Intake of vitamin A, cadmium and lead via liver foods among Finnish women of fertile age – a quantitative risk assessment

Kuvailulehti

Julkaisija	Elintarviketurvallisuusvirasto Evira
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Tekijä	Tiina Lavikainen, Ulla Karlström, Christina Bäckman, Tero Hirvonen
Julkaisun nimi	**Suomalaisten hedelmällisessä iässä olevien naisten A-vitamiinin, kadmiumin ja lyijyn saanti maksaruokien välityksellä — kvantitatiivinen riskinarviointi**
Tiivistelmä	Maksa on monipuolinen ruoka-aine, mutta maksansyönnin turvallisuus raskauden aikana on askarruttanut pitkään niin kuluttajia kuin terveydenhuoltoakin. Maksa sisältää runsaasti retinoidimuotoista A-vitamiinia, joka suurina annoksina voi lisätä sikiövaurioiden ja keskenmenon riskiä. Lisäksi maksa voi sisältää runsaasti kadmiumia ja lyijyä, joilla voi olla haitallinen vaikutus sikiöön. Tämän vuoksi odottaville äideille suositellaan, että maksan syöntiä vältetään koko raskauden ajan. Maksaruokien käytön todellisista riskeistä raskauden aikana ei ole ollut tutkittua tietoa, ja syöntirajoituksen tarpeellisuudesta keskustellaan toistuvasti. Tämän vuoksi Elintarviketurvallisuusvirasto Evirassa on tehty riskinarviointi hedelmällisessä iässä olevien suomalaisnaisten altistumisesta retinoideille maksaruokien välityksellä. Arvioinnissa tarkasteltiin riskiä suositukset ylittävälle retinoidien saannille, mikäli maksaruuat, tai vain jotkin niistä, säilyisivät osana raskauden ajan ruokavaliota. Työssä arvioitiin myös muiden kuin syöntirajoituksiin perustuvien toimenpiteiden vaikutusta retinoidien saantiin. Lisäksi arvioitiin maksaruokien kautta tapahtuvaa kadmium- ja lyijyaltistusta. Arvioinnin pohjana käytettiin maksaruokien kulutustietoja (Finravinto 2002 -tutkimus, KTL), maksaruokien reseptitietoja sekä tuoreita tietoja maksojen retinoidi-, kadmium- ja lyijypitoisuuksista. Arvioinnin välineeksi rakennettiin altistusta kuvaava matemaattinen simulaatiomalli. Altistusta arvioitiin pitkäaikaissaantina (keskimääräinen päivittäinen saanti), ja retinoidien osalta myös altistuksena kerta-annoksesta. Mallin tuloksia verrattiin olemassa oleviin saantisuosituksiin ja saannin ylärajoihin. Riskinarvioinnissa päädyttiin seuraaviin johtopäätöksiin: 1. Maksaruokien käyttö voi altistaa suuremmille retinoidiannoksille kuin mitä pidetään turvallisena raskauden aikana. Liikasaannin riski näyttäisi kuitenkin koskevan lähinnä pääruokana syötäviä maksaruokia, kuten maksalaatikkoa, jauhemaksapihviä ja maksakastiketta. Kohtuullinen maksamakkaran tai maksapasteijan syönti raskauden aikana ei näyttäisi altistavan sellaisille retinoidiannoksille, joiden voidaan katsoa lisäävän sikiövaurioiden riskiä. 2. Tehokkain keino retinoidien liikasaannin riskin vähentämiseksi on välttää etenkin pääruokina käytettyjen maksaruokien syöntiä raskauden aikana. Muiden tarkasteltujen skenaarioiden vaikuttavuus oli selvästi heikompi. Emakonmaksan elintarvikekäytön lopettamisella olisi vain vähäinen vaikutus retinoidien saantiin. Vastaavasti maksojen retinoidipitoisuuksien pitäisi vähentyä noin neljännekseen, jotta vaikutus vastaisi osittaisen syöntirajoituksen (ei pääruokana syötäviä maksaruokia) tehokkuutta. 3. Kun A-vitamiinin saantia tarkastellaan ravitsemuksellisesta näkökulmasta, maksansyönti auttaa saantisuositusten täyttymisessä. Maksa sisältää A-vitamiinin lisäksi myös monia muita tärkeitä hivenaineita ja vitamiineja, kuten foolihappoa ja rautaa. Maksansyönnillä saavutettavat ravitsemukselliset hyödyt voitaneen kuitenkin korvata runsaasti kasviksia ja kohtuullisesti lihaa sisältävällä monipuolisella ruokavaliolla, jolloin retinoidien liikasaannin riskiä ei ole. 4. Maksaa syövillä naisilla kadmiumin ja lyijyn saanti maksaruokien välityksellä on melko vähäistä verrattuna altistukseen muista ravintolähteistä. Maksan sisältämä rauta saattaa vähentää kadmiumin ja lyijyn imeytymistä maksaruuista.

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Författare	Tiina Lavikainen, Ulla Karlström, Christina Bäckman, Tero Hirvonen
Verkets titel	**Intag av A-vitamin, kadmium och bly hos finska kvinnor i fruktbar ålder via maträtter som innehåller lever — en kvantitativ riskbedömning**
Resume	Lever är ett mångsidigt födoämne, men tryggheten i att äta lever under graviditeten har länge varit en osäkerhetsfaktor för både konsumenter och hälsovårdspersonal. Lever innehåller höga halter av A-vitamin som retinoider, som i höga doser kan öka risken för fosterskador och missfall. Lever kan också innehålla höga halter av kadmium och bly som kan ha negativ inverkan på foster. Därför rekommenderas att gravida kvinnor undviker att äta lever under hela graviditeten. Inga forskningsresultat om de verkliga riskerna i användning av leverrätter under graviditeten har funnits, och det diskuteras gång på gång om behovet av att begränsa intaget. På grund av detta har Livsmedelssäkerhetsverket Evira gjort en riskbedömning över hur finländska kvinnor i reproduktiv ålder utsätts för retinoider via levermaträtter. I bedömningen betraktades riskerna att få mer än det rekommenderade intaget av retinoider, ifall leverrätterna, eller endast en del av dem, skulle fortsätta vara en del av graviditetsdieten. Under arbetet bedömdes även inverkningarna av andra åtgärder än begränsningar i dieten på intaget av retinoider. Dessutom bedömdes riskerna för intag av kadmium och bly via leverrätter. Som grund för bedömningen användes konsumtionsdata för leverrätter (Undersökningen Finravinto 2002, Folkhälsoinstitutet), receptdata för leverrätter samt färsk information om retinoid-, kadmium- och blyhalter i olika levertyper. Som redskap för bedömningen byggde man upp en matematisk simulationsmodell som beskriver intaget. Intaget bedömdes som långtidsintag (genomsnittligt dagligt intag), och för retinoidernas del även som intag per engångsdos. Resultaten från modellen jämfördes med existerande rekommendationer och övre gränser för intag. Vid riskbedömningen kom man till följande slutsatser: 1. Användning av leverrätter kan utsätta individen för större retinoiddoser än de som anses vara trygga under graviditeten. Risken för överdoser ser ändå ut att närmast gälla leverrätter som äts som huvudrätt, såsom leverlåda, leverfärsbiff och leversås. Ett rimligt intag av leverkorv eller leverpastej under graviditeten ser inte ut att utsätta kvinnor för sådana retinoiddoser som kunde anses öka risken för fosterskada. 2. Det effektivaste sättet att minska risken att få för mycket retinoider är att undvika att äta särskilt sådana leverrätter som används som huvudrätt under graviditeten. De övriga scenariernas inverkan var tydligt svagare. Att sluta använda sugglever som livsmedel skulle endast ha en liten inverkan på intaget av retinoider. På samma sätt borde retinoidhalterna i olika leversorter minska till ca en fjärdedel för att inverkan skulle motsvara effekten hos en partiell begränsning av intaget (inga leverrätter som huvudrätt). 3. Leverförtäring hjälper till att uppfylla rekommendationerna för intaget av A-vitaminer. Levern innehåller utom A-vitamin även många andra viktiga spårämnen och vitaminer såsom folsyra och järn. Näringsnyttorna som uppnås genom att äta lever kan troligen ändå ersättas med en mångsidig diet, som innehåller gott om vegetabilier och måttliga mängder kött, där ingen risk för överdos av retinoider finns. 4. Kadmium- och blyintaget hos kvinnor som äter lever är rätt litet via leverrätter jämfört med intaget från andra näringskällor. Järnet som finns i levern kan minska upptaget av kadmium och bly ur leverrätter.

Sökord	A-vitamin, retinoider, graviditet, kadmium, bly, risk, teratogenitet
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Description

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Authors	Tiina Lavikainen, Ulla Karlström, Christina Bäckman, Tero Hirvonen
Title	**Intake of vitamin A, cadmium and lead via liver foods among Finnish women of fertile age — a quantitative risk assessment**
Abstract	Liver is a good source of many nutrients, but safety of liver consumption during pregnancy has long plagued the minds of both consumers and healthcare personnel. Liver contains a lot of vitamin A in retinoid form, which can increase the risk of foetal malformations and miscarriages if ingested in large amounts. Liver may also contain high amounts of potentially toxic heavy metals like cadmium and lead. Therefore, it is recommended that pregnant women should not eat liver. There has been no assessment of the true risk, if any, of maintaining liver in the diet of a woman while pregnant, and the need of avoiding liver during pregnancy in present day is regularly discussed. To re-evaluate current recommendations, the Finnish Food Safety Authority Evira made a risk assessment of retinoid intake from liver foods among Finnish women of fertile age. The objectives of the risk assessment were to estimate the risk of intolerably high retinoid intake if all or some liver products were maintained in the diet of women during pregnancy. The effects of scenarios other than diet restriction to lower the retinoid intake were also studied. In addition, the intake of cadmium and lead via liver foods was estimated. Risk assessment was based on liver food consumption data (FINDIET 2002 study, KTL), obtained recipe information and recent results of vitamin A, cadmium and lead in liver. To estimate intake, a mathematic simulation model was designed. Intake was estimated as an average daily intake and for retinoids also as an intake from single liver meal. The simulation model results were compared with intake recommendations and upper intake limits. Based on risk assessment, the following conclusions were made 1. Liver consumption may predispose women to retinoid intakes higher than what is considered safe during pregnancy. However, the risk seems to pertain mainly to liver foods eaten as a main course (i.e., liver casserole, liver patties and liver stew). Safety thresholds are not likely to be exceeded if only liver sausage or pâté is eaten in moderate amounts. 2. The most efficient way to manage the risk is for women to avoid main course liver foods during pregnancy. The effect of the other scenarios examined was notably smaller. Exclusion of sow livers from food production would have only a minor effect on retinoid intakes from liver foods. If effects similar to a restricted consumption scenario (no main course liver foods) were tried to achieve by reducing liver retinoid contents, the retinoid content of livers should be less than 25% of the present level. 3. When considering the total daily vitamin A intake from the nutritional point of view, liver consumption has a positive effect. In addition to vitamin A, liver contains also other beneficial nutritional elements like folic acid and iron. However, the benefits of eating liver can probably be substituted by a well-balanced diet with plenty of vegetables and a reasonable amount of meat without the risk of an excess intake of retinoids. 4. Cadmium and lead exposure from liver foods is relatively low when compared with other dietary sources. The high iron content of liver may reduce the absorption of cadmium and lead from liver foods.

Key words	Vitamin A, retinoids, pregnancy, cadmium, lead, risk, teratogenicity
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Contens

Risk assessment

Definitions and abbreviations

AR
Average requirement, *keskimääräinen tarve.* Nutrient intake value estimated to meet the average physiological requirement of the selected population group.

Bioavailability
Hyötyosuus. The fraction of ingested dose of compound that is available to mediate biological effects in the body.

Carotenoids
Karotenoidit. Organic pigments naturally occuring in photosynthetic organisms. Some carotenoids are vitamin A precursors (provitamin A carotenoids).

Chylomicron
Kylomikroni. Large lipoprotein particle that are created by the absorptive cells of the small intestine. Chylomicrons transport absorbed lipids to target tissues.

Dietary supplement
Ravintoainevalmiste. Concentrated sources of nutrients marketed in dose form (capsules, tablets).

EELA
National Veterinary and Food Reseach Institute of Finland, *Eläinlääkintä- ja elintarviketutkimuslaitos.* United to Finnish Food Safety Authority (Evira) in 2006.

EMEA
European Agency for the Evaluation of Medicinal Products

EVI
→ NFA

EVM
Expert Group of Vitamins and Minerals, Food Standard Agency, UK.

FAO
Food and Agriculture Organization of the United Nations.

FINDIET survey
Finravinto-tutkimus. A national study to measure the average food and nutrient intake among Finnish adult population. The FINDIET study has been carried out by the National Public Health institute every fifth year since 1982.

FNB
Food and Nutrition Board. Unit of the institute of medicine, part of the National Academy of Sciences, USA.

Homeobox genes
Genes with homeobox DNA-sequence. Homeobox genes are involved in the regulation of development of organisms.

HPLC
High-performance liquid chromatography, *korkean erotuskyvyn nestekromatografia*

IPCS
The International Programme on Chemical Safety. A joint programme of ILO (International Labour Organization), UNEP and WHO, implementing activities related to chemical safety.

IU
International unit, *kansainvälinen yksikkö.* A unit of measurement for the amount of a substance, based on measured biological activity. For vitamin A, 1 IU equals the biological activity of 0.3 μg retinol (0.3 μg RE).

KTL
National Public Health Institute of Finland, *Kansanterveyslaitos*

LI
Lower level of intake, *alin hyväksyttävä saanti.* A level below which an nutrient intake could lead to deficiency symptoms of some individuals.

NCM
Nordic Council of Ministers, *Pohjoismaiden ministerineuvosto*

NFA
National Food Agency of Finland, *Elintarvikevirasto.* United to Finnish Food Safety Authority (Evira) in 2006.

NRCP
National Residue Control Program, *kansallinen vierasainevalvontaohjelma.* The NRCP is carried out annually in accordance with both national and European Union legislation. The aim of the NRCP is to assure that food of animal origin is free from drug recidues and unauthorized or banned substances.

Organogenesis
The formation and development of organs and other physical features during embryonic development.

PTWI
A provisional tolerable weekly intake, *tilapäinen siedettävä viikoittainen saanti*

RBP
Retinol-binding protein

RE
→ Retinol equivalent

Retinoids
Retinoidit. A class of chemical compounds consisting of six-carbon ring structure with a polyprenoid side chain and a terminating carbon-oxygen functional group. In this risk assessment, term refers to natural and synthetic retinoid derivatives with biological activity of retinol.

Retinol equivalent
Retinoliekvivalentti. The specific biological activity of 1.0 microgram of all-trans retinol.

Retinol
Retinoli. The most common natural retinoid (as free retinol or esterified to fatty acids), and the key molecule in body retinoid metabolism.

RI
Recommended intake, *suositeltava saanti.* The nutrient intake over time that theoretically would fulfil the needs of practically all (97.5%) of healthy individuals in a selected population group. RI is calculated by adding a safety margin equal to two standard deviations to the estimated average requirement (AR).

RSD%
Relative standard deviation, *suhteellinen keski-hajonta.* Expressed in percent. RSD% is obtained by multiplying the standard deviation (S) by 100 and dividing this product by the mean of the series ($\bar{x}$).
RSD % = 100S/$\bar{x}$

SCF
Scientific Committee on Food, European Commission.

Skewed distribution
Probability distribution in which an unequal number of observations lie below and above the mean.

Teratogen
Teratogeeni. Any agent that can disturb the development of an embryo or foetus. Teratogens may cause a birth defect in the child.

Teratogenesis
Teratogeneesi. Congenital malformation as a result of exposure of the foetus to a teratogen.

UNEP
United Nations Environment Programme

Urogenital system
Relating to both the urinary system, and to the internal and external genitalia.

UL
Upper tolerable level, *suurin hyväksyttävä saanti.* The maximum daily intake of a nutrient unlikely to pose a risk of adverse health effects to humans.

WHO
World Health Organisation of the United Nations

Vitamin A
A-vitamiini. Retinoids and provitamin A carotenoids that exhibit biological activity of retinol.

VRN
National Nutrition Council, *Valtion ravitsemusneuvottelukunta*

1. Yhteenveto

1.1 Johdanto

Maksa sisältää runsaasti A-vitamiinia ja monia muita ravintoaineita. Vaikka maksa on monipuolinen ruoka-aine, sen käytössä on varjopuolensa. A-vitamiini esiintyy maksassa retinoidimuodossa, joka voi suurina annoksina aiheuttaa myrkytysoireita. Retinoidien saantiin tulee kiinnittää erityistä huomiota raskauden aikana, sillä liikasaanti voi aiheuttaa sikiönkehityksen häiriöitä. Suuren retinoidipitoisuuden lisäksi maksa voi sisältää runsaasti kadmiumia ja lyijyä, joilla voi olla haitallinen vaikutus sikiöön.

Vuonna 1989 havaittiin suomalaisen sianmaksan sisältävän erittäin korkeita A-vitamiinipitoisuuksia (Heinonen 1990). Mahdollisten haitallisten sikiövaikutusten vuoksi viranomaiset laativat suosituksen, jonka mukaan raskaana olevien tai raskautta suunnittelevien naisten ei tulisi syödä maksaa (Julkunen ym. 1990). Sikojen rehujen koostumusta muuttamalla ja luopumalla perusrehujen ohella käytettävien vitamiinivalmisteiden liiallisesta käytöstä sianmaksan A-vitamiinipitoisuudet saatiin pian laskemaan. Raskaana oleville annettua maksankäyttösuositusta ei kuitenkaan muutettu. Maksaruokien käytön todellisista riskeistä raskauden aikana ei ole ollut tutkittua tietoa, ja syöntirajoituksen tarpeellisuudesta keskustellaan toistuvasti. Syöntisuosituksen tarpeellisuuden arvioimiseksi ja riskinhallinnan suunnittelun tueksi Elintarvikevirasto (EVI) pyysi Eläinlääkintä- ja elintarviketutkimuslaitosta (EELA) arvioimaan raskaudenaikaiseen maksankäyttöön liittyviä riskejä. Riskinarviointi aloitettiin keväällä 2005.

Tutkimuksen tavoitteena oli

1. määrittää suomalaisen sian-, naudan- ja broilerinmaksan nykyiset retinoidipitoisuudet
2. arvioida hedelmällisessä iässä olevien suomalaisnaisten altistumista retinoideille maksaruokien välityksellä
3. arvioida riskiä suositukset ylittävälle retinoidien saannille raskauden aikana mikäli maksaruuat, tai vain jotkin niistä, säilyisivät osana ruokavaliota
4. arvioida muiden kuin syöntirajoituksiin perustuvien riskinhallintatoimenpiteiden vaikutusta retinoidien saantiin
5. arvioida hedelmällisessä iässä olevien suomalaisnaisten altistumista kadmiumille ja lyijylle maksaruokien välityksellä

Riskinarvioinnin päätavoitteena oli A-vitamiinin saannin arviointi, ja raskasmetallien saannin tarkastelun painoarvo oli suhteessa vähäisempi. Arviointi keskittyy ainoastaan suomalaisesta sian-, naudan- tai broilerinmaksasta valmistettuihin tuotteisiin. Maksan tuonti ulkomailta Suomeen on hyvin vähäistä (alle 1,5 % maksan kulutuksesta vuonna 2003) (Finfood 2005), eikä tuontimaksaa siksi sisällytetty arviointiin. Myöskään maksankäyttösuosituksen toteutumista raskauden ajan ruokavaliossa ei tarkasteltu. Aikaisempien tutkimusten mukaan suositusta noudatetaan varsin huonosti: neljännes naisista käyttää maksaa raskauden aikana (Erkkola ym. 2001).

Riskinarvioinnin kohderyhmäksi valittiin hedelmällisessä iässä olevat naiset, koska retinoidien teratogeeniset vaikutukset ovat suurimmat alkuraskaudessa, jolloin raskauden ajan ravitsemusneuvontaa ei ole välttämättä vielä saatu.

Riskinarviointi noudattaa Codex Alimentarius Commissionin periaatteita (CAC 2004). Arvioinnissa on neljä osaa: vaaran tunnistaminen, vaaran kuvaaminen, altistuksen arviointi ja riskin kuvaaminen.

1.2 Vaaran tunnistaminen

1.2.1 A-vitamiini

Määritelmä

A-vitamiini on välttämätön ravintoaine, jolla on tärkeä merkitys monissa biologisissa toiminnoissa. A-vitamiini ei ole yksittäinen kemiallinen yhdiste, vaan yleisnimitys sellaisille retinoidiyhdisteille, joilla on retinolin biologinen aktiivisuus (IUPAC-IUB 1981). Ravitsemuksellisessa mielessä A-vitamiiniksi voidaan lukea myös A-vitamiinin esiasteina toimivat karotenoidit. Karotenoidit muuttuvat elimistössä retinoideiksi, jotta ne ilmentäisivät A-vitamiiniaktiivisuutta. Yhdisteiden A-vitamiiniaktiivisuus ilmoitetaan retinoliekvivalentteina (RE), jolloin 1 µg RE vastaa biologiselta aktiivisuudeltaan 1 µg retinolia.

Tässä riskinarvioinnissa A-vitamiinilla tarkoitetaan kaikkea ravinnossa esiintyvää A-vitamiinia sisältäen sekä retinoidit että A-vitamiinin esiasteina toimivat karotenoidit. Termiä retinoidit käytetään yhteisnimikkeenä retinolille ja sen luonnollisille ja synteettisille johdoksille, joilla on retinolin biologinen aktiivisuus.

Saantilähteet

Ihmiselimistö ei pysty itse valmistamaan A-vitamiinia, joten sitä on saatava ravinnosta. Ravinnossa A-vitamiini esiintyy kahdessa muodossa: retinoideina eläinperäisissä ruoka-aineissa ja karotenoideina kasvikunnan tuotteissa. Maksan retinoidipitoisuudet ovat moninkertaisesti korkeammat kuin minkään muun ruoka-aineen (Heinonen 1990; KTL 2005). Ruoka-aineista saatavan A-vitamiinin lisäksi A-vitamiinia voidaan saada ravintoainevalmisteista joko retinoidi- tai karotenoidimuodossa.

Metabolia

Retinoidit

Retinoidit ovat ravinnossa pääasiassa retinyyliestereinä. Suolessa retinyyliesterit hydrolysoituvat retinoliksi, joka siirtyy suolen seinämän soluihin. Suolen seinämän soluissa retinoli esteröityy uudelleen ja muodostuneet esterit liittyvät kylomikroneihin. Kylomikronit kulkeutuvat imusuoniston kautta verenkiertoon. Pääosa kylomikronien retinyyliestereistä siirtyy maksaan. Maksassa retinyyliesterit hydrolysoituvat retinoliksi, joka sitoutuu erityiseen kantajaproteiiniin (RBP). Kantaja-proteiiniin sitoutunut retinoli voi erittyä verenkiertoon tai siirtyä maksan varastoiviin soluihin. Tarvittaessa retinoli voi nopeasti vapautua varastoivista soluista verenkiertoon. Hyvässä ravitsemustilassa maksassa olevat retinoidit kattavat 90 % koko elimistön retinoidivarastoista (Olson 1996).

Retinolin vapautuminen maksasta on tarkasti säädeltyä, joten plasman retinoidipitoisuus pysyy tasaisena huolimatta vaihtelusta retinoidien saannissa (Olson 1996). Retinoli kuljetetaan kohdekudoksiin kantajaproteiiniinsa sidottuna. Kohdesolussa retinoli hapettuu retinaaliksi ja retinolihapoksi, jotka ovat retinolin aktiivisia metaboliitteja.

Karotenoidit

Ravinnosta imeytyneet karotenoidit muuttuvat pääosin retinoliksi suolen seinämän soluissa (Harrison 2005). Muodostunut retinoli kulkeutuu maksaan kuten edellä on kuvattu. Pieni osa karotenoideista kulkeutuu muuttumattomana kudoksiin plasman lipoproteiineihin sitoutuneena (Debier & Larondelle 2005). Elimistössä karotenoidit varastoituvat pääasiassa rasvakudokseen (Goodman 1984; Olson 1984). Karotenoidit voivat muuttua kudoksissa retinoideiksi (Debier & Larondelle 2005).

Hyötyosuus

Ravinnon retinoideista imeytyy noin 80 % (Olson 1996). Karotenoidien hyötyosuus on retinoideja pienempi johtuen heikommasta imeytymisestä ja melko tehottomasta muuntumisesta retinoideiksi (Thurnham & Northrop-Clewes 1999; Burri ym. 2004). Karotenoidien hyötyosuus riippuu monista tekijöistä, kuten ruuan koostumuksesta ja valmistusmenetelmistä sekä karotenoidien tyypistä ja määrästä ravinnossa. (FNB 2001; van Lieshout et al. 2001). Karotenoideista ß-karoteenilla on suurin A-vitamiiniaktiivisuus. On laskettu, että 12 µg ravinnon ß-karoteenia tai 24 µg muita A-vitamiinin esiasteena toimivia karotenoideja vastaa biologiselta aktiivisuudeltaan 1 µg RE:a (1 µg retinolia) (FNB 2001).

Fysiologiset vaikutukset

A-vitamiinilla on keskeinen merkitys monissa elimistön toiminnoissa. Se vaikuttaa mm. näkökykyyn, kasvuun, solujen erilaistumiseen, elimistön puolustusmekanismeihin, lisääntymiseen ja sikiön kehityksen säätelyyn (Gerster 1997; Clagett-Dame & DeLuca 2002). A-vitamiinin vaikutuksia elimistössä välittävät lähinnä retinolihappojohdokset, jotka aktivoivat tiettyjä tumareseptoreja ja aikaansaavat muutoksia geenien ilmentymisessä (Debier & Larondelle 2005). A-vitamiinin saannissa sekä puute että liikasaanti voivat aiheuttaa monenlaisia haittavaikutuksia. Suurin osa oireista on palautuvia, mutta huomattava saannin epätasapaino voi johtaa pysyviin vaurioihin, joista vakavimpia ovat sikiön epämuodostumat.

Saantisuositukset ja niiden toteutuminen

Suomessa A-vitamiinin suositeltava saanti (RI) on naisilla yleisesti 700 µg RE päivässä, mutta raskauden aikana 800 µg RE päivässä (VRN 2005). Suositeltava saanti tyydyttää A-vitamiinin tarpeen lähes kaikilla terveillä naisilla. Jos päivittäinen saanti alittaa 400 µg RE (alin hyväksyttävä saanti, LI), seurauksena voi olla puutosoireita (NCM 2004). Länsimaissa A-vitamiinin saanti on suosituksiin nähden riittävää (SCF 2002; NCM 2003; Ervin 2004).

