Aerobic and resistance training do not influence plasma carnosinase content or activity in type 2 diabetes.

Authors:
Sanne Stegen1, Ronald J. Sigal2,3,4, Glen P. Kenny3,4, Farah Khandwala5, Benito Yard6, Emile De Heer7, Hans Baelde7, Wim Peersman8, Wim Derave1

Affiliation:
1 Department of Movement and Sport Sciences, Ghent University, Ghent, Belgium
2 Departments of Medicine, Cardiac Sciences and Community Health Sciences, Cumming School of Medicine, Faculties of Medicine and Kinesiology, University of Calgary, Calgary, Canada
3 School of Human Kinetics, Faculty of Health Sciences, University of Ottawa, Ottawa, ON, Canada.
4 Clinical Epidemiology Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada.
5 Alberta Health Services, Calgary, Canada.
6 5th Medical Department, University Hospital Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.
7 Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands.
8 Department of Family Medicine and Primary Health Care, Ghent University, Ghent, Belgium.

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Address for Correspondence: Wim Derave, Watersportlaan 2, B-9000 Ghent, Belgium. Tel: +32 (0)9 264 63 26. Fax: +32 (0)9 264 64 84. E-mail: wim.derave@ugent.be.
A particular allele of the carnosinase gene (CNDP1) is associated with reduced plasma carnosinase activity and reduced risk for nephropathy in diabetic patients. On the one hand, animal and human data suggest that hyperglycemia increases plasma carnosinase activity. On the other hand, we recently reported lower carnosinase activity levels in elite athletes involved in high-intensity exercise, compared to untrained controls. Therefore, this study investigates whether exercise training and the consequent reduction in hyperglycemia can suppress carnosinase activity and content in adults with type 2 diabetes. Plasma samples were taken from 243 males and females with type 2 diabetes (mean age = 54.3 yr, SD = 7.1) without major microvascular complications before and after a 6-month exercise training program (4 groups: sedentary control (n=61), aerobic exercise (n=59), resistance exercise (n=63) and combined exercise training (n=60)). Plasma carnosinase content and activity, hemoglobin A1c, lipid profile and blood pressure were measured. A 6-month exercise training intervention, irrespective of training modality, did not decrease plasma carnosinase content or activity in type 2 diabetic patients. Plasma carnosinase content and activity showed a high inter-individual but very low intra-individual variability over the 6-month period. Age and sex, but not HbA1c, were significantly related to the activity or content of this enzyme. It can be concluded that the beneficial effects of exercise training on the incidence of diabetic complications is probably not related to a lowering effect on plasma carnosinase content or activity.

**Keywords:** CNDP1, carnosine, histidine-containing dipeptides, carnosinase activity and content
INTRODUCTION

Chronic hyperglycemia increases the risk of long-term microvascular complications of diabetes, and the carnosine-carnosinase system may play a role in mediating this association. Human plasma carnosinase, encoded by the CNDP1 gene, belongs to the M20 family of metalloproteinases and is secreted by the liver or brain into the circulation. It is highly active in human adults (18; 37), hydrolyzing the histidine-containing dipeptides carnosine, homocarnosine and anserine into their constituent amino acids, with the highest specificity for carnosine (28; 29). Both human genetic association studies and animal models suggest a pivotal role for the carnosine-carnosinase system in microvascular complications of diabetes (3; 19; 33; 34; 40).

Genome-wide linkage scans and subsequent fine-mapping studies have indicated a strong association between a CTG trinucleotide repeat in CNDP1 and the susceptibility for developing diabetic nephropathy (14; 19; 26; 40). More specifically, Caucasian type 2 diabetic mellitus (T2D) patients homozygous for the (CTG)₅ allele are less susceptible to develop diabetic nephropathy as compared to T2D patients with a different genotype. In addition, it is known that homozygosis for the (CTG)₅ allele correlates with a significantly lower plasma carnosinase activity (12; 13; 19). It has been hypothesized that low carnosinase activity might afford protection against diabetic nephropathy as a consequence of a higher availability of circulating carnosine upon dietary ingestion of carnosine from meat and fish (9; 13).

