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Analytical Methods

Contribution to explanation of the effect of supplemented creatine in human metabolism

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ABSTRACT

Simple voltammetric determination of thiodiglycolic acid (TDGA) offers the possibility to follow individual deviations in metabolism of thiocompounds and one-carbon (1c) and two-carbon (2c) units, which take part in endogenous synthesis of creatine (CR). In three groups of young men the levels of TDGA in urine were followed after application of CR given as food supplement in 5 g daily doses. In the first group (7 men) it was found that the level of TDGA increased independently of the day time of application of CR. In the second group (9 men) the level of TDGA increased within an interval of 3–8.5 h after CR application and then dropped during 2 h to the normal level (20 mg L^{-1}). In the third group (11 men), in 4 days' study the effects of CR were compared in alternation to vitamin B_{12}. Vitamin B_{12} was given in the evening of the 1st and 3rd day and CR in the morning of the 3rd and 4th day. CR increased the excretion of TDGA in all men, while B_{12} only in four men independently of CR application.

1. Introduction

At present time the voltammetric method (among other methods, e.g., Chylkova & Fadrna, 2004; Samcova, Kvasnicova, Urban, Jelinek, & Coufal, 1999) is being used in toxicology (Dlaskova, Navratil, Heyrovsky, Pelclova, & Novotny, 2003) to measure levels of thiodiglycolic acid (TDGA) in urine. It helps to monitor the exposure of workers in chemical plants to some carcinogenic compounds, e.g., to vinylchloride monomer (VCM) or to ethylene dichloride (EDC) in factories producing polyvinyl chloride (Dlaskova et al., 2003; Senholdova-Dlaskova, 2002). Similarly as in the case of VCM exposure, the TDGA level in urine increases after intake of some remedies (Navratil et al., 2004), victuals (Navratil et al., 2004), thiolic compounds (Steventon, 1999) or of compounds, which affect oxidative metabolic pathways accompanied by release of two-carbon (2C) units (e.g., ethanol, VCM) (Navratil et al., 2004). In these oxidative pathways coenzyme P-450 may participate (Ambrosi, Soleo, Elia, & Attimotelli, 1989) as well.

The oxidative degradation of xenobiotics via TDGA decreases the cell pool of glutathione (GSH). In some critical cases all disposable GSH can get exhausted (Murray, Granner, Mayes, & Rodwell, 2003). This endangers all metabolic pathways dependent on the presence of GSH. Increased level of TDGA in urine indicates a sign of disbalanced cooperation among thiocompounds, 2C units and supply of oxygen radicals (Ermakova et al., 2002a, 2002b). The TDGA concentrations determined in samples of urine in healthy individuals (Dlaskova et al., 2003) do not normally exceed 20 mg L^{-1}.

According to our earlier papers, the appearance of TDGA in urine resulted from the disturbance of metabolic processes coordinated by betaine and vitamins B_{12} and folic acid (Navratil et al., 2007; Pristoupilova et al., 2005). They include transformation of homocysteine – the source of cysteine, and glycine (the source of 2C units), which both take part in endogenous formation of CR.

Creatine (CR) (methyl guanidine acetic acid) represents one of the most important nitrogen containing compounds playing role in energetic metabolism (Webber, 2006). CR is not an essential component of food, because it is formed naturally in human body. CR can be supplied by meaty food or by special food supplementation too. Two thirds of CR are present in the human body as creatine phosphate (PCR), the rest as free CR (Webber, 2006). In normal, healthy man, the turnover of CR is about 1–2 g daily, which is covered by its endogenous synthesis from amino acids (arginine,

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glycine, methionine) in liver and kidneys (Murray et al., 2003), and by food (from animal sources as meat). Under identical conditions the same amount (about 2 g daily) of CR is degraded by non-enzymatic dehydration to creatinine and excreted into urine (Murray et al., 2003).

It is recommended to apply CR as food supplement in amount corresponding to its natural level in meaty food. It is supposed that the human organism uses it for formation of CR phosphate, which is necessary as energetic source for muscular work.

More than 5 million kg of CR is sold yearly (Hespel, Op't Eijnde, Derave & Richter, 2001), mostly for purposes of food supplement for sportsmen. Exogenously applied CR is also used in treatment of neurodegenerative diseases – dystrophy, myalgia, rheumatoid arthritis, etc. (Felber et al., 2000; Petr, 2007; Tarnopolsky & Martin, 1999).

TDGA molecule is built up of similar subunits as CR. The changes in TDGA levels in urine after loading tests with different substances may reveal hitherto unknown relationships among metabolic pathways.

In this study we wanted to elucidate: (1) how rapidly does the organism respond to the administered CR by the release of TDGA into urine; (2) whether there exists a difference between the release of TDGA after separate administration of either CR or of vitamin B_{12}.

