TNO Nutrition and Food Research

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.

© 2003 TNO
## Contents

<table>
<thead>
<tr>
<th>Reason for the amendment</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Revised text</td>
<td>3</td>
</tr>
<tr>
<td>2. Synopsis</td>
<td>4</td>
</tr>
<tr>
<td>11. Study parameters</td>
<td>5</td>
</tr>
<tr>
<td>15. Data management and statistics</td>
<td>6</td>
</tr>
<tr>
<td>23. Approval of protocol amendment 02</td>
<td>7</td>
</tr>
<tr>
<td>24. List of appendices</td>
<td>8</td>
</tr>
</tbody>
</table>
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*

- Total homocysteine will be measured in *fasting* plasma samples on 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
- Total cholesterol, HDL cholesterol, LDL-cholesterol (calculated), triacylglycerols 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
- Plasma vitamin B6, and serum vitamin B12 and folic acid 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

Total homocysteine 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.

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

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

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

Betaine, dimethylglycine and choline 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.
15 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 data-entry 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 transformations 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 Food Research

E.J. Brink, PhD __________________ - -
Principal investigator Signature Date (dd-mm-yy)

W.A.A. Klöpping, MD, PhD __________________ - -
Medical investigator Signature Date (dd-mm-yy)

C. Kistemaker, BSc __________________ - -
Statistician Signature Date (dd-mm-yy)

A.F.M. Kardinaal, PhD __________________ - -
TNO Management Signature Date (dd-mm-yy)
24 List of appendices

P4963 B07 : Distribution list