While formal proof for this assumption is still lacking in humans, studies in type 1 and 2 diabetic rodent models revealed that carnosine supplementation caused a later onset of hyperglycemia and a milder course of diabetes with significantly less renal damage (30; 31; 33; 34; 36). This beneficial effect can be explained in part by carnosine’s physiological and biochemical properties (9). First, carnosine reduces glycoxidative stress, by for example quenching reactive aldehydes, and thereby preventing the formation of other reactive metabolites (3; 4; 11; 16; 24). Second, carnosine may
increase insulin secretion in rodent diabetic models (27; 34), thereby improving glycemic control in these animals. Finally, carnosine supplementation in streptozotocin-induced diabetic and dyslipidemic Balb/cA mice reduces triglyceride and cholesterol levels in heart and liver (21). The latter findings have been confirmed and extended in several other animal models of metabolic diseases (3; 23; 25). Whether the protective effects of carnosine ingestion in rodents can be translated to humans is not known, because rodents, unlike humans, have no carnosinase in their plasma (18). However, it can be expected that the normal dietary intake of carnosine in humans is more protective in people with low carnosinase activity. Carnosinase activity level is thought to be a stable, intrinsic characteristic of individuals. Therefore, a hypothesis of the current study is that T2D patients with low plasma carnosinase have a healthier lipid profile than T2D patients with high plasma carnosinase.

Although in the past decade the number of genetic studies on CNDP1 in relation to diabetic complications has increased, little attention has been given to actual plasma carnosinase activity and content levels in diabetic patients. In healthy subjects, sex, age and particular polymorphisms of CNDP1 are known determinants of plasma carnosinase activity (12; 22; 29). In one study with diabetic patients (type 1 and 2), carnosinase activity was increased compared to genotype matched healthy controls (32).

It has been established that prolonged aerobic exercise training could protect different diabetic rodent models against pathophysiological characteristics of diabetic nephropathy (17; 39). Our lab recently demonstrated that elite athletes, involved in high-intensity exercise, have lower plasma carnosinase content (-31%) and activity (-17%) compared to untrained controls, raising the possibility that exercise training may reduce both the content and activity of the carnosinase enzyme (7). In this light, we hypothesized that exercise training in diabetic patients, either directly, or indirectly through
lowering hyperglycemia, could decrease plasma carnosinase content and activity and thereby partly underlie the protective effects of exercise training on the development of diabetic complications.

For these purposes, we investigated both plasma carnosinase content and activity in a large cohort of males and females with T2D, before and after a 6-month intervention of exercise training (aerobic training only, resistance training only, and combined aerobic and resistance training) compared to sedentary controls.
METHODS

Plasma was taken from 243 T2D participants (153 males, 90 females, mean age = 54.3 yr, SD = 7.1) from the DARE (Diabetes Aerobic and Resistance Exercise) clinical trial (35) to measure plasma carnosinase content and activity before and after a 6-month exercise training program. The DARE trial was originally designed to determine the effect of aerobic and resistance training alone (6 months) versus a sedentary control group, and the incremental effects of performing both types of exercise (combined exercise training) versus aerobic or resistance training alone, on glycemic control and other risk factors for cardiovascular disease (35). Initially, the DARE trial contained 251 patients, but samples of eight individuals were out of stock, leaving 243 subjects with plasma samples. The DARE trial design, participant characteristics and intervention are briefly described below and the reader is referred to the full manuscript of Sigal R.J. et al. (35) for further details.

