subjected to flight exercise. L-carnitine supplementation had significantly ( $p < 0.05$ ) lower glucose levels compared with the L-lysine group subjected to long-distance flight. In this respect, no significant changes were observed after short-distance flight. Serum fructosamine level significantly ( $p < 0.05$ ) decreased after flight independently from the flying

**Table 5** Blood parameters

| Treatment            | n  | GLU                  |      | SEFA              |      | CHOL     |      | TG                |      | TBARS          |      |
|----------------------|----|----------------------|------|-------------------|------|----------|------|-------------------|------|----------------|------|
|                      |    | (mmol/l)             |      | (mmol/l)          |      | (mmol/l) |      | (mmol/l)          |      | ( $\mu$ mol/l) |      |
|                      |    | LSM                  | SEM  | LSM               | SEM  | LSM      | SEM  | LSM               | SEM  | LSM            | SEM  |
| FøTø                 | 6  | 16.61 <sup>a</sup>   | 0.78 | 3.85 <sup>b</sup> | 0.34 | 4.84     | 0.51 | 3.34 <sup>a</sup> | 0.50 | 2.10           | 0.52 |
| FSTø                 | 6  | 15.66 <sup>abc</sup> | 0.96 | 2.49 <sup>a</sup> | 0.42 | 6.57     | 0.63 | 2.41 <sup>b</sup> | 0.62 | 2.62           | 0.64 |
| FSTC                 | 6  | 15.62 <sup>abc</sup> | 0.77 | 2.28 <sup>a</sup> | 0.34 | 6.58     | 0.51 | 2.90 <sup>b</sup> | 0.50 | 2.26           | 0.52 |
| FSTL                 | 8  | 14.46 <sup>c</sup>   | 0.85 | 2.46 <sup>a</sup> | 0.37 | 6.56     | 0.56 | 2.77 <sup>b</sup> | 0.55 | 2.69           | 0.57 |
| FLTø                 | 6  | 12.96 <sup>d</sup>   | 0.77 | 2.42 <sup>a</sup> | 0.34 | 6.15     | 0.51 | 1.75 <sup>b</sup> | 0.50 | 2.06           | 0.52 |
| FLTC                 | 6  | 14.69 <sup>bc</sup>  | 0.80 | 2.53 <sup>a</sup> | 0.35 | 6.53     | 0.53 | 1.68 <sup>b</sup> | 0.52 | 1.75           | 0.54 |
| FLTL                 | 6  | 15.67 <sup>ab</sup>  | 0.67 | 2.32 <sup>a</sup> | 0.29 | 5.12     | 0.44 | 1.86 <sup>b</sup> | 0.43 | 2.33           | 0.45 |
| P <sub>(Group)</sub> |    | 0.048                |      | 0.031             |      | 0.085    |      | 0.032             |      | 0.905          |      |
| Sex                  |    |                      |      |                   |      |          |      |                   |      |                |      |
| Male                 | 19 | 15.34                | 0.49 | 2.64              | 0.21 | 5.64     | 0.38 | 2.52              | 0.32 | 2.06           | 0.33 |
| Female               | 25 | 14.86                | 0.38 | 2.60              | 0.17 | 6.17     | 0.25 | 2.40              | 0.25 | 2.46           | 0.26 |
| P <sub>(Sex)</sub>   |    | 0.448                |      | 0.877             |      | 0.212    |      | 0.766             |      | 0.348          |      |

GLU, glucose; FRA, fructosamine; CHOL, cholesterol; TG, triglycerides; TBARS, 2-thiobarbituric acid reactive substances.

Group 1: FøTø = no flight–no treatment; Group 2: FSTø = Flight: short distance–no treatment; Group 3: FSTC = Flight: short distance–treatment:

L-carnitine; Group 4: FSTL = Flight: short distance–treatment: L-lysine; Group 5: FLTø = Flight: long distance–no treatment; Group 6: FLTC = Flight:

long distance–treatment: L-carnitine; Group 7: FLTL = Flight: long distance–treatment: L-lysine

<sup>abcd</sup>Means bearing different superscripts in the same column differ significantly.

distance and treatments. Cholesterol was neither affected by the treatments nor by the flying distance. The TG level was the lowest (1.68 mmol/l) in the Group 6 (FLTC), but significantly ( $p < 0.05$ ) decreased after flight independently from the flying distance. No significant changes could be observed in TBARS.