1.2.2 Kadmium ja lyijy

Kadmium ja lyijy ovat raskasmetalleja, jotka kertyvät kudoksiin. Kadmium ja lyijy eivät ole elimistölle välttämättömiä, ja altistus näille raskasmetalleille voi aiheuttaa monenlaisia haittavaikutuksia. Tilapäinen siedettävä viikoittainen saanti (PTWI) on kadmiumille 7 µg ruumiinpainokiloa kohden viikossa ja lyijylle 25 µg ruumiinpainokiloa kohden viikossa (WHO 1989b; WHO 1993).

Ruuansulatuskanavassa kadmiumista imeytyy noin 5 % ja lyijystä noin 10 % (IPCS 1992; IPCS 1995; WHO 2000). Imeytymiseen vaikuttavat monet ravitsemukselliset tekijät. Esimerkiksi niukka raudan saanti voi lisätä merkittävästi kadmiumin ja lyijyn imeytymistehokkuutta (IPCS 1992; IPCS 1995).

Imeytynyt kadmium ja lyijy kertyy kudoksiin. Kadmiumilla suurimmat pitoisuudet löytyvät munuaisista ja altistuksen kasvaessa myös maksasta (WHO 1989a). Elimistön lyijykuormasta 90 % on luustossa (IPCS 1995). Luustoon kertynyt lyijy on metabolisesti aktiivista ja nopeasti vapautettavissa vereen. Sen vuoksi raskaus ja muut luun aineenvaihduntaa kiihdyttävät tilat voivat aiheuttaa veren lyijypitoisuuden nousua. Kadmiumin puoliintumisaika kudoksissa on 15-30 vuotta (WHO 1989a). Lyijyn puoliintumisaika luustossa on 20-30 vuotta ja muissa kudoksissa noin kuukausi (IPCS 1995).

Tupakoimattomalla aikuisväestöllä tärkein kadmiumin ja lyijyn lähde on ravinto (IPCS 1992; IPCS 1995). Ruoka-aineista kadmiumia on eniten sisäelimissä, kun taas lyijy on jakautunut melko tasaisesti eri ruoka-aineisiin (EVI 2002). Suomalaiset saavat ravinnosta keskimäärin 10 µg kadmiumia ja 20 µg lyijyä vuorokaudessa (Mustaniemi & Hallikainen 1994; Mustaniemi ym. 1994). Valtaosa suomalaisista jää alle WHO:n antamien saantirajojen.

1.3 Vaaran kuvaaminen

1.3.1 A-vitamiini

A-vitamiinin puute

A-vitamiinin puute on harvinaista länsimaissa mutta se on yleisimpiä ravintoainepuutoksia kehitysmaissa. Puutosoireet ilmenevät vasta pitkään jatkuneen riittämättömän saannin seurauksena, kun maksan A-vitamiinivarastot ovat ehtyneet ja plasman retinoidipitoisuus laskee alle 0,7 µmol/l (SCF 1993).

Tunnetuimmat A-vitamiinin puutteesta johtuvat oireet ovat hämäränäön heikkeneminen sekä silmän sidekalvon ja sarveiskalvon haavaumat, jotka arpeutuessaan voivat johtaa sokeuteen (McLaren & Frigg 2001; Sommer & Davidson 2002). Jo ennen silmäoireiden esiintymistä havaittavia puutosoireita ovat anemia, kasvun hidastuminen ja immuunipuolustuksen heikentyminen (McLaren & Frigg 2001; West 2002).

Monilla nisäkäslajeilla A-vitamiinin puute sikiöaikana johtaa sikiön kuolemaan tai vaikeiden epämuodostumien kehittymiseen (Clagett-Dame & DeLuca 2002). Ihmisillä yhteys A-vitamiinin puutteen ja sikiövaurioiden välillä on edelleen epäselvä, koska A-vitamiinin puutteeseen liittyy yleensä myös muiden sikiönkehityksen kannalta keskeisten ravintoaineiden riittämätön saanti (Gerster 1997).

Retinoidien liikasaanti

Teollisuusmaissa pääasiallisin A-vitamiinin saantiin liittyvä ongelma on retinoidien liikasaannin vaara. Retinoidien liikasaanti ilmenee lapsilla ja aikuisilla A-vitamiinimyrkytyksenä ja sikiöaikana kehityksen häiriintymisenä (retinoidien aiheuttama teratogeneesi). Karotenoidien liikasaannin ei ole osoitettu aiheuttavan A-vitamiinimyrkytystä tai sikiönkehityksen häiriöitä (Bendich 1988; Dawson 2000). Syynä saattaa olla se, että karotenoidien muuttuminen retinoideiksi elimistössä on tarkkaan säädeltyä.

A-vitamiinimyrkytys on yleensä seurausta retinoideja sisältävien ravintoainevalmisteiden väärinkäytöstä, ja ruoka-aineista johtuvat myrkytystapaukset ovat harvinaisia (Bendich & Langseth 1989; Myhre ym. 2003; NCM 2003). Myrkytys voi olla akuutti aiheutuen yksittäisestä hyvin suuresta A-vitamiiniannoksesta, tai krooninen aiheutuen jatkuvasta liikasaannista kuukausien tai jopa vuosien aikana (Hathcock ym. 1990). Aikuisilla akuutin myrkytyksen voi aiheuttaa annos, joka on noin tuhatkertainen suositeltuun päiväannokseen verrattuna, ja kroonisen myrkytyksen annos, joka on noin 15-kertainen suositeltuun päiväannokseen verrattuna (Myhre ym. 2003). A-vitamiinimyrkytyksen oireita ovat yleisoireet, kuten päänsärky ja väsymys, sekä ruuansulatuskanavan, ihon, hermoston ja tuki- ja liikuntaelimistön oireet. Oireet ovat yleensä ohimeneviä, ja häviävät melko nopeasti liikasaannin päätyttyä (Bendich & Langseth 1989).

Teratogeneesi

Monet koe-eläimillä tehdyt tutkimukset ovat osoittaneet, että raskauden aikana tapahtuva retinoidien liikasaanti voi aiheuttaa sikiövaurioita ja että vaurioita syntyy jo pitoisuuksilla, jotka eivät aiheuta emotoksisuutta (Tzimas & Nau 2001). Sikiövaurioita voi syntyä lähes kaikkiin elinjärjestelmiin (Miller & Hayes 1982; Bendich & Langseth 1989). Vaurioiden tyyppi ja vaikeusaste riippuu altistuksen suuruudesta ja sikiönkehityksen vaiheesta (Biesalski 1989). Vaikka havaittavia rakenteellisia epämuodostumia ei syntyisikään, liiallinen retinoidialtistus voi johtaa käyttäytymismuutoksiin, jotka ilmenevät vasta myöhemmällä iällä. Koe-eläimillä retinoidien liikasaantiin on havaittu liittyvän mm. oppimisvaikeuksia ja aktiivisuustason muutoksia (Holson ym. 1997). Retinoidien liikasaanti lisää myös sikiökuolleisuutta (Akase ym. 2003). Yksittäinenkin suuri retinoidiannos raskauden aikana voi olla haitallinen sikiölle (Biesalski 1989; Piersma ym. 1996).

Ihmisillä retinoidien aiheuttamat sikiövauriot ovat tunnettuja terapeuttisesti käytettyjen retinoidijohdosten (isotretinoiini, etretinaatti) yhteydessä (Rosa ym. 1986). Sen sijaan luonnollisten retinoidien (retinoli ja sen esterit) teratogeenisyydestä on enemmän epävarmuutta. Ravintoainevalmisteista saadut suuret retinoidiannokset on liitetty synnynnäisten epämuodostuminen kehittymiseen noin 20 tapausselostuksessa (Rosa ym. 1986). Niiden lisäksi on raportoitu yksi tapaus, jossa sikiön epämuodostumat saattoivat liittyä maksaruuista saatuun suureen retinoidialtistukseen (Buss ym. 1994). Retinoidien aiheuttamat epämuodostumat sijoittuvat tyypillisimmin pään ja kasvojen alueelle, keskushermostoon ja verenkiertoelimistöön (taulukko 1). Ihmisellä herkin aika retinoidien aiheuttamien rakenteellisten epämuodostumien synnylle näyttäisi olevan sikiönkehityksen 2.–5. viikko (Rosa ym. 1986). Rakenteellisten epämuodostumien lisäksi terapeuttisten retinoidijohdosten on havaittu aiheuttavan älyllisen suorituskyvyn alenemista, jonka astetta ei voida suoraan ennustaa rakenteellisten epämuodostumien olemassaolon tai puuttumisen perusteella (Vorhees 1994).

Taulukko 1. Sikiöaikana suurille retinoidiannoksille altistuneiden lasten tyypillisimpiä rakenteellisia epämuodostumia.

Pään ja kasvojen alue
- pienikokoiset tai puuttuvat ulkokorvat ja korvakäytävät
- alas sijoittuneet korvat
- pienileukaisuus
- kitalakihalkio

Keskushermosto
- vesipäisyys
- pienipäisyys
- aivojen vajaakehitys
- verkkokalvon tai näköhermon epämuodostumat

Verenkiertoelimistö
- aortan kaaren epämuodostumat
- valtasuonten transpositio
- kammioväliseinän puutokset

Kateenkorva
- kateenkorvan vajaakehitys

Lähteet: Lammer et al. 1985; Rosa et al. 1986

Teratogeneesin mekanismit perustuvat muutoksiin geenien transkriptiossa. Normaali sikiönkehitys on tulos geenitoiminnan tarkasta säätelystä ajan ja paikan suhteen. Liiallinen retinoidialtistus voi aiheuttaa aktivoitumista niissä geeneissä, joiden tulisi olla hiljaisia, tai estymistä niissä geeneissä, joiden tulisi toimia (Ross ym. 2000). Muutokset solun aineenvaihdunnassa ja solunvälisessä viestinnässä aiheuttavat häiriöitä solujen jakautumisessa, liikkumisessa ja erilaistumisessa, mikä heijastuu koko sikiönkehitykseen.

Teratogeneesin annos-vaste

Epidemiologiset selvitykset ovat tällä hetkellä ainoa käyttökelpoinen tapa selvittää retinoidien teratogeneesin annos-vaste–suhdetta ihmisellä. Eläinkokeista saadut tulokset ovat vaikeasti sovellettavissa ihmiseen, koska retinoidien aineenvaihdunta, kinetiikka ja toksikodynamiikka vaihtelevat suuresti eläinlajeittain (Tzimas & Nau 2001).

Käytettävissä olevat epidemiologiset tiedot ovat hyvin rajalliset, minkä vuoksi selkeän annos–vaste-suhteen määrittäminen ihmisellä ei ole mahdollista (Rosa ym. 1986; SCF 2002; NCM 2003). Myös epämuodostumien syntyyn vaadittavan altistuksen kesto on tuntematon. Tapausselostuksissa, joissa synnynnäiset epämuodostumat on liitetty suureen ravintoperäiseen retinoidialtistukseen, retinoidien saanti oli ollut vähintään 5400 μg RE/vrk, ja käyttö oli jatkunut usean raskausviikon ajan (Rosa ym. 1986). Tapaus–verrokki-tutkimuksissa retinoidialtistuksen ei ole havaittu lisäävän epämuodostumien riskiä, mikäli annos on 3000 μg RE/vrk tai vähemmän (NCM 2003). Monet tahot suosittelevatkin, että hedelmällisessä iässä olevien naisten retinoidien saanti ruuasta ja ravintoainevalmisteista tulisi olla korkeintaan 3000 μg RE/vrk (FNB 2001; SCF 2002; EVM 2003). Koska myös yksittäinen suuri retinoidiannos saattaa olla teratogeeninen, retinoideja sisältäviä ravintoainevalmisteita ei suositella käytettäväksi raskauden aikana yli 7500 μg RE (25 000 IU) sisältävinä kerta-annoksina (WHO 1998).

1.3.2 Kadmium ja lyijy

Kadmium

Pitkäaikainen kadmiumaltistus voi aiheuttaa monenlaisia terveydellisiä haittavaikutuksia. Munuaiset, etenkin munuaisten kuoriosa, näyttäisi olevan keskeisin vaikutuskohta ympäristöperäisesti altistuneessa väestössä (WHO 2001). Munuaisvaikutusten lisäksi kadmium voi aiheuttaa luuntiheyden laskua ja neurologisia oireita sekä altistaa syövän kehittymiselle (WHO 2001). Raskauden aikainen kadmiumaltistus on liitetty ennenaikaisiin synnytyksiin ja lapsen alhaiseen syntymäpainoon (Nishijo ym. 2002). Vain pieni osa äidin veressä olevasta kadmiumista läpäisee istukan (Korpela ym. 1986). Kadmium vaikuttaa kuitenkin istukan hormonituotantoon, mikä saattaa altistaa ennenaikaiselle synnytykselle (Henson & Chedrese 2004).

Kadmiumaltistusta voidaan mitata virtsan tai veren kadmiumpitoisuuden avulla. On arvioitu, että kad-

miumin aiheuttamien munuaisvaurioiden esiintyvyys väestössä kasvaa kun virtsan kadmiumpitoisuus ylittää 2,5 μg/l (2,5 μg/g kreatiniinia) (Järup ym. 1998). Taso vastaa tilannetta, jossa munuaisten kadmiumpitoisuus on 50 μg/g ja kadmiumin päivittäinen saanti on noin 50 μg/vrk. Myös kadmiumin aiheuttamien raskauskomplikaatioiden riski kasvaa, kun virtsan kadmiumpitoisuus on yli 2 μg/l (Nishijo ym. 2002).

Lyijy

Lyijyllä on haittavaikutuksia moniin elimiin ja elinjärjestelmiin (IPCS 1995). Hemin muodostuksen häiriintymisestä johtuva anemia ja hermostolliset vaikutukset ovat pitkäaikaisen lyijyaltistuksen tunnetuimpia oireita. Muita lyijyn haittoja ovat munuaisvauriot, verenpaineen nousu ja kalsiumaineenvaihdunnan häiriöt (IPCS 1995; Papanikolaou ym. 2005). Lyijy vaikuttaa monien elimistön entsyymijärjestelmien toimintaan ja siten moniin solunsisäisiin tapahtumiin (IPCS 1995).

Lyijy kulkeutuu helposti istukan läpi ja kertyy sikiöön 12. raskausviikosta lähtien (Papanikolaou ym. 2005; Harville ym. 2005). Lyijy voi aiheuttaa sikiönkehityksen häiriöitä. Herkimmin vaikutus näkyy sikiön neurolopsykologisen kehityksen häiriintymisenä (UNEP 1998; Schnaas 2006). Lyijy lisää myös sikiökuolemien ja keskenmenon riskiä ja saattaa aiheuttaa lievien rakenteellisten poikkeavuuksien kehittymistä (WHO 2003).

Lyijyaltistusta voidaan mitata veren lyijypitoisuuden avulla. Vuonna 1994 suomalaisten naisten veren lyijypitoisuus oli keskimäärin 0,11 μmol/l (23 μg/l) tulosten vaihdellessa välillä 0–0,36 μmol/l (0–74 μg/l) (Alfthan ym. 1994). Kroonisen lyijymyrkytyksen oireet ilmenevät veripitoisuuksilla 400–800 μg Pb/l (UNEP 1998; WHO 2003). Biokemiallisia vaikutuksia hemin synteesiin havaitaan veripitoisuuksilla 30–300 μg Pb/l (WHO 2003). Niillä raskaana olevilla naisilla, joilla veren lyijypitoisuus on yli 140 μg/l, ennenaikaisen synnytyksen riski on nelinkertainen verrattuna niihin naisiin, joilla veren lyijypitoisuus on 80 μg/l tai vähemmän (WHO 2003). Sen sijaan yhdelle lyijyn vakavimmista haittavaikutuksista, sikiön kognitiivisen kehittymisen häiriintymiselle, ei näyttäisi olevan veripitoisuuden kynnysarvoa (Schnaas ym. 2006).

Koska lyijy voi aiheuttaa haittavaikutuksia jo pieninäkin pitoisuuksina eikä vaikutusten ilmenemiselle ole voitu osoittaa selkeää kynnysarvoa (UNEP 1998), lyijyaltistusta voidaan pitää haitallisena altistuksen määrästä riippumatta.

1.4 Saannin arviointi

Saannin arvioimiseksi kerättiin neljänlaista tietoa:

1. Maksaruokien kulutustiedot suomalaisten 25–44-vuotiaiden naisten osalta saatiin Finravinto 2002 -tutkimuksesta (Männistö ym. 2003). Myös tiedot A-vitamiinin saannista muista lähteistä kuin maksaruuista perustuvat Finravinto 2002 -tutkimukseen.

2. Käytetyissä maksaruuissa olevan maksan tyyppi ja määrä arvioitiin perustuen elintarviketeollisuudelta ja Kansanterveyslaitokselta saatuihin reseptitietoihin.

3. Raaka-ainemaksan A-vitamiinipitoisuuden selvittämiseksi laboratoriossa analysoitiin näytteitä sian-, naudan- ja broilerinmaksoista.

4. Maksojen kadmium- ja lyijypitoisuudet arvioitiin vierasainevalvontaohjelman (NRCP) yhteydessä vuosina 2000–2004 tehtyjen pitoisuusmääritysten perusteella.

Kerätyn tiedon perusteella rakennettiin matemaattinen simulaatiomalli, jonka avulla arvioitiin naisten altistumista A-vitamiinille, kadmiumille ja lyijylle maksaruokien välityksellä. Mallinnustuloksia verrattiin A-vitamiinin saantisuosituksiin sekä retinoidien ja raskasmetallien saantirajoihin.

1.4.1 Maksan käyttö

Finravinto-tutkimuksessa kulutustiedot kerättiin 48 tunnin ruuankäyttöhaastattelulla. Osa osallistujista täytti lisäksi kolmen päivän ruokapäiväkirjan kahdesti (8 päivän tutkimus). Valitun ikäryhmän naisista 529 osallistui 48 tunnin ruuankäyttöhaastatteluun, ja heistä 62 täytti myös ruokapäiväkirjat. Kaiken kaikkiaan 60 (11,3 %) 529 naisesta raportoi maksaruokien käyttöä. 48 tunnin haastattelussa 44 naista (8,3 %) söi maksaruokia. 8 päivän tutkimuksessa maksansyöjiä oli 24 (38,7 %). Yleisimmin käytetty maksaruoka oli maksamakkara tai -pasteija, ja seuraavaksi yleisin oli maksalaatikko. Vain muutama naisista raportoi muiden maksaruokien (jauhemaksapihvi tai maksakastike) käyttöä. Jokaiselle maksaruualle tuotettiin jakaumat annoskoosta ja tuotteen käyttötiheydestä.

1.4.2 Maksa maksaruuissa

Käytettyjen maksaruokien maksapitoisuudet määritettiin reseptitietojen avulla. Maksapitoisuudet ilmoitettiin raakamaksan painoprosentteina lopputuotteesta. Einesten (maksamakkara ja -pasteija, maksalaatikko) osalta reseptit saatiin elintarviketeollisuudelta. Maksan osuus tuotteessa vaihteli eri valmistajilla. Maksamakkarassa ja -pasteijassa maksan osuus oli 8–42 % ja maksalaatikossa 10–21 %. Simulaatiomallia varten eineksille laskettiin keskimääräinen maksapitoisuus siten, että kunkin tuot-

teen maksapitoisuutta painotettiin tuotteen myyntiosuudella (vuoden 2004 tilanne). Näin laskettuna keskimääräinen raaka-ainemaksan osuus suomalaisessa maksamakkarassa tai -pasteijassa oli 20,5 % ja maksalaatikossa 13,6 %. Maksamakkarassa ja -pasteijassa valmistajat käyttivät lähes yksinomaan sianmaksaa, maksalaatikossa puolestaan seoksia eri eläinlajien maksoista.

Jauhemaksapihvin ja maksakastikkeen yleisesti käytetyt reseptit saatiin Kansanterveyslaitokselta. Saadun tiedon perusteella jauhemaksapihvin maksapitoisuudeksi oletettiin 50 % ja maksakastikkeen maksapitoisuudeksi 33 %. Molemmissa ruuissa raaka-aineena oletettiin käytettävän naudan ja sian maksaa suhteessa 2:1.

Resepteissä käytetty sianmaksa sisältää sekä lihasikojen että emakkojen maksan. Suomalaisen elintarviketeollisuuden mukaan emakkojen osuus käytetystä sianmaksasta on korkeintaan 10 %. Emakoilla maksan retinoidipitoisuus on selvästi suurempi kuin lihasioilla, ja siksi näitä kahta maksatyyppiä käsiteltiin riskinarvioinnissa erikseen

Valmistuserän koko voi vaikuttaa tuotteen retinoidipitoisuuden vakauteen eri valmistuserien välillä. Suurissa valmistuserissä matalan ja korkean retinoidipitoisuuden maksat kompensoivat toistensa vaikutuksia. Sen sijaan pienissä valmistuserissä lopputuotteen retinoidipitoisuudessa on enemmän sattuman aiheuttamaa vaihtelua. Suomalaisessa elintarviketeollisuudessa yhteen erään maksamakkaraa tai -pasteijaa käytetään noin 20–120 kg maksaa. Yhteen erään maksalaatikkoa suurimmat valmistajat käyttävät noin 170 kg maksaa. Jauhemaksapihvejä ja maksakastiketta tarjoillaan ravintoloissa ja työpaikkaruokaloissa, joissa valmistuserän koko vaihtelee asiakasmäärän mukaan. Maksaruokia valmistetaan pienissä erissä myös kotitalouksissa.

1.4.3 Maksan A-vitamiini

Maksanäytteet sian-, naudan- ja broilerinmaksoista kerättiin suomalaisilta teurastamoilta keväällä 2005. Sian- ja naudanmaksoja käsiteltiin yksittäisinä näytteinä, mutta kukin broilerinäytteistä koostui kymmenestä maksasta, jotka olivat peräisin saman tuottajan linnuista. A-vitamiinimääritykset tehtiin 76 nautanäytteestä, 128 sikanäytteestä (91 lihasikoja, 37 emakkoja) ja 27 broilerinäytteestä. Kaikki broilerit oli teurastettu 35 päivän ikäisinä ja lihasiat 6 kuukauden ikäisinä. Nautojen ikä vaihteli 13 kuukaudesta 12 vuoteen. Emakkojen ikää ei määritelty. Mahdollista vuodenaikaisvaihtelua maksojen A-vitamiinipitoisuuksissa ei tutkittu eikä otettu huomioon simulaatiomallilla tehdyissä laskelmissa.

Maksanäytteistä analysoitiin retinoli ja retinolin esterit. Analyysit tehtiin korkean erotuskyvyn nestekromatografialla (HPLC) käyttäen diodirividetektoria 325 nm aallonpituudella. Havaitsemisraja oli retinolille 0,02 μg/g ja retinolin estereille 0,03 μg/g. Suhteellinen keskihajonta (RSD%) toistettavuudelle oli naudanmaksassa 12 %, sianmaksassa 6 % ja broilerinmaksassa 11 %. Kunkin näytteen kokonais-A-vitamiiniaktiivisuus (μg RE/g maksaa) laskettiin analysoitujen retinoidiyhdisteiden aktiivisuuden summana. A-vitamiinipitoisuuden keskiarvo oli lihasianmaksassa 170 μg RE/g (mediaani 164 μg RE/g, vaihteluväli 28–549 μg RE/g), emakonmaksassa 443 μg RE/g (mediaani 337 μg RE/g, vaihteluväli 50–2123 μg RE/g), naudanmaksassa 213 μg RE/g (mediaani 175 μg RE/g, vaihteluväli 44–744 μg RE/g) ja broilerinmaksassa 106 μg RE/g (mediaani 97 μg RE/g, vaihteluväli 34–277 μg RE/g). Ero maksojen retinoidipitoisuuksissa oli tilastollisesti merkittävä eri lajien välillä (log-muunnos, Kruskallin-Wallisin testi, $X^2 = 46,7$; $P < 0,001$). Parittaisissa vertailuissa ero oli merkitsevä ($P \leq 0,001$) kaikkien muiden parien paitsi sian- ja naudanmaksan välillä ($P = 0,16$) (Mannin-Whitneyn testi).

Analysoidut keskimääräiset pitoisuudet olivat naudan- ja sianmaksassa samaa tasoa kuin 1990-luvulla. Sianmaksassa keskimääräiset pitoisuudet olivat selvästi alhaisempia kuin vuonna 1989 julkistetut tulokset (600 μg RE/g, Heinonen 1990). Naudalla eroa vuoden 1989 tuloksiin ei käytännössä ollut. Broilerinmaksan retinoidipitoisuudet ovat laskeneet 1990-luvulta tähän päivään: vuonna 1989 broilerinmaksan keskimääräinen retinoidipitoisuus oli 370 μg RE/g (Heinonen 1990) ja vuonna 1991 180 μg RE/g (Hirvi ym. 1992). Nyt mitatut maksojen retinoidipitoisuudet vastaavat suomalaisessa kirjallisuudessa esitettyjä pitoisuuksia (KTL 2005; Reinivuo ym. 2005).

Itävallassa mitatut naudan- ja broilerinmaksojen retinoidipitoisuudet (naudalla 41 μg RE/g ja broilerilla 56 μg RE/g, Majchrzak ym. 2006) ovat selvästi alhaisemmat kuin Suomessa ja muissa pohjoismaissa. Syynä ovat todennäköisesti erot tuotantotavoissa. Itävallan matalat retinoidipitoisuudet terveillä eläimillä kuitenkin viittaavat siihen, että nykyisenkaltaisen retinoidien käytön tarpeellisuutta suomalaisessa kotieläinkasvatuksessa olisi mahdollista arvioida uudelleen.

1.4.4 A-vitamiini muista lähteistä

Arviot A-vitamiinin saannista muista lähteistä kuin maksasta perustuivat tuloksiin 8 päivän Finravinto 2002 -tutkimuksesta ($n = 62$). A-vitamiinin saanti maksaa sisältämättömistä elintarvikkeista ja ravintoainevalmisteista laskettiin käyttäen elintarvikkeiden kansallista koostumustietokantaa (Fineli®) (KTL 2005). Tavanomainen päivittäinen saanti arvioitiin käyttäen menetelmää, jonka on kuvannut Nusser ym. (1996). A-vitamiinin saannin mediaani oli 440 μg RE/vrk (5.–95. persentiilin vaihteluväli 260–720 μg RE/vrk), mikäli mukaan oli laskettu vain saanti ruuasta, ja 490 μg RE/vrk (5.–95. persentiilin vaihteluväli 240–970 μg RE/vrk), mikäli mukaan oli laskettu myös ravintoainevalmisteista saatava A-vitamiini. Retinoidien saannin mediaani oli 250 μg RE/vrk riip-

pumatta eroista saantilähteissä. Retinoidien saannissa 5.–95. persentiilin vaihteluväli oli 140–430 µg RE/vrk, kun huomioitiin vain saanti ruuasta, ja 120–530 µg RE/vrk, kun huomioitiin myös ravintoainevalmisteiden retinoidit.

