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ORIGINAL CONTRIBUTION

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Diurnal pattern of choline concentrations in serum of pigs as influenced by dietary choline or lecithin intake

Diurnaler Verlauf des Serumcholinspiegels beim Schwein nach oraler Applikation von Cholinchlorid oder Lecithin

Summary Athletes especially experience a significant decrease in plasma choline concentrations during exercise which can be compensated in part by consumption of lecithin, a natural source of choline. In addition, the effect of lecithin on plasma choline concentrations in humans is obviously considerably greater and more prolonged than that of an equivalent amount of choline salts. Serum choline acts as a precursor for the synthesis of acetylcholine, which, in turn, acts as a neurotransmitter. The effect of dietary choline derived from either choline chloride or lecithin on the diurnal pattern of free choline concentrations in serum was studied using the pig as a potential model for humans.

Six barrows, average initial body weight 120 kg, were fitted with permanent catheters in the jugular vein to determine the diurnal pattern of serum choline concentrations as affected by dietary choline or lecithin intake. The pigs were fed two semi-purified diets twice daily (1,500 g each meal) that contained corn, casein and a mineral-vitamin premix supplemented with equal amounts of choline (480 mg/kg) from either choline chloride or lecithin (BIOFOSFATIN®). The diets supplemented with choline were fed at 08.00 h in the morning and the experiment was carried out according to a 3 x 2 cross-over design. All pigs received the basal diet that contained 450 mg/kg choline at the evening feeding (20.00 h). Following an adaptation period of 6 d, blood was collected on d 7; 0.5 h before the morning feeding and 1, 2, 4, 6, 8, 10 and 12 h postprandially. The determination of serum choline concentrations was carried out by tandem-mass spectroscopy.

There were no differences ($p > 0.05$) between the two diurnal patterns of the serum choline concentrations. Both diurnal patterns showed a postprandial peak at 0.5 h (2.71 mg/l for choline chloride and 2.35 mg/l for lecithin supplementation) and decreased after 2 h postprandially to the preprandial concentrations. In conclusion, there were no differences ($p > 0.05$) in the diurnal patterns of serum choline concentrations in pigs after consumption of dietary choline chloride or lecithin, which is in contrast to corresponding studies in humans.

Zusammenfassung Das bei Ausdauersportlern festgestellte Absinken des Cholinspiegels im Plasma kann durch Aufnahme von Cholin aus Lecithin vermindert werden. Darüber hinaus zeichnet sich ab, daß die alimentäre Lecithinversorgung eine nachhaltigere Wirkung auf den Plasmacholinspiegel ausübt als die Aufnahme von Cholinsalzen. Freies Serumcholin ist Precursor für die Synthese von Acetylcholin, das als Neurotransmitter bei der Erregungsübertragung fungiert. In der vorliegenden Untersuchung wurde die Eignung des Schweins als Modelltier für den Menschen überprüft, indem die Auswirkungen einer alimentären Cholinversorgung in Form von Cholinchlorid oder Lecithin auf Höhe und Verlauf des

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Serumcholinspiegels mit entsprechenden Literaturdaten aus Studien am Menschen verglichen wurden. Es wurden zwei halbsynthetische Versuchsrationen auf der Basis von Mais, Kasein sowie Mineralstoffen und Vitaminen konzipiert, die mit Cholinchlorid oder Lecithin (BIOFOSFATIN®) dosisäquivalent (480 mg Cholin/kg) supplementiert wurden. Diese Versuchsrationen wurden im „cross-over design" zur Morgenfütterung (08.00 h) an insgesamt 6 männliche Kastraten mit einer mittleren Lebendmasse von 120 kg verabreicht (1,5 kg/Fütterung). Zur Abendfütterung (20.00 h) erhielten alle Tiere die Basisration ohne Cholinergänzung; der Gehalt an nativem Cholin in der Basisration betrug 450 mg/kg. Über Venenverweilkatheter wurde 0,5 h vor der Morgenfütterung und anschließend 0,5; 1; 2; 4; 6; 8; 10 und 12 h postprandial Blut entnommen. Die Bestimmung von freiem Cholin erfolgte mittels Tandem-Massenspektrometrie. Die Wiederfindungsraten der angewandten Methode lagen zwischen 93-98 %. Die Intra-assay-Präzision lag bei wiederholter Bestimmung einer Probe bei 1,6 %.