2. Experimental

2.1. Analytical methods

Urine samples were analyzed for TDGA by the voltammetric technique described in our previous papers (e.g., Dlaskova et al., 2003; Senholdova-Dlaskova, 2002). The preparation of the sample was realized in a column of powdered PVC, the urine sample was transferred to the top of the column and eluted by 0.2 M perchloric acid. The resulting eluate was introduced into the electrolytic cell, deaerated by a stream of nitrogen (purity 99.999%), and then subjected to direct current (D.C.) voltammetric analysis. The measurement was started by accumulation for 10 s under stirring at initial potential of −800 mV vs. Ag/AgCl/ 1 mol L^{-1} KCl, followed by rest period of 15 s, and then by potential scan at the rate of −10 mV·s^{-1} to the final potential of −1200 mV. The values of potentials given in this paper are referred to that Ag/AgCl/1 mol L^{-1} KCl reference electrode, which is at 25 °C by 9 mV more negative than the SCE. For quantitative evaluation the method of double standard addition appeared best suited. The determination of TDGA was carried out by the computer-controlled Eco-Tribo Polarograph using the software polar 5.1 version for Windows (Polaro-Sensors, spol. s r. o., Czech Republic), on pen type hanging mercury drop electrode (HMDE) (Polaro-Sensors, spol. s r. o., Czech Republic), on mercury meniscus modified silver solid amalgam electrode (e.g., Barek et al., 2003; Barek, Fischer, Navratil, Peckova, & Yosypchuk, 2006; Yosypchuk & Novotny, 2002, 2002b), or on solid composite electrodes (e.g., Barek et al., 2007; Navratil & Kopanica, 2002a, 2002b; Navratil, Kopanica, & Krista, 2003; Sebkova, Navratil, & Kopanica, 2004; Sebkova, Navratil, & Kopanica, 2005; Yosypchuk, Navratil, Lukina, Peckova, & Barek, 2007). The results, achieved using all three above mentioned working electrodes, were equivalent. More precisely, the calculated confidence intervals of results overlapped with probability higher than 95%. Nevertheless, the hanging mercury drop electrode was most "user friendly", its surface was the easiest and the fastest renewable, the repeatability of results achieved by it was the best. Smaller amount of mercury on the electrode surface (mercury meniscus, mercury film, polished amalgam surface or amalgam composite surface) causes smaller sensitivity to TDGA concentration. Responses of such electrodes are lower and worse reproducible. Therefore in most experiments presented in this manuscript, the HMDE electrode was used.

Under identical conditions of elution as in case of TDGA model samples, cysteine and carboxymethyl of the same and of 10 times higher concentrations, glutathione (incidentally, the presence of glutathione in urine cannot be expected under normal conditions) and phytochelatins PC2 and PC3 in micromolar range concentrations were determined. The parameters of voltammetric determinations were equal as well. None of all tested substances did yield any voltammetric signal in potential range in which TDGA did. Furthermore, TDGA was determined in presence of these substances and they did not affect TDGA determination (Senholdova-Dlaskova, 2002). From the experiments with thiols it is evident that these compounds are chemisorbed at about zero potential on the mercury electrode surface and desorbed at about −500 mV (in presence of Cu^{2+} ions even at more positive potentials). Because the accumulation potential of TDGA was −800 mV, and the scan was in negative direction, the thiols cannot affect TDGA determination, as it was proven experimentally. CR and creatinine were determined using Specord 200 (Bardodej, David, Sedivec, Skramovsky, & Teisinger, 1989). Compound levels in blood were determined in a commercial laboratory by usual methods. Urine sample was alkalified by NaOH and mixed with saturated solution of picric acid. The reaction lasted 10 min. The solution was analyzed spectrophotometrically at the wave length 530 nm against blank. pH was measured by digital laboratory pH-meter Inolab (Benella CZ, Praha).

2.2. Reagents and materials

The volunteers were supplemented with (a) folic acid with vitamin B_{12} and (b) CR.

Supplementation with folic acid and vitamin B_{12} per os (p.o.) was realized using the food supplement "Kyselina listová, Forte" (in Czech) - "Folic acid, Forte", produced by Agrochemie Ltd., Zlín, containing 0.2 mg of folic acid and 1 μg of vitamin B_{12} of natural origin in 1 tablet (1 dose = 1 tablet).

"Creatine-monohydrate – a special nutritional supplement for athletes" (Plutino, CR) was administered p.o. in 5 g doses, diluted in tepid water.

Other chemicals used were of analytical reagent purity grade. Doubly distilled water was applied throughout the work (conductivity < 1 μS cm^{-1}). All experiments were carried out at room temperature (25 ± 2 °C).