1.4.5 Maksan kadmium ja lyijy

Tiedot maksan kadmium- ja lyijypitoisuuksista saatiin Eläinlääkintä- ja elintarviketutkimuslaitoksesta (EELA). Tiedot perustuivat mittauksiin, jotka oli tehty kansallisen vierasainevalvontaohjelman (NRCP) yhteydessä vuosina 2000–2004. Kunakin vuonna raskasmetallit oli analysoitu 5-10 broilerinmaksasta ja noin 30 naudan- ja sianmaksasta. Riskinarvioinnissa käytettiin maksojen kadmium- ja lyijypitoisuuksien keskimääräisiä vuosikeskiarvoja. Kadmiumin keskimääräinen pitoisuus oli naudoilla ja sioilla 25 µg Cd/kg maksaa ja broilereilla 10 µg Cd/kg maksaa. Lyijyn keskimääräinen pitoisuus oli kaikilla lajeilla noin 45 µg Cd/kg maksaa.

1.4.6 Simulaatiomalli

Suomalaisten hedelmällisessä iässä olevien naisten A-vitamiinin, kadmiumin ja lyijyn saantia maksaruuista arvioitiin matemaattisen simulaatiomallin avulla. Simulaatiomallin rakenne on esitetty kuvassa 1. Lähtöarvoihin liittyvä epävarmuus on sisällytetty malliin.

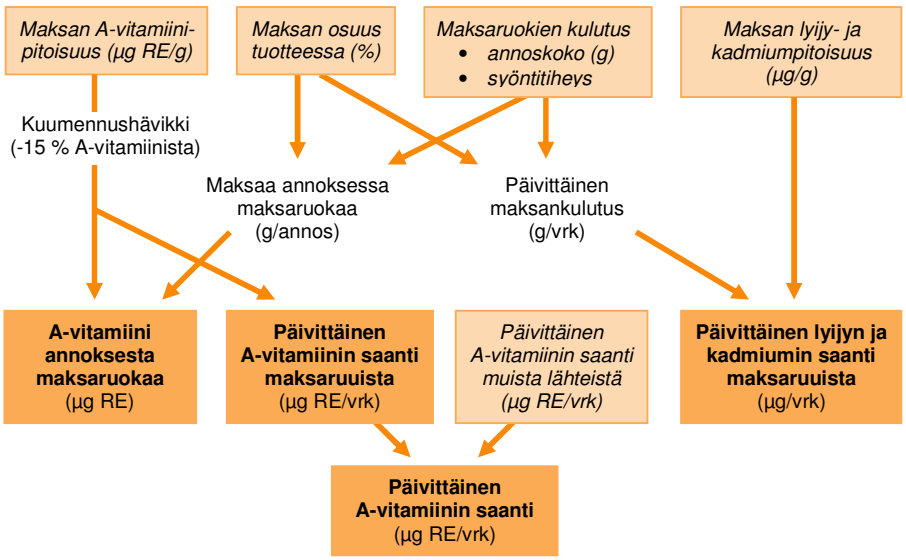
Mallissa maksaperäinen retinoidien saanti laskettiin sekä keskimääräisenä päiväsaantina että saantina kerta-annoksesta. Kokonais-A-vitamiinille laskettiin vain keskimääräinen päiväsaanti.

Kerta-annoksesta saatava retinoidialtistus voi vaihdella suuresti annoksittain riippuen raaka-aineena käytettyjen maksojen retinoidipitoisuuksista. Tämän vuoksi retinoidien kerta-annossaanti arvioitiin kahdessa tilanteessa:

1. Raaka-aineena käytetyt maksat on poimittu satunnaisesti retinoidipitoisuuden jakaumalta (keskimääräisen retinoidipitoisuuden tilanne).

2. Raaka-aineena käytetyt maksat on poimittu retinoidipitoisuuden jakauman ylimmästä kymmenyksestä (korkean retinoidipitoisuuden tilanne).

Mallissa käytettiin Monte Carlo -tekniikkaa, jonka avulla tulokset altistumisesta saatiin ilmaistua todennäköisyysjakaumina. Malli rakennettiin Excel-taulukkolaskentaohjelmassa (Microsoft Corporation, USA), ja mallinnus suoritettiin kaupallisella ohjelmalla (@Risk, versio 4.5, Palisade Corporation, USA, 2004). Monte Carlo -simulaatiossa lähtöarvot voidaan esittää jakaumina piste-estimaattien sijaan. Mallinnuksen aikana tapahtumaketjua toistetaan lukuisia kertoja lähtötietojen jakaumista satunnaisesti poimituilla arvoilla.



Kuva 1. A-vitamiinin, kadmiumin ja lyijyn saannin arvioimiseen käytetyn mallin rakenne. Yhdistelemällä käytettävissä olevaa empiiristä aineistoa (*kursivoitu*) voitiin laskennallisesti arvioida A-vitamiinin ja raskasmetallien saantia maksaruuista sekä A-vitamiinin kokonaissaantia maksansyöjillä (**lihavoidut** osat).

Mallissa oletettiin, että kulutustiedot ja laboratoriotulokset edustavat todellisia populaatioita, ja että emakonmaksat ovat tasaisesti jakautuneet ruuantuotannossa käytettyihin sianmaksaeriin. A-vitamiinin saannin arvioinnissa käytettiin seuraavia viitearvoja:

- A-vitamiinin saanti: Pohjoismaiset ravitsemussuositukset (NCM 2004)
  - Suositeltava saanti (RI): 800 µg RE/vrk (raskaana oleville naisille)
  - Keskimääräinen saantitarve (AR): 500 µg RE/vrk (naisille)
  - Alin hyväksyttävä saanti (LI): 400 µg RE/vrk (naisille)
- Ylin hyväksyttävä päivittäinen saanti (UL) retinoideille: 3000 µg RE/vrk (SCF 2002)
- Ylin hyväksyttävä saanti retinoideille kerta-annoksesta: 7500 µg RE. Raja asetettiin perustuen tapausselostuksiin retinoidien aiheuttamista sikiövaurioista (Rosa ym. 1986) ja annettuihin ohjeisiin retinoidivalmisteiden käytöstä (WHO 1998).

1.4.7 Skenaariot

Riskinhallintatoimenpiteiden vaikutus

Mallin avulla arvioitiin paisi suomalaisten naisten tämänhetkistä A-vitamiinin saantia, myös eri riskinhallintatoimenpiteiden vaikutusta A-vitamiinin saantiin. Riskinhallintatoimenpiteet voivat kohdistua elintarviketuotantoon tai kuluttajaan. Tämän vuoksi riskinarvioinnissa tarkasteltiin kahta toimenpidevaihtoehtoa:

1. Emakonmaksaa ei käytetä elintarvikkeiden raaka-aineena.
2. Raskauden aikana syödään maksamakkaraa ja -pasteijaa, mutta ei muita maksaruokia.

Maksojen retinoidipitoisuuden alentaminen

Eläinperäisten tuotteiden retinoidipitoisuus on riippuvuussuhteessa eläinten ruokinnassa käytettyjen rehujen retinoidipitoisuuteen. Eläinten ravitsemusta käsittelevä tiedekomitea toteaa äskettäin julkaistussa raportissaan, että tuotantoeläinten rehujen retinoidipitoisuuksien alentamisen mahdollisuutta ja sen vaikutuksia kuluttajien retinoidien saantiin tulisi selvittää (SACN 2005). Tämän taustan huomioiden mallinsimme ravinnosta saatavan A-vitamiinin määrää tilanteessa, jossa maksojen retinoidipitoisuus olisi 10–90 % nykyisestä tasosta. Skenaariossa mallinnettiin vain seurauksia ottamatta kantaa siihen, olisiko retinoidipitoisuuden alentaminen käytännössä mahdollista. Skenaariossa oletettiin, että A-vitamiinipitoisuudet muissa eläinperäisissä elintarvikkeissa kuin maksassa säilyisivät muuttumattomina.

1.5 Riskin kuvaaminen

1.5.1 A-vitamiinin ja retinoidien päivittäinen saanti

Simulaatiomallin mukaan A-vitamiinin saannin mediaani on maksaa syövillä 1170 µg RE/vrk (5.–95. persentiilin vaihteluväli 500–4700 µg RE/vrk) ja muilla 490 µg RE/vrk (5.–95. persentiilin vaihteluväli 240–970 µg RE/vrk), kun mukaan on laskettu sekä ruuasta että ravintoainevalmisteista saatava A-vitamiini. Neljännes maksansyöjistä jäi alle raskaudenaikaisen A-vitamiinin saantisuosituksen (800 µg RE/vrk), mutta keskimääräinen saantitarve (500 µg RE/vrk) täyttyi lähes kaikilla. Sitä vastoin maksaa käyttämättömistä suurin osa jäi alle suositeltavan saannin, ja kolmanneksella saanti alitti jopa alimman hyväksyttävän saannin rajan (400 µg RE/vrk). Maksansyöntiin verrattuna ravintoainevalmisteiden käytöllä oli vain vähäinen vaikutus A-vitamiinin saantiin.

Mallin mukaan retinoidien saannin mediaani on maksaa syövillä 880 µg RE/vrk (5.–95. persentiilin vaihteluväli 310–4430 µg RE/vrk) ja muilla 250 µg RE/vrk (5.–95. persentiilin vaihteluväli 120–530 µg RE/vrk), kun mukaan on laskettu sekä ruuasta että ravintoainevalmisteista saatavat retinoidit. 11 % maksansyöjistä ylittää ylimmän hyväksytyn saantitason (3000 µg RE/vrk) riippumatta siitä, käyttävätkö he lisävitamiineja vai eivät. Niillä naisilla, jotka eivät syö maksaa, ei ole riskiä ylittää ylintä hyväksyttyä saantitasoa.

Skenaarioiden vaikutus A-vitamiinin ja retinoidien keskimääräiseen päiväsaantiin

Skenaarioiden vaikutusta maksansyöjien A-vitamiinialtistukseen arvioitiin tilanteessa, jossa A-vitamiinia saadaan sekä ruuasta että ravintoainevalmisteista.

Tulosten mukaan emakonmaksan elintarvikekäytöstä luopuminen ei juuri vaikuttaisi retinoidien saantiin maksansyöjillä (taulukko 2). Jos emakonmaksan käytöstä luovuttaisiin, retinoidien saannin mediaani laskisi tasolta 880 µg RE/vrk tasolle 810 µg RE/vrk (5.–95. persentiilin vaihteluväli 300–4260 µg RE/vrk). Retinoidien ylimmän hyväksytyn saantitason (3000 µg RE/vrk) ylittävien naisten osuus laskisi 11 %:sta 10 %:iin (taulukko 3). Emakonmaksan käytöstä luopuminen ei vaikuttaisi merkittävästi myöskään kokonais-A-vitamiinin saantiin (taulukot 2 ja 3).

Sen sijaan maksansyönnin osittaisella rajoittamisella olisi huomattava vaikutus A-vitamiinin ja retinoidien saantiin (taulukot 2 ja 3). Mikäli naiset söisivät maksamakkaraa ja -pasteijaa, mutta eivät muita maksaruokia, vain alle 1 %:lla naisista retinoidien saanti ylittäisi 3000 µg RE/vrk. Retinoidien saannin mediaani olisi 580 µg RE/vrk (5.–95. persentiilin vaihteluväli 270–1510 µg RE/vrk), eli selvästi alhai-

Taulukko 2. Simulaatiomallin avulla lasketut A-vitamiinin ja retinoidien keskimääräiset päiväsaannit ruuasta ja ravintoainevalmisteista suomalaisilla 25–44-vuotiailla naisilla. Maksaa syövien osalta taulukossa on esitetty myös tutkittujen riskinhallintaskenaarioiden vaikutus saantiin.

	A-vitamiini (µg RE vrk^{-1})		Retinoidit (µg RE vrk^{-1})	
Lähde/skenaario	Mediaani	5.–95. persentiili	Mediaani	5.–95. persentiili
Ei syö maksaa	490	240–970	250	120–530
Syö maksaa				
Nykytila [1]	1170	500–4700	880	310–4430
Ei emakkoja [2]	1090	470–4540	810	300–4260
Rajoitettu käyttö [3]	850	420–1850	580	270–1510

[1] Mukana kaikkien maksatuotteiden käyttö. Käytetystä sianmaksasta 90 % lihasianmaksaa ja 10 % emakonmaksaa.
[2] Kaikki käytetty sianmaksa lihasianmaksaa
[3] Vain maksamakkaraa ja -pasteijaa, ei muita maksaruokia (maksalaatikko, jauhemaksapihvi, maksakastike)

sempi kuin mitä saavutettaisiin emakonmaksan käytöstä luopumalla. Kokonais-A-vitamiinin saanti vähenisi myös huomattavasti (mediaani 850 µg RE/vrk, 5.–95. persentiilin vaihteluväli 420–1850 µg RE/vrk). Tämän seurauksena 45 % naisista jäisi alle suositeltavan saannin rajan (800 µg RE/vrk) ja 4 % naisista jäisi alle alimman hyväksyttävän saantitason (400 µg RE/vrk).

Mikäli maksansyönnin osittaista rajoittamista vastaava tilanne haluttaisiin aikaansaada maksojen retinoidipitoisuutta vähentämällä, pitoisuuksien tulisi laskea alle neljännekseen nykyisestä tasosta. Jos retinoidipitoisuudet vähenisivät puoleen nykyisestä, 2,5 % maksaa syövistä naisista ylittäisi edelleen 3000 µg RE/vrk saantirajan.

1.5.2 Retinoidien kerta-annossaanti

Kerta-annokseksi katsottiin päivän aikana syödyn tuotteen yhteenlaskettu määrä. Saantiarviot laskettiin erikseen kullekin tutkitulle maksaruualle.

Yksittäiset annokset maksamakkaraa ja -pasteijaa eivät näyttäisi keskimäärin altistavan haitallisille retinoidiannoksille (taulukko 4). Sen sijaan retinoidialtistus annoksesta maksakastiketta tai etenkin annoksesta jauhemaksapihviä ylittää selvästi sen rajan, mikä on katsottu raskauden aikana turvalliseksi. Maksalaatikko jää näiden ääripäiden väliin, ja sen kohdalla saantirajan ylittyminen on riippuvainen raaka-aineena käytettyjen maksojen retinoidipitoisuuksista (taulukko 4).

Emakonmaksan käytön lopettamisella olisi vain vähäinen vaikutus retinoidien saantiin kerta-annoksesta maksaruokaa (taulukko 4). Jauhemaksapihvistä

Taulukko 3. A-vitamiinin ja retinoidien päivittäiset saannit suhteessa saantisuosituksiin ja -rajoihin suomalaisilla 25–44-vuotiailla naisilla. Simulaatiomallin avulla lasketut tulokset, jossa saantilähteenä olivat ruoka ja ravintoainevalmisteet. Maksaa syövien osalta taulukossa on esitetty myös tutkittujen riskinhallintaskenaarioiden vaikutus.

	Osuus (%) naisista, joilla A-vitamiinin saanti on			Osuus (%) naisista, joilla retinoidien saanti on > UL [7]
Lähde/skenaario	< LI [4]	< AR [5]	< RI [6]	
Ei syö maksaa	32	52	88	0
Syö maksaa				
Nykytila [1]	2	5	26	11
Ei emakkoja [2]	2	6	29	10
Rajoitettu käyttö [3]	4	11	45	< 1

[1] Mukana kaikkien maksatuotteiden käyttö. Käytetystä sianmaksasta 90 % lihasianmaksaa ja 10 % emakonmaksaa.
[2] Kaikki käytetty sianmaksa lihasianmaksaa
[3] Vain maksamakkaraa ja -pasteijaa, ei muita maksaruokia (maksalaatikko, jauhemaksapihvi, maksakastike)
[4] LI = Alin hyväksyttävä saanti, 400 µg RE vrk^{-1} (Nordic Council of Ministers 2004)
[5] AR = Keskimääräinen tarve, 500 µg RE vrk^{-1} (Nordic Council of Ministers 2004)
[6] RI = Suositeltava saanti raskauden aikana, 800 µg RE vrk^{-1} (Nordic Council of Ministers 2004)
[7] UL = Suurin hyväksyttävä saanti raskauden aikana, 3000 µg RE vrk^{-1} (Scientific Committee on Food 2002)

Taulukko 4. Nykyinen retinoidien saanti (µg RE) kerta-annoksesta eri maksaruokia ja emakonmaksan käytöstä luopumisen vaikutus saantiin suomalaisilla 25–44-vuotiailla naisilla. Taulukossa on esitetty retinoidien saannin mediaanit ja 5.–95. persentiilin vaihteluvälit sekä niiden naisten osuus, joilla saanti ylittää raskaudenaikaisen ylimmän hyväksytyn kertasaannin (7500 µg RE). Tulokset on laskettu simulaatiomallin avulla, ja laskelmissa on huomioitu vain maksasta saatava retinoidiannos.

	Maksan retinoidipitoisuus					
	Keskimääräinen [3]			Korkea [4]		
Lähde ja skenaario	Mediaani	5.–95. persentiili	Osuus (%), joilla saanti ylittää 7500 µg RE	Mediaani	5.–95. persentiili	Osuus (%), joilla saanti ylittää 7500 µg RE
Maksamakkara ja -pasteija						
Nykytilanne [1]	1150	340–3420	< 1	2760	840–8010	6
Ei emakoita [2]	1010	310–2920	< 1	2260	690–6480	3
Maksalaatikko						
Nykytilanne [1]	2910	1430–5580	1	6880	3420–12780	41
Ei emakoita [2]	2640	1310–4860	1	5950	2980–10850	26
Jauhemaksapihvi						
Nykytilanne [1]	10430	5550–21440	81	26370	22240–39950	100
Ei emakoita [2]	9930	5070–20820	76	24720	20790–38280	100
Maksakastike						
Nykytilanne [1]	8030	4270–16500	57	20290	17100–30730	100
Ei emakoita [2]	7640	3900–16010	52	19020	16000–29450	100

[1] Käytetystä sianmaksasta 90 % lihasianmaksaa ja 10 % emakonmaksaa
[2] Kaikki käytetty sianmaksa lihasianmaksaa
[3] Raaka-aineena käytettyjen maksojen retinoidipitoisuudet on poimittu satunnaisesti pitoisuusjakaumalta
[4] Raaka-aineena käytettyjen maksojen retinoidipitoisuudet on poimittu pitoisuusjakauman ylimmästä kymmenyksestä

ja maksakastikkeesta saatavat retinoidiannokset ovat niin suuria, että keskimääräisessä tilanteessa maksojen retinoidipitoisuuden tulisi laskea noin 70 % nykyisestä, jotta saanti jäisi suurimmalla osalla alle suositellun raja-arvon (7500 µg RE). Jos maksat poimittaisiin vain retinoidipitoisuuden ylimmästä kymmenyksestä, retinoidipitoisuuksien tulisi vähentyä noin 85 % nykyisestä tasosta.

Annoskoon ja saantirajan suhde

Taulukossa 5 on esitetty kunkin maksaruuan osalta simuloitu annoskoko (mediaani), joka muun saannin ohella riittää joko retinoidialtistukseen 3000 µg RE/vrk (ylin hyväksyttävä päivittäinen saanti raskauden aikana) tai retinoidialtistukseen 7500 µg RE (ylin hyväksyttävä kertasaanti raskauden aikana). Taustasaantina laskelmissa käytettiin ei-maksaperäisen retinoidialtistuksen 95. persentiiliä (530 µg RE/vrk).

1.5.3 Kadmium ja lyijy

Simulaatiomallin mukaan maksasta saatava kadmiumannos maksaa syövillä on keskimäärin 0,17 µg/vrk (mediaani 0,09 µg/vrk, 90 % vaihteluväli 0,01–0,64 µg/vrk) ja lyijyannos keskimäärin 0,32 µg/vrk (mediaani 0,16 µg/vrk, 90 % vaihteluväli 0,03–1,14 µg/vrk). Arvioitu saanti koskee vain niitä kahdeksan päivän jaksoja, jolloin henkilö syö vähintään yhtä maksaruokaa. Jos maksaa syödään harvemmin, maksan kautta saatavan keskimääräisen raskasmetallialtistuksen määrä on vastaavasti pienempi. Maksaruokien kautta tapahtuva kadmiumin ja lyijyn saanti on suhteellisesti melko vähäistä, kun tuloksia verrataan ravinnon kautta tapahtuvaan kokonaisaltistukseen (kadmium 10 µg/vrk, lyijy 20 µg/vrk) (Mustaniemi & Hallikainen 1994; Mustaniemi ym. 1994).

1.6 Johtopäätökset

1. Tämän riskinarvioinnin perusteella maksaruokien käyttö voi altistaa suuremmille retinoidipitoisuuksille kuin mitä pidetään turvallisena raskauden aikana. Liikasaannin riski näyttäisi kuitenkin koskevan lähinnä pääruokana syötäviä maksaruokia, kuten maksa-laatikkoa, jauhemaksapihviä ja maksakastiketta. Kohtuullinen maksamakkaran tai maksapasteijan syönti raskauden aikana ei näyttäisi altistavan sellaisille retinoidiannoksille, joiden voidaan katsoa lisäävän sikiövaurioiden riskiä.

2. Tehokkain keino retinoidien liikasaannin riskin vähentämiseksi on välttää etenkin pääruokina käytettyjen maksaruokien syöntiä raskauden aikana. Muiden tarkasteltujen skenaarioiden vaikuttavuus oli selvästi heikompi. Emakonmaksan elintarvikekäytön lopettamisella olisi vain vähäinen vaikutus retinoidien saantiin. Vastaavasti maksojen retinoidipitoisuuksien pitäisi vähentyä noin neljännekseen, jotta vaikutus vastaisi osittaisen syöntirajoituksen tehokkuutta.

3. Kun A-vitamiinin saantia tarkastellaan ravitsemuksellisesta näkökulmasta, maksansyönnistä on selvästi hyötyä saantisuositusten täyttymisessä. Maksa sisältää A-vitamiinin lisäksi myös monia muita tärkeitä hivenaineita ja vitamiineja kuten foolihappoa ja rautaa. Maksansyönnillä saavutettavat ravitsemukselliset hyödyt voitaneen kuitenkin korvata runsaasti kasviksia ja kohtuullisesti lihaa sisältävällä monipuolisella ruokavaliolla, jolloin retinoidien liikasaannin riskiä ei ole.

4. Kadmiumin ja lyijyn saanti maksaruokien välityksellä on melko vähäistä verrattuna altistukseen muista ravintolähteistä. Maksan sisältämä rauta saattaa vähentää kadmiumin ja lyijyn imeytymistä maksaruuista.

Taulukko 5. Simulaatiomallin avulla arvioidut maksaruokien annoskoot (mediaani), joilla retinoidialtistuksen taso vastaa raskaudenaikaisen saannin hyväksyttävää ylärajaa (päivittäinen saanti ja kerta-annossaanti), kun taustalla on myös muista lähteistä tuleva retinoidien saanti. Taustasaantina on käytetty suomalaisten 25–44-vuotiaiden naisten ei-maksaperäisen retinoidialtistuksen 95. persentiiliä (530 µg RE vrk^{-1}). Annoskoot on laskettu tilanteessa, jossa raaka-aineena käytettyjen maksojen retinoidipitoisuudet on poimittu satunnaisesti pitoisuusjakaumalta (keskimääräinen pitoisuus), ja tilanteessa, jossa maksojen retinoidipitoisuudet on poimittu pitoisuusjakauman ylimmästä kymmenyksestä (korkea pitoisuus).

	Annos (g) joka vastaa saantia			
	3000 µg RE päivittäin [1]		7500 µg RE annoksessa [2]	
	Maksojen retinoidipitoisuus		Maksojen retinoidipitoisuus	
Tuote	Keskimääräinen	Korkea	Keskimääräinen	Korkea
Maksamakkara tai -pasteija	70	30	210	90
Maksalaatikko	130	50	370	150
Jauhemaksapihvi	30	10	90	30
Maksakastike	50	20	140	50

[1] Suurin hyväksyttävä päivittäinen saanti raskauden aikana (Scientific committee on Food 2002)
[2] Suurin hyväksyttävä kerta-annossaanti raskauden aikana

2. Summary

2.1 Introduction

Liver is a good source of vitamin A and many other nutrients like folic acid and iron. In contrast to many beneficial nutritional effects, liver consumption has some potential disadvantages. Liver contains vitamin A in retinoid form, which can be toxic if ingested in large amounts. Certain population groups, like pregnant women and young children, should pay special attention to prevent excess retinoid intake. Liver may also contain high amounts of potentially toxic heavy metals like cadmium and lead.

At the turn of 1989, very high concentrations of vitamin A in livers of pigs were reported in Finland (Heinonen 1990). Due to the possible risk of retinoid teratogenesis, the National Board of Health of Finland recommended that women who were pregnant or who were planning to become pregnant should not to consume liver. By changing the composition of animal feeds and cutting down the overuse of additional vitamin preparations, the vitamin A level in pig livers was soon lowered. Despite reduced vitamin A contents in livers, the recommendations of liver consumption for pregnant women remained unchanged. The need of avoiding liver during pregnancy in present day is regularly discussed. To re-evaluate current recommendations and to provide a better basis for risk management, the National Food Agency of Finland (NFA) asked the National Veterinary and Food Research Institute (EELA) to assess the risks of maternal liver consumption to an unborn child. The assessment started at the beginning of 2005.

The objectives of this research were

1. to determine the present retinoid concentrations in swine, bovine and broiler livers in Finland

2. to estimate retinoid exposure via liver products among Finnish women of fertile age

3. to assess risk of intolerably high retinoid intake if all or some liver products were maintained in the diet of women during pregnancy

4. to evaluate the effects of scenarios other than diet restriction to lower the retinoid intake

5. to estimate the intake of cadmium and lead via liver foods among Finnish women of fertile age.

The main focus of this risk assessment was on vitamin A intake, with a lesser emphasis on cadmium and lead. The importing of liver into Finland is very marginal (less than 1.5% of liver consumed in the country in 2003) (Finfood 2005) and was therefore not included in the assessment. As well, compliance with maternity clinic advice to restrict the diet was excluded from this research. According to previous studies, 25% of pregnant women eat liver despite the current recommendations (Erkkola et al. 2001).

