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Effects of Acute, Heavy-Resistance Exercise on Urinary Peptide Hormone Excretion in Humans

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To examine physical exercise-related changes in urinary excretion of protein/peptide hormones and to correlate modifications with the general increase in post-exercise proteinuria, urine C-peptide, insulin and insulin-like growth factor-I (IGF-I) and their plasma concentrations were measured. Plasma and urinary C-peptide, insulin and IGF-I before (B_{ex}) and at the end (E_{ex}) of physical exercise (a 2.5-hour competition, 102 km) were analysed in 20 young cyclists. At E_{ex} compared with B_{ex}, concentration of urinary C-peptide decreased slightly but significantly (21.3±2.7 *vs.* 13.5±1.7 nmol/l), but urinary insulin and urinary IGF-I concentrations significantly increased at E_{ex} (92.5±4.2 *vs.* 131.4±15.7 pmol/l and 10.0±2.1 *vs.* 33.6±3.8 pmol/l, respectively). Plasma insulin and plasma C-peptide significantly decreased, whereas plasma IGF-I was unchanged. Urinary concentrations of total proteins and creatinine significantly increased. Both E_{ex} urinary C-peptide/urinary protein and urinary C-peptide/urinary creatinine ratios were significantly reduced. The correlation between C-peptide and insulin in plasma was confirmed at B_{ex} as well as E_{ex}, but in urine only at B_{ex}. An increased renal tubular reabsorption of C-peptide at the end of exercise might be suggested, but the expected values considering creatinine excretion were almost three times less. The E_{ex} urinary insulin concentration was higher than expected, considering the circulation levels, but lower when compared with the expected concentration considering creatinine excretion. Physical exercise proteinuria, related to an increased protein filtration and a saturation of the mechanisms responsible for the reabsorption, does not appear similar for all peptide hormones. Clin Chem Lab Med 2003; 41(10):1308–1313

Key words: Physical exercise; Peptide hormones; Proteinuria; Urine.

Abbreviations: B_{ex}, before exercise; CE, capillary electrophoresis; CFRD, circulation/filtration/reabsorption/degradation; E_{ex}, end of exercise; GH, growth hormone; IGF-I, insulin-like growth factor I; pIGF-I, plasma IGF-I; uIGF-I, urinary IGF-I.

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Introduction

The increase of the rate of urinary protein/peptide excretion after strenuous physical exercise is a studied phenomenon in healthy individuals (1–3). Urinary albumin analysis aims to distinguish globular filtration impairment (4, 5), and the measurements in urine of low molecular weight proteins aid the tubular reabsorption failure investigations (6–9). A special role has been hypothesised for the protein/peptide hormones in studying the behaviour of renal handling of peptide/protein urinary excretion with physical exercise. A correlation between serum and urine was reported for protein/peptide hormones, but these findings were mainly related to steady-state conditions (10, 11). It has been proposed that protein/peptide hormones, like other protein/polypeptide compounds, are cleared from the circulation by glomerular filtration with subsequent tubular reabsorption and degradation.

Recently an increased urinary excretion of growth hormone (GH) and insulin-like growth factor (IGF-I), well known protein/peptide hormones, has been demonstrated after strenuous physical exercise (12). Furthermore, IGF-I influences renal functions (13). These findings require further studies, in particular to clarify whether the increased urinary excretion of GH and IGF-I is only related to the general increase in post-exercise proteinuria, or whether it has a hormonal role.

C-peptide measurement has been considered as a marker of daily insulin synthesis/secretion and of hepatic insulin extraction. Furthermore, urinary C-peptide was mainly measured in 24-hour urine (14). This measurement was also suggested by the fact that C-peptide was generally accepted as possessing little or no biological activity, but new investigations have provided interesting results and demonstrate that this peptide, namely a hormone fragment, called "connecting peptide", could have a function (15). Therefore, contrary to early beliefs, C-peptide possesses a favourable biological effect (16), and this idea is still reinforced by the demonstration of a specific binding evidenced in several cultured human cell types (17, 18). Moreover, the kidney is the major site for extraction of C-peptide and insulin (15), and insulin effects in the rat collecting duct have also been demonstrated (19).