2.3. Proband group characterization, sampling and CR supplementation

There were three groups of volunteers involved in our study; the men were students of Faculty of Physical Education and Sport, Charles University in Prague of the age from 20 to 37. Women were not subjected to the present study due to complicated hormonal changes during month, which affect cell water content substantially. All probands were young, healthy, physically active persons, dealing with sportive activities (ice hockey, football, horsemanship, and athletics) on professional level. Sportive activities were very carefully observed, however, they were not consistent. All probands signed the informed consent. None of volunteers in the second and in the third experimental group applied any other food supplements, stimulants, drugs, vitamin preparations, they did not consume victuals containing onion, garlic etc., alcohol, remedies containing carboxymethyl cysteine (CMC) (ACC 100 etc.), and vitamin B_{12}, folic acid, during this study and one day before. They registered all administered remedies and important victuals. They drank about 2 L of fluids per day (Petr, 2007).

CR was administered p.o. in 5 g doses, diluted in tepid water.

The first group consisted of seven young men (from 20 to 27 years). All individuals were not informed about the experiment for the case of getting prepared for it in advance. Three or four samples of urine were taken from each one. The first sample was collected in the morning (at about 8 a.m.). Later on a single dose of CR was administered (between 9 a.m. and 5 p.m.). Further urine samples were collected individually in time intervals from 3 to 5 h after CR intake.

The second group consisted of nine young men (from 22 to 37 years). The spot urine samples were collected from each individual during two days according to the following protocol. First day: The urine was collected in the morning when fasting. Second day (called "creatine day"): The urine was collected in the morning when fasting, then, after 2 h, a single CR dose (5 g) was administered. Consequently spot urine samples were collected 2, 3, 4, 5, 6, 7, 8 and 9 h after CR administration. If the sampling was not realized at given time (e.g., sufficient amount of urine), the real time of each sample taking was recorded.

The third group consisted of 11 young men (from 21 to 28).

The spot urine samples were collected during four days' period according to the following scheme:

First day: The urine was sampled in the morning between 8 and 11 a.m. when fasting, in the afternoon at 2 p.m. and in the evening at 10 p.m. Vitamin supplement, containing folic acid and vitamin B_{12}, was administered immediately after the last urine sample had been taken. During the second day no food supplement was given. Urine samples were collected at the same time as on the first day. The third day: urine was taken at 9 a.m. followed by a single dose of CR at 10 a.m. Spot urine samples were collected 3, 4, 5, 6, 7, 8 and 9 h after CR administration. Provided that the sampling was not realized at a certain time (e.g., insufficient amount of urine), the real time of each sample was recorded. On the same day, before sleeping time (about 10 p.m.), the food supplement, containing folic acid and vitamin B_{12}, was administered. Schedule of the 4th day was the same as that of the 3rd day, but the evening vitamins were not administered.

3. Results

For reasons, which are explained at the end of this chapter, all values of TDGA concentration in this paper, are recalculated to the urine specific gravity (the simple recalculation procedure is described, e.g., in Bardodej et al., 1989; Pristoupilova et al., 2005).

The corrections for specific gravity were calculated by means of Eq. (1). In calculations it is assumed that the mean reference value of the specific gravity of human urine is 1.020 g mL^{-1}.

$$X_{cor} = X\frac{0.020}{\text{sp.g.} - 1} \quad (1)$$

where X_{cor} denotes the corrected value (TDGA/sp.g. [mg L^{-1}], CR/sp.g. [g L^{-1}], etc., respectively), X denotes the value without corrections (TDGA [mg L^{-1}], CR [g L^{-1}], creatinine [g L^{-1}], respectively), sp.g. denotes the urine specific gravity, which represents density of urine [g mL^{-1}]. If not otherwise specified, the values of TDGA, creatinine and CR in the whole text are given after corrections per specific gravity.

The value "0.02" in Eq. (1) was calculated from normal urine specific gravity in g cm^{-3} (according to the above mentioned literature sources 1.02 g cm^{-3}), from which the value 1 was subtracted. Therefore, this value 0.02 has the same unit as specific gravity of analyzed urine and their quotient is dimensionless. In all experiments realized by us in this field of research till now, we evaluated specific gravity in 519 different urine samples. The average specific gravity amounted to 1.01743 ± 0.00054 g mL^{-1}; median 1.0180 ± 0.0010 g mL^{-1}; minimum 1.002 g mL^{-1} and maximum 1.035 g mL^{-1}; the results exhibited normal (i.e., Gaussian) distribution. The in literature used value of specific gravity 1.020 g mL^{-1} was not included in calculated 95% confidence interval. Nevertheless, for consistency of our results with other earlier data, published by us and by other authors, the value 1.020 g mL^{-1} in Eq. (1) was used.

Corrections realized per creatinine (e.g., TDGA/Creatinine) were realized using division of TDGA concentration by creatinine concentration.