Fertile age women were chosen to be the subject of the study because teratogenic effects of retinoids are strongest at the very beginning of the pregnancy when the nutritional guidance from maternity clinics has probably not yet been received.

Risk assessment follows the principles of the Codex Alimentarius Commission (CAC 2004). The assessment consists of four parts: hazard identification, hazard characterisation, exposure assessment and risk characterisation.

2.2 Hazard identification

2.2.1 Vitamin A

Definition

Vitamin A is an essential nutrient that plays a very important role in many biological processes. Vitamin A is not a single chemical substance but a generic term referring to retinoids that exhibit the biological activity of retinol (IUPAC-IUB 1981). In a nutritional context, provitamin A carotenoids can also be included in the vitamin A family. Provitamin A carotenoids are vitamin A precursors that have to be converted into retinoids in the body to express vitamin A activity. Vitamin A activity of the compounds is defined as retinol equivalents (RE), where 1 μg RE is equal to the biological vitamin A activity of 1 μg retinol.

In this assessment, the term vitamin A refers to the total amount of vitamin present in the diet, including retinoids and provitamin A carotenoids. The term retinoids is used to combine retinol and its natural and synthetic derivatives that have the biological activity of retinol.

Dietary sources

Vitamin A cannot be synthesised *de novo* by the human body, and has to be provided in the diet. Vitamin A in foods is derived from two sources: retinoids from foods of animal origin and provitamin A carotenoids mainly from plant-derived foods. The retinoid content of liver is several times higher than in any other foodstuffs (Heinonen 1990; KTL 2005). In addition to intake from foods, vitamin A can be taken as dietary supplements in the form of retinoids or carotenoids.

Metabolism

Retinoids

In foods, retinoids occur mainly as retinyl esters. Retinyl esters are hydrolysed to retinol in the intestinal lumen and taken up by intestinal cells. In the intestinal cells, retinol is re-esterified and incorporated into chylomicrons. Chylomicrons are released into the blood circulation via the lymph. Most of the retinyl esters in the chylomicrons are taken up by the liver. In liver, retinol esters are hydrolysed to free retinol, which then binds to specific transport protein, retinol-binding protein (RBP). Retinol bound to RBP (holo-RBP) can be secreted directly into the circulation or transferred to storage cells in the liver. When needed, stored retinol is rapidly released into the circulation. Under well-nourished conditions, hepatic vitamin A represents over 90% of the total body reserves (Olson 1996).

The release of retinol from the liver is tightly controlled, so the normal concentration of retinol in plasma is ensured despite fluctuations in retinol intake (Olson 1996). Retinol is transported to the target cells mainly as holo-RBP. When it enters the target cell, retinol is oxidised to retinal and retinoic acid, which are the active retinoid metabolites.

Carotenoids

Absorbed dietary provitamin A carotenoids are mainly converted to retinol in intestinal cells (Harrison 2005). Retinol is then transported to the liver as described above. Some intact carotenoids can be transported to peripheral tissues bound to plasma lipoproteins (Debier & Larondelle 2005). Carotenoids are stored in particular in adipose tissue (Goodman 1984; Olson 1984). Carotenoids can be further transformed into retinoids in peripheral tissues (Debier & Larondelle 2005).

Bioavailability

Approximately 80% of retinoids in the diet is absorbed (Olson 1996). The bioefficacy of provitamin A carotenoids is lower than retinoids due to poor absorption and relatively inefficient conversion to retinoids (Thurnham & Northrop-Clewes 1999; Burri et al. 2004). The absorption efficacy and proportion converted to retinol is affected by a number of factors, as the food matrix and food processing, the amount and type of carotenoids consumed in a meal (FNB 2001; van Lieshout et al. 2001). Among carotenoids, ß-carotene is most efficiently converted to retinol. It has been calculated that 12 μg of dietary ß-carotene or 24 μg of other dietary provitamin A carotenoids corresponds to the biological vitamin A activity of 1 μg RE (1 μg retinol) (FNB 2001).

Physiological functions

Vitamin A has an essential role in many biological processes like vision, growth, cell differentiation, immune functions, reproduction and embryonic development (Gerster 1997; Clagett-Dame & DeLuca 2002). The biological effects of vitamin A are mainly mediated by retinoic acid derivatives, which activate specific nuclear receptors and thereby lead to changes in gene expression (Debier & Larondelle 2005). Both excessive and insufficient intake of vitamin A can cause a wide spectrum of adverse health effects. Most symptoms are reversible, but significant intake imbalance can lead to permanent damages, one of the most severe being abnormal foetal development.

Intake recommendations and current intake

The Finnish recommendation for vitamin A intake is 700 μg RE/day for non-pregnant women and 800 μg RE/day during pregnancy (VRN 2005). The recommended intake (RI) fulfils the nutritional needs of practically all healthy individuals in a selected population group. Deficiency symptoms may occur if daily intake is below 400 μg RE/day (lower level of intake, LI) (NCM 2004). In the western world, the intake recommendations for vitamin A are met by the general population (SCF 2002; NCM 2003; Ervin et al. 2004).

2.2.2 Cadmium and lead

Cadmium and lead are heavy metals that accumulate in the body. Cadmium and lead are not essential to humans, and exposure to these metals can cause adverse health effects. A provisional tolerable weekly intake (PTWI) is 7 μg/kg body weight for cadmium and 25 μg/kg body weight for lead (WHO 1989b; WHO 1993).

About 5% of ingested cadmium and about 10% of ingested lead is absorbed in the digestive tract (IPCS 1992; IPCS 1995; WHO 2000). Intestinal absorption is affected by many nutritional factors, for example, low intake of iron may markedly increase the absorption efficiency of cadmium and lead (IPCS 1992; IPCS 1995).

Absorbed cadmium and lead are accumulated in body tissues. Highest cadmium concentrations are found in the kidneys, but, as exposure levels increase, also in the liver (WHO 1989a). Over 90% of the body lead burden is found in the skeleton (IPCS 1995). Lead in bone is not metabolically inert and is readily mobilised to blood. Therefore pregnancy and other conditions that increase bone turnover may cause blood lead levels to rise. The excretion rate of absorbed lead is low (about 30-40 μg/day) with the most significant route being via urine (IPCS 1977). The biological half-life for cadmium is 15-30 years (WHO 1989a). Lead has a half-life of

20-30 years in bone and about one month in other tissues (IPCS 1995).

Food is the major source of cadmium and lead exposure for the non-smoking general population (IPCS 1992; IPCS 1995). Among foods, the highest cadmium concentrations are found in edible organs, but lead is distributed quite evenly between different foods (EVI 2002). In Finland, the average dietary intake is 10 μg/day for cadmium and 20 μg/day for lead (Mustaniemi & Hallikainen 1994; Mustaniemi ym. 1994). Cadmium and lead exposure of the majority of Finnish population is below the level of PWTI.

2.3 Hazard characterisation

2.3.1 Vitamin A

Vitamin A deficiency

Vitamin A deficiency is rare in the western world, but a major nutritional problem in developing countries. Deficiency signs begin to appear when liver reserves of retinyl esters are exhausted due to long-term vitamin A insufficiency and the plasma retinol concentration falls below 0.7 μmol/l (SCF 1993).

The best known consequences of vitamin A deficiency are blindness due to insufficient formation of visual pigments and corneal ulcers and scars that can eventually lead to permanent blindness (McLaren & Frigg 2001; Sommer & Davidson 2002). Deficiency symptoms that can occur before eye disease include anaemia, poor growth, and reduced immune competence (McLaren & Frigg 2001; West 2002).

Vitamin A deficiency during pregnancy leads to foetal death or severe congenital malformations in many mammal species (Clagett-Dame & DeLuca 2002). In humans, the causality between vitamin A deficiency and congenital malformations is less clear, because the insufficient availability of vitamin A is usually accompanied by the deficiency of other nutrients essential to normal development (Gerster 1997).

Retinoid toxicity

In western countries, the main concern regarding vitamin A intake is excess retinoid intake. Excess intake of retinoids can produce toxicity appearing as hypervitaminosis A among adults and children or as teratogenicity during foetal life. Excess carotene intake has not been shown to cause vitamin A toxicity, possibly because its cleavage to retinoids is tightly controlled (Bendich 1988; Dawson 2000).

Cases of hypervitaminosis A caused by natural food sources are rare, and illness is usually caused by misuse of retinoid supplements (Bendich & Langseth 1989; Myhre et al. 2003; NCM 2003). Hypervitaminosis can be divided into two categories: acute, resulting from ingestion of very high doses over a short period of time, and chronic, resulting from continued ingestion of high doses for months or even years (Hathcock et al. 1990). In adults, acute toxicity can be caused by doses around thousand times the recommended daily intake, and chronic toxicity by doses around 15 times the recommended daily intake (Myhre et al. 2003). The symptoms of retinoid toxicity include general symptoms like headache and fatigue, and symptoms from digestive tract, skin, nervous system and musculoskeletal system. The symptoms are usually reversible and rapid recovery results when excessive intakes are discontinued (Bendich & Langseth 1989).

Teratogenicity

Numerous experimental animal studies have shown that excess retinoids can cause embryotoxic effects if administrated during pregnancy and that teratogenicity can occur at doses that do not induce any detectable toxicity symptoms in the mother (Tzimas & Nau 2001). Excess retinoid exposure has been shown to produce congenital malformations in almost all organ systems (Miller & Hayes 1982; Bendich & Langseth 1989). The type and severity of defects depend on the amount of exposure and the stage of gestation (Biesalski 1989). Even in the absence of overt structural abnormalities, embryonic retinoid exposure can lead to minor brain defects, growth disturbances and behavioural abnormalities that become evident later during postnatal life (Miller & Hayes 1982; Holson et al. 1997). Behavioural effects in experimental animals include, for example, increased or reduced activity and learning deficits (Holson et al. 1997). Excessive retinoid intake also increases foetal mortality (Akase et al. 2003). Even a single high dose of retinoids during pregnancy can cause toxic effects in foetus (Biesalski 1989; Piersma et al. 1996).

In humans, retinoid teratogenesis is well-known in the context of therapeutically used retinoid derivatives (isotretinoin, etretinate) (Rosa et al. 1986). However, the teratogenicity of natural retinoids (retinol and its esters) in humans is not so obvious. The consumption of large doses of retinoid supplements has been reported to be associated with congenital malformations in around twenty separate case reports (Rosa et al. 1986). In addition to these reports, there has been one reported case of congenital malformations that may have been linked to the high intake of retinoids from liver foods (Buss et al. 1994). Malformations caused by retinoid teratogenicity are typically placed in head and facial area, in central nervous system and in circulatory system *(Table 1)*. For major malformations, the most critical period for exposure seems to be between the second and fifth week of embryonic develop-

Table 1. Typical malformations observed in infants with excess prenatal retinoid exposure.

Craniofacial area
- small or absent external ears and auditory canals
- low set ears
- micrognathia
- cleft palate
Central nervous system
- hydrocephalus
- microcephalia
- brain hypoplasia
- anomalies of retina or optic nerve
Cardiovascular system
- aortic arch abnormalities
- transposition of the large vessels
- ventricular septal defects
Thymus
- thymic hypoplasia

References: Lammer et al. 1985; Rosa et al. 1986

ment (Rosa et al. 1986). In addition to birth defects, children exposed to therapeutically used retinoids often have reduced intelligence, but this impairment is not always accompanied by other malformations (Vorhees 1994).

The molecular mechanism of retinoid teratogenesis is based on alterations in gene transcription. Normal embryonic development is a result of strictly controlled changes in gene activity regarding time and space. Excess retinoid exposure can lead to activation of genes that should be silent or inhibition of genes that should be expressed (Ross et al. 2000). Alterations in cell metabolism and intercellular interactions cause failures in division, migration and differentiation of cells, thus disturbing the ground plan of embryonic development.

Dose-response in retinoid teratogenesis

In humans, epidemiological data is so far the only usable tool to assess the dose-response relationship for the teratogenic effects of retinoids. Animal studies provide a lot of dose-response data, but due to the species variation in retinoid metabolism, kinetics and toxicodynamics, the risk extrapolation of animal data to humans is difficult (Tzimas & Nau 2001).

The available epidemiological data is very limited and therefore it is not possible to establish a clear dose-response curve for the teratogenic effects of retinoids in humans (Rosa et al. 1986; SCF 2002; NCM 2003). As well, the duration of exposure needed to produce teratogenic effects is unknown. Case reports linking high retinoid intake and birth defects involved supplemental retinoid exposure of 5,400 μg RE/day or more for several weeks during pregnancy (Rosa et al. 1986). No associations have been found in the case-control studies between the intake of 3,000 μg RE/day or less and foetal malformations (NCM 2003). Many authorities recommend that the intake of retinoids from food and dietary supplements for women of childbearing age should not exceed 3,000 μg RE/day (FNB 2001; SCF 2002, EVM 2003). Moreover, since a high single dose of retinoids can be teratogenic, a single supplemental dose over 7,500 μg RE (25,000 IU) is not advisable during pregnancy.

2.3.2 Cadmium and lead

Cadmium

Chronic cadmium exposure is responsible for a broad spectrum of adverse health effects. The kidney, in particular the renal cortex, is the critical organ in environmentally exposed populations (WHO 2001). In addition to renal effects, exposure to cadmium may cause a decrease in bone mineral density, neurotoxic effects and contribute to the development of cancers (WHO 2001). In human pregnancy, maternal exposure to cadmium associated with early delivery and low birth weight (Nishijo et al. 2002). Only small amount of the cadmium in maternal circulation crosses human placenta (Korpela 1986). However, cadmium affects placental hormone secretion, which might contribute to premature delivery (Henson & Chedrese 2004).

Cadmium exposure can be measured by cadmium concentration in blood or urine. It has been estimated that the prevalence of renal toxicity in the general population increases when urinary cadmium con-

centrations exceeds 2.5 μg/l (2.5 μg/g creatinine) (Järup et al. 1998). This corresponds to a renal cadmium concentration of 50 μg/g and a long-term cadmium intake of about 50 μg/day. The risk of adverse pregnancy outcomes are also increased with urinary cadmium concentration over 2 μg Cd/l (Nishijo et al. 2002).

Lead

Lead adversely affects several organs and organ systems (IPCS 1995). Anaemia caused by inhibition of heme synthesis and neurotoxic effects are probably the best known symptoms of lead toxicity. Other health effects include renal toxicity, increased blood pressure and effects on calcium homeostasis (IPCS 1995; Papanikolaou et al. 2005). Lead exposure alters many enzyme systems, thus affecting various cellular processes (IPCS 1995).

Lead is readily transported across the placenta, and is taken up by the foetus from 12th week of pregnancy (Papanikolaou et al. 2005; Harville et al. 2005). Lead can cause toxic effects in the foetus. The most critical effect is the impairment of neurological development of the foetus (UNEP 1998, Schnaas et al. 2006). Exposure to lead also increases the risk of foetal death and preterm delivery, and is associated with many minor malformations in the baby (WHO 2003).

Exposure to lead can be monitored by blood lead concentration. In 1994, the average blood lead concentration among Finnish women was 0.11 μmol/l (23 μg/l) with a range of 0 to 0.36 μmol/l (0-74 μg/l) (Alfthan et al. 1994). Symptoms of chronic lead toxicity may appear in adults at blood lead levels of 400-800 μg/l (UNEP 1998; WHO 2003). Biochemical effects in heme synthesis are seen at exposure levels of 30-300 μg Pb/l (WHO 2003). Among pregnant women, the risk of preterm delivery is reported to be fourfold among women with blood lead levels above 140 μg/l compared with women with blood lead levels of 80 μg/l or less (WHO 2003). Unfortunately, there seems to be no threshold for one of the most critical effects of lead exposure: reduced intellectual development of the foetus (WHO 2003, Schnaas et al. 2006).

Since lead is shown to be toxic at very low exposures, and because no safe level of lead exposure has been found (UNEP 1998), any exposure should be considered to be a potential concern.

2.4 Exposure assessment

For exposure assessment, four different datasets were collected:

1. The consumption of liver products among women at ages between 25-44 years was obtained from the FINDIET 2002 survey (Männistö et al. 2003). Data of vitamin A intake of vitamin A from non-liver sources was also based on the FINDIET 2002 study.

2. The amount and type of liver in the consumed liver foods were estimated relative to the market shares of all Finnish liver products. The information was obtained from the food industry and the National Public Health Institute of Finland.

3. Laboratory analyses of swine, bovine and chicken liver samples were carried out to obtain information on the vitamin A content in the raw material of liver foods.

4. Cadmium and lead concentrations in livers were obtained from analyses made in connection with the National Residue Control Program (NRCP) between 2000 and 2004.

Based on the collected data, a mathematical model was built to simulate women's exposure to vitamin A, cadmium and lead whether liver foods are eaten or not. Simulated results were compared with the intake recommendations for vitamin A and with the intake limits for retinoids and heavy metals.

2.4.1 Liver consumption

In the FINDIET study, the consumption data was collected using a 48-hour recall interview. A subsample of the participants also filled in a three-day food record twice (eight-day study). A 48-hour recall was filled by 529 women of a given age interval, and 62 of them participated in the eight-day study. In total, 60 (11.3%) of 529 women reported liver consumption. In the 48-hour recall, 44 women (8.3%) ate liver foods. During the eight-day study period, 24 women (38.7%) ate liver foods. The most commonly eaten liver food was liver sausage or pâté, followed by liver casserole. Only a few women ate liver patties or liver stew. Distributions for portion sizes and eating frequencies were generated for each liver food.

The consumption data included no information about the proportion of liver eaters in the real population. Therefore, no reliable calculations could be done to estimate the general intake of vitamin A from liver foods among liver eaters, and estimated liver consumption reflects only those eight-day periods when at least one liver food is eaten.

2.4.2 Liver in liver foods

To quantify the liver content of consumed liver foods, recipe information was used. The amount of liver was reported as a percentage of weight of the end product. For commercially available prepared liver foods (liver casserole and liver sausage and pâté), recipes were obtained from Finnish food industry. The liver content of the products varied between manufacturers. The range in liver content was 8-42% in liver sausage and pâté and 10-21% in liver casserole. For simulation model calculations, the liver contents were averaged out relative to the market shares of the products (situation in 2004). The mean liver content in liver sausage or pâté was 20.5% and in liver casserole 13.6%. Mainly swine liver was used in production of liver sausage and pâté. In production of liver casserole, mixtures of livers of different species were commonly used.

The customary recipes for liver patties and liver stew were received from the National Public Health Institute of Finland. Based on this information, the liver content was assumed to be 50% for liver patties and 33% for liver stew. In both foods, the liver used as an ingredient was assumed to be bovine liver and swine liver in a proportion of 2 to 1.

In recipes, swine liver includes both finishing pig liver and sow liver. According to Finnish food manufacturers, sow livers make up no more than 10% of swine livers used. Sow livers have significantly higher retinoid content compared with finishing pig livers, and therefore these two liver types were dealt separately in this risk assessment.

The size of the production run can affect the run-to-run stability of the final retinoid content of the product. If a large amount of food is produced at a time, the amount of liver used is also large, and livers with high and low retinoid content compensate for each other. Meanwhile, if the production run is small with only one or a few livers in a run, the final retinoid content varies more due to chance. Among Finnish food manufacturers, one batch of liver sausage or pâté can use 20-120 kg of liver. For liver casserole, one batch uses around 170 kg of liver among the large manufacturers. Liver patties and liver stew are served in canteens and restaurants in different volumes. Liver foods are also made in small quantities at home.

2.4.3 Vitamin in liver

Liver samples from bovine, swine and chicken were collected from Finnish slaughterhouses during the spring of 2005. Bovine and swine livers were treated as individual samples, but, among chickens, each sample comprised of the livers of ten birds from the same producer. Vitamin A content was analysed from 76 bovine samples, 128 swine samples (91 finishing pigs, 37 sows) and 27 chicken samples. All the chickens were slaughtered at the age of 35 days and pigs at the age of six months. The age of bovines varied from 13 months to 12 years. The age of the sows was not defined. A possible seasonal variation in liver vitamin A content was not studied or taken into account in the simulation model calculations.

Retinol, retinyl esters and ß-carotene (only from bovines) were analysed from liver samples with high-performance liquid chromatography (HPLC) using diode array detection at 325 nm. The limit of detection for retinol was 0.02 μg RE/g and 0.03 μg RE/g for retinyl esters. Relative standard deviation (RSD%) calculated for repeatability was 12% for bovine liver, 6% for pig liver and 11% for chicken liver. Liver vitamin A content (μg RE/g) in each sample was calculated as a sum of the analysed vitamin A activity of retinol and retinyl esters. The analysed mean liver retinoid content was 170 μg RE/g in pigs (median 164 μg RE/g, range 28-549 μg RE/g), 443 μg RE/g in sows (median 337, range 50-2,123 μg RE/g), 213 μg RE/g in bovines (median 175 μg RE/g, range 44-744 μg RE/g) and 106 μg RE/g in chickens (median 97 μg RE/g, range 34-277 μg RE/g). There was a statistically significant difference in liver retinoid content between the species (log-transformation, Kruskall-Wallis test, $X^2 = 46.7$, $P < 0.001$). In a pair-wise comparison, the significant difference (Mann-Whitney test, $P \leq 0.001$) was found in all pairs except pigs and bovines (Mann-Whitney test, $P = 0.16$).

Current retinoid contents in pig and bovine livers are about the same as in the 1990s. In pig liver, the mean retinoid content was clearly below the results published in 1989 (600 μg RE/g liver, Heinonen 1990). The mean retinoid content of bovine liver has not changed markedly since 1989. In chickens there has been a clear decline in liver retinoid content since the 1990s. In 1989, the mean liver retinoid content in chicken was 370 μg RE/g (Heinonen 1990) and in 1991 180 μg RE/g (Hirvi et al. 1992). The current results of liver retinoid contents are equivalent to the levels reported in the Finnish literature (KTL 2005; Reinivuo ym. 2005).

In Austria, the retinoid contents of bovine and chicken livers (41 μg RE/g in bovines and 56 μg RE/g in chickens, Majchrzak ym. 2006) are notably lower than in Finland or other Nordic countries. This is probably due to the differences in feeding and production circumstances. However, such low retinoid levels in healthy animals might indicate that retinoid supplementation practices in animal husbandry in Finland could be further reconsidered.

2.4.4 Vitamin A from non-liver sources

Intake estimates of Vitamin A intakes from non-liver sources were based on eight-day FINDIET 2002 data (n = 62). The intakes were calculated by the National Food Composition Database Fineli® (KTL 2005). The long-run average of daily intakes (usual daily intake) was estimated using the method of Nusser and co-

workers (1996). The median intake of vitamin A was 440 µg RE/day (5th–9th range 260-720 µg RE/day) when derived from non-liver foods only, and 490 µg RE/day (5th–9th range 240-970 µg RE/day) when derived from both non-liver foods and dietary supplements. For retinoids, the median intake was 250 µg RE/day irrespective of the differences in sources. The 5th–9th range in retinoid intake was 140-430 µg RE/day when intake was from foods only and 120-530 µg RE/day when intake of dietary supplements was included.

2.4.5 Cadmium and lead in liver

Data on cadmium and lead contents of livers were obtained from the National Veterinary and Food Research Institute of Finland (EELA). Data used were from routine analyses made in connection with the National Residue Control Program between 2000 and 2004. Five to ten chicken livers and around 30 livers of bovine and swine were analysed annually. In the simulation model, the averages of the annual means were used. For cadmium, the average of the annual means was 25 µg Cd/kg liver among bovines and swine and 10 µg Cd/kg liver among chickens. For lead, the average of annual mean was around 45 µg Pb/kg liver among all species.

2.4.6 Simulation model

The intake of vitamin A, cadmium and lead via liver foods among Finnish women of fertile age was estimated by mathematic simulation model. Basic structure of the simulation model is shown in Figure 1. Uncertainties in the used input data were included in the model.In the model, retinoid intake was estimated as an average daily intake and as intake of retinoids from single liver meal. In the case of total vitamin A, only average daily intake was estimated.

In the case of single meals, retinoid intake can vary greatly from meal to meal depending on the retinoid content of the livers used as an ingredient. Therefore, retinoid intakes from single portions of liver foods were simulated in two situations:

1. Livers used as an ingredient are randomly sampled from retinoid concentration distribution (average retinoid content situation).

2. Livers used as an ingredient are sampled from the highest decile of retinoid concentration distribution (high retinoid content situation).

The Monte Carlo simulation method was used to produce probability distributions for intakes. The simulation model was designed using commercial spreadsheet software (Microsoft Office Excel 2003, Microsoft Corporation, USA), with a commercial add-in module for risk analysis (@Risk, version 4.5.2, Palisade Corporation, USA, 2002). In the Monte Carlo model, input values can be described as probability distributions instead of point estimates. During the simulation process, the chain of events is repeated many times (iterations). New input values are randomly sampled from probability distributions for each iteration.

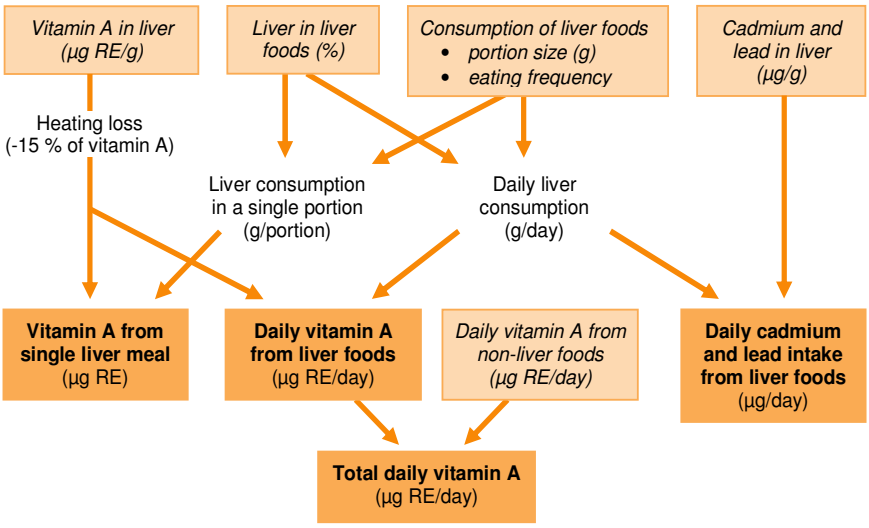


Figure 1. Design of the simulation model used to estimate vitamin A, cadmium and lead intakes. By combining the obtained empirical data (*italicized*), mathematic calculations could be performed to estimate the intakes of vitamin A and heavy metals from liver foods and total vitamin A intake among liver consumers (**bolded** parts).