Design

A 26-week, single-center, randomized, controlled trial with parallel-group design was performed. The trial included a 4-week run-in phase plus a 22-week intervention phase. Participants were randomly assigned to four groups: aerobic training, resistance training, combined aerobic and resistance training, or a control group. Before and after the 6-month intervention period, heparinized plasma blood samples were taken in a fasted state and participants were instructed not to exercise 48 hours before each visit. The study was approved by the Ottawa Hospital Research Ethics Board, and all participants gave informed consent.
Participants

Baseline characteristics are presented in table 1. Inclusion criteria included type 2 diabetes (as defined by the American Diabetes Association (1)) for more than 6 months and a baseline hemoglobin A1c value of 6.6% to 9.9% (normal range, 4.0% to 6.0%). Exclusion criteria were current insulin therapy; participation in exercise more than twice a week for more than 20 minutes at a time during the previous 6 months; changes during the previous 2 months in oral hypoglycemic, antihypertensive, or lipid-lowering agents or body weight (≥5%); serum creatinine level of 200 µmol/L or greater (≥2.26 mg/dL); proteinuria greater than 1 g/d; blood pressure greater than 160/95 mm Hg; restrictions in physical activity because of disease; or presence of other medical conditions that made participation inadvisable. The exercise intervention took place at eight community-based exercise facilities in the Ottawa–Gatineau, Canada, region. Exercise was supervised by personal trainers with equal frequency in all exercising groups. Individual exercise supervision was provided weekly for the first 4 weeks after randomization, biweekly for the next 2 months and every 4 weeks thereafter.

Intervention

Exercise group participants exercised 3 times weekly, and training progressed gradually in duration and intensity. The aerobic training group exercised on treadmills or bicycle ergometers. Participants progressed from 15 to 20 minutes per session at 60% of the maximum heart rate to 45 minutes per session at 75% of the maximum heart rate, as determined by using a maximal treadmill exercise test. Heart rate monitors (Polar Electro Oy, Kempele, Finland) displaying the participant’s heart rate were used to standardize exercise intensity. Throughout the resistance training program, participants alternated between two groups of 7 exercises performed on weight machines each session targeting all major muscle groups. These were as follows: Group A: abdominal crunch, seated row, biceps curl, bench press, leg press, shoulder press and leg extension; and, Group B: abdominal crunch, lateral pulldown, triceps pushdown, chest press, leg press, upright row and leg curls. Participants performed
2 to 3 sets of each exercise at the maximum weight that could be lifted 7 to 9 times. When the participant could perform more than 8 repetitions while maintaining proper form, the weight or the resistance of the exercise was increased by 5-10 pounds with guidance from the personal trainer. The combined exercise training group carried out the full aerobic training program plus the full resistance training program to ensure an adequate dose of each type of exercise. More details on the exercise training programs are available online (http://www.annals.org/cgi/content/full/147/6/357). Control participants were asked to revert to pre-study activity levels, had the same dietary intervention and spent the same time with the research coordinator and diettian as did the participants from the exercise groups.

Plasma analyses

Glycemic control, lipid profile and blood pressure. Hemoglobin A1c was measured by turbidimetric immunoinhibition, and total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride levels were measured using enzymatic methods on a Beckman-Coulter LX20 analyzer (Beckman Instruments, Brea, California). Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald equation (15). Blood pressure was measured after 10 minutes at rest; the mean of 2 readings obtained 2 minutes apart was used in statistical analysis.

Plasma carnosinase content and activity.

Plasma carnosinase concentrations (content) were determined by a sandwich ELISA (enzyme-linked immunosorbent assay) as described in Adelmann et al. (2). Plasma carnosinase activity was determined according to a method described by Teufel et al. (2003) (37). Plasma carnosinase specific activity was calculated by dividing carnosinase activity by its content (the activity of an enzyme per milligram of total protein) and expressed as μmol/min/mg.
Statistical analysis.

The effect of a 6-month aerobic training, resistance training or combined exercise training on plasma carnosinase content and activity was investigated by a linear mixed model design, adjusted for sex, age and body mass index (BMI). Within the mixed models, we calculated 95% confidence intervals and p-values for pre-specified intergroup contrasts, and for change in carnosinase and carnosinase activity within each group over time. For this model, SAS (version 9.2, Institute, Cary, North Carolina) was used. To investigate the intra-individual variation of plasma carnosinase content and activity over a 6-month period, the intraclass correlation coefficient (ICC) (a two-way mixed model with measures of absolute agreement) was calculated for control subjects (remaining sedentary) only. In addition, Pearson correlation coefficients were calculated to study the relationship between plasma carnosinase content and its activity within subjects at baseline.