3.1. First experimental group

TDGA was determined in the morning urine samples with the following results: Zero value of TDGA was found only in two men. The others had increased TDGA levels from 30 to 155 mg L^{-1}. During the day the level of TDGA dropped to normal before CR supplementation (20 mg L^{-1}). Approximately 4–5 h after CR supplementation, a sharp increase of TDGA levels was registered. The heights of those peaks, from 105 to 250 mg L^{-1}, were independent of the TDGA values in the morning urine and independent of the day time of CR administration. Only in one man, who had a zero value of TDGA in the morning urine, the TDGA level did not increase after CR supplementation.

The amount of excreted creatinine increased or decreased individually and was independent of the amount of excreted TDGA (e.g., Navratil et al., 2004; Pristoupilova et al., 2005).

3.2. Second experimental group

On the 1st day in the morning, five men had TDGA level within normal limit, one man had slightly increased value of it (30 mg L^{-1}) and three men had their values elevated up to 60, 60, and 90 mg L^{-1}. On the 2nd day in the morning, all men had TDGA levels below the normal limit (20 mg L^{-1}). After CR administration, the TDGA values subsequently increased and then decreased again in intervals of 3–4.5 h (similarly as in the 1st experimental group). In two men a doubled peak and a prolonged excretion time of TDGA to 8.5 h was observed. The decrease of TDGA level was the fastest in men with the fastest increase of TDGA. The prolonged excretion of TDGA into urine was registered in individuals with the doubled TDGA peak mentioned above.

Similarly as in the 1st experimental group, the administration of CR led to individual changes in creatinine excretion. In some cases the maximal excretion of TDGA corresponded to maximal excretion of creatinine, in other cases the mutual positions of their maxima were shifted in time (Fig. 1A).

The urinary pH values in the morning before CR administration differed only negligibly among the men. A rapid increase of pH (by 0.1–2.2 pH-units) was registered at the time of the highest TDGA level. This increase of pH was followed by subsequent decrease, which in some cases dropped to the values lower than that before supplementation (Fig. 1B).

3.3. Third experimental group

On the 1st day, the TDGA levels in the morning urine were elevated over normal limit in three persons (Nos. 9, 10, and 11) (up to 40, 70, and 150 mg L^{-1}, respectively), nevertheless these levels decreased to the normal values during the day in all men.

The vitamin B_{12} administered in the evening of the 1st day caused an increase of the TDGA level in the morning urine of the 2nd day up to 200, 260, 40, and 260 mg L^{-1} in men Nos. 4, 6, 9, and 10, respectively. Only man No. 9 with mild hyperhomocysteinemia had increased level of TDGA in the morning urine of each day. The others had the levels of TDGA in normal limit during the whole 2nd and 3rd days.

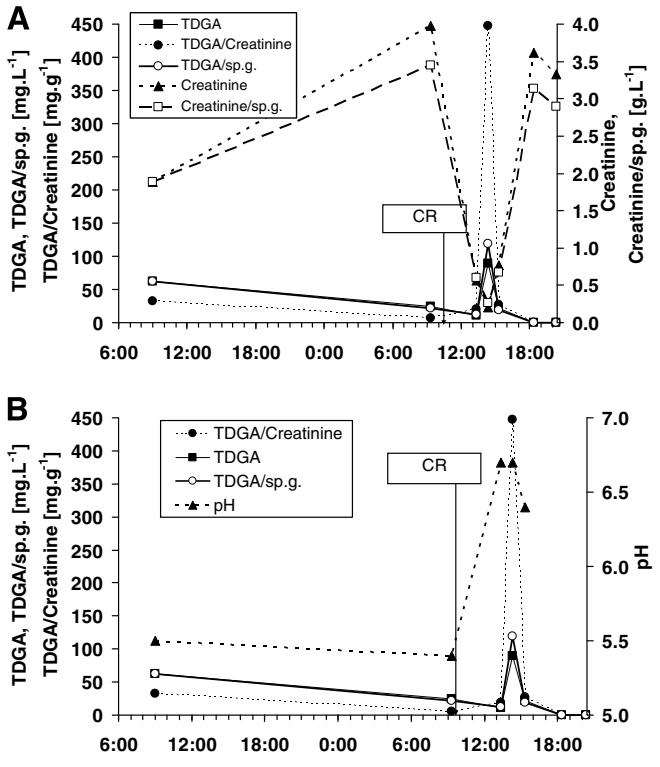

Fig. 1. Effect of 5 g CR supplementation (p.o.) (label [CR] indicates the time of its application) in two days' experiment on excretion of TDGA, on excretion of TDGA corrected per specific gravity (TDGA/sp.g.), on excretion of TDGA corrected per creatinine (TDGA/Creatinine), (A) on excretion of creatinine and of creatinine corrected per specific gravity (Creatinine/sp.g.), (B) on urine pH in a healthy man (weight 83 kg, height 178 cm, 25 years old) from the second experimental group.

