Biochemical and genetic aspects of mevalonate kinase and its deficiency
Houten, S.M.

Citation for published version (APA):
Houten, S. M. (2002). Biochemical and genetic aspects of mevalonate kinase and its deficiency
Temperature dependency of mevalonate kinase enzyme activity is a pathogenic factor in Hyper-IgD and periodic fever syndrome


Submitted to The Journal of Clinical Investigation.
Hyper-IgD and periodic fever syndrome (HIDS) and mevalonic aciduria (MA) are autosomal recessive disorders characterized by recurrent episodes of fever and generalized inflammation. Both syndromes are caused by specific mutations in the gene encoding mevalonate kinase (MK), resulting in a depressed enzymatic activity mainly due to reduced protein levels. We studied the effect of temperature on the activity of wildtype and several mutant MKs in fibroblasts. All fibroblast cell lines originating from HIDS patients and harboring the common V377I MVK allele, displayed substantially higher MK activities at 30°C as compared to 37°C. As shown by temperature inactivation experiments this resulted in a protein nearly as stable as in control cell lines, indicating that primarily the maturation of the protein is affected. Accordingly, when HIDS cell lines were cultured at 39°C, MK activity decreased further. This triggered a compensatory increase in 3-hydroxy-3-methylglutaryl-CoA reductase activity indicating that MK becomes progressively rate-limiting. A similar phenomenon occurs in vivo. MK activity in peripheral blood mononuclear cells drops 2-6-fold when HIDS patients experience febrile attacks. Thus, minor elevations in temperature, can set off a chain of events, with MK becoming progressively rate-limiting, leading to a temporary deficiency of isoprenoid end-products, followed by inflammation and fever.

Introduction

Recurrent fever and generalized inflammation are the hallmarks of auto-inflammatory diseases, which include the hereditary periodic fever syndromes such as familial Mediterranean fever, TNF-receptor associated periodic syndromes, hyper-IgD and periodic fever syndrome (HIDS, MIM 260920) and mevalonic aciduria (MA, MIM 251170)[1]. HIDS and MA are relatively rare autosomal recessive diseases and are caused by a depressed activity of the enzyme mevalonate kinase (MK, EC 2.7.1.36)[2-4]. In both MA and the more benign HIDS, patients suffer from recurrent fever episodes associated with lymphadenopathy, arthralgias, vomiting, diarrhea and skin rash [5, 6]. Fever episodes can be triggered by minor infections, physical and emotional stress, and childhood immunizations. However, most attacks occur without a clear precipitating event [5]. Inflammation in MA may be more severe, since it has been fatal in some cases [6]. In addition to the fever episodes, MA patients have variable degrees of psychomotor retardation, facial dysmorphism, failure to thrive, hepatosplenomegaly, and anemia [6].

In MA, MK enzyme activity in patient cells is usually virtually undetectable [6, 7]. In HIDS, however, residual MK activity is measurable, and varies between 1% and 7% in cultured skin fibroblasts and lymphocytes [3, 8]. MK deficiency in both MA and HIDS is caused by mutations in the MVK gene which encodes MK [2-4, 7-11]. One particular missense mutation has been identified exclusively in HIDS. This mutation, 1129 G>A, leads to the substitution of the valine at position 377 for an isoleucine (V377I) and has been found in ~90% of HIDS patients analyzed so far [8, 11]. Most HIDS patients are compound heterozygotes for this mutation and a second missense mutation. The second mutation often is one that has been identified also in MA, implying that it results in a non-functional enzyme. This indicates that the V377I substitution is responsible for the HIDS phenotype.

It is unclear how mevalonate kinase deficiency leads to inflammation. Neither is it clear, why this inflammation is episodic rather than continuous. Previously, we and others demonstrated that heterologous expression of the V377I mutant MK protein in Escherichia
coli yielded considerable residual enzyme activity [3, 12]. In contrast, cultured human skin fibroblasts homozygous for this mutation showed less than 8% residual enzyme activity. Furthermore, upon immunoblotting, hardly any MK protein could be detected [3], while Northern blot analysis demonstrated that this was not due to lowered MVK gene expression [8]. Together, this suggested that the V377I mutation affects the stability and/or maturation of the mutant MK.