## Discussion

The active hyperaemia in the lung and dilatation of bronchial tubes of pigeons participating in the long-distance race (Group 5: FLTø, 6: FLTC and 7: FLTL) could be because of an intense exercise load. Accumulation of brownish black substances in the cytoplasm of macrophages was the residue of the contamination from the air. The focal lymphohistiocytoid infiltration in the interstitium of lung, liver and kidneys and the occurrence of the chondroid islet can be treated as individual subclinical disorders. Diminishing content of glycogen and lipid in the liver cells indicates the expenditure of those substances during flight. Under physiological conditions, lipids could not be detected by light microscopic histochemical examination in the cells of heart and skeleton muscles.

Neither L-carnitine nor L-lysine administration influenced the body weight loss during flight. Similar effect of flight was observed by Janssens et al. (1998), when the effect of L-carnitine (90 mg/day for 1 week)

on the energy metabolism of trained male racing pigeons during flight simulation by electrostimulation of the breast muscles was investigated in a respiration chamber test. L-carnitine supplementation did not influence the weight loss. Also in another experiment, when pigeons were put on a low-energy diet for 2 months (Janssens et al., 2000a), no significant effects of L-carnitine were seen on body weight loss. Although, when investigating growing pigeons squabs (Janssens et al., 2000b), significant effects of L-carnitine supplementation were referred.

The differences in DM content can be explained by the different body fat contents. Regarding these parameters, the main differences can be shown between pigeons that were raced and pigeons which did not fly. During long-distance flight therefore, the effect of the better fat mobilization because of L-carnitine and L-lysine supplementation can be supposed. No similar conclusion can be drawn when evaluating the results after the short-distance flight. Because of these, the effect of L-carnitine or L-lysine administration may also depend on the extent of exercise (flying distance). According to the similar results of the Group 6 and the Group 7, a sufficient conversion rate of L-lysine to L-carnitine can be supposed. In humans, 30–50% of total  $\epsilon$ -N-trimethyllysine (from diet and endogenous production) is converted to carnitine, and the remainder is excreted in urine (Rebouche et al., 1989). Administration of 5 g L-lysine orally to normal adults produced a

significant increase in plasma L-carnitine level within 6 h followed by a further rise by 48 h (Khan-Siddiqui and Bamji, 1983). Levels remained high up to 72 h. Similar changes in plasma L-carnitine were not observed if blood was sampled without L-lysine load or after administering a load of other amino acids such as tryptophan or threonine. These observations suggest that there may be a rapid *in vivo* conversion of orally administered L-lysine to L-carnitine. According to the present results, no major differences were detected between the males and the females. It corresponds to previous results when the effect of breed and sex on the adult body composition of four pigeon breeds were investigated (Fekete *et al.*, 1999).

As an effect of long-distance flight, glucose level significantly ( $p < 0.05$ ) decreased. The result is different from that of other animals such as mammals which showed a lower rate of decrease of the average glucose after exercise (Viguie *et al.*, 1993). Because the glucose level did not change significantly during the short-distance flight, it can be supposed that previous exercise training and also the extent of exercise can alter the results. Cholesterol did not change significantly but the TG level was the lowest in the Group 6 (FLTC) which is related to the physiological function of L-carnitine. For long-distance flight, L-lysine supplementation had a comparable effect on plasma TG level with L-carnitine supplementation which might indicate a similar action pathway. Surprisingly, TBARS content of blood did not increase but slightly decreased after long-distance flight. There are some data relating to the higher rate of lipid peroxidation, higher TBARS formation as a result of exercise mainly in mammals (Avellini *et al.*, 1995). The lack of this tendency in this study is supposed to be the effect of the diminishing plasma lipid content which has a marked effect on the actual TBARS level (Dworschák *et al.*, 1988). The other possible explanation of that result would be the lower rate of free radical formation during long-term exercise, in this case the long-distance flight, a hypothesis that supports the results of a previous investigation where lower amount of oxidatively modified guanine was found during exercise (Viguie *et al.*, 1993). Borghijs and De Wilde (1992) investigated whether *per os* supplementation of L-carnitine to trained pigeons had beneficial effects on the use of free fatty acids (FFA) as an energy source and on the stability of muscles during prolonged exercise. They noticed a significant influence of L-carnitine on the course of plasma creatine phosphokinase, FFA and lactate. They

concluded that L-carnitine supplementation to trained pigeons is beneficial to maintain the oxidative metabolism and to decrease the muscular destruction during prolonged exercise.

In conclusion, during long-distance flight, the effect of the better fat mobilization because of the L-carnitine and L-lysine supplementation can be supposed. No similar conclusion can be drawn when evaluating the results after the short-distance flight. The main changes in the measured blood parameters were caused by the extent of exercise. This experiment considered the extent of exercise as a factor potentially modulating L-carnitine supplementation effects. In conclusion, flight distance affected several parameters, but the supplements of L-carnitine and L-lysine were not effective in the tested situations. Sex did not affect the measured parameters.

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