In the simulation model, it was assumed that the consumption data used and the results of laboratory analyses represent true populations and that sow livers are uniformly distributed among swine liver used. To proportion simulated vitamin A intakes, the following reference values were used:

■ Total vitamin A intake: Nordic nutrition recommendations (NCM 2004).

- Recommended intake (RI): 800 μg RE/day for pregnant women
- Average requirement (AR): 500 μg RE/day for women
- Lower level of intake (LI): 400 μg RE/day for women

■ Upper tolerable daily dose (UL) for retinoids: 3,000 μg RE/day (SCF 2002).

■ Upper tolerable dose for retinoids from single liver meal: 7,500 μg RE. The limit was set based on reported cases of retinoid teratogenesis (Rosa 1986) and on recommendations made for retinoid supplementation (WHO 1998).

2.4.7 Scenarios

Risk management scenarios

In addition to modelling present vitamin A intake with and without liver foods, simulations were done to estimate the effects of some risk management options to vitamin A intake. Risk management options may involve actions at the food processing level or in consumer level. Therefore, the following two risk management scenarios were studied:

1. Sow livers are not used as an ingredient in liver foods (action at the food processing level).

2. Pregnant women are allowed to eat liver sausage or pâté but not liver casserole, liver patties or liver stew.

Reduction of liver retinoid levels

Retinoid concentrations in animal products are strongly correlated with the level of retinoids in animal feeds. It has been recently recommended by the Scientific Committee for Animal Nutrition that the possibility of reduction in retinoid content of animal feeds and the effect of this action on retinoid intake among consumers should be explored (SACN 2005). On this basis, we simulated dietary retinoid intake in a situation where the retinoid content of the livestock livers were 10-90% of the present level. The scenario focused only on effects and no evaluation was done whether the reduction is possible in practice. An assumption was made that retinoid levels in other animal products than liver will not be reduced.

2.5 Risk characterisation

According to the simulation model, the median intake of total vitamin A among liver eaters is 1,170 μg RE/day (5th-95th range 500-4,700 μg RE/day), and among non-liver eaters 490 μg RE/day (5th-95th range 240-970 μg RE/day), when both dietary and supplemental intake is taken into account. One of four liver eaters fall below the recommended vitamin A intake for pregnant women (800 μg RE/day), but the average requirement (500 μg RE/day) is achieved by almost everyone. However, the majority of non-liver eaters remain below the recommended intake, and one-third has the intake even below the lower intake limit (400 μg RE/day). Supplement use has a minor effect on vitamin A intake when compared with liver consumption.

The median retinoid intake among liver eaters is 880 μg RE/day (5th-95th range 310-4,430 μg RE/day), and among non-liver eaters 250 μg RE/day (5th-95th range 120-530 μg RE/day), when both dietary and supplemental intake is taken into account. Eleven percent (11%) of liver eaters exceeds the recommended upper intake limit. The result is independent of the use of dietary supplements. Among non-liver eaters, there is no risk of exceeding the maximum intake limit.

Effects of scenarios on daily vitamin A and retinoid intake among liver consumers

The effects of selected scenarios were estimated in a situation where intake of vitamin A and retinoids from both foods and dietary supplements was taken into account.

If sow livers were not used in liver foods, the effect on daily retinoid intake would be small. (Table 2). Median retinoid intake would drop from 880 μg RE/day to 810 μg RE/day (5th-95th range 300-4,260 μg RE/day). The proportion of women with retinoid intake more than the upper tolerable level (3,000 μg RE/day) would fall from 11% to 10% (Table 3). The exclusion of sow livers would not have significant effects on total vitamin A intake (tables 2 and 3).

However, the partial restriction in liver consumption would have a clear effect on intake of vitamin A and retinoids. If women ate liver sausage/pâté but not main course liver foods, only less than 1% of women would exceed the upper tolerable level of 3,000 RE/day (Table 3). Median retinoid intake would be 580 μg RE/day with 5th-95th range 270-1,510 μg RE/day, i.e., significantly lower than in the sow exclusion scenario (Table 2). Intake of total vitamin A would be clearly reduced too (median 850 μg RE/day, 5th-95th range 420-1,850 μg RE/day). As a consequence, 45 % of women would receive less than the recommended 800 μg RE/day, and 4 % of women would remain below the minimum intake limit (400 μg RE/day) (Table 3).

Table 2. Simulated daily intakes of vitamin A and retinoids from foods and dietary supplements among Finnish women of 25-44 years. Among liver consumers, the effects of the studied risk management scenarios are also shown.

	Vitamin A (µg RE d^{-1})		Retinoids (µg RE d^{-1})	
Source/scenario	Median	5th-95th pecentile	Median	5th-95th pecentile
Without liver	490	240-970	250	120-530
With liver				
Present situation [1]	1,170	500-4,700	880	310-4,430
No sows [2]	1,090	470-4,540	810	300-4,260
Restricted intake [3]	850	420-1,850	580	270-1,510

[1] Includes consumption of all liver foods. Swine livers from finishing pigs (90 %) and from sows (10 %).
[2] All swine livers are finishing pig livers
[3] Liver derived intake only from liver sausage/pâté. (Intakes from liver casserole, liver patties and liver stew are excluded.)

If effects similar to a restricted consumption scenario were tried to achieve by reducing liver retinoid contents, the retinoid content of livers should be less than 25% of the present level. If liver retinoid content were decreased to 50% of present level, 2.5% of women would still have retinoid intake more than the recommended limit of 3,000 µg RE/day.

2.5.2 Single-meal retinoid intake

Single meal intake of retinoids from different liver foods

A single meal was defined as an amount of certain liver product eaten in one day. Intake estimates were calculated separately for every liver food studied.

On average, single portions of liver sausage or pâté does not seem to expose women to harmful doses of retinoids (Table 4). However, intakes from liver stew and especially from liver patties clearly exceed the level considered safe during pregnancy. Liver casserole falls between these end points, the safety being dependent of the retinoid concentration of the liver used as an ingredient (Table 4).

The exclusion of sow livers would have only a minor effect on single-meal retinoid intake (Table 4). The high retinoid intakes from single meals of main course liver foods are difficult to reduce also by decreasing liver retinoid contents. In an average situation, liver retinoid contents should be 70% lower than the present level so that the majority of population would remain below the upper tolerable intake of 7,500 µg RE per portion. If livers in the product are sampled from the highest decile of retinoid distribution, the reduction should be 85%.

Table 3. Daily intakes of total vitamin A and retinoids in proportion to reference levels in Finnish women of 25-44 years. Simulated intakes from foods and dietary supplements. Among liver consumers, the effects of the studied risk management scenarios are also shown.

	% of women below the reference levels for vitamin A			% of women above the UL [7] for retinoids
Source/scenario	< LI [4]	< AR [5]	< RI [6]	
Without liver	32	52	88	0
With liver				
Present situation [1]	2	5	26	11
No sows [2]	2	6	29	10
Restricted intake [3]	4	11	45	< 1

[1] Includes consumption of all liver foods. Swine livers from finishing pigs (90 %) and from sows (10 %).
[2] All swine livers are finishing pig livers
[3] Liver derived intake only from liver sausage/pâté. (Intakes from liver casserole, liver patties and liver stew are excluded.)
[4] LI = Lower level of intake, 400 µg RE d^{-1} (Nordic Council of Ministers 2004)
[5] AR = Average requirement, 500 µg RE d^{-1} (Nordic Council of Ministers 2004)
[6] RI = Recommended intake for pregnant women, 800 µg RE d^{-1} (Nordic Council of Ministers 2004)
[7] UL = Upper tolerable intake level for pregnant women, 3,000 µg RE d^{-1} (Scientific Committee on Food 2002)

Table 4. Present retinoid intakes (µg RE) from single portions of different liver products and the effect of exclusion of sow livers among Finnish women aged 25-44 years. The table shows the medians and 5th-95th percentile ranges of retinoid intakes and the proportions of women who exceed the upper tolerable level for single dose during pregnancy (7,500 µg RE). Simulation model estimates based on the retinoid content of liver only.

	Liver retinoid content					
	Average [3]			High [4]		
Source and scenario	Median	5th-95th percentile	% of women above the intake of 7,500 µg RE	Median	5th-95th percentile	% of women above the intake of 7,500 µg RE
Liver sausage and pâté						
Present situation [1]	1,150	340-3,420	< 1	2,760	840-8,010	6
No sows [2]	1,010	310-2,920	< 1	2,260	690-6,480	3
Liver casserole						
Present situation [1]	2,910	1,430-5,580	1	6,880	3,420-12,780	41
No sows [2]	2,640	1,310-4,860	1	5,950	2,980-10,850	26
Liver patties						
Present situation [1]	10,430	5,550-21,440	81	26,370	22,240-39,950	100
No sows [2]	9,930	5,070-20,820	76	24,720	20,790-38,280	100
Liver stew						
Present situation [1]	8,030	4,270-16,500	57	20,290	17,100-30,730	100
No sows [2]	7,640	3,900-16,010	52	19,020	16,000-29,450	100

[1] Swine livers from finishing pigs (90 %) and from sows (10 %).
[2] All swine livers are finishing pig livers
[3] Retinoid concentrations of livers are randomly sampled from concentration distribution
[4] Retinoid concentrations of livers are above 90th percentile of concentration distribution

Table 5. The simulated median portion sizes of liver foods needed for pregnant women to achieve the upper limits of retinoid intakes (daily dose and single dose) when retinoid exposure from liver is additive to retinoid exposure from non-liver sources. As a background intake, the 95th percentile of the non-liver intake of Finnish women aged 25-44 years (530 µg RE d^{-1}) was used. Portion sizes are estimated in a situation where retinoid concentrations of livers are randomly sampled from concentration distribution (average liver retinoid content) and in a situation where retinoid concentrations of livers are sampled only from the upper decile of concentration distribution (high liver retinoid content).

	portion size (g) that equals intake			
	3,000 µg RE daily [1]		7,500 µg RE per portion [2]	
	Liver retinoid content		Liver retinoid content	
Product	Average	High	Average	High
Liver sausage or pâté	70	30	210	90
Liver casserole	130	50	370	150
Liver patties	30	10	90	30
Liver stew	50	20	140	50

[1] Upper tolerable daily intake for pregnant women (Scientific committee on Food 2002)
[2] Upper tolerable single dose for pregnant women

Portion sizes needed to achieve retinoid intake limits

Simulated portion sizes of different liver products needed to achieve the intake limits of 3,000 µg RE/day (upper daily dose for pregnant women) and 7,500 µg RE per portion (upper single dose for pregnant women) are shown in Table 5. The 95th percentile of non-liver retinoid intake (530 µg RE/day) was used as a background intake.

2.5.3 Cadmium and lead

Exposure to cadmium and lead via liver products was estimated in the present situation among liver consumers only. According to the simulation model, average intake of cadmium from liver is 0.17 µg/day (median 0.09 µg/day, 5th-95th range 0.01-0.64 µg/day) and average intake of lead 0.32 µg/day (median 0.16 µg/day, 5th-95th range 0.03-1.14 µg/day). Estimated exposure reflects only intake during those eight-day periods when at least one liver food is eaten. If liver is eaten less frequently in the long run, the average heavy metal exposure from liver foods is smaller. Cadmium and lead exposure from liver foods is relatively low when compared with the total dietary intakes of 10 µg Cd/day and 20 µg Pb/day (Mustaniemi & Hallikainen 1994; Mustaniemi et al. 1994).

2.6 Conclusions

1. This risk assessment model indicates that liver consumption may predispose women to retinoid intakes higher than what is considered safe during pregnancy. However, the risk seems to pertain mainly to liver foods eaten as a main course (i.e., liver casserole, liver patties and liver stew). Safety thresholds are not likely to be exceeded if only liver sausage or pâté is eaten in moderate amounts.

2. The most efficient way to manage the risk is for women to avoid main course liver foods during pregnancy. The effect of the other scenarios examined was notably smaller. Exclusion of sow livers from food production would have only a minor effect on retinoid intakes from liver foods. If effects similar to a restricted consumption scenario (no main course liver foods) were tried to achieve by reducing liver retinoid contents, the retinoid content of livers should be less than 25% of the present level.

3. When considering the total daily vitamin A intake from the nutritional point of view, liver consumption has a positive effect. In addition to vitamin A, liver contains also other beneficial nutritional elements like folic acid and iron. However, the benefits of eating liver can probably be substituted by a well-balanced diet with plenty of vegetables and a reasonable amount of meat without the risk of an excess intake of retinoids.

4. Cadmium and lead exposure from liver foods is relatively low when compared with other dietary sources even during those weeks when liver is eaten. The high iron content of liver may further reduce the relative impact of cadmium and lead exposure from liver foods.

Risk assessment

3. Introduction

3.1 Liver as food

Liver is a highly regarded food due to its nutritional value. Liver is a good source of vitamin A, many B-group vitamins, iron, and trace elements like selenium and zinc. Proteins in liver are high quality with essential amino acids.

In contrast to many beneficial nutritional effects, liver consumption has some potential disadvantages. Liver contains vitamin A in retinoid form, which can be toxic if ingested in large amounts. Certain population groups, like pregnant women and young children, should pay special attention to prevent excess retinoid intake. Liver may also contain high amounts of potentially toxic heavy metals like cadmium and lead.

3.2 Liver foods and pregnancy

In Finland, over 55,000 women give birth every year and nearly 58,000 children are born. Safety of liver foods during pregnancy has been a long-time issue among healthcare personnel and consumers. The main concern is the high amount of retinoids in the liver. Retinoids are essential for embryonic development, but excessive exposure can lead to teratogenic effects such as abortions, birth defects and learning disabilities of the offspring. Although the dose-response of retinoid teratogenesis has not been established, animal studies and human case reports have shown its potency to cause abnormalities in foetal development. The other safety issue is the possible harm caused by cadmium and lead accumulated in the liver. Cadmium and lead can have detrimental effects on foetal development and outcomes of pregnancy.

Since 1990, Finnish maternity clinics have been advised women not to eat liver or liver products during pregnancy because of the possible risk of retinoid teratogenesis (Julkunen et al. 1990; Hasunen et al. 2004). The same recommendation was made in 1991 by the European Commission (SCF 1992), and it is followed by several EU states (EMEA 1998). In addition to retinoid load, Finnish recommendations bring out the point of high heavy metal exposure via liver foods (Hasunen et al. 2004). However, until now there has been no assessment of the true risk, if any, of maintaining liver in the diet of a woman while pregnant.

3.3 Project history

At the turn of 1989, very high concentrations of retinoids in livers of pigs were reported in Finland (Heinonen 1990). The issue of the safety of liver foods evoked an intensive and widespread public discussion. Soon after the discovery, the National Board of Health of Finland came up with recommendations for consumption of liver and liver products (Julkunen et al. 1990). Women who were pregnant or who were planning to become pregnant were advised not to consume liver foods at all.

High retinoid levels in livers were due to the overuse of vitamin A in animal feeds and healthcare. Heavy vitamin A supplementation was believed to ensure good growth and health of animals. Action was taken to cut down the inexplicable overuse. New instructions for vitamin A additions to the feed were given to feed manufacturers, and veterinarians and farmers were informed of the use of retinoid supplements (Hirvi et al. 1992).

Retinoid contents in liver samples were monitored monthly during 1990 and 1991 (Hirvi et al. 1992). Since the retinoid levels were found to be significantly reduced by the end of 1991, intensive monitoring ended. Thereafter liver samples were analysed routinely in connection with the National Residue Control Program.

Despite reduced retinoid contents in livers, the recommendations of liver consumption for pregnant women remained unchanged. The need of avoiding liver during pregnancy in present day is regularly discussed. To re-evaluate current recommendations and to provide a better basis for risk management, the National Food Agency of Finland (NFA) asked the National Veterinary and Food Research Institute (EELA) to assess the risks of maternal liver consumption to an unborn child. The assessment started at the beginning of 2005.

3.4 Objectives

The objectives of this research were

1. to determine the present retinoid concentrations in swine, bovine and broiler livers in Finland

2. to estimate retinoid exposure via liver products among Finnish women of fertile age

3. to assess risk of intolerably high retinoid intake if all or some liver products were maintained in the diet of women during pregnancy

4. to evaluate the effects of scenarios other than diet restriction to lower the retinoid intake

5. to estimate the intake of cadmium and lead via liver foods among Finnish women of fertile age.

The main focus of this risk assessment was on vitamin A intake, with a lesser emphasis on cadmium and lead. The importing of liver into Finland is very marginal (less than 1.5% of liver consumed in the country in 2003) (Finfood 2005) and was therefore not included in the assessment. As well, compliance

with maternity clinic advice to restrict the diet was excluded from this research. According to previous studies, 25% of pregnant women eat liver despite the current recommendations (Erkkola et al. 2001).

Fertile age women were chosen to be the subject of the study because teratogenic effects of retinoids are strongest at the very beginning of the pregnancy when the nutritional guidance from maternity clinics has probably not yet been received.

3.5 Parts of risk assessment

This risk assessment follows the format of the Codex Alimentarius Commission (CAC 2004) and consists of four parts:

1. Hazard identification: Describes the general properties of vitamin A, cadmium and lead such as chemistry, sources and metabolism, and physiological functions. Hazard identification includes the current intake recommendations.

2. Hazard characterisation: Describes the toxicological properties of vitamin A, cadmium and lead. The common symptoms occurring due to the excessive or insufficient exposures are characterised. The toxicity for healthy adults and the teratogenic effects are described, and recommended safety limits are discussed.

3. Exposure assessment: Evaluates the exposure of retinoids, cadmium and lead from liver foods among Finnish women aged 25-44 years. This part consists of four subsections: 1) The consumption of food products containing liver is estimated based on data obtained from the FINDIET 2002 study. 2) Laboratory analyses were conducted to obtain the concentrations of retinoids in swine, bovine and chicken livers. Analyse results are presented, and the sampling procedure along with the analytical method used is described. 3) The average liver content and the type of liver in consumed liver products are estimated based on recipe information and market shares. 4) The intake of retinoids, cadmium and lead via liver products is then estimated by using a simulation model designed for this study.

4. Risk characterisation: This step brings together the preceding three sections. The effects of liver consumption on intake of total vitamin A, retinoids, cadmium and lead are studied. The results are compared with the intake recommendations and safety levels for pregnant women.

4. Hazard identification

4.1 Vitamin A

4.1.1 Chemistry and definitions

Vitamin A is an essential nutrient that plays a very important role in many biological processes. Vitamin A is not a single chemical substance but a generic term referring to retinoids that exhibit the biological activity of retinol (IUPAC-IUB 1981). Retinoids are a class of compounds consisting of six-carbon ring structure with a polyprenoid side chain and a terminating carbon-oxygen functional group (Figure 2). Differences in the functional group and alterations in the molecular skeleton give about six hundred different retinoid analogues with different chemical properties and potentially different biological effects (Gundersen & Blomhoff 2001).

In a nutritional context, provitamin A carotenoids can also be included in the vitamin A family. Provitamin A carotenoids are vitamin A precursors that have to be converted into retinoids in the body to express vitamin A activity. Provitamin A carotenoids are derived from a 40-carbon polyene chain, which is terminated by one or two cyclic end-groups (Sklan 1987) (Figure 3). Carotenoids with provitamin A activity are ß-carotene, α-carotene, γ-carotene and ß-cryptoxanthin.

Since vitamin A family consists of several chemical compounds with different structure and potency, it is essential to have a standard measurement for vitamin A activity. Vitamin A activity is defined as retinol equivalents (RE), where 1 μg RE is equal to the biological vitamin A activity of 1 μg retinol.

In this assessment, the term vitamin A refers to the total amount of vitamin present in the diet, including retinoids and provitamin A carotenoids. The term retinoids is used to combine retinol and its natural and synthetic derivatives that have the biological activity of retinol.

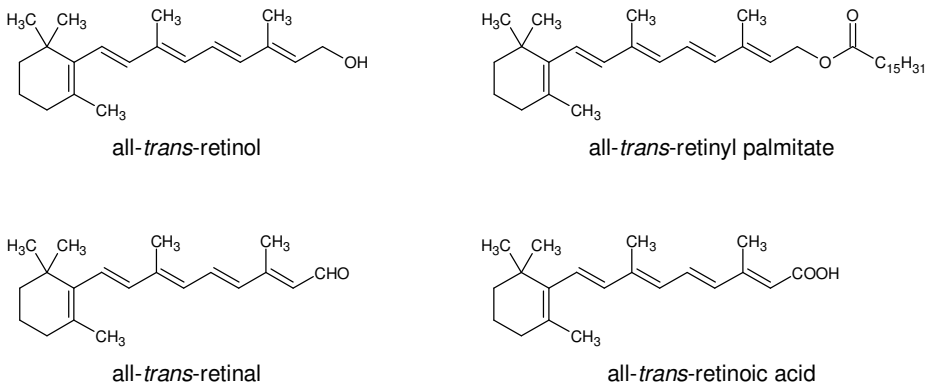


Figure 2. Chemical structures of some natural retinoids.

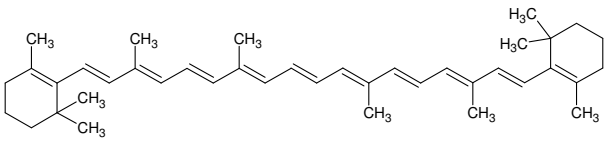


Figure 3. Chemical structure of β-carotene.

Table 6. Mean vitamin A content in some Finnish foods. In foods of animal origin, the vitamin A is mainly in retinoid form. In plant-derived foods, the vitamin A is in carotenoid form.

Food group	Mean vitamin A content (µg RE/100 g)[1]
Liver (average)	18,000
Rose hip puree	1,230
Margarine, fat spread (average)	850
Carrot	770
Butter	710
Cheese (average)	300
Egg	260
Tomato	70
Rainbow trout (fillet)	19
Orange	10
Beef steak	5
Potato	< 1
Rye bread	< 1

[1] KTL 2005

4.1.2 Dietary sources

Vitamin A cannot be synthesised *de novo* by the human body, and has to be provided in the diet. Vitamin A in foods is derived from two sources: retinoids from foods of animal origin and provitamin A carotenoids mainly from plant-derived foods. In addition to intake of vitamin A from foods, vitamin A can be taken as dietary supplements in the form of retinoids or carotenoids. The vitamin A content of some foods is shown in Table 6.

In foods, the highest concentrations of retinoids are found in liver. Retinoids are also present in dairy products, egg yolk, dietary fats and fatty fish, but the concentrations are only a fraction of those found in liver (Heinonen 1990; KTL 2005). In foods, retinoids are mostly found as retinyl esters and on lesser amounts as free retinol (Olson 1996; Majchrzak et al. 2006).

Significant sources of provitamin A carotenoids are dark green leafy vegetables, red and yellow vegetables, and yellow and orange non-citrus fruits (Booth et al. 1992). ß-carotene is the most important provitamin A carotenoid in terms of its relative provitamin A activity and quantitative contribution to the diet (Heinonen et al. 1990).

4.1.3 Metabolism and distribution

Absorption and storage

In foods, retinoids occur mainly as retinyl esters. Retinyl esters are hydrolysed to retinol in the intestinal lumen and taken up by intestinal cells. In the intestinal cells, retinol is re-esterified to long chained fatty acids before its incorporation into large lipoproteins called chylomicrons. Chylomicrons and associated retinyl esters are released into the blood circulation via the lymph. Most of the retinyl esters in the chylomicrons are taken up by the liver. In liver parenchymal cells, retinol esters are hydrolysed to free retinol, which then binds to specific transport protein, retinol-binding protein (RBP). Retinol bound to RBP (holo-RBP) can be secreted directly into the circulation or transferred to liver stellate cells for storage. In stellate cells, retinol is esterified and stored in cytosolic lipid droplets. When needed, stored retinol is transferred back to parenchymal cells and released into the circulation as holo-RBP.

Liver is the main site of body retinoid storage. Under well-nourished conditions, hepatic vitamin A represents over 90% of the total body reserves (Olson 1996). The rest is stored in adipose tissue and extrahepatic stellate cells in lungs, kidneys and intestine (Tsutsumi et al. 1992; Nagy et al. 1997).

Absorbed dietary provitamin A carotenoids are mainly converted to retinol in intestinal cells (Harrison 2005). Retinol is then transported to the liver as described above. Some intact carotenoids can be transported to peripheral tissues bound to plasma lipoproteins (Debier & Larondelle 2005). Carotenoids are stored in particular in adipose tissue (> 80%) and liver (10%), but relatively high levels are also found in the adrenals and the testes (Goodman 1984; Olson 1984). Carotenoids can be further transformed into retinoids in peripheral tissues (Debier & Larondelle 2005).

Blood transport and tissue uptake

The release of retinol from the liver is tightly controlled, so the normal concentration of retinol in plasma is ensured despite fluctuations in retinol intake (Olson 1996). Retinol is transported to the target cells mainly as holo-RBP. When it enters the target cell, retinol is oxidised to retinal and retinoic acid, which are the active retinoid metabolites.

4.1.4 Bioavailability and vitamin A activity

It is commonly stated that, under normal physiological conditions, approximately 80% of retinol in the diet is absorbed (Olson 1996). However, there is some disagreement between these statements and the data available, and it is possible that the true bioavailability is not as high as generally supposed (Blomhoff et al. 1991). The vitamin A activity of 1 μg retinol is equal to 1 μg RE. The retinyl esters are believed to be as bioavailable as retinol, hence a simple adjustment based on molecular weight gives the appropriate amounts of specific esters equivalent to 1 ug RE.

The bioefficacy of provitamin A carotenoids is lower than retinoids due to poor absorption and inefficient metabolism (Thurnham & Northrop-Clewes 1999; Burri et al. 2004). The absorption efficacy and proportion converted to retinol is affected by a number of factors, as the food matrix and food processing, the amount of carotenoids consumed in a meal, effectors of absorption, and the species of carotenoid (FNB 2001; van Lieshout et al. 2001). Among carotenoids, ß-carotene is most efficiently converted to retinol. It has been calculated that 12 μg of dietary ß-carotene or 24 μg of other dietary provitamin A carotenoids corresponds to the biological vitamin A activity of 1 μg RE (1 μg retinol) (FNB 2001).

4.1.5 Physiological functions of vitamin A

Vitamin A has an essential role in many biological processes like vision, growth, cell differentiation, immune functions, reproduction and embryonic development (Gerster 1997; Clagett-Dame & DeLuca 2002). The biological effects of vitamin A are mainly mediated by retinoic acid derivatives, which activate specific nuclear receptors and thereby lead to changes in gene expression (Debier & Larondelle 2005). The resulting activation or inhibition in gene expression mediates the biological effects of retinoids. Several hundred genes have so far been shown to be induced or depressed by retinoids (Debier & Larondelle 2005).