At baseline, the effect of age on plasma carnosinase content and activity was explored by linear regression analysis, with age as independent variable and carnosinase as dependent variable. In sensitivity analyses, duration of diabetes was taken as a covariate and analyzed for males and females separately. To study the effect of sex on plasma carnosinase, a two-sided independent-samples T-test was performed to compare plasma carnosinase content and activity between males and females at baseline. A linear regression analysis was performed to investigate the relationship between plasma carnosinase content/activity (independent variable) and glycemic control (HbA1c), the lipid profile (triglycerides, total cholesterol, HDL-C, LDL-C and the ratio total cholesterol/HDL-C) and blood pressure (SBP and DBP) (dependent variables) at baseline. Patients with missing values at baseline (N=2 for plasma carnosinase content and N=4 for activity because of technical measurement errors) were excluded from the analyses. In sensitivity analyses, patients on glucose-, lipid- and blood pressure lowering medication were excluded while investigating the relationship with respectively HbA1c, lipid profile and blood pressure. In addition, age and sex were taken as covariates and the natural logarithm (Ln) of plasma carnosinase content and activity was used because distributions of
the independent variables (plasma carnosinase content and activity) were skewed and were normalized after logarithmic transformation. Standardized beta-coefficients are reported. All analyses were done with SPSS statistical software (SPSS 20, Chicago, IL), except for the linear mixed model design (as mentioned above). The statistical significance was set at p<0.05.
RESULTS

The effect of exercise training and glycemic control on plasma carnosinase

Table 2 provides the changes in plasma carnosinase protein content and activity, by time and exercise training for 6 months, and adjusted by age, sex and BMI. Neither carnosinase protein content, nor its activity was influenced by the 6-month exercise program, irrespective of exercise training modality (aerobic or resistance exercise alone, or the combination of both types). At baseline, there was no association between HbA1c and plasma carnosinase protein content or activity in the overall population (table 3) or when patients on glucose lowering medication were excluded (N=58; \( B=0.13, p=0.328 \) for content; \( B=0.15, p=0.266 \) for activity). The aerobic and resistance training programs decreased HbA1c compared to the control group, and the combined training program did so to a greater extent than either aerobic or resistance training alone (pre-exercise HbA1c values: see table 1, post-exercise values: control: 7.69%; aerobic: 7.23; resistance: 7.47 and combined: 6.71%). In addition, fasting plasma glucose was only significantly decreased by the combined exercise training group (pre- and post-exercise training values respectively for control: 9.40 and 9.19, \( p=0.491 \); aerobic: 9.30 and 9.11, \( p=0.574 \); resistance: 9.33 and 8.79, \( p=0.096 \); combined: 9.11 and 7.88 mmol/l, \( p<0.001 \)).

We repeated all analyses above (effect of exercise training on plasma carnosinase and on glycemic control) separately for each sex and all results were essentially identical (data not shown).

Test-retest for plasma carnosinase content and activity

The ICC for plasma carnosinase content (figure 1A) and activity (figure 1B) over a 6-month period in the sedentary control group were 0.91 (\( p<0.001 \)) and 0.63 (\( p<0.001 \)) respectively, demonstrating an excellent stability for plasma carnosinase content, but only a moderate stability for its activity. The lower and upper bound of the 95% confidence interval (CI) for plasma carnosinase content were respectively 0.86 and 0.95, and 0.44 and 0.75 for carnosinase activity. In addition, a low, but
significant, positive correlation was observed between carnosinase content and activity at baseline for the entire group ($r=0.22$, $p=0.001$, figure 1C).

**Influence of sex and age on plasma carnosinase**

**Sex:** At baseline, plasma carnosinase protein content was 32% higher in females compared to males, but there was no sex difference in carnosinase activity (figure 2A). As consequence, the specific activity of the enzyme was 24% higher in the males compared to the females (figure 2B). **Age:** carnosinase protein content was negatively correlated with age in females ($r=-0.21; p=0.049$), but not in males ($r=-0.12, p=0.132$). Plasma carnosinase activity was negatively correlated with age, for both males ($r=-0.19; p=0.022$) and females ($r=-0.27; p=0.011$).