We now report that the common V377I mutation and a second mutation found in a patient with HIDS indeed affect the stability and/or maturation of MK in patient cells in a temperature sensitive manner. The decrease in MK activity occurring at elevated temperatures may provide an explanation for the episodic nature of the fever episodes in HIDS.

**Patients and Methods**

**Patient cell lines and cells**

After approval by the institutional ethical review board and written informed consent by the patients’ parents, skin fibroblasts were obtained by skin biopsy and/or blood was drawn by venipuncture in sterile pyrogen-free heparinized plastic tubes. Peripheral blood mononuclear cells (PBMC) were isolated by ficoll hypaque (Amersham Pharmacia Biotech, Uppsala, Sweden) density gradient centrifugation, washed twice with phosphate buffered saline (PBS), snap frozen in liquid nitrogen in aliquots of 3-10 x10^6 PBMC and stored at -80°C until assay.

Fibroblast cell lines were cultured in Nutrient Mixture Ham’s F-10 with L-glutamine and 25 mM HEPES (Gibco, Invitrogen, Carlsbad, CA) supplemented with 10% Fetal Calf Serum (Gibco) at the temperatures indicated. For enzyme and immunoblot analysis cells were harvested and washed twice with PBS after trypsinization, and either used directly or snap-frozen in liquid nitrogen and stored at -80°C until use.

All patients and patient cell lines had low MK enzyme activity in combination with mutations in the encoding MVK gene.

**MK and 3-hydroxy-3-methylglutaryl-CoA reductase enzyme analysis**

MK activity was measured radiochemically in cell lysates using ^14^C-labeled mevalonate (NEN, Perkin Elmer Life Sciences, Boston, MA)[13]. 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) was measured radiochemically in cell lysates using ^14^C-labeled 3-hydroxy-3-methylglutaryl-CoA (Amersham Pharmacia Biotech, HMG-CoA)[14]. When necessary, the obtained batch of ^14^C-HMG-CoA was further purified by cellulose thin layer chromatography using as solvent system 1-butanol : acetic acid : water (5 : 2 : 3). Cell pellets were lysed by sonication (twice, 40 J at 8 W output, with cooling between the pulse periods). This was done in PBS when only MK was assayed or in HMGR assay buffer [14] when both HMGR and MK were measured simultaneously. In the latter case, extra MgCl2 (12.7 mM instead of 6 mM) was added to assay MK in order to compensate for the EDTA and EGTA present in the HMGR assay buffer. The thermal inactivation experiments were performed in PBS containing 10 mM dithiothreitol (DTT) in order to prevent inactivation of MK due to oxidation [15]. Samples were removed on time points indicated and assayed for activity. In every sample the activity of MK and/or HMGR was determined in duplicate.

**Immunoblot analysis**

Immunoblot analysis was performed in the same samples used for MK enzyme analysis. Antibodies were generated as described in Hogenboom et al. [14]. Detection by
Temperature affects MK activity in HIDS

Chemiluminescence was performed using the Western Light system (Tropix, Applied Biosystems, Foster City, CA) or the enhanced chemiluminescence kit (ECL, Amersham Pharmacia Biotech).

Statistical methods
The medians were compared by either an unpaired two tailed Mann-Whitney test, or a Kruskal-Wallis nonparametric ANOVA test followed by Dunn's multiple comparisons test. A p-value < 0.05 was considered significant. The mean values are displayed ± the standard deviation.

Results
The V377I mutation affects MK activity in a temperature sensitive manner
Since episodic fever is a prominent symptom of HIDS and MA, we studied whether temperature has an effect on MK enzyme activity. To this end, skin fibroblast cell lines of control subjects, MA and HIDS patients with different genotypes were cultured at 30°C, 37°C or 40°C for 3 days. The HIDS cell lines used for this study were from patients who are compound heterozygous for the V377I and either the H20P mutation or the I268T mutation. The H20P mutation has been shown to result in an unstable and fully inactive protein and the I268T mutation in homozygous state results in MA [7, 10]. The MA cell lines were from patients which are homozygous for the I268T and for a V310M mutation and compound heterozygous for a H20P and an A334T mutation [7, 9, 10]. Enzyme analysis in the various cell lines revealed that the residual MK enzyme activity in the HIDS cells cultured at 37°C varied between 1.1% and 3.0%, whereas the residual activity in the MA cells was virtually undetectable (figure 1). When the same cells were cultured at 30°C, the residual MK enzyme activity in the HIDS cells increased up to 9%, whereas the activity in these cells grown at 40°C became virtually undetectable. No change in the residual MK enzyme activities in the MA cells was detected. Although the MK activity in the control cells also increased at 30°C and decreased at 40°C, these differences were smaller than in the HIDS cell lines.