Retinoids are also able to modulate cellular events by mechanisms independent of retinoic receptors. Best-known example is the visual cycle, where 11-cis-retinal is part of visual pigments. Retinoids can also form a covalent bond with some proteins, which can modify the properties of target protein and thus its activity as signal mediator (Debier & Larondelle (2005). Generally, the retinoic receptor independent effects besides vision are still poorly understood (Perrotta et al. 2003).

Both excessive and insufficient intake of vitamin A can cause a wide spectrum of adverse health effects. Most symptoms are reversible, but significant intake imbalance can lead to permanent damages, one of the most severe being abnormal foetal development. Vitamin A deficiency and retinoid excess are more extensively discussed in Chapter 5.

4.2 Vitamin A intake

4.2.1 Recommendations for vitamina A intake

A diet should contain sufficiently but not too much vitamin A because both excess and deficiency can cause health problems. Therefore, different countries and different health organisations have made recommendations on dietary intakes of vitamin A. Due to varying safety factor calculations and considerations, a range of recommended values exists for vitamin A. Some intake recommendations are given in Table 7. Finnish recommendations for vitamin A intake (VRN 2005) are equal to Nordic recommendations (NCM 2004), i.e., 700 μg RE/day for non-pregnant women and 800 μg RE/day during pregnancy. Recommended intake (RI) is defined as the intake over time that theoretically would fulfil the nutritional needs of practically all healthy individuals in a selected population group (NCM 2004). RI is set at a level above the average physical need with no biochemical signs of deficiency (average requirement, AR) but significantly lower than toxicological data indicates any adverse effects. Lower level of intake (LI) refers to the level below which intake could lead to clinical deficiency symptoms.

The need of vitamin A is increased during the pregnancy, and therefore intake recommendations during pregnancy are different from that of the non-pregnant stage. However, the increment needed is relatively small and is confined mostly to the last trimester (Olson 1987; NCM 2004). In western countries, the increased need can usually be provided from maternal reserves (Olson 1987). Chronically inadequate intake must take place in critically deplete maternal body stores before detrimental effects occur in the mother and foetus (Underwood 1994).

4.2.2 Current knowledge on intake of vitamin A

In the western world, the intake recommendations for vitamin A (Table 7) are met by the general population. In the USA and various European countries, the mean vitamin A intakes are well above the recommended level (SCF 2002; NCM 2003; Ervin et al. 2004). However, median intakes remain below the reference values indicating a skewed distribution of intakes. Some foods contain significantly higher amounts of vitamin A than others. Individuals consuming products with high vitamin A

Table 7. Vitamin A intake recommendations (μg RE d^{-1}).

Source	Women		Men
	Not pregnant	Pregnant	
FAO/WHO [1]	500	800	600
USA [2]	700	770	900
Nordic countries [3]			
Recommended intake [4]	700	800	900
Average requirement [5]	500		600
Lower level of intake [6]	400		500

[1] FAO/WHO 2002
[2] Institute of Medicine (2001)
[3] Nordic Council of Ministers (2004)
[4] Intake value estimated to meet the needs of 97.5 % of the population group
[5] Intake value estimated to meet the needs of 50 % of the population group
[6] Intake below which deficiency symptoms may occur

content can have intakes two to four times higher than population means (SCF 2002; NCM 2003).

The proportion of vitamin A consumed as retinoids varies worldwide. In the Nordic countries, the intake of retinoids is higher than in most other areas of the world (NCM 2003). In Finland, retinoids constitutes two thirds of the dietary vitamin A intake (VRN 2005).

4.3 Cadmium and lead

4.3.1 Cadmium

General properties and occurrence

Cadmium is a heavy metal that accumulates in the body. Cadmium is not essential to humans, and exposure to cadmium can cause adverse health effects. The kidney is the critical organ in long-term cadmium exposure.

Cadmium occurs naturally at very low concentrations in the lithosphere and in topsoil (IPCS 1992). Cadmium has been dispersed through the modern environment mainly as a result of pollution from variety of sources. Most of the released cadmium originates from the metal industry (IPCS 1992). Cadmium is also used in many consumer products such as batteries, pigments and plastics.

Recommended intake limits

A provisional tolerable weekly intake (PTWI) for cadmium established by the FAO/WHO Expert Committee on Food Additives is 7 μg per kg of body weight corresponding to 1 μg/kg body weight/day (WHO 1989b). This means a daily maximum intake of 60 μg for a 60 kg person. However, recent studies suggest that toxic effects occur at lower exposure levels than previously thought (Järup et al. 1998). However, the WHO has determined that the new data do not provide a sufficient basis for revising the tolerable intake limit (WHO 2004).

Kinetics

Exposure to cadmium occurs via inhalation of air and ingestion of food and water. The absorbed amount is about 5-50% in the lungs and about 5% in the digestive tract (IPCS 1992). Intestinal absorption is affected by many nutritional factors. Diets with low levels of iron, calcium, protein and fibre may increase the cadmium uptake. In individuals with low body iron stores, the cadmium absorption efficiency is on average twice as high (about 10%) as the general population (IPCS 1992).

Absorbed cadmium accumulates in body tissues, principally in kidneys and liver (WHO 1989a). Highest cadmium concentrations are generally found in the renal cortex, but, as exposure levels increase, a greater proportion of the absorbed cadmium is stored in the liver. The cadmium excretion rate is normally low, and the biological half-life is 15-30 years (WHO 1989a).

Cadmium intake

Food is the main source of cadmium in the general adult non-smoking population (IPCS 1992). In Finland, the average dietary cadmium intake is 10 μg/day (Mustaniemi & Hallikainen 1994; Soininen et al. 2002). Among foods, the highest concentrations of cadmium are found from edible organs, shrimps, shellfish and mushrooms (Table 8). Yet, the greatest part (on average 80%) of the total dietary cadmium taken in by Finns originates from crops, especially from cereals (Table 8).

The distribution of dietary cadmium intake is skewed to the right with maximum intakes of two to three times higher than the average intake, mainly due to

Table 8. Mean cadmium content in some Finnish foods and their contribution to total dietary cadmium intake.

Food group	Mean cadmium content (μg/g)[1]	% of total dietary intake
Cereals	0.03-0.05	57
Vegetables and fruits	0.001-0.02	23
Fish and canned fish	0.01	4
Edible organs	0.1	5
Other		11
Meat	0.001	
Dairy products	0.001	
Mushrooms	0.5	
Bivalve molluscs	0.3	
Crustaceans	0.1	

[1] Mustaniemi & Hallikainen 1994, SCOOP 2004

differences in dietary habits (IPCS 1992; Louekari 1989). It has been suggested that, for about 5% of the Finnish population, the dietary intake is at least two-fold compared with the average intake of 10 μg/day (Louekari et al. 2000).

Tobacco is an important source of cadmium in smokers. One cigarette contains 1-2 μg cadmium, and it has been estimated that a person smoking 20 cigarettes a day will absorb about 1 μg of cadmium (Järup et al. 1998). The absorbed amount of cadmium among Finnish smokers can therefore be assumed to be approximately three-fold compared with the average non-smoker with absorbed amounts of 0.5 μg/day (5% of the average dietary intake of 10 μg/day).

The cadmium exposure of the majority of the Finnish population is below the level of PWTI. However, a sub-population with high dietary cadmium intake, increased absorption or smoking may be at risk from adverse health effects caused by cadmium (Louekari et al. 2000, Vahteristo et al. 2003).

4.3.2 Lead

General properties and occurrence

Lead is a bioaccumulative heavy metal with no nutritional value. Exposure to lead can cause adverse health effects in virtually all organ systems in the body.

Lead is found in mineral deposits and is the most common heavy metal in the earth's crust (WHO 2003). Lead is released into the environment due to natural causes as well as through human industrial activity. Human activities such as mining, manufacturing and the burning of fossil fuels are the major sources of environmental lead (Papanikolaou et al. 2005). The removal of lead from gasoline in 1990s reduced significantly the lead discharge, consequently lowering body lead burden (Penttinen et al 2002).

Recommended intake limits

A provisional tolerable weekly intake (PTWI) for lead established by the FAO/WHO Expert Committee on Food Additives is 25 μg/kg body weight, i.e., 3.6 μg/kg body weight/day (WHO 1993). This means a daily maximum intake of 215 μg for a 60-kg person. The recommendation was initially made for children, but, because foetuses are at least as sensitive to lead as small children, an intake limit of 3.6 μg/kg body weight/day was set for all population groups (WHO 1993).

Kinetics

Exposure to lead may occur as a result of inhalation of air or ingestion of food and water. Among adults the absorbed amount is 30-50% in lungs and an average 10% in the digestive tract (IPCS 1995; WHO 2000). Intestinal absorption is influenced by nutritional status and type of diet consumed. Under fasting conditions, absorption may be several times higher than in general. Absorption is also increased with deficiency of calcium, phosphate, selenium, zinc, iron or vitamin D (IPCS 1995).

Absorbed lead is distributed to blood, bone and soft tissues. In adults, lead has an estimated half-life of 20-30 years in bone and 35-40 days in other tissues (IPCS 1995). Due to the very long half-life in bone, over 90% of the body lead burden is found in the skeleton (IPCS 1995). Lead in bone is not metabolically inert and is readily mobilised to blood. Therefore conditions that increase bone turnover, such as pregnancy, may cause blood lead levels to rise. The excretion rate of absorbed lead is low (about 30-40 μg/day) with the most significant route being via urine (IPCS 1977).

Table 9. Mean lead content in some Finnish foods and their contribution to total dietary lead intake.

Food group	Mean lead content (µg/g)[1]	% of total dietary intake
Cereals	0.01-0.02	15
Vegetables, fruits and berries	0.005-0.01	17
Fish and canned fish	0.03-0.1	22
Dairy products	0.002-0.03	11
Meat and meat products	0.01-0.03	4
Edible organs	0.02	4
Alcohol	0.01	8
Non-alcohol beverages	0.0006-0.02	13
Other		6

[1] Mustaniemi et al. 1994; SCOOP 2004

Lead intake

Food is the major source of lead exposure for the general population (IPCS 1995). In Finland, the average dietary lead intake is 20 µg/day (Mustaniemi et al. 1994). Among women in Lapland, the intake is reported to be higher with the mean intake of 50 µg Pb/day and range of 20-120 µg Pb/day (Soininen et al. 2002). Dietary lead comes equally from various food sources (Table 9). Lead in foods is mainly result of the settling of airborne dust on the aboveground plant parts, and the following accumulation in food chain. Part of the dietary exposure originates from food handling and storage (lead in water pipes, tins and kitchenware). The highest lead concentrations are found in tinned foods, old vines, edible organs and unwashed vegetables (EVI 2002).

The distribution of dietary lead intakes among Finnish population is skewed to the right with maximum intakes of 2.5 times higher than the average intake (Louekari et al. 1989, Soininen et al. 2002). The estimated maximum intakes are still below the level of PTWI.

5. Hazard characterisation

5.1 Vitamin A deficiency

5.1.1 Definition and epidemiology

Normally the reserves of retinyl esters in the liver can be used to maintain a constant plasma retinol concentration. Deficiency signs begin to appear only when liver reserves of retinyl esters are exhausted and the plasma retinol concentration falls below 0.7 μmol/l (SCF 1993).

Vitamin A deficiency is rare in the western world, but a major nutritional problem in developing countries: 140 million children and more than 7 million pregnant women suffer from vitamin A deficiency every year (West 2002). The main reason to deficiency is insufficient amount of vitamin A in the diet (FAO/WHO 2002). Unlike the western world, in developing countries people consume most of their vitamin A needs from provitamin carotenoid sources. The initially low bioavailability of carotenoids is yet reduced by the low fat content of the diet. Children and pregnant or lactating women are most susceptible to deficiency due to high vitamin A requirements (FAO/WHO 2002).

5.1.2 Consequences of vitamin A deficiency

The best known consequence of vitamin A deficiency is the eye disease xerophthalmia (Sommer & Davidson 2002). The first sign of xerophthalmia is night blindness due to insufficient formation of visual pigments. In the next stages, the cornea becomes dry and ulcerated, and corneal scars can eventually lead to permanent blindness (McLaren & Frigg 2001). Nowadays it is clear that vitamin A deficiency causes health problems long before the clinical eye symptoms appear (West 2002). These early consequences include anaemia, poor growth and reduced immune competence resulting in increased infectious morbidity and mortality (McLaren & Frigg 2001).

Vitamin A deficiency during pregnancy leads to foetal death or severe congenital malformations in many mammal species (Clagett-Dame & DeLuca 2002). Described malformations include a large array of defects affecting eyes, lungs, face, limbs, ears, and cardiovascular, urogenital and central nervous systems (Maden 2000, Clagett-Dame & DeLuca 2002; Perrotta et al. 2003). Therefore, it is obvious that the accurate embryonic development is dependent upon adequate vitamin A supply. Normally the supply of vitamin A to the foetus is carefully regulated and is relatively insensitive to maternal vitamin A status, so emergence of mal-formations requires continuous vitamin A deprivation and exhaustion of tissue stores (Underwood 1994).

In humans, the causality between vitamin A deficiency and congenital malformations is less clear, because the insufficient availability of vitamin A is usually accompanied by the deficiency of other nutrients essential to normal development (Gerster 1997). There are some case reports suggesting that symptomatic vitamin A deficiency in the mother may be linked to ocular defects in offspring (Miller et al. 1998). However, in the absence of controlled studies, no definitive conclusions can be drawn.

5.2 Retinoid toxity

Excess intake of retinoids can produce toxicity appearing as hypervitaminosis A among adults and children or as teratogenicity during foetal life. The chance for toxicity is caused by the fact that, while retinoids are effectively absorbed, their clearance is slow (Hathcock et al. 1990). Thus, the body lacks the mechanism to rapidly destroy excessive loads. Excess carotene intake has not been shown to cause vitamin A toxicity, possibly because its cleavage to retinoids is tightly controlled (Bendich 1988; Dawson 2000). The potential risks of high dietary intake of retinoids in Nordic countries have raised increasing concern (NCM 2003).

As mentioned previously (Chapter 4.1.3), the liver is able to store large amounts of retinoids, serving as a reservoir for the steady supply of retinoids according to the body's needs. Controlled mobilisation ensures normal concentration of retinoids in plasma despite fluctuations in intake. However, if the consumption of retinoids is excessive, the liver stores become saturated, and excess retinoids spill over to blood circulation (NCM 2003).

5.2.1 Hypervitaminosis A

Epidemiology

Hypervitaminosis A is a minor problem compared with the incidence of vitamin A deficiency — worldwide, an estimated 200 cases of symptomatic hypervitaminosis A occur annually (Bendich & Langseth 1989). Hypervitaminosis can be divided into two categories: acute, resulting from ingestion of a very high doses over a short period of time, and chronic, resulting from continued ingestion of high doses for months or even years (Hathcock et al. 1990). Based on reported human cases, chronic hypervitaminosis is more common than acute hyper-vitaminosis (Myhre et al. 2003). Acute cases are mostly reported among small children 0-2 years old, when chronic hypervitaminosis is more common in older age groups (Myhre et al. 2003).

Cases of hypervitaminosis A caused by natural food sources are rare, and illness is usually caused by

Table 10. The most commonly reported symptoms of hypervitaminosis A.

General symptoms
- loss of apetite, weight loss
- fever
- fatigue, tiredness
- impaired immunity

Gastrointestinal system
- nausea, vomiting
- abdominal pain
- liver dysfunction
- hepatomegaly
- cirrhosis
- splenomegaly

Musculoskeletal system
- reduced bone mineral density
- skeletal pain
- muscular pain
- joint pain

Skin and hair
- hair loss
- skin scaling
- mouth and lip fissures
- pruritus
- pigmentation/paleness
- haemorrhages
- exanthema

Nervous system and vision
- intracranial hypertension
  - headache
  - bulging of fontanelles
  - papillary oedema
- cerebral irritability
- ataxia (lack of coordination and balance or a disturbance of gait)
- blurred or doubled vision

References: Bendich & Langseth 1989; Dawson 2000; Myhre et al. 2003

misuse of retinoid supplements (Bendich & Langseth 1989; Myhre et al. 2003; NCM 2003). In the Nordic countries, 17 cases of suspected hypervitaminosis A have been described in the scientific literature, all of which were chronic hypervitaminosis due to intake of retinoid supplements (NCM 2003). Case reports were published in nine articles between 1959 and 1983. None of these reports was from Finland.

Biochemical basis and symptoms of hypervitaminosis A

Retinoids are normally transported in plasma as retinol bound to specific transport protein, retinol-binding protein (RBP) (Kanai et al. 1968). With surplus retinoid supply, the demand for RBP is greater than what is available (Mallia et al. 1975). The result is that excess retinol is associated with plasma lipoproteins in esterified form. Esterified retinol not bound to RPB is a surface-active compound, and is believed that many toxic effects are due to its ability to damage biological membranes (Mallia et al. 1975). However, some toxic consequences, especially histopathologic effects like cirrhosis and osteoporosis due to chronic retinoid exposure, are mediated by alterations in gene expression and by modulation of humeral and intercellular signalling (Biesalski 1989; NCM 2003).

There is no consensus which symptoms or biomarkers should be fulfilled to be classified as hypervitaminosis A. Due to altered retinoid metabolism, high levels of retinyl esters in plasma is a good marker of vitamin A toxicity (NCM 2003). The pattern of symptoms may vary depending on the age of the victim and the duration of exposure. The multiple clinical symptoms most commonly associated to acute and chronic hypervitaminosis A are shown in Table 10. The symptoms are usually reversible and rapid recovery results when excessive intakes are discontinued (Bendich & Langseth 1989). However, damages in bone mineral density and in liver functions may be permanent (EVM 2003).

5.2.2 Teratogenicity

Animal studies

Numerous experimental animal studies have shown that excess retinol, retinoic acid or other natural or synthetic retinoids can cause embryotoxic effects if administrated during pregnancy (Tzimas & Nau 2001). Embryotoxic effects have been seen in every animal species tested, including mammals, birds, amphibians and fishes. Teratogenicity can occur at doses that do not induce any detectable toxicity symptoms in the mother (Tzimas & Nau 2001). Teratogenic effects are seen independent of the type of source of retinoids, i.e., given out as chemical preparation or foods rich of retinoids (Biesalski 1989; Akase et al. 2003).

Excess retinoid exposure has been shown to produce congenital malformations in almost all organ systems, and more than 70 types of anomalies have been described (Miller & Hayes 1982; Bendich & Langseth 1989). The type and severity of defects depend on the amount of exposure and the stage of gestation (Biesalski 1989). The most critical period for exposure is the time of organogenesis. Exposure during early organogenesis can result in anomalies of the craniofacial area, central nervous system and cardiovascular system, whereas later exposure is associated with defects of the limbs and urogenital system (Biesalski 1989). Even in the absence of overt structural abnormalities, embryonic retinoid expo-

sure can lead to minor brain defects, growth disturbances and behavioural abnormalities that become evident later during postnatal life (Miller & Hayes 1982; Holson et al. 1997). Behavioural effects in experimental animals include, for example, increased or reduced activity and learning deficits (Holson et al. 1997). Excessive retinoid intake also increases foetal mortality (Akase et al. 2003). Retinoids can cause embryotoxic effects given either as single doses or over several days during organogenesis (Biesalski 1989; Piersma et al. 1996).

Human studies

The first observations of human teratogenicity caused by retinoids were published in the 1980s, after retinoic acid derivatives had come to medical use as a treatment for dermatological diseases (Rosa et al. 1986). Children exposed prenatally to 13-cis-retinoic acid (isotretinoin) exhibit a pattern of congenital malformations including defects of the face and head, central nervous system, heart and thymus (table 11). In addition to birth defects, several spontaneous abortions have been described (Lammer et al. 1985, Rosa et al. 1986). Later it become evident that children exposed to isotretinoin often have reduced intelligence, but this impairment is not always accompanied by other malformations (Vorhees 1994). For major malformations, the most critical period for exposure seems to be between the second and fifth week of embryonic development (Rosa et al. 1986), but caution should be taken from the very beginning and up to the 60th day of pregnancy (SCF 2002). In contrast to animal experiments, in humans no clear dependency has been found between the severity of malformations and the retinoid dose (Biesalski 1989). As well, the duration of the exposure needed for development of teratogenic effects is unknown (NCM 2003).

While therapeutically used retinoid derivatives are well-known human teratogens, the teratogenicity of natural retinoids in humans is not so obvious. The consumption of large doses of retinoid supplements has been reported to be associated with congenital malformations in around twenty separate case reports (Rosa et al. 1986). It has been claimed, however, that in some of the cases the pattern of malformations described is not typical to retinoid exposure (Azaïs-Braesco & Pascal 2000). Nonetheless, there is sufficient evidence to consider natural retinoids as human teratogens. The evidence is supported by several animal studies clearly demonstrating the link between excessive intake of natural retinoids and teratogenesis. To further investigate the teratogenicity in humans, several epidemiological studies have been performed (as a review, see NCM 2003). Unfortunately, only limited conclusions can be drawn from these studies due to varying study designs and criteria, and the low number of women consuming high amounts of natural retinoids.

In the scientific literature, there are no reports of human teratogenesis due to retinoid intake of retinoids from foods. However, in Britain there has been one reported case of congenital malformations that may have been linked to the foodborne retinoids. In this case, the mother consumed a diet containing abnormally large amounts of liver, and the baby showed birth defects similar to those described in babies who were prenatally exposed to medically used retinoids like isotretionin (Buss et al. 1994).

Mechanisms of retinoid teratogenesis

Embryonic exposure to either an excess of retinoids or a deficiency of vitamin A leads to abnormal foetal development. Thus, it is obvious that retinoids are essential regulators of embryogenesis. Most of the teratogenic effects of retinoid excess become obvious during the early stages of pregnancy, when the major organ systems differentiate. In humans, the time of organogenesis is from the late third to eighth weeks of embryonic development (Larsen 1997).

No single mechanism is likely to explain retinoid teratogenesis. Many of the malformations observed due to retinoid excess appears to result from abnormal migration of neural crest cells (Hart et al. 1990; Morriss-Kay et al. 1993; Li et al. 2001; Williams et al. 2004). The neural crest is a special population of cells arising at the margins of the neural plate (Larsen 1997). These cells migrate to specific locations in the body and differentiate into several cell types. Neural crest cells give rise to many structures typically deformed by retinoid excess, like the bones and cartilages of the skull and the outflow of heart (Larsen 1997). Retinoids affect also the expression of homeobox genes that control the positional organisation of the developing embryo (Ross et al. 2000). Homeobox genes are involved in differentiation of many essential structures, for example, the head and neck, brain, heart, urogenital system and limbs (Innis 1997; Larsen 1997). Part of the teratogenic effect of excess retinoid exposure may be due to disruption of regional retinoid gradients in the embryo (Ross et al. 2000).

The molecular mechanism of retinoid teratogenesis is based on alterations in gene transcription. Normal embryonic development is a result of strictly controlled changes in gene activity regarding time and space. Excess retinoid exposure can lead to activation of genes that should be silent or inhibition of genes that should be expressed (Ross et al. 2000). Alterations in cell metabolism and intercellular interactions cause failures in division, migration and differentiation of cells, thus disturbing the ground plan of embryonic development.

Table 11. Typical malformations observed in infants with excess prenatal retinoid exposure.

Craniofacial area
- small or absent external ears and auditory canals
- low set ears
- micrognathia
- cleft palate
Central nervous system
- hydrocephalus
- microcephalia
- brain hypoplasia
- anomalies of retina or optic nerve
Cardiovascular system
- aortic arch abnormalities
- transposition of the large vessels
- ventricular septal defects
Thymus
- thymic hypoplasia

References: Lammer et al. 1985; Rosa et al. 1986

5.2.3 Dose-response in retinoid toxity from food or dietary supplements

Factors affecting the toxicity of retinoids

Dietary retinoids are derived from both foods and dietary supplements, and the toxicity of a given dose may vary depending on the source. Retinoid in foods such as liver or supplemental retinoids in oily solution seems to be less toxic than those preparations where retinoids are blended with water or packed in dry tablets (NCM 2003). One reason for the difference may be the more efficient absorption of water-based retinoids compared with retinoids in oily solutions. Different sources also result in different sets of retinoid metabolites, which may have a different toxicological potential (NCM 2003).

The toxicity of a given dose is also affected by age, general health status (protein status, liver and kidney function etc.) and interactions of retinoids with other dietary factors or xenobiotics (Bendich & Langseth 1989, NCM 2003).

Hypervitaminosis A

Acute toxicity is reported with supplemental doses of 6,000-37,600 μg RE/kg in adults (Myhre et al. 2003). Onset of chronic toxicity is dependent on the dose and the length of exposure. For oil-based preparations, intakes over 2,000 μg RE/kg/day for weeks or months is needed to induce hypervitaminosis A. If administrated in a water-miscible or emulsified form, the threshold dose reduces and the toxic symptoms appear after an intake of 200 μg RE/kg/day for a few weeks. (Myhre et al. 2003). Hepatic cirrhosis due to vitamin A overdose has not been reported below intakes of 7,500 μg RE/day taken continuously over several years (SCF 2002).

The symptoms of hypervitaminosis A include a reduction in bone mineral density (Table 10) indicating that high doses of retinoids interfere with bone metabolism. The evidence of association is established by many animal studies, where very high doses of retinoids have caused bone lesions and spontaneous fractures in laboratory animals (EVM 2003; SACN 2005). While the connection between bone abnormalities and very high doses of retinoids is clear, the skeletal effects of retinoid intake in the physiological range are poorly known. Some recent epidemiological data suggest that intakes of 1,500 μg RE/day or more may be associated with an increased risk of bone fracture, but the evidence is inconsistent (SACN 2005). Although evidence is insufficient to make any general dietary recommendations, the Scientific Advisory Committee on Nutrition sees that it may be advisable for population subgroups at increased risk of osteoporosis not to consume more than 1,500 μg RE/day (SACN 2005).

Teratogenicity

Several methods have been used to assess the dose-response relationship for the teratogenic effects of retinoids, or define a threshold above which maternal retinoid intake may be harmful to foetus. The only useable tool so far is human epidemiological data from case reports, case-control studies and cohort studies. Unfortunately, these data are very limited (as a review, see Rosa et al. 1986; SCF 2002; NCM 2003). Animal studies provide a lot of dose-response data, but due to the species variation in retinoid metabolism, kinetics and toxicodynamics, the risk extrapolation of animal data to humans is difficult (Tzimas & Nau 2001). As well, a metabolic approach has been proposed based on the hypothesis that human plasma concentrations of retinol and its metabolites is predictive to teratogenic risk. How-

ever, this method has number of problems that prevent its use for deriving safe levels of intake (SCF 2002).