**Plasma carnosinase, lipid profile and blood pressure (table 3)**

At baseline, the ratio of total cholesterol/HDL-C was positively correlated with plasma carnosinase content ($p<0.01$). Carnosinase activity on the other hand, was not associated with the total cholesterol/HDL-C ratio. In addition, HDL-C alone was negatively correlated with plasma carnosinase protein content ($r=-0.153, p=0.020$), but positively with activity ($r=0.131, p=0.038$). These effects appeared to be sex-specific as the correlations were predominantly present for males (carnosinase content: total cholesterol/HDL-C: $r=0.239, p=0.002$; HDL-C: $r=-0.168, p=0.039$) (carnosinase activity: HDL-C: $r=0.271, p=0.001$), but not for females. Finally, blood pressure and carnosinase protein content or activity were not correlated with one another. In sensitivity analyses, patients on lipid- and blood pressure lowering medication were excluded while investigating the relationships with lipids (N=141) and blood pressure (N=105) respectively. Here, no significant correlations were found between carnosinase content/activity and lipid levels or blood pressure. In addition, if these data were analyzed separately for males and females, low carnosinase content was associated with low total cholesterol/HDL-C ratio ($B=0.22, p=0.048$), and low carnosinase activity was associated with low HDL-C levels in males ($B=0.26, p=0.020$), but not in females.
Adults with T2D who are homozygous for the (CTG)₅ allele of CNDP1, encoding for low plasma carnosinase activity, have reduced risk to develop diabetic nephropathy (19; 26; 40). In this study, the effect of exercise training or lowering hyperglycemia on plasma carnosinase content or activity was investigated. In addition, this large cohort of T2D adults, without advanced microvascular diabetic complications, was used to explore whether there was an association between carnosinase activity levels and other diabetes-related vascular risk factors, including dyslipidemia and increased blood pressure.

Effect of exercise training on plasma carnosinase

We hypothesized that exercise training (aerobic, resistance or combined exercise) would reduce plasma carnosinase content and activity, based on two previous observations. First, we recently reported lower plasma carnosinase levels (both content and activity) in elite athletes, involved in high-intensity exercise training, compared to untrained controls (7). Second, Riedl et al. (32) showed elevated plasma carnosinase activity, due to post-translational modification of carnosinase, in diabetic patients. These reports suggested that exercise training might have a suppressive effect on carnosinase activity levels, either directly or indirectly through improved glycemic control. This would be beneficial for people with diabetes who tend to have higher plasma carnosinase levels than those without diabetes (32). In the DARE trial, HbA1c decreased -0.51% and -0.38% in the aerobic and resistance training groups respectively compared to the control group. Moreover, combined exercise training resulted in additional changes in HbA1c of -0.46% compared with aerobic training alone and -0.59% compared with resistance training alone. However, we now show that exercise training, despite effectively reducing HbA1c, did not reduce plasma carnosinase content or activity. In addition, we did not observe a correlation between carnosinase content or activity and HbA1c at baseline (before the exercise intervention). Therefore, we refute the hypothesis that the protective effect of exercise training on the development of diabetic complications is partly explained by reducing
plasma carnosinase, because 6-month exercise training was unable to reduce carnosinase activity in T2D patients.