In order to investigate the changes in urinary excretion of peptide/protein hormones, C-peptide, insulin and IGF-I have been explored in the present work. The

urine and plasma levels of these peptide hormones were measured before and at the end of physical exercise in well-trained individuals.

Materials and Methods

Subjects and physical exercise

The Ethics Committee of the Medical School of the University of Padova approved this research protocol. Subjects were informed of the nature of the investigation before their consent to participate was obtained.

The athlete group was recruited from well-trained cyclists (20 males), aged 17–18 years, weight 66.3 ± 2.7 kg and height 168.2 ± 7.8 cm. The urine and blood samples were collected from each cyclist at rest (B_{ex}; 10–15 min before exercise) and at the end of exercise (E_{ex}; no later than 10–15 min after arrival). The 102 km race took place late in the morning and lasted about 2.5 hours. All studied cyclists had had a 1350–1500 kcal meal (65% carbohydrate, 25% lipid, 10% proteins) 120–180 minutes before the competition.

EDTA-blood specimens were centrifuged a few minutes after being drawn, and plasma samples were stored together with urine samples at –80 °C until assay.

Analyses and calculations

C-peptide assay

Plasma or centrifuged urine samples, diluted in standard 0 (C-peptide-free human serum), were used for the measurement of C-peptide in a commercial competitive immuno-radiometric kit from DiaSorin-Saluggia (Vercelli, Italy). Sample and tracer (100 µl) were allowed to compete for the monoclonal antibody coated to the tube. After an incubation of 2 hours and a washing procedure, the bound radioactivity was counted. The range of detection was 0.08–4.96 nmol/l for plasma and urine sample analyses. The used method revealed good correspondence with Nauck's tested methods and provided reliable measurements of C-peptide (20). The intra- and inter-assay imprecision data for measurements in three different (low, intermediate and high concentrations) plasma samples were < 4.5, < 3.0, < 3.8% and < 12.7, < 12.9, < 18.8%, respectively. The reference values for normal subjects were 0.12–1.25 nmol/l in plasma and 7.5–40.6 nmol/die in urine.

Insulin assay

Plasma insulin was measured with the DSL Active Insulin ELISA kit (DSL-10-1600; Webster, TX, USA) using two antibodies, one coated to a microtitre plate and the other conjugated to horseradish peroxidase (standard curve 30–698 pmol/l and reference value 14.6–214.9 pmol/l). For the analyses of the urine samples BioSource-Belgium IRMA reagents (Nivelles, Belgium) were used. In short, 50 µl of centrifuged urine (or standard solution) and monoclonal ^{125}I-labelled antibody solution were incubated at room temperature for 2 hours in vials coated with anti-insulin monoclonal antibody. The antigen-antibodies sandwich complex was measured after suitable washing and decanting. A standard curve ranging from 30 to 2128 pmol/l (cpm *vs.* concentration.; linear) was used.

Insulin-like growth factor-I assay

A competitive immuno-radiometric method for total IGF-I from Mediagnost (Reutlingen, Germany) was utilised. For the urinary IGF-I analysis a standardised method was used (21). The range of detection was 20.4–1310.0 pmol/l and 5.1–327.5 pmol/l for plasma (diluted 1:125) and for urine sample analyses, respectively. The reference values obtained from normal young male subjects were 15.0–34.9 pmol/l in plasma and 0.65–31.96 nmol/l in urine.

Urine creatinine

The assay was a capillary electrophoresis (CE) method as described by Gatti *et al.* (22). The detection range was 40–200 µmol/l and urine samples were diluted 1:80 in CE run buffer. The average of urinary creatinine of 83 healthy young male athletes was 13.0 ± 6.5 mmol/l.

Total urine proteins

The assay utilised the colorimetric Coomassie Blue micromethod from Sigma (cod. A-610; Milano, Italy). The reference value was 0.03–0.12 g/die.

Calculations

All results are expressed in molar concentration per litre, where the molecular weight is known.

The at-rest "basal" measured concentrations (*C*), both in plasma ($B_{ex}C_P$) and in urine ($B_{ex}C_U$), were defined as 100%. The measurements in plasma and urine at the end of exercise were $E_{ex}C_P$ and $E_{ex}C_U$, respectively, and the percentage values in plasma (X_P) and urine (X_U) were expressed as percent of the "basal" measurement.