Based on the available epidemiological data, it is not possible to establish a clear dose-response curve for the teratogenic effects of retinoids. Moreover, because of the long biological half-life of retinol and its bioaccumulative properties, retinoid levels in the body depend on the duration of use (Rosa et al. 1986). No associations have been found in the case-control studies between the intake of 3,000 μg RE/day or less and foetal malformations (NCM 2003). Rothman et al. (1995) suggested that retinoid intake over 3,000 μg RE/day increases the risk of teratogenicity, but other studies indicate that the threshold may be higher (EVM 2003; NCM 2003). Case reports linking high retinoid intake and birth defects involved supplemental retinoid exposure of 5,400 μg RE/day or more for several weeks during pregnancy (Rosa et al. 1986). The evidence remains insufficient to exclude the teratogenic risk between 3,000 and 5,400 μg RE/day. Therefore, many authorities recommend that the intake of retinoids from food and dietary supplements for women of childbearing age should not exceed 3,000 μg RE/day (FNB 2001; SCF 2002, EVM 2003). The Scientific Committee on Food (SCF 2002) recommends the same upper level also for men.

A high single dose of retinoids can also be teratogenic. There is one case report of congenital malformations after a massive single-dose exposure of 150 mg RE during the second month of pregnancy (Rosa et al. 1986). However, the threshold for single-dose teratogenesis is unknown. Although most case reports concerning malformations involve a long-time maternal exposure to retinoids, no conclusions can be made if a shorter time can lead to the same outcome. There are several malformation cases associated with maternal supplemental exposure to 7,500 μg RE/day (Rosa et al. 1986), and, according to the WHO (1998), a single supplemental dose over 7,500 μg RE (25,000 IU) is not advisable during pregnancy.

A question has arisen whether pre-existing body stores affect the dose-response for retinoid intake and teratogenesis. The hypothesis has been postulated that the risk of teratogenesis could be higher in mothers with high body retinoid reserves. Piersma et al. (1996) reported that the background vitamin A status seems not to influence the teratogenic potential of single high dose of retinoids in rats. However, the single dose used was so high that almost all foetuses were malformed even in a group with the lowest body retinoid reserves, and therefore the potential additional effect of high body stores cannot be totally ruled out.

5.3 Cadmium and lead toxity

5.3.1 Cadmium

Adverse health effects of chronic low-dose toxicity

Chronic cadmium exposure is responsible for a broad spectrum of adverse health effects. The kidney, in particular the renal cortex, is the critical organ in environmentally exposed populations (WHO 2001). Environmental exposure to cadmium may be sufficient to influence body calcium homeostasis and bone metabolism, thus decreasing bone density and increasing the risk of fractures (WHO 2001; Satarug & Moore 2004). Exposure to cadmium may also contribute to the development of cancers and neurotoxic effects (WHO 2001).

In human pregnancy, maternal exposure to cadmium associated with early delivery and low birth weight (Nishijo et al. 2002). Only a small amount of the cadmium in maternal circulation crosses human placenta, and hence the foetus seems to be protected against cadmium load (Korpela 1986). However, cadmium affects placental hormone secretion, which might contribute to premature delivery (Henson & Chedrese 2004).

Dose-response

Cadmium exposure can be measured by cadmium concentration in blood or urine. In most studies, the adverse health effects have been related to urinary levels of cadmium. It has been estimated that the prevalence of renal toxicity in the general population increases when urinary cadmium concentrations exceeds 2.5 μg/l (2.5 μg/g creatinine) (Järup et al. 1998). This corresponds to a renal cadmium concentration of 50 μg/g and a long-term cadmium intake of about 50 μg/day. Approximately 5% of population would reach their critical renal concentration at a urinary cadmium excretion of 4.75 μg/l, corresponding to the intake of about 100 μg Cd/day (Järup et al. 1998) The risk of adverse pregnancy outcomes is also increased with urinary cadmium concentration over 2 μg Cd/l (Nishijo et al. 2002).

5.3.2 Lead

Adverse health effects of chronic low-dose toxicity

Lead adversely affects several organs and organ systems (IPCS 1995). Anaemia caused by inhibition of heme synthesis and neurotoxic effects are probably the best known symptoms of lead toxicity. Other health effects include renal toxicity, increased blood pressure and effects on calcium homeostasis (IPCS 1995; Papanikolaou et al. 2005). Lead exposure alters many enzyme systems, thus affecting various cellular processes (IPCS 1995).

Lead is readily transported across the placenta, and is taken up by the foetus from 12th week of pregnancy (Papanikolaou et al. 2005; Harville et al. 2005). Lead can cause toxic effects in the foetus. The most critical effect is the impairment of neurological development of the foetus (UNEP 1998; Schnaas et al. 2006). Exposure to lead also increases the risk of foetal death and preterm delivery, and is associated with many minor malformations in the baby (WHO 2003).

Dose-response

Metabolic studies in infants and children indicate that in intakes less than 4 μg/kg body weight per day the excretion of lead exceeded intake. (WHO 2003).

Exposure to lead can be monitored by blood lead concentration. In 1994, the average blood lead concentration among Finnish women and men was 0.11 μmol/l (23 μg/l) and 0.17 μmol/l (35 μg/l), respectively, with a range of 0 to 0.36 μmol/l (0-74 μg/l) in both sexes (Alfthan et al. 1994). Due to reduced lead discharge after the removal of lead from gasoline in the 1990s, the trend in lead exposure has been declining. In 2002, the blood lead concentrations of pregnant women in Lapland were 0.05 μmol/l (11.3 μg/l) with a range of 0.02-0.2 μmol/l (5-58 μg/l) (Soininen et al. 2002).

Symptoms of chronic lead toxicity may appear in adults at blood lead levels of 400-800 μg/l (UNEP 1998; WHO 2003). Biochemical effects in heme synthesis are seen at exposure levels of 30-300 μg Pb/l (WHO 2003). Among pregnant women, the risk of preterm delivery is reported to be fourfold among women with blood lead levels above 140 μg/l compared with women with blood lead levels of 80 μg/l or less (WHO 2003).

One of the most critical effects of lead exposure is reduced intellectual development of the children. Defects may be caused from lead exposure during foetal period or after birth. Unfortunately, there seems to be no threshold for these effects (WHO 2003, Schnaas et al. 2006). In the study of Schnaas et al. (2006), the range of maternal blood lead values were 10 to 320 μg/l, but half of the deleterious effects of lead occurred within the range of 10 to 60 μg Pb/l.

The foetus is exposed not only the exogenous maternal lead intake, but also the endogenous lead accumulated by the mother prior to pregnancy. During pregnancy and lactation, the maternal bone resorption increases to meet the calcium needs of the developing offspring skeleton (Gulson et al. 2004). As a result, the mobilisation of the bone lead increases. Blood lead concentration rises on average 25-30% during the pregnancy and 65% during lactation (Gulson et al. 1997 and 2004). This is contributed by increased absorption of dietary lead and decreased elimination of lead burden during pregnancy (Rothenberg et al. 1994).

Since lead is shown to be toxic at very low exposures, and because no safe level of lead exposure has been found (UNEP 1998), any exposure should be considered to be a potential concern.

6 Exposure assessment

6.1 Design of the exposure assessment

For exposure assessment, four different datasets were collected:

1. The consumption of liver products among women at ages between 25-44 years was obtained from the FINDIET 2002 survey (Männistö et al. 2003). Data of vitamin A intake of vitamin A from non-liver sources was also based on the FINDIET 2002 study.

2. The amount and type of liver in the consumed liver foods were estimated relative to the market shares of all Finnish liver products. The information was obtained from the food industry and the National Public Health Institute of Finland.

3. Laboratory analyses of swine, bovine and chicken liver samples were carried out to obtain information on the vitamin A content in the raw material of liver foods.

4. Cadmium and lead concentrations in livers were obtained from analyses made in connection with the National Residue Control Program (NRCP) between 2000 and 2004.

Based on the collected data, a mathematical model was built to simulate women's exposure to vitamin A, cadmium and lead whether liver foods are eaten or not. Simulated results were compared with the intake recommendations for vitamin A and with the intake limits for retinoids and heavy metals.

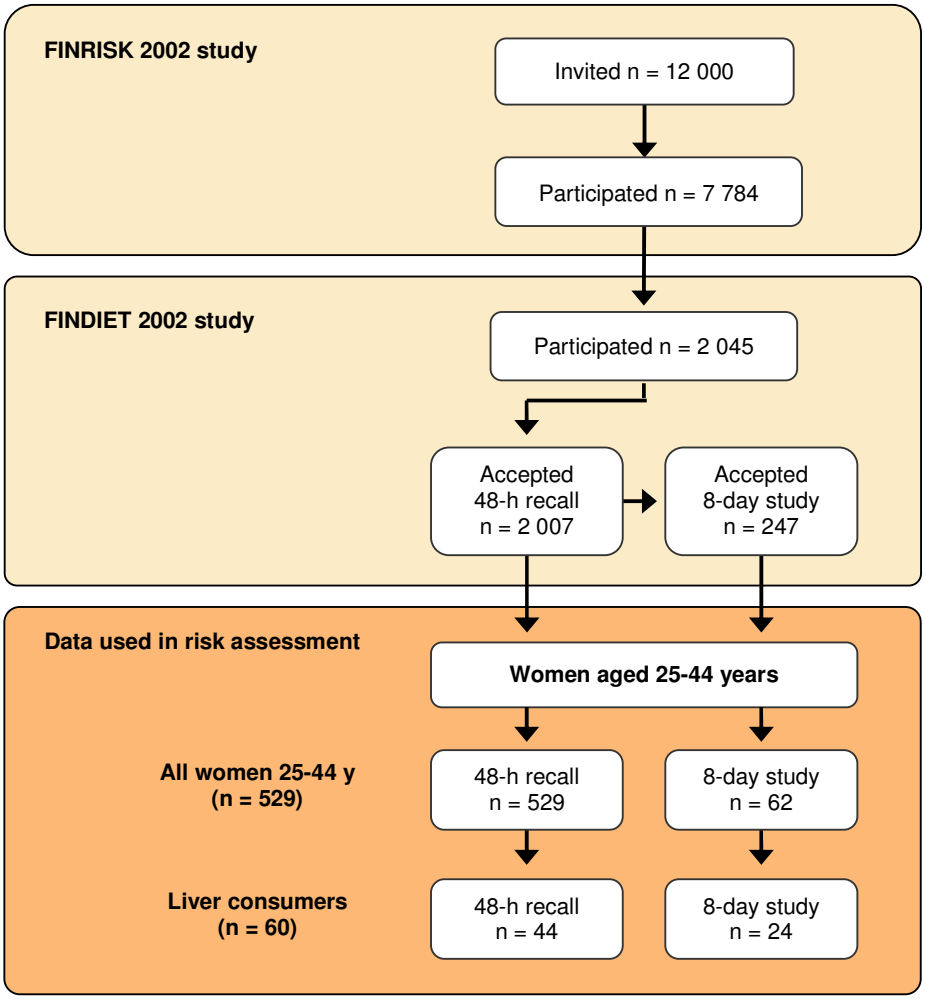


Figure 4. Chart of the structure of FINDIET 2002 study and the part of the data used in risk assessment.

6.1.1 Application of the FINDIET 2002 survey

Data on consumption of liver products and intake of vitamin A from non-liver foods and dietary supplements were obtained from the FINDIET 2002 survey.

FINDIET 2002 study design

The FINDIET 2002 study is a national survey that was carried out as part of the FINRISK 2002 study, which monitors cardiovascular risk factors among Finnish adult population. For FINRISK 2002 study, a random sample of 12,000 persons from 25 to 64 years of age and stratified by sex and 10-year age groups was drawn from the population register. The participation rate was 65% (7,784 subjects) (Figure 4). Of the invited subjects, 32% were randomly selected to participate in the dietary survey. The final number of participants in the dietary survey was 2007 subjects (Figure 4).

The FINDIET 2002 study was carried out in five areas: 1) the Helsinki metropolitan area, 2) the cities of Turku and Loimaa, and some rural communities in southwestern Finland, and in the provinces of 3) North Karelia, 4) North Savo, and 5) Oulu. The participants were interviewed using the 48-hour recall method. The dietary intake data covered all days of the week except Fridays. A sub-sample (n=247) of the participants filled in a three-day food record twice (the first starting the day after the 48-hour recall in early spring, and the second in autumn).

The National Food Composition Database Fineli® (KTL 2005) was used to calculate the intake of vitamin A from non-liver foods and from dietary supplements. Data on the use of dietary supplements during the preceding six months were collected with a questionnaire. Participants filled in the brand name of the supplement, dosage, and frequency of use. The Fineli database was used to calculate the intake of vitamin A from dietary supplements.

Underreporting in the FINDIET 2002 study

In food consumption studies, some participants report energy intakes that are lower than their estimated basic metabolic rate. In the FINDIET 2002 study, 37% of respondents were underreporters (KTL 2006). Underreporting affects the intake estimates of many micronutrients (Hirvonen et al. 1997). However, underreporting is probably unevenly distributed among different food groups, and therefore corrective actions to reduce the bias are difficult to implement.

FINDIET 2002 data used in this risk assessment

In this study, the FINDIET 2002 data on women aged 25-44 years was used. A 48-hour recall was filled by 529 women of a given age interval, and 62 of them participated in the eight-day study (Figure 4). The age distribution of women in the part of the FINDIET 2002 data used (n = 527) and the age distribution of Finnish parturients in 2004 (n = 56,878) are expressed in Figure 5. Due to the age limits in the FINDIET study, the mean age of parturientsis somewhat lower than the mean age of women participated in the FINDIET 2002 study.

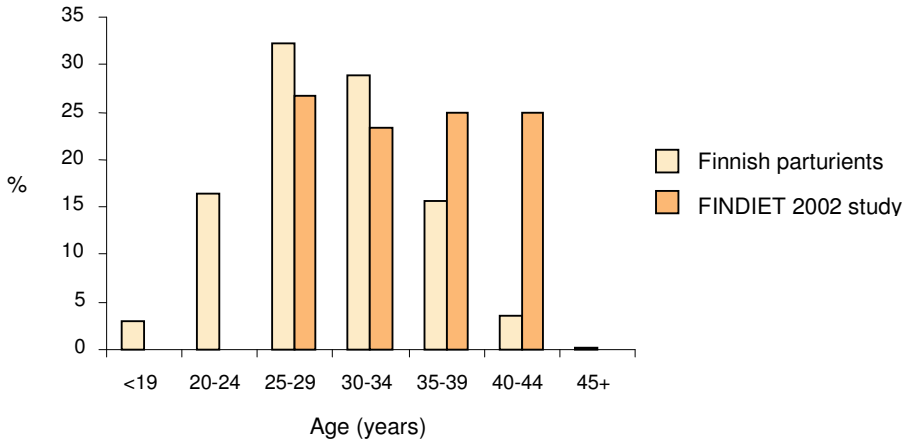


Figure 5. The age distribution of women aged 25-44 years in the FINDIET 2002 study (n = 529) used in risk assessment compared with the age distribution of Finnish parturients in 2004 (n = 56,878) (Stakes 2005).

Table 12. The use of different liver foods among women aged 25-44 years in the FINDIET 2002 study. Based on 48-hour and eight-day food consumption data (n = 529).

Food	Number of consumers	Proportion of consumers (%)	
		Among liver consumers (n = 60)	Among all women (n = 529)
Liver sausage and pâté	44	73.3	8.3
Liver casserole	14	23.3	2.6
Liver patties	4	6.7	0.8
Liver stew	3	5.0	0.6

6.2 Consumption of liver

Consumption estimates were based on the FINDIET 2002 data (Männistö et al. 2003). In total, 60 (11.3%) of 529 women reported liver consumption during the FINDIET study (Figure 4). Four (6.7%) of these 60 women ate more than one liver product during the study period. In the 48-hour recall including 529 women, 44 (8.3%) ate liver foods (Table 12). During the eight-day study period including 62 women, 24 (38.7%) ate liver foods. The most commonly eaten liver food was liver sausage or pâté, followed by liver casserole. Only a few women ate liver patties or liver stew (Table 12).

Estimates of the average daily consumption (g/day) of liver products were based on two variables: eating frequency and portion size.

1. Eating frequency of liver foods

Eating frequency was defined as the number of study days when certain liver products were eaten. Liver products are not consumed daily, and the eight-day study period was estimated to correspond to better the real liver eating frequency than the 48-hour recall. Therefore, the frequency estimate was based on eight-day study period. Eating frequencies are expressed in Table 13.

2. Portion size

Portion size was defined as an amount of liver product consumed during a day (g/day).To estimate portion sizes for consumption estimates, both 48-hour and eight-day data was used, with a view to maximize the sample size. The mean portion size for liver sausage and pâté was 42 g (n = 68, median 32 g, range 10-190 g), for liver casserole 167 g (n = 15, median 164 g, range 70-300 g), for liver patties 119 g (n = 4, median 130 g, range 85-130 g), and for liver stew 138 g (n = 4, median 150 g, range 100-150).

Eating frequency and portion size were treated as unrelated variables. In the case of liver sausage and pâté, eating frequency was independent of portionsize in the eight-day data (Fisher's exact test, n = 31, p = 1.0). In the case of other liver products, either the eating frequency or portion size was constant.

The consumption data included no information about the proportion of liver eaters in the real population, i.e., the size of the sub-population with possible liver consumption in the long run. Therefore, no reliable calculations could be done to estimate the general intake of vitamin A from liver foods among liver eaters, and estimated liver consumption reflects only those eight-day periods when at least one liver food is eaten.

Table 13. Distributions of eating frequencies of liver foods among women aged 25-44 years in the FINDIET 2002 study. Frequencies are based on eight-day food consumption data among those women who ate liver foods (n = 24). Percentages are proportions of women eating each liver food at named frequency.

Product	Eating frequency (eating days during 8-day study)				
	0	1	2	3	4
Liver sausage and pâté	38 %	25 %	17 %	13 %	8 %
Liver casserole	71 %	29 %	-	-	-
Liver patties	83 %	17 %	-	-	-
Liver stew	92 %	4 %	4 %	-	-

6.3 Liver in liver foods

6.3.1 Amount and type of liver in liver foods

To quantify the liver content of consumed liver foods, recipe information was used. For commercially available prepared liver foods (liver casserole and liver sausage and pâté), recipes were obtained from Finnish food industry. For liver patties and liver stew, which were not available as prepared food, the customary recipes were received from the National Public Health Institute of Finland. Information was gathered on both the amount and the type of liver (animal species). The amount of liver was reported as a percentage of weight of the end product, i.e., the amount of raw liver (g) needed to produce 100 g end product. Thus, the water loss during food manufacture was included in the numbers.

In the case of prepared liver foods, recipes were asked from all Finnish liver food manufacturers. Reported liver contents were then averaged out relative to the market shares (situation in 2004). The results are summarised in Table 14. Information was received from all but three products with a small market share (0.3-0.9%). In liver sausage and pâté, the liver used is mainly pork liver, whereas in liver casserole several liver types are mixed and used in very variable rations. Variation in liver types is seen not only between manufacturers but also between production runs in one producer. Although the type of liver used may vary, the total liver content of the products is kept very constant by each manufacturer.

The mean liver content in liver sausage or pâté was 20.5% (range 8-42%) and in liver casserole 13.6% (range 10-21%) (Table 14).

For liver patties and liver stew, the following common recipes were obtained from the National Public Health Institute of Finland:

- liver patties: liver content 50%; one-third swine liver and two-thirds bovine liver.

- liver stew: liver content 33%; one-third swine liver and two-thirds bovine liver.

In recipes, swine liver includes both finishing pig liver and sow liver. According to Finnish food manufacturers, sow livers make up no more than 10% of swine livers used. Sow livers have significantly higher retinoid content compared with finishing pig livers, and therefore these two liver types were dealt separately in this risk assessment.

6.3.2 Size of production run

The size of the production run can affect the run-to-run stability of the final retinoid content of the product. If a large amount of food is produced at a time, the amount of liver used is also large, and livers with high and low retinoid content compensate for each other. Meanwhile, if the production run is small with only one or a few livers in a run, the final retinoid content varies more due to chance.

Among Finnish food manufacturers, the sizes of single production runs vary depending on the production volume and the amount of purchase orders. One batch of liver sausage or pâté can use 20-120 kg of liver. For liver casserole, one batch uses around 170 kg of liver among the large manufacturers. Liver patties and liver stew are served in canteens and restaurants in different volumes. Liver foods are also made in small quantities at home.

Table 14. Liver contents of Finnish industrial liver foods obtained from food manufacturers. Contents are calculated as an arithmetic means and as a weighted means relative to market shares. (n = number of trade names with content information available/number of trade names on sale in 2004.)

Product and statistic	Total liver content (%)[1]	Type of liver (% of total liver content)		
		Swine	Bovine	Chicken
Liver sausage and pâté (n = 24/27)				
Mean (min-max)	18.4 (8-42)	95.8 (0-100)	4.2 (0-100)	–
Weighted mean	20.5	98.9	1.1	–
Liver casserole (n = 7/7)				
Mean (min-max)	14.1 (10-21)	64.5 (0-100)	18.3 (0-100)	17.2 (0-45)
Weighted mean	13.6	67.5	3.4	29.1

[1] Amount of raw liver component as a percentage of weight of the end product.

Table 15. Collected liver samples.

Species	Number of liver samples	Number of slaughterhouses	Age of sampled animals
Bovine	76[1]	8	1.25 -10 years
Swine			
Pig	91[1]	10	6 months
Sow	37[1]	6	not defined
Chicken	27[2]	2	35 days

[1] Samples from single livers
[2] Pooled samples of ten livers

6.4 Vitamin A in liver

6.4.1 Laboratory experiments to determine vitamin A in liver

Sampling

Liver samples from bovine, swine and chicken were collected from Finnish slaughterhouses during the spring of 2005. All bovine samples and part of the swine samples were taken in the context of the National Residue Control Program. Additional swine samples and all chicken samples were collected separately for purposes of this study. These separate samples were randomly taken from animals of different producers. Bovine and swine livers were treated as individual samples, but, among chickens, each sample comprised of the livers of ten birds from the same producer. Samples were sent (frozen at -20˚C) to the laboratory where they were stored at -70˚C until analysed. The number of liver samples analysed and the number of slaughterhouses whose animals were sampled are summarised into *Table 15*. All the chickens were slaughtered at the age of 35 days and pigs at the age of six months. The age of bovines varied from 13 months to 12 years. The age of the sows was not defined. A possible seasonal variation in liver vitamin A content was not studied or taken into account in the simulation model calculations.

Analytical methodology

Pre-treatment of the samples involved sample homogenisation, protein precipitation and liquid-liquid extraction. Retinol, retinyl esters and ß-carotene (only from bovines) were analysed from liver samples with high-performance liquid chromatography (HPLC) using diode array detection at 325 nm. The analysing procedure based on the methods of Barua et al. (1998) and van Merris et al. (2002). In the first step of analysis, 1 g of liver sample was homogenised with 20 ml of tris-HCl buffer, pH of 7.5 (shortly, 0.635 g tris-HCl and 0.118 g tris-base was diluted in 100 ml of water). 500 mg of buffer-sample homogenate was accurately weighted into the Eppendorf vial, 50 µl of internal standard (retinyl acetate, 6.25 µg/ml in injection volume), 150 µl methanol and 50 mg ascorbic acid were added into the vial. Analytes were extracted from precipitated sample homogenate with 3x400 µl di-isopropylether. Solvent phases were collected, evaporated with nitrogen, diluted in 200 µl of methanol:ethyl acetate (3:1) and injected into the HPLC system. Peak identification based on diode array spectroscopic data of analytes (in the range 210 nm–500 nm) and specific retention times with commercially available (retinol, retinol acetate and retinol palmitate) and synthesised standard compounds (retinol stearate, retinol oleate, retinol linoleate. Syntheses were kindly carried out by Helsinki University, Department of Analytical Chemistry). Examples of chromatograms for the samples are shown in Figure 6.

6.4.2 Results of laboratory analyses

Liver vitamin A content (µg RE/g) in each sample was calculated as a sum of the analysed vitamin A activity of retinol and retinyl esters. The vitamin A activity of ß-carotene was not taken into account, because its impact on total vitamin A activity would have been negligible.

The analysed mean liver retinoid content was 170 µg RE/g in pigs (median 164 µg RE/g, range 28-549 µg RE/g), 443 µg RE/g in sows (median 337, range 50-2,123 µg RE/g), 213 µg RE/g in bovines (median 175 µg RE/g, range 44-744 µg RE/g) and 106 µg RE/g in chickens (median 97 µg RE/g, range 34-277 µg RE/g) (Figure 7). There was a statistically significant difference in liver retinoid content between the species (log-transformation, Kruskall-Wallis test, $X^2 = 46.7$, $P < 0.001$). In a pair-wise comparison, the significant difference (Mann-Whitney test, $P \leq 0.001$) was found in all pairs except pigs and bovines (Mann-Whitney test, $P = 0.16$).