Thus, there is an apparent discrepancy between the two observations: on the one hand elite athletes involved in high intensity exercise training had significant lower plasma carnosinase content and activity (7), on the other hand, exercise training (aerobic, resistance or combined training) did not influence plasma carnosinase in T2D adults (table 2). Recent twin data from our lab may provide a possible explanation for this discrepancy. Plasma carnosinase in monozygotic twins is highly correlated (r=0.93, p<0.001 for content and r=0.76, p<0.001 for activity), but not in dizygotic twins (r=0.19, p=0.473 for content and r=0.35, p=0.196 for activity) (unpublished data). This indicates that genetic factors may explain the high variability between healthy humans and the low variability within healthy humans. Thus, the difference between the elite athletes and the untrained (7) was likely due primarily to genetic factors, rather than exercise training. However, it would be premature to exclude the possibility of a training effect in healthy subjects. Preliminary data from our lab shows a decrease in carnosinase activity after a 5-week sprint training program (3 times a week, alternating cycling and running) in 10 healthy, previously untrained females (pre= 7.2 ± 0.3; post= 6.2 ± 1.2 µmol/ml/h; p=0.04), but not in males (pre= 6.3 ± 1.8; post= 5.8 ± 0.7; p=0.38). These analyses were done retrospectively on the study of Baguet et al. (5). It remains to be confirmed whether this training effect on plasma carnosinase activity is indeed present in healthy subjects. In addition, the exercise volume and intensity in the elite athletes were much higher than in the DARE trial. It is conceivable that, with much higher-volume and higher-intensity training, carnosinase content and activity could have been altered in type 2 diabetic individuals. However, to our knowledge there is no physiological intervention that can acutely or even sub-acutely alter plasma carnosinase content and activity.
Similarly to healthy individuals, plasma carnosinase content and activity displayed a very large inter-individual variability in T2D individuals (range content: 22-211 µg/ml; range activity: 2-10 µg/ml). Yet, within the T2D subjects, the variability over a 6-month period (evaluated in the sedentary controls) was very small especially for carnosinase content (ICC=0.91) and to a lesser extent for activity (ICC=0.63). Sex and age are two known determinants of plasma carnosinase in healthy subjects (8; 12; 22; 29). It is worthy to mention that plasma carnosinase behaves slightly differently in individuals with diabetes. Similar to healthy subjects, diabetic females in the present study had higher carnosinase content levels compared to males. In non-diabetic individuals, this sex difference is also present for carnosinase activity, but in our present sample of diabetic individuals, the activity level was equal between T2D males and females. As a consequence, the specific activity of the enzyme was lower for the diabetic females than for the diabetic males, suggesting that an unknown factor was suppressing plasma carnosinase activity in T2D females. Lower carnosinase activity might protect diabetic females to some extent from developing diabetic nephropathy. Indeed, Kamenov et al. (20) showed that diabetic nephropathy develops earlier in males than in females with T2D. In the present study, we found for the first time a negative correlation between age and carnosinase activity (males: r= -0.19; p=0.022 and females: r= -0.27; p=0.011), even if duration of diabetes was adjusted for. However, our study contained middle aged and older (age range: 39-70 years) T2D individuals, in contrast to previous studies with younger (50 years or less) healthy subjects.

Low plasma carnosinase content/activity associated with lipid profile and blood pressure?

Several studies in rodents have recently reported protective effects of carnosine administration on dyslipidemia (lipid profile in plasma (3; 25), cholesterol levels in heart and liver (21; 25), atherosclerosis (23)) and blood pressure (3). In contrast to rodents, humans have the enzyme plasma carnosinase and as we confirm in this study, its activity is highly variable between individuals. We hypothesized that diabetic patients with the lowest plasma carnosinase levels, would have the
highest circulating carnosine levels upon dietary ingestion of carnosine (present in meat and fish) and therefore would have the most healthy lipid profiles. However, the observed correlations did not support the hypothesis.

Conclusion

We found high inter-individual, but low intra-individual variability in plasma carnosinase content and activity over a 6-month period in our group of middle aged and older adults with T2D. Low plasma carnosinase activity was not associated with a more favorable lipid profile. The effect of different exercise training modalities and hyperglycemia (as assessed by HbA1c) were investigated, but no relationship with plasma carnosinase was found. Our study could not confirm the hypothesis that the protective effect of exercise training on development of diabetic complications is somehow related to a training-induced attenuation of plasma carnosinase.
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Table 1. Baseline characteristics of T2D participants.

Table 2 provides the changes in plasma carnosinase protein content and activity over a 6-month period for four different interventions (aerobic training, resistance training, combined aerobic and resistance training and a control group). Results are estimated means from linear mixed-effects models, adjusted by age, sex and BMI. Neither carnosinase protein content, nor its activity was influenced by the 6-month exercise program, irrespective of the exercise training modality.