To determine whether the increase in MK activity at lower temperatures is due to an increase in MK protein levels we performed immunoblot analysis with an MK-specific antibody (figure 1). This revealed that immunoreactive material in lysates of the HIDS cells grown at 30°C becomes readily detectable, while hardly any MK protein is detected in HIDS cells grown at 37°C. When grown at 40°C immunoreactive material is undetectable (figure 1). MK protein levels in the MA cells are very low but detectable and do not change with temperature. Taken together, these results indicate that all mutations tested result in unstable proteins with very low steady-state levels of active and properly folded MK protein. In the case of the V377I allele, however, the levels appear to be dependent on the culturing temperature.

To study this phenomenon in more detail, we transferred control, HIDS (H20P, V377I) and MA (I268T) fibroblast cell lines from 37°C to 30°C and measured MK activity after 24, 48, 96 and 144 hours of culture. Again the activity of MK in the MA cell line remained virtually undetectable at all time points, whereas the activity in the HIDS cell line increased to even 45% after 144 hours (figure 2a). Since this HIDS cell line is heterozygous for the V377I allele and the fully inactive H20P allele, enzyme activity is expected to increase no further than 50%. These results imply that the V377I allele produces a fully stable protein at 30°C.
Figure 1. Relative enzyme activities of MK in fibroblast lysates and immunoblot analysis of MK in the corresponding samples after 3 days of culture at the indicated temperature. The specific activity of the control fibroblast cell line grown at 37°C (598 pmol/min/mg protein) is used as the 100% value. For immunoblot analysis, equal amounts of protein (45 µg) were separated by 10% SDS-PAGE and analyzed on immunoblot with MK-specific antibodies. As a control for equal loading, the blot was analyzed with actin-specific antibodies. The figure includes a control cell line (C), two MA cell lines (M1, I268T; M2, V310M) and three HIDS cell lines (H1, H20P/V377I; H2, H20P/V377I; H3, H20P/V377I). The results for the HIDS and MA cell line with the I268T/V377I and H20P/A334T genotypes are not shown.

Figure 2. a. Relative enzyme activities in fibroblast lysates of control, HIDS (H20P/V377I) and MA (I268T) cell lines after the indicated time grown at 30°C. The specific activity of the WT cell line after 1 day at 30°C (349 pmol/min/mg protein) is used as the 100% value. b. Thermal inactivation of MK enzyme activity in WT and HIDS cell lysates at 50°C. c. Thermal inactivation of MK enzyme activity in WT and HIDS cell lysates at 37°C.
Temperature affects MK activity in HIDS

To study the stability of the matured enzymes, we performed thermal inactivation experiments with MK in control and HIDS fibroblast lysates at 50°C and 37°C. The inactivation process followed first order kinetics and was significantly faster in HIDS cell lysates at both temperatures, although the difference was only 1.3-fold (p<0.05, figure 2b and 2c). This shows that the mutant V377I protein, after it has reached the active and properly folded conformation, is almost as stable as the WT protein. Moreover, it shows that the V377I mutation primarily affects its maturation and not the stability per se.

Temperature sensitivity in another HIDS causing mutation

Although the vast majority of HIDS patients carry the V377I allele, at least three additional mutations appear specific for HIDS as well: A148T (442 G>A), P167L (500 C>T), and T209A (625 A>G)[2, 3, 8, 16]. In order to study whether temperature sensitivity of the MK protein could be a common characteristic for HIDS, we tested a cell line heterozygous for the A148T allele and the I268T allele [8]. To this end, this cell line, a control cell line and a HIDS cell line containing the V377I allele were cultured at 30°C and 37°C for 6 days. Residual activity of MK in the HIDS cell line containing the V377I increased 9-fold, and in the HIDS cell line with the A148T mutation the residual activity increased 8-fold, which indicates that the two mutant alleles produce MKs with similar temperature sensitivity (figure 3).