Im prä- und postprandialen Verlauf des Serumcholinspiegels ergeben sich keine signifikanten ($p > 0,05$) Differenzen, die auf eine unterschiedliche Absorptionskinetik für Cholin aus Cholinchlorid oder Lecithin schließen lassen. Beide Kurven verlaufen parallel und weisen einen postprandialen Peak bei 0,5 h auf (2,71 mg/l bei Cholinchlorid- und 2,35 mg/l bei Lecithinsupplementierung), während sich 12 h postprandial die Serumcholinkonzentrationen sowohl für die Cholinchlorid- als auch für Lecithinration im Bereich von 2 mg/l bewegen. Die vorliegenden Untersuchungen lassen keine eindeutigen Präferenzen für Cholinchlorid oder Lecithin für die Bereitstellung von freiem Cholin zur Acetylcholinsynthese erkennen. Sie stehen damit im Gegensatz zu entsprechenden Untersuchungen, die bei Menschen durchgeführt wurden.

Key words Choline – lecithin – serum – pig – diurnal pattern

Schlüsselwörter Cholin – Lecithin – Serum – Schwein – diurnaler Verlauf

Introduction

Choline is an important nutrient as a source of labile methyl groups, although it is not a true vitamin in the classical sense (1). Three different important functions of choline are known in metabolism: as the acetylester, acetylcholine, it serves as a neurotransmitter; it is metabolized to phosphatidylcholine (lecithin), an important structural part of cell membranes, and it is also oxidized to betaine (2). Especially in animal nutrition the effect as a substitute for methionine should be mentioned (3), (4). Usually, pig diets are supplemented with choline in the form of choline chloride. However, lecithin is suggested as a source of choline as well. The lecithin molecule consists of hydrophilic phosphate ester groups and hydrophobic fatty acid chains (5) and plays an important role as an emulsifying agent in biological systems (6).

Differences in the kinetics of absorption of choline derived from either choline chloride or lecithin have been reported in studies with humans. Lecithin was considerably more efficient in raising choline concentrations in human serum than equivalent quantities of choline chloride. After consumption of a diet containing lecithin as a choline source the serum choline concentration rose by 33 % after 30 min and continued to rise to 265 % ($p < 0.001$) compared to the preprandial concentration 12 h postprandially. Consuming the same amount of choline from choline chloride, serum choline concentrations rose by 86 % ($p < 0.01$) 12 h postprandially, attaining peak values after 30 min. (7), (8).

No studies have been carried out yet in which the pig, as a model for human nutrition and medicine, has been used to study the effect of dietary choline derived from either choline chloride or lecithin on the diurnal pattern of free choline concentrations in serum.

Materials and methods

Experimental procedure

Studies were carried out with six barrows with an average initial body weight of 120 kg. The animals were housed individually in pens. For blood sampling, a silastic catheter was implanted in the *V. jugularis* and exteriorised in the neck (9). Between sampling, the catheters were filled with a heparin solution (1 g heparin/l saline solution) and flushed with the heparin solution daily to avoid precipitations in the catheter.

Three semi-purified diets consisting of corn and casein were supplemented with minerals and vitamins. The choline concentrations of the two experimental diets were increased by supplementing the basal diet with 480 mg/kg choline from lecithin or choline chloride resulting in dietary levels of 884 and 855 mg/kg choline, respectively (Table 1).

The pigs were fed twice daily; 1500 g each meal at 08.00 h and 20.00 h. All pigs received the basal diet (without choline supplementation) during the evening feeding whereas during the morning feeding the experimental diets supplemented with either lecithin or choline were fed. The experiment was carried out according to a 3 x 2 cross-over design. Feeding a diet relatively low in choline content during the evening feeding might provoke a more defined elevation of the serum choline concentra-

Table 1 Formulation of the experimental diets and choline content

Diets	Basal	Basal + Lecithin	Basal + Choline chloride
Ingredients, %			
Corn	87	87	87
Casein	10	10	10
Vitamin-mineral-premix	3	3	3
BIOFOSFATIN[1], %	–	3	–
Choline chloride, %	–	–	0,064[2]
Choline[3], mg/kg	456	884	855

[1] Commercial lecithin product, Biolinol GmbH, Hamburg
[2] Equivalent to 3% Biofosfatin
[3] Determined according to a microbiological assay (LUFA Kiel)

tions after the morning meals (with choline supplementation) were fed.