Table 3. Results of the linear regression analysis between plasma carnosinase content and activity (taken as natural logarithm) and glycemic control, lipid profile and blood pressure, adjusted by age and sex (baseline measurements for the 243 participants with T2D). Beta-coefficients and p-values are given.

Figure 1. The ICC for plasma carnosinase content (1A) and activity (1B) over a 6-month period in the sedentary control group is 0.91 (p<0.001) and 0.63 (p<0.001) respectively (line of identity is given). Figure 1C represents a low, but significant, positive correlation between plasma carnosinase content and activity at baseline for all participants (regression line is given).

Figure 2. At baseline, plasma carnosinase protein content is higher in females compared to males, but there is no sex difference in carnosinase activity (figure 2A). As consequence, the specific activity of the enzyme is higher in the males compared to the females (figure 2B).* p<0.001.
Table 1. Baseline characteristics of T2D participants.

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Control group</th>
<th>Aerobic training group</th>
<th>Resistance training group</th>
<th>Combined exercise training group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/Females, n/n</td>
<td>38/23</td>
<td>39/20</td>
<td>39/24</td>
<td>37/23</td>
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<tr>
<td>BMI (SD), kg/m²</td>
<td>33.6 (5.7)</td>
<td>34.2 (5.8)</td>
<td>32.6 (5.5)</td>
<td>33.7 (6.1)</td>
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<tr>
<td>Mean age (SD), y</td>
<td>54.6 (7.1)</td>
<td>54.0 (6.6)</td>
<td>54.8 (7.6)</td>
<td>53.6 (7.2)</td>
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<tr>
<td>Mean duration of diabetes, y</td>
<td>5.2 (4.9)</td>
<td>5.3 (3.5)</td>
<td>5.1 (4.6)</td>
<td>6.1 (4.7)</td>
</tr>
<tr>
<td>Mean hemoglobin A1c value (SD), %</td>
<td>7.66 (0.88)</td>
<td>7.69 (0.86)</td>
<td>7.72 (0.86)</td>
<td>7.66 (0.92)</td>
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<td>Medication use</td>
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<td></td>
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<tr>
<td>Patients on oral hypoglycemic agents, n</td>
<td>49</td>
<td>49</td>
<td>47</td>
<td>40</td>
</tr>
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<td>34</td>
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<td>lipid-lowering agents, n</td>
<td>27</td>
<td>24</td>
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</table>

Abbreviations: BMI: body mass index, SD: standard deviation.
Table 2 provides the changes in plasma carnosinase protein content and activity over a 6-month period for the four different interventions (aerobic training, resistance training, combined aerobic and resistance training and a control group). Results are estimated means from linear mixed-effects models, adjusted by age, gender and BMI. Neither carnosinase protein content, nor its activity was influenced by the 6-month exercise program, irrespective of the exercise training modality.