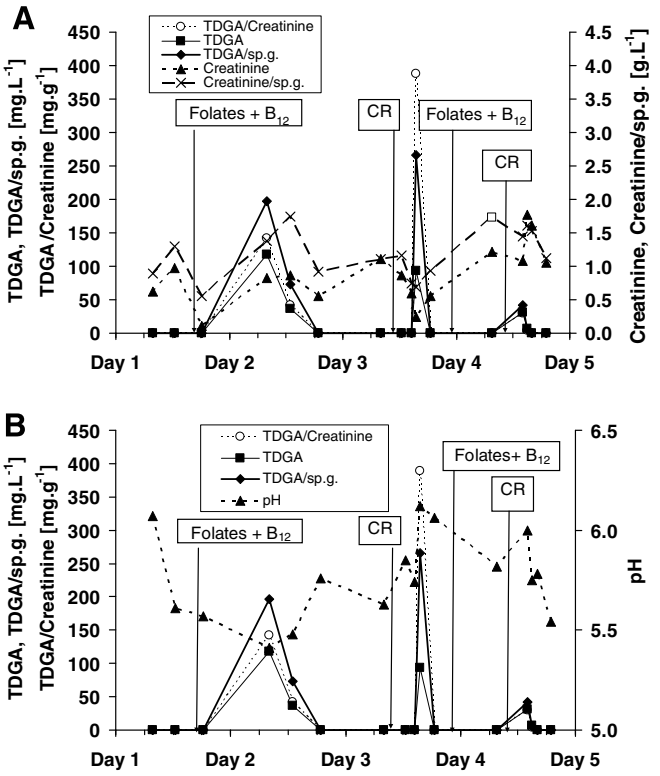

Fig. 2. Effect of 5 g CR (p.o.) and 1 μg of vitamin B_{12} (p.o.) supplementation (labels [CR] and [Folates + B_{12}] indicate the time of their applications) in five days' experiment on excretion of TDGA, on excretion of TDGA corrected per specific gravity (TDGA/sp.g.), on excretion of TDGA corrected per creatinine (TDGA/Creatinine), and (A) on excretion of creatinine and of creatinine corrected per specific gravity (Creatinine/sp.g.), (B) on urine pH in a healthy man (weight 80 kg, height 187 cm, 23 years old) from the second experimental group.

The 2nd application of vitamin food supplement in the evening of the 3rd day increased the TDGA level in the morning urine of the 4th day in men Nos. 1, 3, 6, and 9 (70, 30, 70, and 40 mg L^{-1}, respectively).

CR, administered in the morning of the 3rd and 4th day, affected the TDGA excretion, similarly as was observed in the 1st and 2nd experimental groups. This effect was represented by a sharp peak of TDGA level (in time interval from 3 to 8.5 h after CR administration, followed by a rapid decrease in the next one or 2 h) in all men but the man No. 8. His level of TDGA remained in the normal limit in course of the whole study with exception of the 2nd application of CR, when it increased to 35 mg L^{-1}. Men Nos. 4, 5, 7, 9, and 11 had more elevated levels of TDGA after the 1st application of CR (280, 60, double peak 30 and 390, 40, and 60 mg L^{-1}, respectively). Men Nos. 2, 3, 6, and 10 exhibited higher concentrations of TDGA after the 2nd application of CR (90, 150, 470, double peak 40 and 120 mg L^{-1}, respectively). In the man No. 1 both peaks of TDGA were of the same height (50 mg L^{-1}).

Similarly as in the 2nd experimental group, the maxima of TDGA concentration corresponded in some cases to minimal concentrations of creatinine excreted into urine, in other cases the mutual positions of the minima were shifted in time (Fig. 1B). .

Excretion of creatinine varied individually, and its maxima and minima did not correspond mostly to the peaks of TDGA concentrations, caused either by vitamin B_{12} or by CR administration. Therefore, for better illustration, the values of TDGA, as well as of CR or creatinine were recalculated to specific gravity (Bardodej et al., 1989; Pristoupilova et al., 2005) (Fig. 1B).

pH values in the morning urine of men of all three groups as well as those before CR and vitamins administration differed only negligibly among the individuals. Administration of vitamin B_{12} did not affect the urinary pH level substantially (the registered changes amounted to ±0.5 pH-units). The rapid increase of pH (by 0.3–2.0 pH-units) was registered after CR administration at the moment of the highest TDGA concentration. This increase was followed by a decrease, which was in some cases below a baseline level registered before the supplementation (Fig. 2B).