Each experimental period consisted of a 6 d adaptation period. Blood samples were obtained on d 7; 0.5 h preprandially and 0.5, 1, 2, 4, 6, 8, 10 and 12 h postprandially. Blood (10 ml) was centrifuged at 3,000 rpm immediately after sampling; the serum was frozen at -20 °C until analyses.

Analyses

A stock solution containing 1 mg/ml choline was prepared by dissolving 15.2 mg choline bromide (Sigma, C 1754, Deisenhofen, Germany) in 10 ml distilled water. This stock solution was used to produce standards with concentrations of 2, 5 and 10 mg/l choline, respectively. Cholinebromide-d_{13} (Cambridge-Isotopes Laboratories, Andover, MA, US) with a concentration of 100 mg/l in distilled water was used as the internal standard.

All samples were processed in 1.5 ml reaction vessels. Processing began by adding 20 µl internal standard and 200 µl acetonitrile (Acetonitril gradient grade, Merck, No. 100030, Darmstadt, Germany) to 25 µl of the samples and standards. The tubes were vortexed for 10 sec. and thereafter centrifuged for 5 min. at 10,000 rpm. Hundred µl of the supernatant was transferred into a 1.5 ml reaction vessel and 200 µl acetonitrile-water (50:50 v/v) with 0.05 % formic acid (Merck, No. 100263, Darmstadt, Germany) were added.

The samples were analyzed in positive ion mode with a Perkin Elmer Sciex Triple Quadrupol Mass Spectrometer API 300 fitted with an IonSpray ion-source. For control of the system and quantification of the data an Apple Power Mac using the PE Sciex Software SampleControl and MacQuan 1.5 was installed. A solution of acetonitrile-water (50:50 v/v) with 0.05 % formic acid was sprayed into the IonSpray ion-source with a constant flow of 20 µl/min (Shimadzu pump LC 10A). Twenty µl of the processed samples / standards were pipetted by means of an autosampler (Gilson 223 XL) into the solvent flow (flow-injection). The mass spectrometer was calibrated with polypropylenglycol and the resolution was set to 0.7 amu[1] 50% peak hight for both resolving quadrupols. The lens voltage and the collision energy were optimized with the choline standard solution to 14 eV collision energy with nitrogen 2 mtorr as a collision gas. The fragmentation was observed and recorded for choline (m/z[2] 104 to m/z 60) and the internal standard (m/z 177 to m/z 69), respectively.

The detection limit was 0.001 mg/l and the quantification limit 0.05 mg/l. Intra-assay coefficient of variation was 1.63 % and recovery in the effective range up to 10 mg/l was between 93 to 98 %.

Statistical Analyses

Data were analyzed using SAS, (Statistical Analysis System, Version 6.12, 1996, Cary, US). The data were normally distributed ($p<0.05$) and tested with repeated measures analysis of variance with treatment, time and treatment x time interaction in the model. The level of significance was set at $p < 0.05$.

Results and discussion

Compared to previous studies in which tissue samples (10) or human sera (8) were assayed for free choline by a radio-enzymatic method, measurements of free choline in sera of pigs by means of a tandem-mass spectrometer were characterized by improved repeatability, recovery and sensitivity. The radio-enzymatic method (10) showed intra-assay coefficients of variation and recoveries in the range of 3.9 to 8 % and 87 to 106 %, compared to 1.63