<table>
<thead>
<tr>
<th>Variable by Group</th>
<th>No.</th>
<th>Baseline</th>
<th>6 month</th>
<th>Within-Group Change</th>
<th>Between-Group Difference in Change</th>
<th>Mean (SE)</th>
<th>From baseline to 6 months, mean (95% CI)</th>
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<tbody>
<tr>
<td>Plasma carnosinase content, ug/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td>61</td>
<td>69.07 (3.80)</td>
<td>71.45 (4.02)</td>
<td>2.32 (-1.15 to 5.79)</td>
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<tr>
<td>Aerobic</td>
<td>59</td>
<td>67.74 (3.83)</td>
<td>69.14 (4.04)</td>
<td>1.41 (-2.44 to 5.25)</td>
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<tr>
<td>Resistance</td>
<td>62</td>
<td>70.31 (3.79)</td>
<td>72.73 (4.05)</td>
<td>2.32 (-1.33 to 5.97)</td>
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<td>Combined</td>
<td>59</td>
<td>66.95 (3.65)</td>
<td>66.78 (3.72)</td>
<td>-0.17 (-3.88 to 3.53)</td>
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<td>Aerobic vs Control</td>
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<td>NA</td>
<td>NA</td>
<td>-0.91 (-6.08 to 4.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistance vs Control</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.00 (-5.04 to 5.03)</td>
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<tr>
<td>Combined vs Control</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>-2.50 (-7.57 to 2.57)</td>
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<tr>
<td>Plasma carnosinase activity, µmol/ml/h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>61</td>
<td>4.25 (0.15)</td>
<td>4.40 (0.17)</td>
<td>0.15 (-0.15 to 0.46)</td>
<td>NA</td>
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<tr>
<td>Aerobic</td>
<td>57</td>
<td>4.26 (0.15)</td>
<td>4.44 (0.19)</td>
<td>0.19 (-0.15 to 0.52)</td>
<td>NA</td>
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<tr>
<td>Resistance</td>
<td>62</td>
<td>4.54 (0.15)</td>
<td>4.62 (0.18)</td>
<td>0.07 (-0.24 to 0.39)</td>
<td>NA</td>
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<tr>
<td>Combined</td>
<td>59</td>
<td>4.38 (0.15)</td>
<td>4.27 (0.17)</td>
<td>-0.11 (-0.42 to 0.21)</td>
<td>NA</td>
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<td>6 Months - Baseline:</td>
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<tr>
<td>Aerobic vs Control</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.03 (-0.42 to 0.48)</td>
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<tr>
<td>Resistance vs Control</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>-0.08 (-0.51 to 0.36)</td>
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</tr>
<tr>
<td>Combined vs Control</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>-0.26 (-0.70 to 0.18)</td>
<td></td>
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</tbody>
</table>

Abbreviations: SE: standard error, CI: confidence interval, NA: not applicable. Concerning plasma carnosinase content, the numbers of participants at 6 months were 59, 49, 55, 57 in the control group, the aerobic group, the resistance group and the combined exercise training group respectively. Concerning plasma carnosinase activity, the numbers of participants at 6 months were 61, 57, 62 and 59 in the control group, the aerobic group, the resistance group and the combined exercise training group respectively.
Table 3. Results of the linear regression analysis between plasma carnosinase content and activity (taken as natural logarithm) and glycemic control, lipid profile and blood pressure, adjusted by age and gender (baseline measurements for the 243 participants with T2D). Beta-coefficients and p-values are given.

<table>
<thead>
<tr>
<th></th>
<th>Ln plasma carnosinase content</th>
<th>Ln plasma carnosinase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta-coefficient</td>
<td>p-value</td>
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<tr>
<td>Glycemic control</td>
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<tr>
<td>HbA1c</td>
<td>0.036</td>
<td>0.596</td>
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<td>Lipid profile</td>
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<tr>
<td>Triglycerides</td>
<td>0.131</td>
<td>0.055</td>
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<tr>
<td>Total cholesterol</td>
<td>0.056</td>
<td>0.412</td>
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<tr>
<td>HDL-C</td>
<td>-0.153</td>
<td>0.020</td>
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<tr>
<td>LDL-C</td>
<td>0.027</td>
<td>0.706</td>
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<tr>
<td>Total cholesterol/HDL-C</td>
<td>0.182</td>
<td>0.007</td>
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<td>Blood pressure</td>
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<tr>
<td>Systolic</td>
<td>-0.039</td>
<td>0.563</td>
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<tr>
<td>Diastolic</td>
<td>-0.097</td>
<td>0.146</td>
</tr>
</tbody>
</table>

Abbreviations: HbA1c: Hemoglobin A1c, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, Ln: natural logarithm.
**Figure 1.** The ICC for plasma carnosinase content (1A) and activity (1B) over a 6-month period in the sedentary control group is 0.91 (p<0.001) and 0.63 (p<0.001) respectively (line of identity is given). Figure 1C represents a low, but significant, positive correlation between plasma carnosinase content and activity at baseline for all participants (regression line is given).
**Figure 2.** At baseline, plasma carnosinase protein content is higher in females compared to males, but there is no sex difference in carnosinase activity (figure 2A). As consequence, the specific activity of the enzyme is higher in the males compared to the females (figure 2B).* p<0.001.