Plasma percentage with respect to "basal" (line A in Table 3):

$$X_P = 100\ (E_{ex}C_P/B_{ex}C_P)$$

Urine percentage with respect to "basal" (line B in Table 3):

$$X_U = 100\ (E_{ex}C_U/B_{ex}C_U)$$

Expected concentration in urine, having the same percentage measured in plasma (line C in Table 3):

$$X_P \times B_{ex}C_U/100$$

When the values were calculated in relation to urine creatinine concentration, at rest ($B_{ex}Cr_U$) and at the end of exercise ($E_{ex}Cr_U$), the data were obtained calculating the same percentage of the "basal" urinary measurement.

Creatinine variation percentage:

$$CrX_U = 100\ (E_{ex}Cr_U/B_{ex}Cr_U)$$

Expected concentration in urine, having the same variation percentage of the urinary creatinine (line E in Table 3):

$$B_{ex}C_U \times CrX_U/\ 100$$

Statistical analyses

Data are expressed as means ± SEM. Student's t test for paired data and Pearson's correlation were used for statistical analysis; $p < 0.05$ was considered statistically significant.

Results

C-peptide plasma levels in the cyclists at rest, before the physical exercise, were significantly higher than at

Table 1 Plasma biochemical markers measured in the cyclists before (B_{ex}) and at the end of exercise (E_{ex}).

Plasma biochemical marker	B_{ex}	E_{ex}
C-peptide [nmol/l]	1.05 ± 0.06	0.63 ± 0.04*
Insulin [pmol/l]	105.30 ± 17.60	57.20 ± 10.60*
Insulin-like growth factor-I [nmol/l]	34.30 ± 1.90	32.30 ± 1.50

B_{ex} *vs.* E_{ex}: *$p < 0.01$.

Table 2 Urinary biochemical markers measured in the cyclists before (B_{ex}) and at the end of exercise (E_{ex}).

Urine biochemical marker and ratios	B_{ex}	E_{ex}
uC-Peptide [nmol/l]	21.3 ± 2.7	13.5 ± 1.7*
uC-Peptide/uProtein [nmol/mg]	2.03 ± 0.62	0.11 ± 0.02*
uC-Peptide/uCreatinine 10^{-6}	4.26 ± 0.70	1.51 ± 0.21*
uInsulin [pmol/l]	92.5 ± 4.2	131.4 ± 15.7**
uInsulin/uProtein [pmol/mg]	13.53 ± 5.66	0.89 ± 0.12**
uInsulin/uCreatinine 10^{-9}	26.3 ± 6.8	15.5 ± 2.6
uIGF-I [pmol/l]	10.0 ± 2.1	33.6 ± 3.8*
uIGF-I/uProtein [pmol/mg]	0.71 ± 0.25	0.20 ± 0.03
uIGF-I/uCreatinine 10^{-9}	2.02 ± 0.55	3.74 ± 0.50
Creatinine (uCr) [mmol/l]	6.3 ± 1.0	10.0 ± 0.8*
Total proteins (utPr) [mg/l]	29.4 ± 6.7	325.9 ± 95.1*

B_{ex} *vs.* E_{ex}: * $p < 0.01$; ** $p < 0.05$. u: urinary.

the end of exercise (Table 1), and this significant difference was also demonstrated when the urinary levels were measured (Table 2).

As expected, the urinary total proteins and creatinine were significantly increased at the end of exercise ($p < 0.005$). When the ratio between urinary C-peptide and urinary protein and urinary creatinine were measured, they both demonstrated significantly decreased ratios ($p < 0.005$). Insulin plasma levels, as expected, were significantly decreased after exercise, but different behaviour was demonstrated in the urinary concentrations. In fact, urinary insulin concentrations were significantly increased after exercise, and when their ratios with urinary protein and with urinary creatinine were measured, a significant decrease was demonstrated for the urinary protein ratio. When the plasma urine molar ratio was calculated, a significant decrease was measured only for insulin ($p < 0.001$), while C-peptide did not demonstrate this significant difference comparing B_{ex} and E_{ex} (1.2 ± 0.2 *vs.* 0.5 ± 0.1 nmol/l; 0.08 ± 0.02 *vs.* 0.07 ± 0.02 nmol/l, respectively).