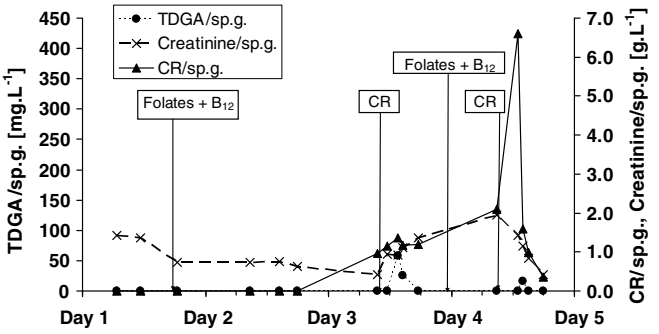

Fig. 3. Effect of 5 g CR (p.o.) and 1 μg of vitamin B_{12} (p.o.) supplementation on excretion of TDGA, of CR and of creatinine, all values corrected per specific gravity (TDGA/sp.g., CR/sp.g., and Creatinine/sp.g., respectively) in a healthy man (weight 87 kg, height 183 cm, 25 years old) from the third experimental group.

CR was determined together with creatinine in urine of men Nos. 5 and 8 only. They had no CR in their urine samples till the morning of the 3rd day. Then the level of CR began to increase and reached its maximum (7 g L^{-1}) (Fig. 3) after the second application of CR, after which it dropped down nearly to 0 mg L^{-1}. The man No. 8 exhibited decreased and increased level of CR according

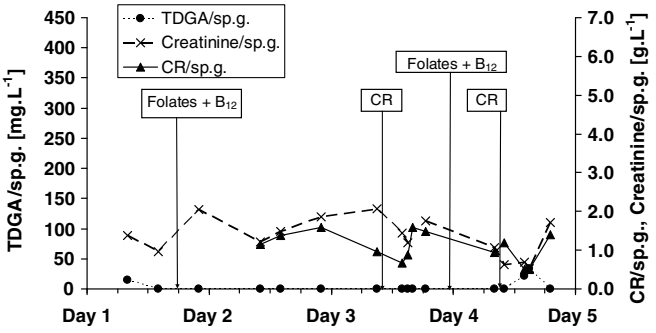

Fig. 4. Effect of 5 g CR (p.o.) and 1 μg of vitamin B_{12} (p.o.) supplementation (labels [CR] and [Folates + B_{12}] indicate the time of their applications) in five days' experiment on excretion of TDGA, of CR and of creatinine, all values corrected per specific gravity (TDGA/sp.g., CR/sp.g., and Creatinine/sp.g., respectively) in a healthy man (weight 72 kg, height 174 cm, 28 years old) from the third experimental group.

to the daily rhythm, similarly as the creatinine level. The maximal concentrations were recorded before midnight and the minimal at noon (Fig. 4).

4. Discussion

Supplementation of humans with CR at any time of the day increased the excretion of TDGA into urine. Only people with well balanced metabolic pathways as men Nos. 1, 3, 5, 8 (as can be judged according to their better physiological parameters in comparison with other men under study (Petr, 2007)) exhibited increased TDGA levels only after the second CR supplementation. This indicates that excessively ingested CR affects metabolic pathways and equilibria, concerning thiolic substances and also other pathways, e.g., those that release or use 1C or 2C units, which all take part in CR and TDGA synthesis (Navratil et al., 2007, 2008). The man No. 6 had high increase of TDGA levels after each supplementation of B_{12} as well as of CR (Fig. 5). In this man the administration of vitamin B_{12} affects probably more intensive pathways, which coordinate the synthesis and utilization of S-adenosylmethionine (S-AM).

Distribution of methionine, the precursor of S-AM and cysteine, into metabolic pathways is dependent more on the vitamin B_{12}-redox changes of the Co atom in its molecule. The usage of 1C and 2C units, the rests of the S-AM molecules, is more dependent on the folate cycle affected by NADPH – H^+ vs. $NADP^+$ equilibrium. That

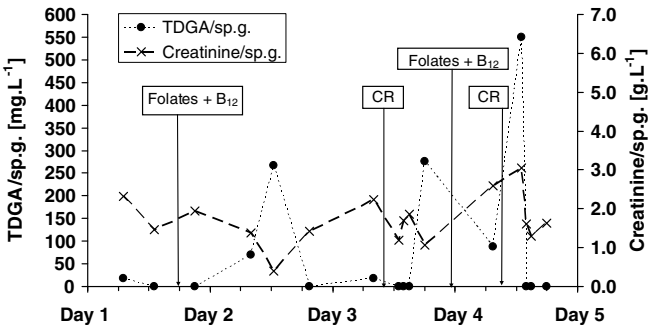

Fig. 5. Effect of 5 g CR (p.o.) and vitamin B_{12} (p.o.) supplementation (labels [CR] and [Folates + B12] indicate the time of their applications) in 5 days' experiment on excretion of TDGA corrected per specific gravity (TDGA/sp.g.) and on excretion of creatinine corrected per specific gravity (Creatinine/sp.g.) in a healthy man (weight 85 kg, height 183 cm, 28 years old) from the third experimental group.

