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Results from in-vivo and in-vitro studies using cocoa and linseeds

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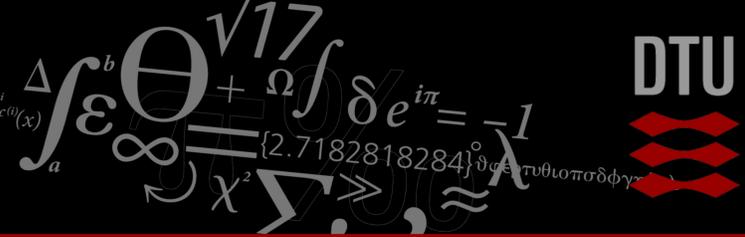
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Bioavailability of cadmium

Results from in-vivo and in-vitro studies using cocoa and linseeds



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INTRODUCTION

Cadmium (Cd) is a toxic element found as an environmental contaminant, both through natural occurrence and from industrial and agricultural sources. Food is the main source of cadmium exposure for the non-smoking general population. Besides foods, tobacco smoking and work place air have been identified as potential significant contributors to cadmium exposure. Upon exposure cadmium is efficiently retained in the kidneys and liver in the human body, with a very long biological half-life ranging from 10 to 30 years. Cadmium is primarily toxic to the kidneys and may cause renal dysfunction. Cadmium can also cause bone demineralisation, either through direct bone damage or indirectly as a result of renal dysfunction. There is limited evidence for the carcinogenicity of cadmium following oral administration. In 2009 the CONTAM Panel of EFSA evaluated the dietary exposure to cadmium in the European population. Here a tolerable weekly exposure (TWI) value of 2.5 µg/kg bw/week was established, based on human studies on kidney effects (EFSA, 2009). This value was maintained in a statement from 2011 (EFSA, 2011) following a renewed evaluation due to a provisional tolerable monthly exposure (PTMI) of 25 µg/kg bw/month established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2010.

AIM

The aim of the study was to investigate the difference in absorption of cadmium from whole linseed, crushed linseed cocoa and CdCl₂ in rats and thereby enable the food authorities to refine the recommendations to the public concerning the intake of these food items.

FEED

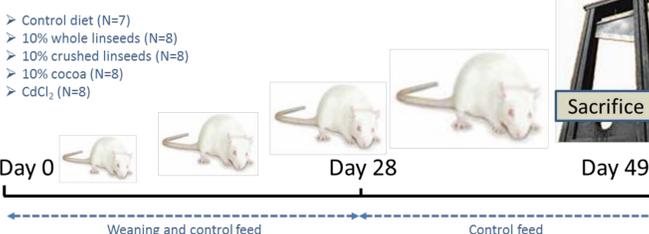
The composition of the feed can be found in table 1. The animals were given 10 % of the feed (determined by weight) as whole linseed, crushed linseed or cocoa (partly replacing corn starch). Furthermore, CdCl₂ was added to one of the feeds.

Feed composition	Mass (g)
Caseinate	180
Potato starch	220
Corn starch	460
Vitamin mix ADEK	50
Mineral mixture	28
B-vitamin	12
Cellulose	50

1 KG FEED

DESIGN ANIMAL STUDY

For the animal study four weaning Wistar rat mothers were acquired each with 10 pups. The pups went directly from breast feed to control feed during the period from day 0 to day 28. Subsequently, the rats were divided into 5 groups and given different diets for 21 days and then sacrificed. Ethical approval was given by the Danish Animal Experiments Inspectorate. The experiments were overseen by the National Food Institutes in-house Animal Welfare Committee for animal care and use.