The correlation between plasma C-peptide with plasma insulin was confirmed before and after exercise (Figure 1). In the case of the urinary levels, the correlation was demonstrated only before exercise (Figure 2).

Considering the plasma and urinary concentrations of the analysed hormones, the variation percentages compared between B_{ex} (defined as 100%) and E_{ex} were calculated (Table 3). These measurements demonstrated that only plasma IGF-I before and at the end of exercise was comparable (96%). The percentage was also calculated for the urinary levels and urinary C-peptide that continued to be decreased (86%), but urinary insulin and urinary IGF-I were clearly higher than 100% (141 and 988%, respectively). Taking into account the measured percentages, the expected concentrations

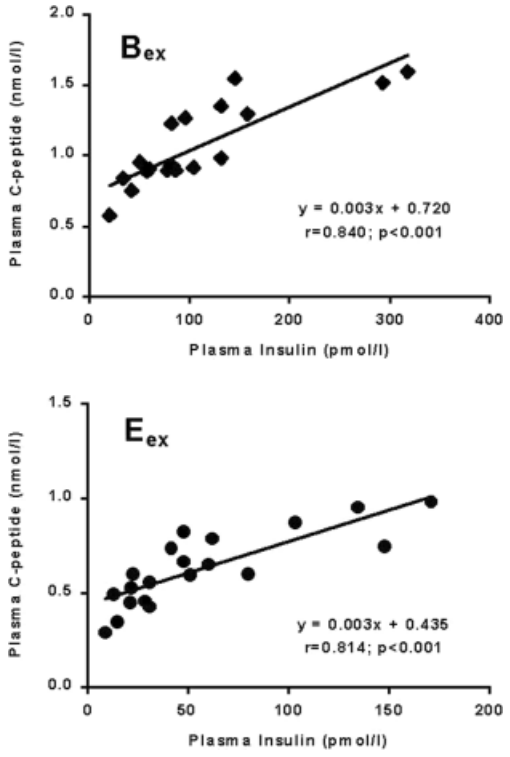

Figure 1 Correlation between plasma C-peptide and plasma insulin before (B_{ex}) and at the end of exercise (E_{ex}).

Table 3 Calculation of the expected concentrations in urine.

	C-peptide	Insulin	IGF-I
A	63.7 ± 6.0%	82.8 ± 25.6%	96.5 ± 2.1%
B	86.4 ± 13.6%	141.1 ± 13.4%**	988 ± 283%**
C	14.5 ± 2.6 (nmol/l)	76.9 ± 23.5 (pmol/l)**	10.2 ± 2.1 (pmol/l)*
D	13.5 ± 1.7 (nmol/l)	131.4 ± 15.7 (pmol/l)	33.6 ± 3.8 (pmol/l)
E	40.5 ± 7.0 (nmol/l)*	250.1 ± 67.3 (pmol/l)	24.6 ± 9.4 (pmol/l)

A. Percentage of variation of plasma C-peptide, insulin and IGF-I, considering 100% the at-rest (B_{ex}) values. B. Percentage of variation of urinary C-peptide, insulin and IGF-I, considering 100% the at-rest (B_{ex}) values. C. Concentration expected in urine if each peptide hormone followed, in the urine, the same variation measured in the circulation. D. Concentration measured in urine. E. Concentration expected in urine if each peptide hormone followed the same percent of variation measured in urinary creatinine. A *vs.* B **p < 0.05; C *vs.* D **p < 0.05; *p < 0.001; E *vs.* D *p < 0.001.