![Figure 3. Relative enzyme activities of one control, one HIDS cell line with V377I (H20P/V377I) and one HIDS cell line without V377I (A148T/I268T) after 6 days of culture at 30°C. The specific activity of the control fibroblast cell line grown at 37°C (543 pmol/min/mg protein) is used as the 100% value.](image)

Temperature elevation induces increased HMGR activity in HIDS fibroblasts

HMGR is among the most tightly regulated enzymes in nature and is the rate-limiting enzyme step in isoprenoid biosynthesis [17]. As previously reported by Gibson et al. [18], we observed an increased HMGR activity in cultured skin fibroblasts of MA patients (table 1). In fibroblasts of HIDS patients, however, HMGR activity was within the normal range. Since we found that MK activity is temperature-sensitive in HIDS cells, we investigated whether an increased temperature induces an increase in HMGR activity. Indeed, when switched to 39°C a marked difference between HIDS and control fibroblasts was observed (table 2). After 6 hours there was a decrease in MK activity and an increase in HMGR activity in most HIDS cells, whereas this did not occur in control cells. After 24 hours this effect was even more pronounced, which is reflected clearly in the 4 to 7-fold decrease in MK/HMGR ratio (table 2).
Table 1. HMGR enzyme activity in fibroblasts and PBMCs from controls, HIDS and MA patients.

<table>
<thead>
<tr>
<th></th>
<th>Fibroblasts</th>
<th>PBMCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>5±2 (24; 5)</td>
<td>2±1 (20; 10)</td>
</tr>
<tr>
<td>HIDS</td>
<td>7±4* (19; 7)</td>
<td>12±4** (15; 7)</td>
</tr>
<tr>
<td>MA</td>
<td>73±46*** (26; 5)</td>
<td>40 (1; 1)</td>
</tr>
</tbody>
</table>

Values are expressed as pmol/min.mg protein.

$**p=0.5,, ns$  
$***p<0.001$  
The number of samples and different subjects, respectively, are displayed in parentheses.

Table 2. MK activity, HMGR activity and the MK/HMGR ratio in fibroblasts of control and HIDS patients cultured at different temperatures.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Condition</th>
<th>MK pmol/min.mg</th>
<th>HMGR pmol/min.mg</th>
<th>MK/HMGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>37°C</td>
<td>394±14.9</td>
<td>6±0.1</td>
<td>64</td>
</tr>
<tr>
<td>C1</td>
<td>39°C, 6h</td>
<td>347±10.0</td>
<td>6±0.1</td>
<td>59</td>
</tr>
<tr>
<td>C1</td>
<td>39°C, 23h</td>
<td>313±2.4</td>
<td>7±0.4</td>
<td>43</td>
</tr>
<tr>
<td>C2</td>
<td>37°C</td>
<td>343±38.8</td>
<td>4±0.4</td>
<td>77</td>
</tr>
<tr>
<td>C2</td>
<td>39°C, 7h</td>
<td>311±11.2</td>
<td>4±0.5</td>
<td>87</td>
</tr>
<tr>
<td>C2</td>
<td>39°C, 25h</td>
<td>274±6.0</td>
<td>6±0.5</td>
<td>48</td>
</tr>
<tr>
<td>C2</td>
<td>37°C</td>
<td>357±20.0</td>
<td>6±0.3</td>
<td>74</td>
</tr>
<tr>
<td>C2</td>
<td>39°C, 7h</td>
<td>310±7.8</td>
<td>6±0.5</td>
<td>51</td>
</tr>
<tr>
<td>C2</td>
<td>39°C, 24h</td>
<td>288±2.4</td>
<td>11±1.0</td>
<td>27</td>
</tr>
<tr>
<td>H1</td>
<td>37°C</td>
<td>19±1.2</td>
<td>7±0.4</td>
<td>2.6</td>
</tr>
<tr>
<td>H1</td>
<td>39°C, 6h</td>
<td>9</td>
<td>12±1.6</td>
<td>0.72</td>
</tr>
<tr>
<td>H1</td>
<td>39°C, 23h</td>
<td>7±0.9</td>
<td>15±0.3</td>
<td>0.47</td>
</tr>
<tr>
<td>H2</td>
<td>37°C</td>
<td>22±0.8</td>
<td>8±0.5</td>
<td>2.7</td>
</tr>
<tr>
<td>H2</td>
<td>39°C, 6h</td>
<td>11±0.1</td>
<td>13±0.0</td>
<td>0.87</td>
</tr>
<tr>
<td>H2</td>
<td>39°C, 23h</td>
<td>10±0.3</td>
<td>26±2.2</td>
<td>0.39</td>
</tr>
<tr>
<td>H3</td>
<td>37°C</td>
<td>21±0.6</td>
<td>4±0.5</td>
<td>4.7</td>
</tr>
<tr>
<td>H3</td>
<td>39°C, 7h</td>
<td>11±0.2</td>
<td>4±0.5</td>
<td>2.4</td>
</tr>
<tr>
<td>H3</td>
<td>39°C, 25h</td>
<td>4±0.5</td>
<td>9±0.2</td>
<td>0.46</td>
</tr>
<tr>
<td>H4</td>
<td>37°C</td>
<td>44±0.6</td>
<td>10±1.0</td>
<td>4.3</td>
</tr>
<tr>
<td>H4</td>
<td>39°C, 7h</td>
<td>15±0.3</td>
<td>19±0.7</td>
<td>0.77</td>
</tr>
<tr>
<td>H4</td>
<td>39°C, 24h</td>
<td>22±0.9</td>
<td>13±0.3</td>
<td>1.62</td>
</tr>
</tbody>
</table>