Foodstuffs used for the production of rat feed



Whole linseed 123 µg/kg Cd
Crushed linseed 151 µg/kg Cd
Cocoa 1380 µg/kg

Cd concentrations in the feed (N=4):

Control group:	6 ± 1 µg/kg
Whole linseed group:	19 ± 1 µg/kg
Crushed linseed group:	22 ± 2 µg/kg
Cocoa group:	164 ± 3 µg/kg
CdCl ₂ group:	950 ± 724 µg/kg

ICPMS ANALYSIS

Feed and kidneys: Subsamples (homogenized feed 0.2-0.4 g and whole single kidneys 0.6-1.5 g) were digested in high-pressure quartz vessels using microwaves (Multiwave 3000, Anton Paar) with 2 ml ultrapure water and 4 ml concentrated nitric acid (PlasmaPure, SCP Science). Prior to analysis the digests were further diluted to a volume of 40 ml with ultrapure water from a Millipore Element apparatus (Millipore).

Simulated gastric juices: The simulated gastric juice suspensions were first centrifuged (4700 rpm, 10°C, 15 min) followed by filtration with single use hydrophilic syringe filters (0.45 µm, Minisart, Sartorius) and prior to analysis aliquots (0.4 ml) were further diluted to a volume of 5 ml with ultrapure water.

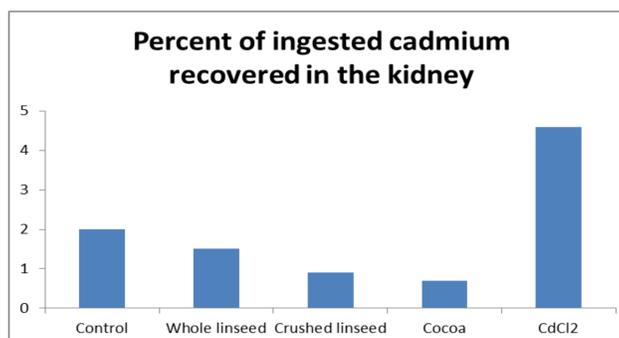
The cadmium content was subsequently determined at m/z 111 by ICP-MS using an Agilent 7500ce instrument (Agilent Technologies). Quantification was done by addition calibration with internal standardisation using ¹⁰³Rh as internal standard at 1 µg/l in all blanks, standards and samples. The trueness was verified from the analysis of the certified reference material BCR186 Pig Kidney (Institute of Reference Materials and Measurements (IRMM), Geel) 2.83±0.17 mg/kg (N=6, mean ± 2sd), which results agreed well with the certified value (2.71±0.15 mg/kg).

RESULTS

The Cd amount in (both of) the rat kidneys following the exposure experiment was calculated from Cd concentration in the kidneys and kidney weight.

	N	mean (µg)	rsd (%)	range (µg)
Control	6	24	49	13 - 46
Crushed linseed	8	36	21	25 - 49
Whole linseed	8	47	24	27 - 64
Cocoa	8	230	31	129 - 328
CdCl ₂	8	2245	20	1622 - 2932

1-2 % of the ingested Cd in the linseeds and cocoa was recovered in the kidneys.



An in-vitro experiment with simulated gastric juices for rat vs human stomach was conducted. 50 g of whole linseed, crushed linseed or cocoa were stirred slowly in 500 ml of HCl at human stomach pH (1.5) or rat stomach pH (4). Subsamples were collected after 0.5, 1 and 2 hours and analysed by ICPMS.

Food type	Simulated gastric juice	
	Human	Rat
Whole linseed	2.6	1.7
Crushed linseed	11	4.3
Cocoa	67	45

Human gastric conditions (pH 1.5) are more efficient to release cadmium from foodstuff compared to rat gastric conditions (pH 4). The mean ratio between human/rat is 1.5 for both whole linseeds and cocoa, but even higher (2.6) for crushed linseeds. This factor needs to be taken into account when transferring data from rat feeding trial to humans.

CONCLUSIONS

1. The study design allowed the measurement of differences in the absorption of cadmium from foodstuffs in rats at human exposure levels
2. Absorption of cadmium from linseed and cocoa is lower than the absorption from CdCl₂
3. The rat experiment did not indicate lower absorption from whole linseed compared to crushed linseed
4. The *in vitro* experiment indicate a much higher release of cadmium from crushed linseed compared to whole linseed at a pH similar to the pH in the human stomach

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Acknowledgements

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