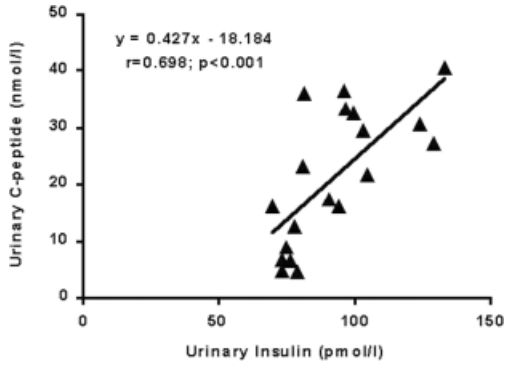

Figure 2 Correlation between urinary C-peptide and urinary insulin before exercise.

are shown in Table 3. These expected values were calculated considering the ratio measured in the circulation as unchanged in urine. Only C-peptide demonstrated no significant variation of this ratio (64% and 86% in plasma and urine, respectively), but the measured values of urinary insulin and urinary IGF-I were higher than expected even when considering the plasma variations. The expected values, considering that the hormones follow the same filtration of creatinine, were also calculated, and only urinary C-peptide concentration was lower than expected (13.5 *vs.* 40.5 nmol/l, respectively).

Discussion

Data reported in this study demonstrated that, concurrently with post-exercise proteinuria, urinary excretion of protein/peptide hormones also changed, and in particular the concentrations of urinary insulin, urinary IGF-I and urinary C-peptide also varied significantly (Table 2) considering their percentages (Table 3). The end-of-exercise plasma levels of C-peptide and insulin, as expected, were decreased, and plasma IGF-I did not demonstrate any variation. The correlation between plasma levels of C-peptide and insulin were confirmed both before and after exercise, but when the analyses were carried out in urine specimens, the correlation was demonstrated only at rest (Figure 1). This result could be related to the fact that at kidney level, at rest, the protein/peptide hormone circulation/filtration/reabsorption/degradation (CFRD) was in a dynamic equilibrium condition. But when exercise was carried out, this CFRD balanced status varied and the renal turnover of these peptide hormones was re-distributed. This suggestion could be confirmed by the fact that at rest, correlation was measured between urinary IGF-I and urinary C-peptide and urinary insulin ($r = 0.7498$, $p < 0.001$; $r = 0.7784$, $p < 0.001$, respectively), but at the end of exercise, there was no correlation. The calculations, reported in Table 3, include the expected values considering the plasma concentrations. The creatinine measurements and the urinary levels of the analysed hormones seem to suggest that CFRD varied with exercise; only "selected" hormones might occupy a role in the kidney, other hormones might have the same behaviour as other non-hormonal peptide compounds.

A different proportion in the plasma-urine concentrations of C-peptide, insulin and IGF-1, following strenuous exercise, was measured. For insulin, this ratio changed from (1:0.8) to (1:2.3), for IGF-I from ($1:29\times10^{-3}$) to ($1:96\times10^{-3}$) comparing B_{ex} with E_{ex}, respectively, while the ratio remained fairly constant for C-peptide (about 1:20). This might suggest a disparity in the functional, regulatory, or hormonal roles. This disparity could also be suggested if a comparison between the changes in the urinary concentrations, with strenuous exercise, is considered.

With regard to the insulin/C-peptide pattern, the idea of a CFRD equilibrium is further reinforced if a comparison by Brundin's ratio is carried out (23). At rest, the molar plasma C-peptide/plasma insulin ratio of the present investigation (9.97) is comparable with Brundin's (10.09) value. Moreover, the present results seem to confirm the C-peptide removal from the circulation by the kidney; in fact the urinary C-peptide/urinary insulin molar ratio was about 230 at rest and decreased to 99 at the end of exercise. The filtered C-peptide could be in part reabsorbed, as its level in urine tended to decrease at end of exercise. In agreement with literature data, which demonstrated that the filtered insulin at rest is more reabsorbed (24, 25), our measurements of insulin concentration in urine were lower than C-peptide, and this result was still present at the end of exercise. In

normal subjects the renal metabolism of C-peptide is characterised by high fractional extraction and low urinary clearance. Also, present data confirmed that the kidney sustained an important function in the homeostasis of circulating C-peptide. The renal tissue processes most of the C-peptide (26), and the present results demonstrated that the plasma:urine ratio was < 1 at rest, as well as at the end of exercise.