The presented data in the table are derived from three independent experiments. The control cell lines are denoted as C, the HIDS cell lines as H1 (I268T/V377I), H2 (H20P/V377I), H3 (H20P/V377I) and H4 (I268T/V377I).

Decrease of MK activity in HIDS PBMCs during fever

To investigate whether temperature also affects in vivo MK activity in patients, we analyzed MK and HMGR activity in PBMCs obtained from four HIDS patients during and between fever attacks. From HIDS patient A (table 3, figure 4), we obtained PBMCs on the first and
Temperature affects MK activity in HIDS

second day of a fever attack and twice after recovery. The generalized inflammation during the fever attack was reflected by neutrophil leukocytosis and elevated C-reactive protein (CRP) levels. We measured MK and HMGR activity in all samples. As shown in figure 4, in this patient the MK activity of ~14 pmol/min.mg (6% of the control value) between fever episodes decreases 3-fold during fever (~5 pmol/min.mg). In patient B and patient C the drop in MK activity during fever was even higher: six- and seven-fold respectively. Finally, in one clinically severely affected patient, MK activity showed no response to the fever and remained low whether or not fever was present. Overall, in the 4 HIDS patients, MK activity during fever episodes was significantly lower than at normal temperature (p<0.05).

![Figure 4.](image)

Table 3. MK activity, HMGR activity and the MK/HMGR ratio in PBMCs of HIDS patients during and in between fever episodes.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Condition</th>
<th>MK activity (pmol/min.mg)</th>
<th>HMGR activity (pmol/min.mg)</th>
<th>MK/HMGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Fever</td>
<td>5</td>
<td>12</td>
<td>0.38</td>
</tr>
<tr>
<td>A</td>
<td>Non febrile</td>
<td>14</td>
<td>15</td>
<td>0.95</td>
</tr>
<tr>
<td>B</td>
<td>Fever</td>
<td>1</td>
<td>20</td>
<td>0.07</td>
</tr>
<tr>
<td>B</td>
<td>Non febrile</td>
<td>6</td>
<td>8</td>
<td>0.74</td>
</tr>
<tr>
<td>C</td>
<td>Fever</td>
<td>1</td>
<td>16</td>
<td>0.07</td>
</tr>
<tr>
<td>C</td>
<td>Non febrile</td>
<td>7</td>
<td>9</td>
<td>0.82</td>
</tr>
<tr>
<td>D</td>
<td>Fever</td>
<td>2</td>
<td>14</td>
<td>0.12</td>
</tr>
<tr>
<td>D</td>
<td>Non febrile</td>
<td>3</td>
<td>9</td>
<td>0.31</td>
</tr>
</tbody>
</table>

The mean MK and HMGR values in controls are 217±51 and 2±1 pmol/min.mg, respectively. All patients had the same genotype, I268T/V377I.
In general, the HMGR activities measured in the PBMCs from the HIDS patients appeared significantly elevated compared to controls, although less than in PBMCs of an MA patient. Analysis of the HMGR enzyme activity in the samples obtained from the four HIDS patients during and between fever attacks revealed a 2-fold rise in HMGR activity during fever in patients B and C, whereas in patients A and D there was only a modest response to fever or none at all. Thus, the patients who showed the most pronounced difference in MK activity also displayed the greatest difference in HMGR enzyme activity. Overall, however, the difference between HMGR activity during and between fever episodes was not significant (p<0.1).