[1] amu = atomical mass unit
[2] m/z = mass / charge

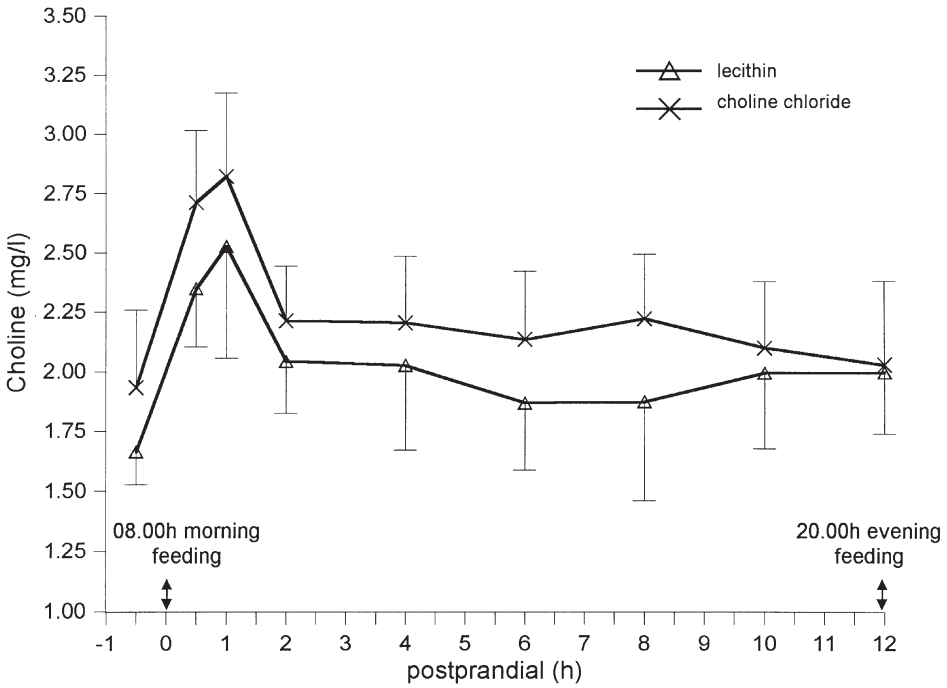

Figure 1 Diurnal patterns of choline concentrations in serum

% and 93 to 98 % in this study, respectively. The sensitivity of the method described in this study is characterized by a detection and quantification limit of 0.001 and 0.05 mg/l, respectively. The radio-enzymatic method (10) does not allow reliable measurements of free choline below concentrations of 2 mg/l.

The pigs remained healthy throughout the experiment and consumed their meal allowances within 10 min. As is shown in Figure 1, the mean fasting serum choline concentrations ranged between 1.66 and 1.93 mg/l 0.5 h preprandially ($p > 0.05$). One hour after consumption of a meal supplemented with lecithin or choline chloride, the serum choline concentrations peaked at 2.53 and 2.82 mg/l, respectively; however these differences were not significant ($p < 0.05$). Within 1 to 2 h thereafter serum choline concentrations approached preprandial concentrations. The statistical procedure with repeated measures analysis of variance with treatment and time proves that there were no differences ($p > 0.05$) between the diurnal patterns of serum choline concentrations as influenced by dietary choline or lecithin intake. The treatment x time interaction was not significant ($p > 0.05$).

In the present study the daily supply of choline was close to requirement (11). Under these conditions there was no influence ($p < 0.05$) of the dietary source of choline on the diurnal pattern of the choline concentration in serum of pigs.

These results are not in agreement with corresponding studies in humans (7), (8). These studies showed that after consumption of a meal enriched with choline chloride, serum choline concentrations rose ($p < 0.01$) by 86% compared to preprandial concentrations, attaining peak values after 0.5 h. When the same subjects consumed a meal containing an equal amount of choline in the form of lecithin, serum choline concentrations rose by 33 % after 0.5 h and continued to rise at least for 12 h, to 265 % over preprandial values ($p < 0.001$). The fact that lecithin more effectively elevates serum choline concentrations than equimolar amounts of choline salts coincides with results described earlier (12). According to these studies dietary choline is rapidly metabolized to di- and trimethylamines by intestinal bacteria. It has also been shown that urinary trimethylamine concentrations are much lower in people consuming lecithin than those consuming choline salts (13), which may suggest that lecithin may be metabolized to a lesser extent by intestinal bacteria than choline chloride.

Furthermore, there are clear indications that the diurnal patterns of choline concentrations in serum are also related to the absolute amount of choline in the diet (8). Consumption of a diet rich in choline caused prolonged elevation in serum choline concentrations while the consumption of a low choline diet resulted in relatively constant low concentrations of choline in serum postprandially. Thus, when only small amounts of dietary choline were consumed serum choline concentrations did not fluctuate diurnally. This applies also to the present study in which the daily supply with choline represents the lower range recommended for pigs. It can be assumed that at higher dietary levels of choline a response on

diurnal patterns of serum choline concentrations could be obtained similar to those in humans. This is of particular interest in the nutrition of athletes who experience a constant decrease in plasma choline concentrations during exercise (14) which can be partly compensated by the consumption of lecithin (15). However, possible mechanisms underlying these responses to the level and source of choline in the diet (choline salts vs. lecithin) have not been studied and thus warrant further investigations.

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