Recently C-peptide has been recognised as possessing a function, and urinary C-peptide level might be related to its turnover in the circulation, but a specific role might also be suggested at the renal level. This role also seems confirmed by the fact that the renal fractional extraction of C-peptide and insulin is influenced by a glucose meal, demonstrating that the kidney accounts for their extraction, playing a role in the C-peptide balance between production and extraction (25, 27). Normal human kidney function plays a quantitatively important role for insulin economy, for example by attenuating the post-prandial accumulation of insulin in the blood. In the literature, however, the data related to this urinary fraction is conflicting. However, our athletes were all studied about 120–180 minutes after a meal, and thus our data seems to be in agreement with Samnegard's results, which demonstrated an increased insulin renal uptake after oral ingestion of glucose (25).

The origin and the role of insulin and IGF-I in urine are not completely clear, and C-peptide renal metabolism still needs further investigation. Hanabusa and coworkers (28) observed that approximately half of the secreted insulin is removed by the kidney by glomerular and peritubular processes. In the present work, however, when physical exercise was carried out, this phenomenon was less evident and insulin urinary concentration increased significantly following the phenomenon of post-exercise proteinuria, and the percentage of urine concentration with respect to plasma concentration changed from 87.8% to 229.7%. However, this did not seem to happen with C-peptide, and the percentage of urine concentration with respect to plasma concentration changed slightly from 2028% to 2142%. In this work, for the first time a clear increase in urinary concentration of IGF-I at the end of exercise was demonstrated, and the percentage of urine concentration with respect to plasma concentration changed three-fold, from 29.1 to 96.1×10^{-3}%. This result might suggest an increased glomerular filtration and a decreased reabsorption, but further investigation is needed to verify any role of the binding protein/free fraction equilibrium. In fact, IGF-1 circulates primarily in a bound form in the plasma and in this work, total IGF-I was measured in the circulation as well as in urine. Excretion may be affected greatly by changes in the small proportion of free IGF-I. It might be that an increased urinary excretion of IGF-I (3 times) could result from an increased leakage of the bound form through the glomerulus or from a rise in the free circulating fraction of IGF-I. Moreover, Wallace's study demonstrated that there was no significant effect of exercise on serum-free IGF-I concentration compared to values before exercise (29).

Finally, it is the first time that a urinary C-peptide decrease after strenuous physical exercise has been observed, in the measured concentrations ($p < 0.01$) as well as in its ratio with total proteins ($p < 0.01$) and with creatinine ($p < 0.01$). This phenomenon seems to be different from insulin, and urinary insulin seems to behave quite differently from urinary IGF-I. An increased renal tubular reabsorption of C-peptide at the end of exercise might be suggested; the excretion followed the plasma changes, but the expected values considering creatinine excretion were almost three times less. The urinary concentration of insulin at the end of exercise was higher than expected considering the circulation levels, but it was lower when compared with the expected concentration considering creatinine excretion. Further investigation would help to explain the significance of this urinary C-peptide result. It might be that C-peptide, more than insulin, plays a role in the reabsorption processes in the kidney and when renal function is changed by physical exercise. With regard to IGF-I, the measured urine concentration was almost three times higher than expected from the circulation, and also the expected concentration considering urinary creatinine was slightly, and not significantly, increased. In conclusion, physical exercise proteinuria, related to an increased protein filtration and a saturation of the mechanisms responsible for the reabsorption, does not appear similar for all peptide hormones. Furthermore, there is a possibility that the tubular reabsorption is not the only mechanism responsible for the urinary concentration-lowering process, but also degradation might be one of the causes.

In any case, urinary protein/peptide hormone measurements might even be used as a non-invasive surrogate of plasma concentration measurements, particularly when a monitoring study requests daily analyses, but this is only possible when a basal condition is monitored, in fact, physical exercise changes fundamentally the urinary concentrations in relation to a CFRD redistribution.

In summary, this work demonstrated that strenuous exercise changed the protein/peptide hormone concentrations in urine: 1) urinary C-peptide decreased and was 86.4% of the basal level, 2) urinary insulin increased and was 141.1% of the basal level, 3) urinary IGF-I increased and was 988% of the basal level. It was also demonstrated that the molar ratio (protein/peptide hormone:creatinine) in urine was: 1) decreased for urinary C-peptide, 2) slightly but not significantly decreased for urinary insulin, 3) slightly but not significantly increased for urinary IGF-I. In conclusion, physical exercise proteinuria, related to an increased protein filtration and a saturation of the mechanisms responsible for the reabsorption, does not appear similar for all peptide hormones.

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