#### TNO Nutrition and Food Research

Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek / Netherlands Organisation for Applied Scientific Research



Nutritional Physiology Location Zeist Utrechtseweg 48 P.O. Box 360 3700 AJ Zeist The Netherlands

www.tno.nl

P +31 30 694 41 44 F +31 30 695 72 24 infofood@voeding.tno.nl

#### **TNO Protocol Amendment 02**

P4963 | Amendment 02 / Final |

Effect of choline on post-methionine loading and fasting concentrations of plasma homocysteine in healthy volunteers

Date 8th September, 2003

At the request of Wageningen Centre for Food Sciences

Drafted by Dr ir E.J. Brink

TNO project number 010.21110 TNO study code 4963

Status Final

Previous versions ---

Number of pages 8 Number of appendices 1

#### All rights reserved.

No part of this publication may be reproduced and/or published by print, photoprint, microfilm or any other means without the previous written consent of TNO.

In case this report was drafted on instructions, the rights and obligations of contracting parties are subject to either the Standard Conditions for Research Instructions given to TNO, or the relevant agreement concluded between the contracting parties. Submitting the report for inspection to parties who have a direct interest is permitted.

© 2003TNO

# Contents

	Page		
Reason for the amendment			
Revised text	3		
2. Synopsis	4		
11. Study parameters	5		
15. Data management and statistics	6		
23. Approval of protocol amendment 02	7		
24. List of appendices	8		

## Reason for the amendment

This amendment describes a change of the revised final protocol dd 19 March 2003 with respect to the study parameters.

Fasting and post-methionine loading homocysteine concentrations in plasma are the main outcome parameters of this study. Betaine, dimethylcholine and choline concentrations in plasma were planned to be measured to elucidate a possible mechanism. At this stage it is decided by the sponsor not to measure betaine, dimethylglycine and choline concentrations in plasma, but first await and report the results of the effect of choline on homocysteine concentrations. The sponsor will decide in a later stage whether or not betaine, dimethylglycine and choline (as well as for the other additional parameters as mentioned in section 11.2.1 of the protocol) will be measured. This decision will be documented in an amendment and filed in the study dossier.

### **Revised text**

Only changed text is presented in this amendment. The presented text replaces the text as given in the protocol. Text which is not applicable anymore is indicated by a single strikethrough.

# 2 Synopsis

### Test parameters in study

Blood analysis

- <u>Total homocysteine</u> will be measured in <u>fasting</u> plasma samples on days 01, 13, 15, 29, 41, 43 and in <u>non-fasting plasma samples collected at 6h</u> after methionine loading on day 01, 15, 29 and 43
- <u>Total cholesterol, HDL cholesterol, LDL-cholesterol (calculated), triacylglycerols</u> in fasting serum samples on day 13, 15, 41 and 43
- gamma-GT, ALAT, ASAT, ALP, creatinine in fasting serum samples on days 13, 15, 41 and 43
- <u>Plasma vitamin B6, and serum vitamin B12 and folic acid</u> in fasting samples on days 13, 15, 41 and 43
- Betaine, dimethylglycine and choline in fasting and non-fasting plasma samples collected at 6h after methionine loading on day 01, 15, 29, 43

## 11 Study parameters

### 11.2 In-study

Blood analysis

<u>Total homocysteine</u> will be measured in fasting plasma samples at days 01, 13, 15, 29, 41, 43 and in non-fasting plasma samples collected at 6h after methionine loading on day 01, 15, 29 and 43

<u>Total cholesterol, HDL cholesterol, LDL-cholesterol (calculated), triacylglycerols</u> will be measured in fasting serum samples on days 13, 15, 41 and 43

<u>Gamma-GT, ALAT, ASAT, ALP, creatinine</u> will be measured in fasting serum samples on days 13, 15, 41 and 43

<u>Plasma vitamin B6 and serum vitamin B12 and folic acid concentrations</u> will be measured in fasting blood samples on days 13, 15, 41 and 43

<u>Betaine, dimethylglycine and choline</u> will be measured in fasting and non-fasting plasma samples collected at 6h after methionine loading at day 01, 15, 29, 43.

Blood lipids and liver enzymes will be analysed after each treatment period. The remaining blood parameters will be analysed after completion of the study.

## Data management and statistics

#### 15.1 Data management

The following SAS datasets will be prepared:

- 1. Pre-screening parameters
- 2. Body weight (days 01, 15, 29, 43)
- 3. Fasting homocysteine (days 01, 13, 15, 29, 41, 43)
- 4. Homocysteine after methionine (days 01, 15, 29, 43)
- 5. Blood lipids and liver enzymes (days 13, 15, 41 and 43)
- 6. Vitamin B6, B12 and folic acid (days 13, 15, 41 and 43)
- 7. Betaine, dimethylglycine, choline (days 01, 15, 29 and 43)

For data set 1 raw data will partly be delivered on paper and entered using double dataentry and partly in electronic format. For data set 2 raw data will be delivered on paper and entered using double data-entry. For data set 3, 4, 5, 6 and 7 raw data will be delivered in electronic format /on-line.

Raw data will be registered and will be converted to SAS data. All tranformations and changes in datasets will be controlled and registered. Locked datasets will be electronically transferred to the sponsor.

# 23 Approval of protocol amendment 02

23.1	Sponsor		
	P. Verhoef, PhD Project Manager		
	, ,	Signature	Date (dd-mm-yy)
23.2	TNO Nutrition and Foo	d Research	
	E.J. Brink, PhD Principal investigator	Signature	Date (dd-mm-yy)
	W.A.A. Klöpping, MD, F Medical investigator	PhDSignature	Date (dd-mm-yy)
	C. Kistemaker, BSc Statistician	Signature	Date (dd-mm-yy)
	A.F.M. Kardinaal, PhD TNO Management	Signature	<u></u> Date (dd-mm-yy)

# 24 List of appendices

P4963 B07 : Distribution list