Taken together, these results indicate that although MK activity is lowered at all times in PBMCs from HIDS patients, this deficiency becomes more prominent during fever.

Discussion

Three years after the identification of the genetic defect in HIDS, the pathogenesis of the fever attacks that characterize the disease remains elusive. It is not known how the metabolic defect leads to inflammation. Neither is it known why inflammation is episodic rather than continuous. Patients often report attacks after trivial events such as minor infections, vaccinations, or vigorous exercise, events that also in otherwise healthy individuals temporarily raise body temperature. We hypothesized that such an increase in temperature may be involved in triggering the attacks. Since most MVK mutations result in deficient MK activity mainly due to reduced protein levels, we studied the effect of changes in temperature on the activity of normal and mutant mevalonate kinase.

We found that two MVK alleles exclusively associated with HIDS (V377I and A148T) display temperature sensitivity with respect to MK activity. For mutant alleles found in MA we did not observe such temperature sensitivity. When grown at 30°C the residual MK activity in cultured HIDS fibroblasts increases much more than in control cells, whereas at 40°C the residual activity diminishes. Importantly, this change in enzyme-activity correlates nicely with similar changes in the MK protein-levels as determined by immunoblotting. Since thermal inactivation of the mutant MK protein appears comparable with the WT protein, the observed low steady-state protein level in cells of HIDS patients is most probably due to inefficient or incorrect folding of the mutant protein at 37°C leading to its degradation.

Recently, Ríos et al [12] characterized the V377I mutant protein expressed in E. coli. They found only modest kinetic differences in comparison with the WT enzyme (6-fold inflation of the \( K_m \) for mevalonate) and, as we did in patient fibroblast cell lines, normal thermal inactivation of the mutant protein. From these results these authors concluded that the V377I mutation is unlikely to provide an explanation for the observed depressed MK protein levels and catalytic activity in HIDS. However, our results with the fibroblast lysates show that the V377I allele encodes a polypeptide that apparently is not capable of folding in the correct conformation at higher temperatures and subsequently undergoes degradation. These results together with all the genetic evidence such as the occurrence of this specific mutation in the vast majority of HIDS patients [8, 11], its absence in control subjects [2], the results of linkage analysis [2] and the autosomal recessive mode of inheritance, unequivocally demonstrate that the V377I allele is the cause of the depressed MK activity in HIDS.

When we measured HMGR enzyme activity in cultured HIDS and MA fibroblasts, we found the activity elevated in MA fibroblasts as previously reported by Gibson et al. [18], whereas in HIDS cells, it was within the normal range. These results indicate that under the
Temperature affects MK activity in HIDS

tested culture conditions MA cells compensate for their reduced MK activity, whereas in HIDS cells no compensation occurs. When HIDS fibroblasts are cultured at 39°C, however, the decrease in the enzymatic activity of MK is associated with an increased HMG R activity, in contrast to the control cells. This indicates that due to the increase in culture temperature MK becomes progressively rate-limiting, resulting in an increased HMG R enzyme activity in order to compensate for the decrease in MK activity. These results thus provide an explanation for the observed increase in mevalonate excretion during fever episodes in HIDS [19]. Together with the reported difference in mevalonate excretion in urine between HIDS and MA [3, 6, 19], these observations also imply that in MA, MK performs the rate-limiting step instead of HMG R, whereas in HIDS the control of the pathway, at least at normal temperatures, remains at the level of HMG R.