is the reason, why the effect of vitamin B_{12} addition differs from that of CR on TDGA excretion into urine. However, both B_{12} and CR introduce imbalance in utilization of cysteine and glycine, or serine respectively, in different points of metabolic pathways connected with release of TDGA. Leucovorin and betaine suppress the effect of vitamin B_{12} on TDGA excretion (Navratil et al., 2007). In our previous papers (Navratil et al., 2007; Pristoupilova et al., 2005) it was proved that the food supplement labeled by its producer as "Folic acid" (containing vitamin B_{12}) affected the release of TDGA similarly as vitamin B_{12} given in injections in 1000 fold higher doses. The dose applied intramuscularly (i.m.) (is stored in livers immediately and only small part of the dose (comparable with the dose applied p.o.) is released into body. The individual effect of vitamin B_{12} on the TDGA excretion may be explained by individual mode of its utilization. The time of deposition and of release of this vitamin to and from the stores is individual. It seems to be dependent on the function of thyroid and related endocrine glands (Wernisova, 2006).

It can be supposed that CR affects the metabolic pathways of its precursors, glycine and arginine, the substrates of urea cycle (UC) and that of S-AM. The main goal of UC is to remove from human body the surplus of amino groups in the form of urea, as well as of creatinine (to maintain acido–basic equilibrium). That is the reason, why administration of CR affects pH of urine.

It was proved in studies with volunteers given CMC in different day-times that the excretion of TDGA is day-time dependent. CMC is the natural precursor of TDGA, of the catabolic pathway of 2C unit bound to cysteine. TDGA is the main product of CMC degradation in the time interval between midnight and morning. Different forms of sulfoxides are excreted into urine instead of TDGA during the day (Steventon, 1999). It can be judged from the experiments with bacterial cultures used for detoxication of mustard gas that insufficient supply of oxygen supports the release of TDGA from the cells before it undergoes further oxidation (Ermakova et al., 2002a; Ermakova et al., 2002b). The possible metabolic pathway from creatine to TDGA is suggested in Fig. 6. At the beginning the depicted metabolic pathway includes formation of creatine (Murray et al., 2003) and its oxidative decomposition to glycine (via sarcosine). Under the metabolic conditions connected with TDGA formation, glycine is further transaminated to glyoxylic acid and then reduced to glycolic acid, which is further attached to cysteine part of GSH. The fate of this 2C unit, derived from creatine, follows the metabolic pathway of 2C units derived from xenobiotics (Ambrosi et al., 1989). Our results confirm that supplemented CR is not excreted from the body only in the form of creatinine (Fig. 6). This supports the earlier published hypothesis that supplementation of CR reduces its endogenous production, however, upon termination of CR supplementation, its normal production is resumed (Poortmans et al., 2005; Walker, 1979). It was proved that supplementation of CR increases the quantity of CR in muscle cells (Walker, 1979).

Therefore, in accordance with literature findings (e.g., Burke, Smith-Palmer, Holt, Head, & Chilibeck, 2001), we can suppose that supplemented CR is partly metabolized in another way and not via CR dehydration and creatinine formation.

Nevertheless, it is not possible to increase the amount of CR in the body over a certain limit. After exceeding some biological saturation limit (probably this limit is given by the rate constants of the metabolic processes), practically all surplus of supplemented CR is excreted into urine in unchanged form (Willer, Stucki, Hoppeler, Bruhlmann, & Krahenbuhl, 2000).

The voltammetric determination of TDGA in urine might be a useful tool for control of optimal CR doses to be used as a safety means not only for sportsmen (to get better physiological properties), but also for medical cure (Ozkan et al., 2005; Willer et al., 2000).

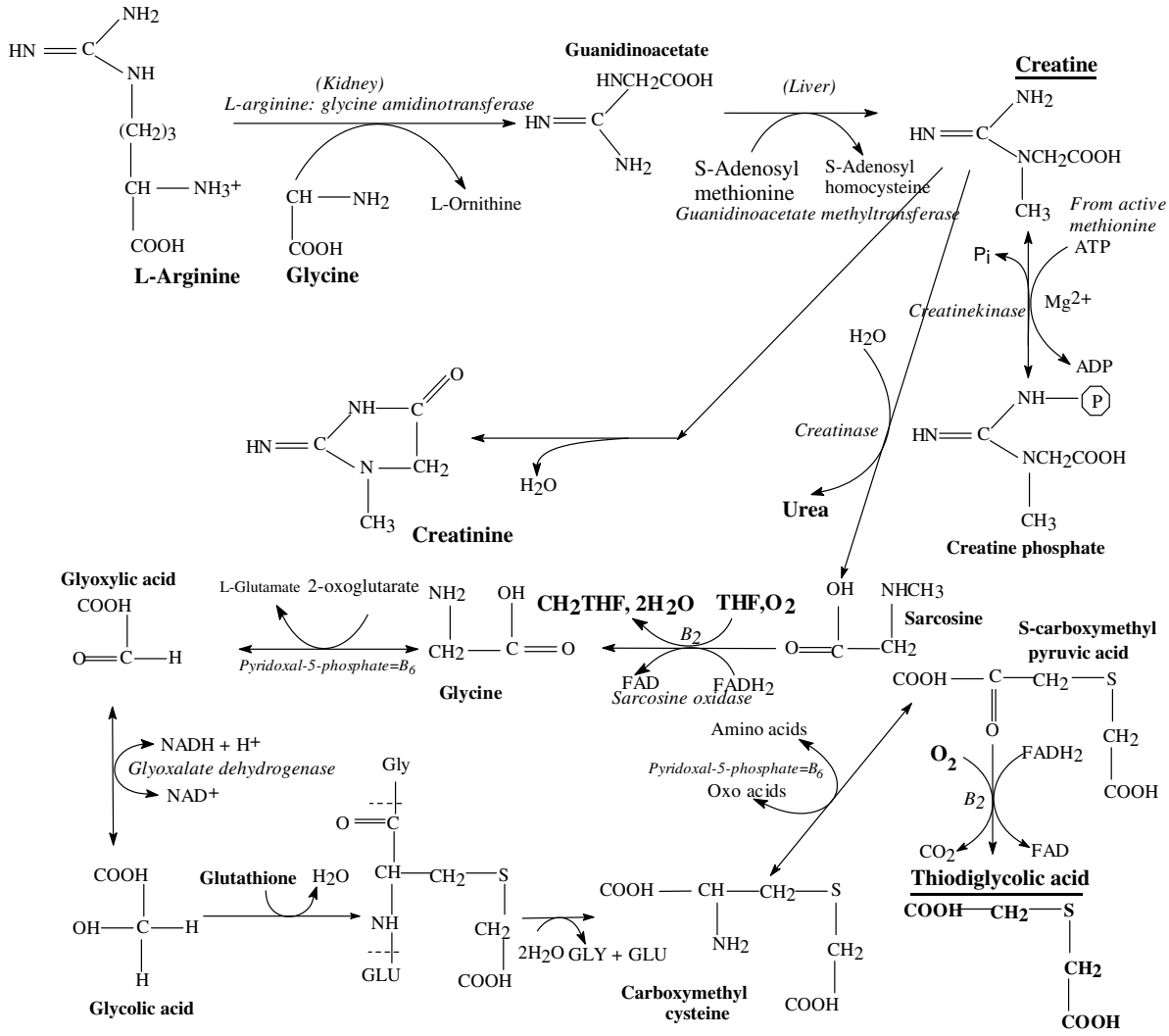

Fig. 6. Suggested metabolic pathways describing the transformation of arginine, glycine, and S-Adenosyl methionine (S-AM) to CR. Oxidative pathway of CR into urea involves vitamins B_2, B_6, tetrahydrofolate (THF) and glutathione, with urea and TDGA (besides others) as final products. The other (non oxidative) metabolic pathways aim one to the non-enzymatic creatinine formation (excreted from the human body) and the other one to creatine phosphate.

5. Conclusions

CR, consumed regularly as a food supplement, increases the TDGA level in the morning urine. This concentration normally does not exceed 20 mg L^{-1}. After single application of CR at any time of day, a sudden increase of urinary TDGA appears as a one hour lasting peak, starting individually 4–8 h after the CR administration.

The amount of excreted TDGA is specific for different individuals. It depends on the stability in metabolic equilibria concerning thiolic substances, vitamin B_{12} and folates. Added CR affects the pH-value and the excretion of creatinine: supplemented CR mostly decreased the amount of excreted creatinine and increased the pH value of urine in the time of maximal TDGA excretion. Therefore the values of TDGA (as well as that of CR and creatinine) were recalculated to specific gravity. Because metabolic pathways of their formation are not independent, the recalculations to excreted creatinine seem not to be suitable. We can suppose that supplemented CR reduces its endogenous production in humans and saves its precursors for other purposes.

In our experiments, presented in this paper in accordance with our previous findings (Navratil et al., 2004; Navratil et al., 2007; Pristoupilova et al., 2005), vitamin B_{12}, applied p.o., increased the TDGA level independently of CR supplementation, and did not affect the pH-value of urine. We can conclude that the stimulation of TDGA excretion after vitamin B_{12} application is caused by disturbance of redox equilibria induced by changes in supply of thiolic substances. Otherwise they are used in the molecule of S-AM for CR synthesis. On the other hand, supplementation of CR can increase the input of 1C and 2C units into the system of metabolic pathways, connected with TDGA synthesis.

It is evident that there are more ways of 2C units and thiolic group metabolism leading to formation of TDGA and to its excretion into urine. We can only state that an increased level of TDGA in urine indicates a disturbance of redox equilibria in human body. The presented results should be further studied and considered in more detail.

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