We investigated whether the thermosensitivity of MK activity observed in vitro also plays a role in HIDS patients during and between fever episodes. Therefore we analyzed MK and HMG R activity in PBMCs obtained from four HIDS patients during and between fever attacks. We observed that MK enzyme activity drops 2-6-fold when HIDS patients experience febrile attacks. In contrast to the normal HMG R enzyme activity in fibroblasts from HIDS patients, we observed an elevated HMG R enzyme activity in PBMCs from these patients. Although this elevation was not as high as in a sample obtained from an MA patient, it indicates that there is a derangement in isoprenoid biosynthesis in PBMCs from HIDS patients. No significant difference was observed in HMG R enzyme activity during and between a fever episode. However, the two patients who showed the most pronounced difference in MK activity also displayed an additional elevation in HMG R enzyme activity during fever. This could suggest that an elevation in HMG R enzyme activity may be missed in the other patients due to a delay between the onset of the attack and sampling. Our results show that during fever, MK deficiency in PBMCs from HIDS patients becomes even more prominent. From our in vitro data we conclude that this is caused by the impaired maturation of MK especially at higher temperatures.

There are several indications that isoprenoid biosynthesis plays a role in inflammation. For example, it has been reported that administration of LPS, TNF-α, or IL-1β to Syrian hamsters triggers a rapid upregulation of hepatic HMG R and a downregulation of squalene synthase, the enzyme catalyzing the first committed enzyme step of sterol biosynthesis [20-22]. These observations suggest a higher demand for non-sterol isoprenoids during inflammation. Examples of non-sterol isoprenoids are the farnesyl- and geranylgeranyl-groups used for protein isoprenylation, isopentenyl tRNAs, dolichol, ubiquinone-10, and heme A. Furthermore, statins, a class of lipid-lowering drugs that are competitive inhibitors of HMG R and accordingly lead to decreased production of isoprenoids, have been reported to have both anti-inflammatory [23-26] and pro-inflammatory effects [27-29]. Our data reported here suggest that in HIDS patients, the fever that accompanies inflammation enhances the MK deficiency, which will result in a temporary reduction in non-sterol isoprenoid production. This will affect especially isoprenoids with a high turnover, such as ubiquinone-10 in plasma, which is decreased in most MA patients [30], prenylated small G-proteins like Rho [31], which are involved in multiple cellular processes like signal transduction or cytoskeletal organization, and the prenylated guanylate-binding proteins (GBP) which are specifically synthesized in response to IFN-γ and LPS [32]. It is conceivable that this shortage of non-sterol isoprenoids is responsible for the pro-inflammatory phenotype of HIDS and MA. Thus, even minor elevations in temperature, due to exercise or infections could set off a
Chapter 8

chain of events, with MK becoming progressively rate limiting, leading to a temporary deficiency of anti-inflammatory isoprenoids, followed by inflammation and fever.

Strong support for this hypothesis is provided by the negative outcome of a therapeutic trial in which two MA patients were treated with low doses of lovastatin in order to block the production of mevalonate (speculated to be pathogenic). This trial had to be stopped because of the development of severe clinical crises [6]. The outcome of this trial strongly suggests that the symptoms of MA are not caused by an excess of mevalonate but by a shortage of isoprenoid endproducts. The enormous difference in urinary mevalonate excretion in HIDS and MA patients also argues against a causative role of mevalonate in the pathogenesis of the clinical crises. MA patients have much higher mevalonate levels, but fever episodes occur as frequently as in HIDS.

The observed differences in stability and temperature sensitivity of mutant MKs may explain why HIDS patients display episodic fever. Since such fever episodes are prominent not only in HIDS but also in MA, the same may be true for MA. Indeed, the fact that mevalonate excretion in urine of both HIDS and MA patients correlates with disease severity points to a similar mechanism [6, 19]. Unfortunately, the extremely low and already hardly detectable MK activity levels in MA cells do not allow the demonstration of a similar temperature sensitive phenomenon. However, the finding that the increase in temperature does not only affect the mutant MKs, but also the wild-type MK activity renders it highly plausible. Even a small additional decrease in the already extremely low MK activity in MA cells may have far-reaching consequences. Since HMGR already appears maximally induced to compensate for the MK deficiency, a further induction to establish even higher mevalonate levels may not be possible. Furthermore, in MA cells the MK enzyme will be saturated already, thus an additional increase in the mevalonate concentration will have no effect on the flux through the pathway. These reasons may provide an explanation for the reported fatal outcome of a fever episode in several MA patients [6].

Acknowledgements
The authors thank Gerrit Jan Romeijn for expert help with the mevalonate kinase enzyme assays, Saskia Mandey for the isolation of PBMCs, Katja Klumpers for help with mathematics and Bert Groen for critical reading of the manuscript.

References

104
Temperature affects MK activity in HIDS