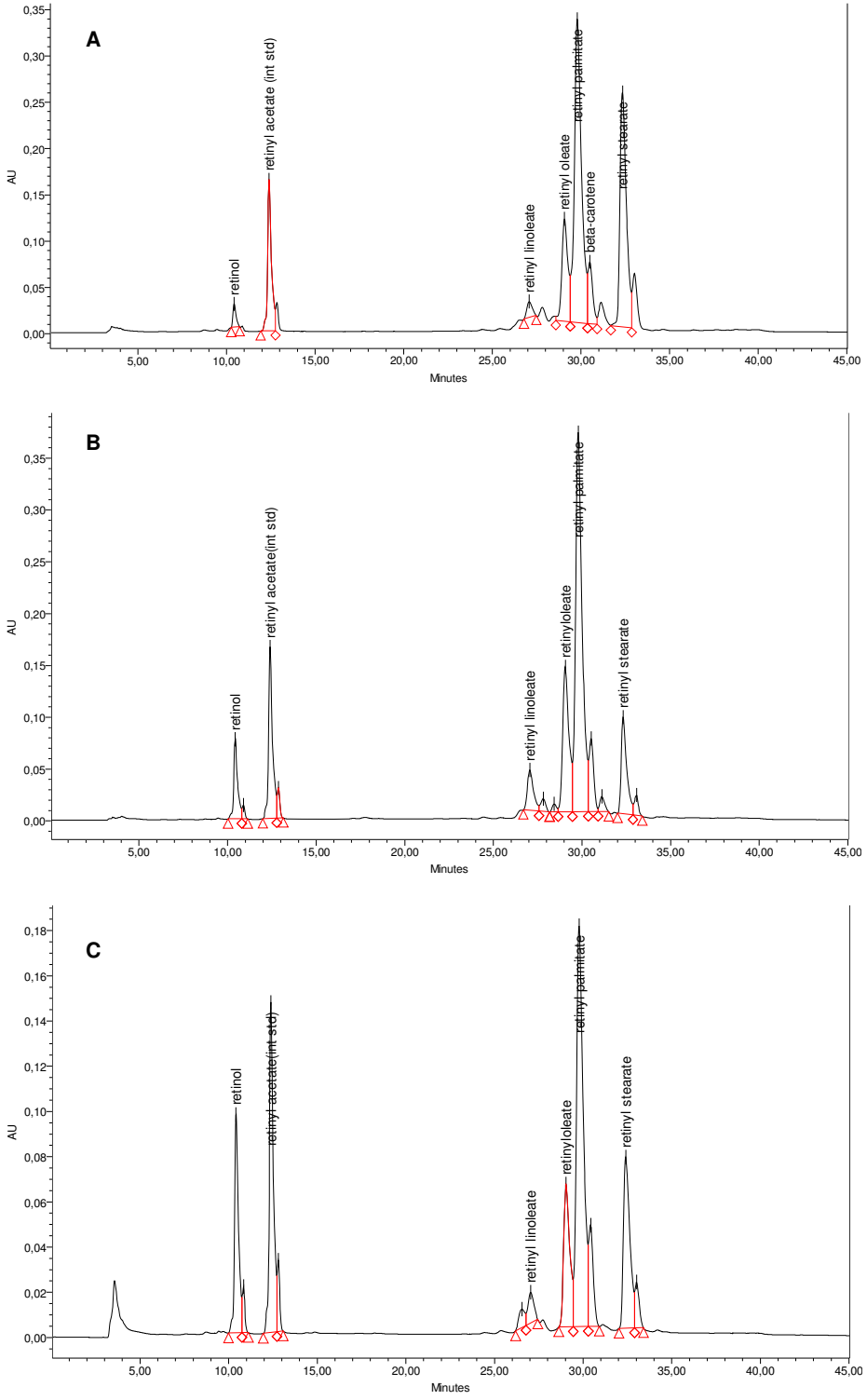


Figure 6. Examples of chromatograms of bovine liver (A), pig liver (B) and chicken liver (C) at the wavelength of 325 nm.

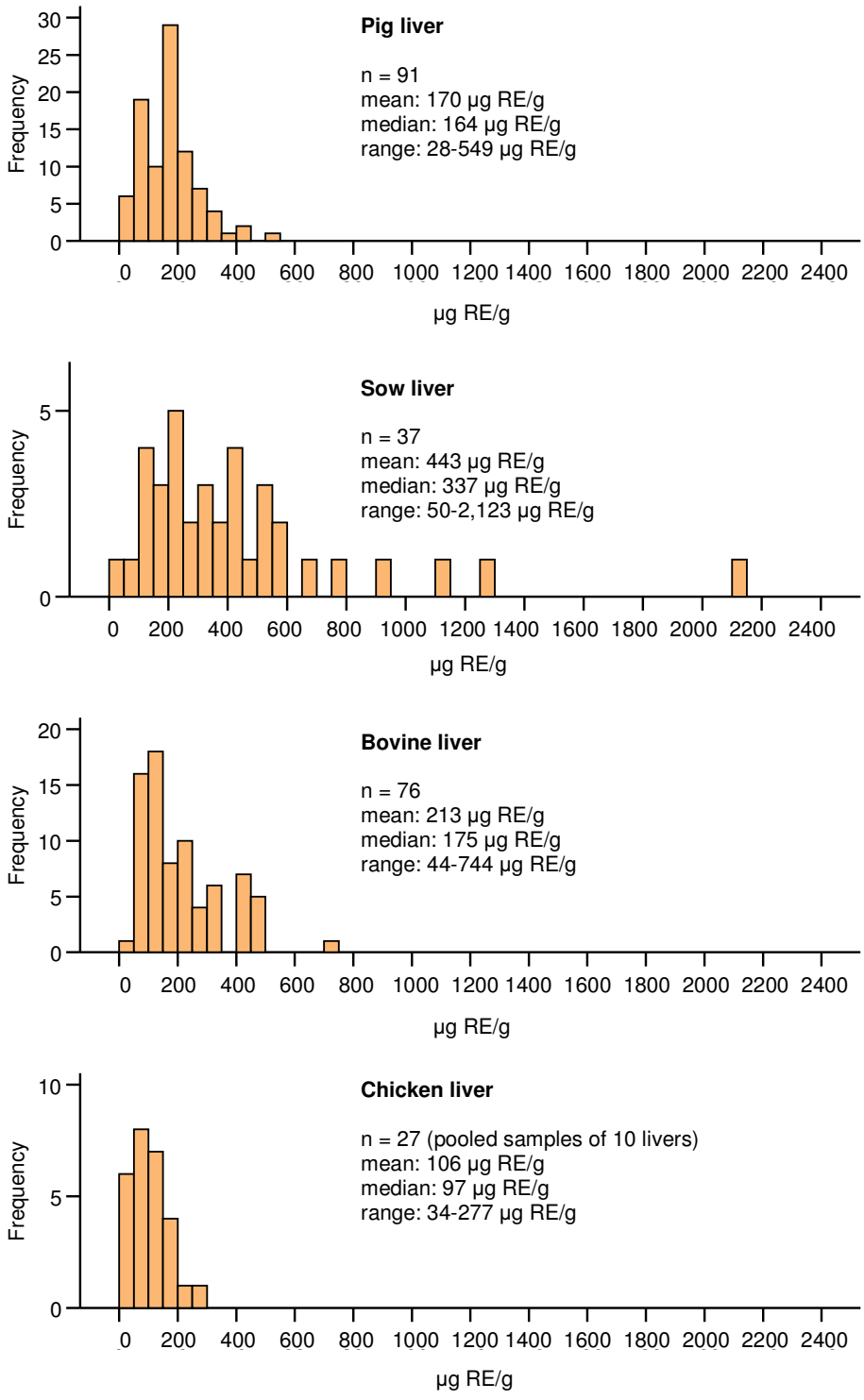


Figure 7. Histograms of liver retinoid content in pigs, sows, bovines and chickens with some distribution statistics. Results of laboratory analyses made in EELA in 2005.

Current retinoid contents in pig livers are about the same as in the 1990s. In the analyses made between August 1990 and October 1991, the mean retinoid content of pig livers was 200 μg RE/g with a range of 28 to 540 μg RE/g (Hirvi et al. 1992). These values are clearly below the results made in 1989, when mean retinoid content was about 600 μg RE/g liver (Heinonen 1990), and there was public concern of the safety of liver as food. The rapid decline in pig liver retinoid content took place at the beginning of 1990, probably due to the new recommendations concerning reduced use of vitamin A in feeds (Hirvi et al. 1992).

The mean retinoid content of bovine liver has not changed markedly since 1989 and 1991. In 1989, the mean concentration was 270 μg RE/g liver (Heinonen 1990). In 1991, the mean concentration was 150-300 μg RE/g liver, depending on the age of the animal (Hirvi et al. 1992). Unlike bovines, in chickens there has been a clear decline in liver retinoid content. In 1989, the mean liver retinoid content in chicken was 370 μg RE/g (Heinonen 1990) and in 1991 180 μg RE/g (Hirvi et al. 1992), when the current result is 106 μg RE/g liver.

The retinoid contents (mean and variation) of pig, cattle and chicken livers in Finland and some other European countries are compared in *Table 16.* The retinoid contents are notably lower in Austria than in the Nordic countries, especially in the case of cattle and chickens. This is probably due to the differences in feeding and production circumstances. However, such low retinoid levels in healthy animals might indicate that retinoid supplementation practices in animal husbandry in Finland could be further re-considered.

Table 16. Vitamin A content (μg RE/g) of bovine, pig and chicken livers in different European countries.
N = number of samples; NA = data not available.

A. Bovine liver

	Mean	Range	N
Finland[1]	213	44-744	76
Sweden[2]	144	NA	NA
Norway[3]	232	227-242	NA
Denmark[4]	155	31-360	24
Austria[5]	41*	11-67*	18*

* Animals less than 2 years old

B. Pig liver

	Mean	Range	N
Finland[1]	170	28-549	91
Sweden[2]	130	NA	NA
Norway[3]	236	163-227	NA
Denmark[4]	141	30-520	253
Austria[5]	111	65-189	18

C. Chicken liver

	Mean	Range	N
Finland[1]	106*	34-277*	27*
Sweden[2]	95	NA	NA
Norway[3]	NA	NA	NA
Denmark[4]	180	20-537	7
Austria[5]	56	16-166	18

* Pooled samples of 10 livers

[1] Analyses made in EELA in 2005
[2] Livsmedelsverket 2006
[3] Borgejordet 2006
[4] Møller et al. 2005
[5] Majchrzak et al. 2006

6.5 Vitamin A from non-liver sources

Intake estimates of Vitamin A intakes from non-liver sources were based on the eight-day FINDIET 2002 data (Männistö et al 2003). Both natural and supplemental intake was estimated using the method of Nusser and co-workers (1996). The method gives the long-run average of daily intakes (usual daily intake) by taking into account day-to-day–correlation and nuisance effects (such as day-of-week and interview sequence). It also allows exceptions from normality through grafted polynomial transformations and recognises the measurement error associated with one-day dietary intakes. Estimates were done using SAS based SIDE® program.

In carotenoid intake calculations, following ratios were used: 1 μg RE = 12 μg of dietary ß-carotene = 24 μg of other dietary provitamin A carotenoids.

Based on eight-day food consumption records of women aged 25-44 years (n = 62), the median intake of total vitamin A was 440 μg RE/day (5th-9th range 260-720 μg RE/day) when derived from non-liver foods only, and 490 μg RE/day (5th-9th range 240-970 μg RE/day) when derived from both non-liver foods and dietary supplements. For retinoids, the median intake was 250 μg RE/day irrespective of the differences in sources. The 5th-9th range in retinoid intake was 140-430 μg RE/day when intake was from foods only and 120-530 μg RE/day when intake of dietary supplements was included. The intake distributions are shown in Figure 8. The most important natural sources are aggregated in Table 17.

Non-liver derived intake was similar among liver eaters (n = 38) and non-liver eaters (n = 24) for both total vitamin A (t-test, intake of vitamin A from foods, t = -0.54, p = 0.59; intake of vitamin A from dietary supplements, t = 1.61, p = 0.12) and retinoids (t-test, intake of retinoids from foods, t = 0.02, p = 0.98; intake of retinoids from dietary supplements, t = 1.61, p = 0.12).

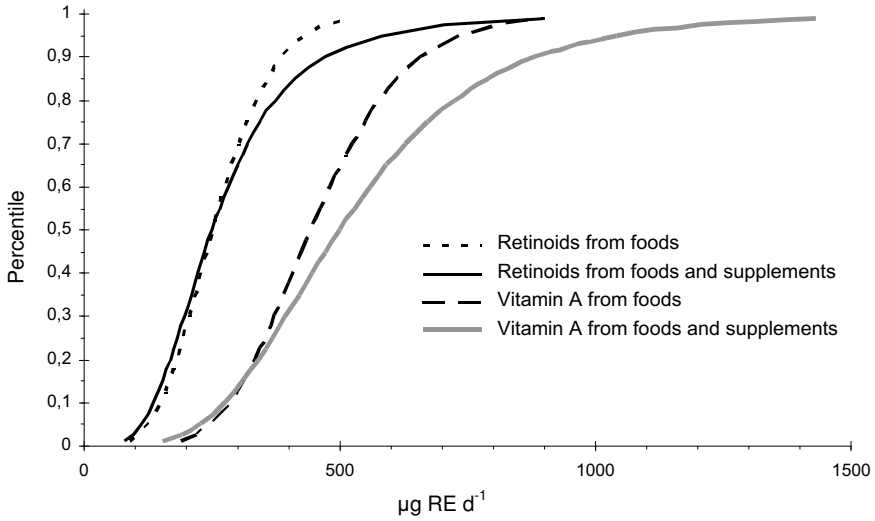


Figure 8. Average daily intakes of non-liver derived vitamin A and retinoids among women aged 25-44 years in FINDIET 2002 study. Cumulative distributions based on eight-day food consumption data (n = 62). Intakes from foods only and from foods and dietary supplements are shown separately.

Table 17. The most important non-liver food sources of vitamin A (a) and retinoids (b) among women aged 25-44 years in the FINDIET 2002 study (n = 529).

a. Vitamin A

Food	% of total intake
Roots	32.2
Cheese	7.8
Fruit vegetables	7.2
Butter and butter spread	6.3
Vegetable fat	6.2

b. Retinoids

Food	% of total intake
Cheese	24.3
Butter and butter spread	13.2
Egg	9.1
Cream	6.1
Vegetable fat	5.4

Table 18. Mean cadmium and lead contents ($\bar{x}$, μg/kg) in bovine, swine and chicken livers between 2000 and 2004. Data from the National Veterinary and Food Research Institute of Finland (EELA). N = number of samples analyzed.

	Cadmium						Lead					
	Bovine		Swine		Chicken		Bovine		Swine		Chicken	
Year	$\bar{x}$	N	$\bar{x}$	N	$\bar{x}$	N	$\bar{x}$	N	$\bar{x}$	N	$\bar{x}$	N
2000	35	31	29	33	11	13	60	31	60	33	70	13
2001	34	31	29	34	14	6	50	31	50	34	40	6
2002	26	30	25	32	8	5	40	30	40	32	50	5
2003	20	30	20	31	8	5	50	30	50	31	50	5
2004	21	30	26	29	12	5	40	31	20	29	20	5
Mean of years 2000-2004	**27**		**26**		**11**		**48**		**44**		**46**	

6.6 Cadmium and lead in liver

Data on cadmium and lead contents of livers were obtained from the National Veterinary and Food Research Institute of Finland (EELA). Data used was from routine analyses made in connection with the National Residue Control Program between 2000 and 2004. Five to ten chicken livers and around 30 livers of bovine and swine and were analysed annually. To assess the intake of heavy metals from liver foods, the averages of the annual means were used. For cadmium, the average of the annual means was 25 μg Cd/kg liver among bovines and swine and 10 μg Cd/kg liver among chickens. For lead, the average of annual mean was around 45 μg Pb/kg liver among all species (Table 18).

6.7 Simulation model

6.7.1 Aims of the model

The simulation model was designed to quantitatively estimate the intake of total vitamin A and retinoids from liver and non-liver sources in Finnish women of fertile age. The main objectives of the model were:

1. to estimate retinoid exposure via liver foods among Finnish women aged 25-44 years

2. to estimate the risk of intolerably high retinoid intake if liver foods were eaten similarly during pregnancy

3. to estimate the effects of different scenarios on the risk of excess dietary retinoid intake during pregnancy

In addition to possible retinoid toxicity, liver consumption is not recommended during pregnancy due to the capacity of liver to accumulate some heavy metals. Therefore, rough estimates on exposure to cadmium and lead were also included in the model.

6.7.2 Principles of the model

The simulation model was designed using commercial spreadsheet software (Microsoft Office Excel 2003, Microsoft Corporation, USA), with a commercial add-in module for risk analysis (@Risk, version 4.5.2, Palisade Corporation, USA, 2002). @Risk uses Monte Carlo method to produce possible outcomes.

The results of simulation model depend on used input values. In the Monte Carlo model, possible input values can be described as probability distributions instead of point estimates. During the simulation process, the chain of events is repeated many times (iterations). New input values are randomly sampled from probability distributions for each iteration. Individual results are then combined to produce a probability distribution for outcome values. In this risk assessment, simulation run with 30,000 iterations was used.

6.7.3 Design of the simulation model

Vitamin A intake

Basic structure of simulation model for vitamin A intake is shown in Figure 9. In the model, retinoid intake was calculated as an average daily intake and as an intake of retinoids from a single liver meal. A single meal was defined as an amount of certain liver product eaten in one day. In the case of total vitamin A, only average daily intake was estimated.

In the case of single meals, retinoid intake can vary greatly from meal to meal depending on the retinoid content of the livers used as an ingredient. Therefore, retinoid intakes from single portions of liver foods were simulated in two situations:

1. Livers used as an ingredient are randomly sampled from retinoid concentration distribution (average retinoid content situation). Since all liver meals were assumed to be parts of a large batch with several livers combined, livers with high and low retinoid content compensate for each other, and the result show a tendency to an average retinoid content of that product.

2. Livers used as an ingredient are sampled from the highest decile of retinoid concentration distribution (high retinoid content situation). This situation gives a conception of the most excessive but still possible retinoid intake within the limits of consumption data used.

Inputs used in the model and the empirical parameters on the background of fitted distributions are presented in Table 19. Uncertainties in the empirical data are included in used input distributions. Characteristics of input data parameters are explained in detail in previous chapters, except of "size of production run" and "heating loss", which are explained below.

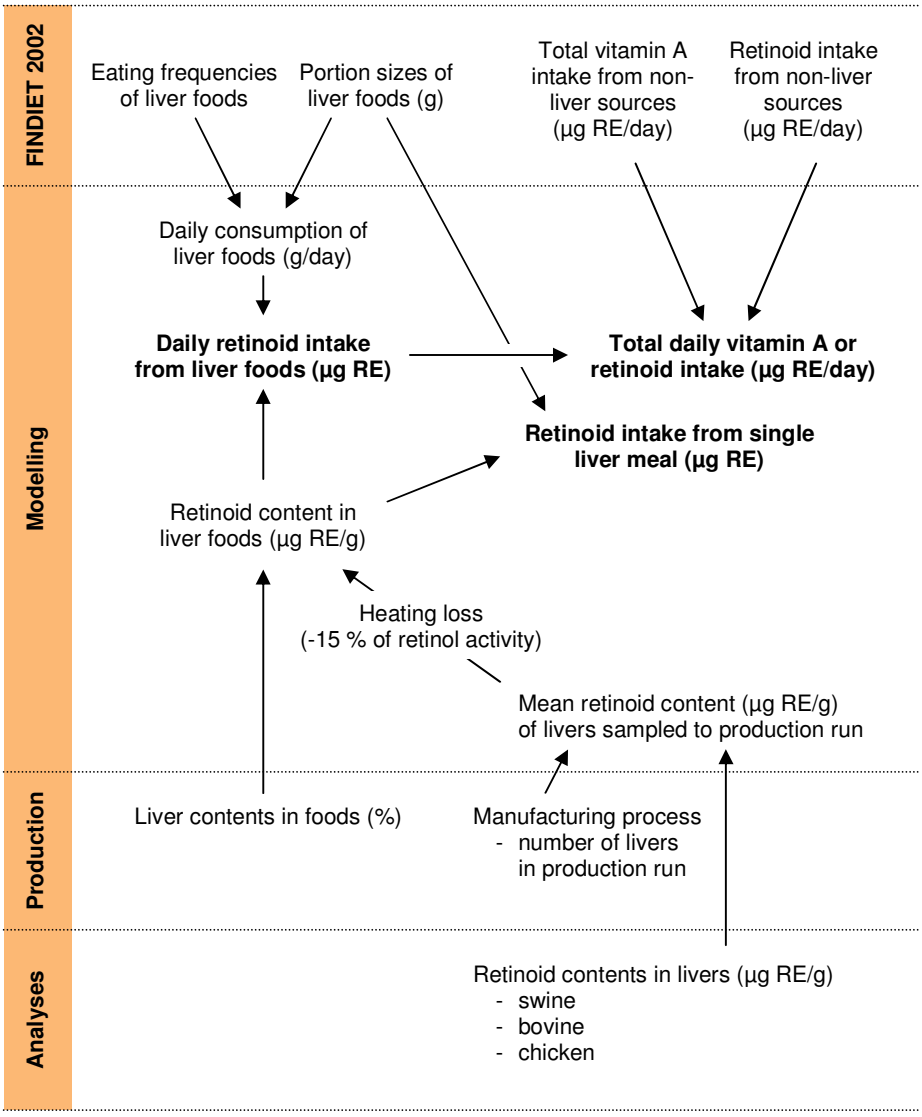


Figure 9. Basic structure of the simulation model for vitamin A exposure. Model outputs are bolded.

Table 19. Inputs of vitamin A model.

Input	Distribution[1] or value in model	Parameters of empirical data or obtained distributions					Data source
Eating frequencies (eating days/8 days)		N	Mean	Median	5th-95th range	Min-max	FINDIET 2002 study, KTL
Liver sausage or pâté	Poisson (λ = 2.07; truncated to min 1)	15	2.07	2		1-4	
Liver casserole	Poisson (λ = 1; truncated to min 1)	7	1	1		1	
Liver patties	Poisson (λ = 1; truncated to min 1)	4	1	1		1	
Liver stew	Poisson (λ = 1.5; truncated to min 1)	2	1.5	1.5		1-2	
Portion sizes (g)							FINDIET 2002 study, KTL
Liver sausage or pâté	Lognormal (μ = 41.37; σ = 30.17)	68	42	32	10-100	10-190	
Liver casserole	Lognormal (μ = 159.89; σ = 60.54)	15	160	200	70-300	70-300	
Liver patties	Point estimate (median) 130	4	119	130		85-130	
Liver stew	Point estimate (median) 150	4	138	150		100-150	
Vitamin A and retinoid intake from non-liver sources (μg RE/day). F = intake from foods, S = intake from dietary supplements							FINDIET 2002 study, KTL
Vitamin A (F+S)	Lognormal (μ = 533.21; σ = 233.42)	529		499	227-1,042		
Vitamin A (F)	Lognormal (μ = 457.41; σ = 144.15)	529		444	250-737		
Retinoids (F+S)	Lognormal (μ = 275.58; σ = 133.79)	529		249	113-580		
Retinoids (F)	Lognormal (μ = 260.07; σ = 92.62)	529		252	127-431		
Retinoid contents in livers (μg RE/g)							EELA
Swine, finishing pig	Lognormal (μ = 171.5; σ = 95.7)	91	170	164	44-330	28-549	
Swine, sow	Lognormal (μ = 448.9; σ = 369.7)	37	443	337	103-1,143	50-2,123	
Bovine	Lognormal (μ = 215.4; σ = 139.5)	76	213	175	59-467	44-744	
Chicken	Lognormal (μ = 107.6; σ = 62.2)	27	106	97	38-224	34-277	
Liver contents in foods (amount of raw liver component as a percentage of weight from the end product). Weighted means relative to market shares.							
Liver sausage or pâté	Swine 20.4 %, bovine 0.1 %						Finnish food industry
Liver casserole	Swine 8.5 %, bovine 0.5 %, chicken 4.5 %						Finnish food industry
Liver patties	Swine 16.7 %, bovine 33.3 %						KTL
Liver stew	Swine 11.1 %, bovine 22.2 %						KTL
Heating loss	15 %						Bergström 1994
Size of production run (liver units [2])							
Liver sausage or pâté	10						Assumption based on information obtained from food industry and literature
Liver casserole	20						
Liver patties	1.5						
Liver stew	1.5						

[1] Includes uncertainties in input distributions
[2] 1 liver unit = 1 bovine liver = 4 swine livers = 15 * 10 broiler livers (pooled)

Size of production run

In the simulation model, different liver types were proportioned by weight to liver units so that one bovine liver (3.4-9.2 kg (Nickel et al. 1979)) equals four swine livers (1-2.5 kg per liver (Nickel et al. 1979)) and 15 pooled chicken samples of ten livers (35-51 g per liver (Nickel et al. 1977)). Thus, the weight of one liver unit was around 6 kg. These liver units were randomly sampled from concentration distributions in each iteration, and the resulting means were used to calculate simulation outputs.

The lot size of ten liver units was chosen to represent industrial food production of liver sausage and pâté (based on information of mean lot size of 70 kg liver per lot, see Chapter 6.2.2). Sampling was made for pig liver pool of nine units (36 livers) and for one bovine liver unit (Table 20), corresponding to the mean proportions of these two liver types in the product (see Table 14). For liver casserole, the average lot size was estimated based on information obtained from large manufacturers. Modelling was done to lot size of 20 liver units (120 kg liver) consisting of one beef liver unit, 13 swine liver units and six chicken liver units (Table 20). In the case of both liver sausage or pâté and liver casserole, 10% of pig liver pool was replaced by sow liver in those scenarios where sow livers were included. Sow livers were sampled directly from concentration distribution as single livers.

For liver patties and liver stew, a volume of 1.5 liver units was chosen to the model. This corresponds to about 150 standard portions (Sääksjärvi & Reinivuo 2004), representing a volume of small canteen. In addition, the small lot size makes the simulation to fit the household cookery. Sampling was done to one bovine liver unit (one bovine liver) and 0.5 pig liver units (2 pig livers) to correspond to the 2:1 ratio in liver types in recipes. If sow livers were included, it was done similarly as in the case of industrial liver foods.

Heating loss

Heating loss is the decrease of retinoid activity due to high temperatures in food processing. It is estimated to be 15% based on studies of Bergström (1994). The effect of heating loss is compensated by simultaneous rise in retinoid concentration due to water loss from the product. In this study, water loss was included in the data of the liver content of the products, and therefore it is not separately on view in input value list.

Heavy metal exposure

Basic structure of simulation model for heavy metal exposure is shown in Figure 10. Liver consumption data and liver contents of foods were same as in vitamin A model (see Table 19 for input description). For heavy metal contents of livers, the averages of annual means in Table 18 were used (analyses made by the National Veterinary and Food Research Institute of Finland in years 2000-2004).

6.7.4 Assumptions of the model

Following assumptions were made:

- The consumption data used and the results of laboratory analyses represent true populations
- Sow livers are uniformly distributed among swine liver used

To proportion simulated vitamin A intakes, the following reference values were used:

- Total vitamin A intake: Nordic nutrition recommendations by Nordic Council of Ministers (NCM 2004).
  - Recommended intake (RI): 800 μg RE/day for pregnant women
  - Average requirement (AR): 500 μg RE/day for women
  - Lower level of intake (LI): 400 μg RE/day for women
- Upper tolerable daily dose (UL) for retinoids: 3,000 μg RE/day (SCF 2002).
- Upper tolerable dose for retinoids from single liver meal: 7,500 μg RE. The limit was set based on reported cases of retinoid teratogenesis (Rosa 1986) and on recommendations made for retinoid supplementation (WHO 1998) (see Chapter 5.2.3).

Possible underreporting in consumption data was not taken into account in the simulation model calculations.

Table 20. Sizes of production runs used to calculate simulation outputs.

	Number of liver units[1] sampled to one production run			
Product	Bovine	Swine	Chicken	*Total*
Liver sausage and pâté	1	9	-	*10*
Liver casserole	1	13	6	*20*
Liver patties	1	0.5	-	*1.5*
Liver stew	1	0.5	-	*1.5*

[1] 1 liver unit = 1 bovine liver = 4 swine livers = 15 * 10 broiler livers (pooled)

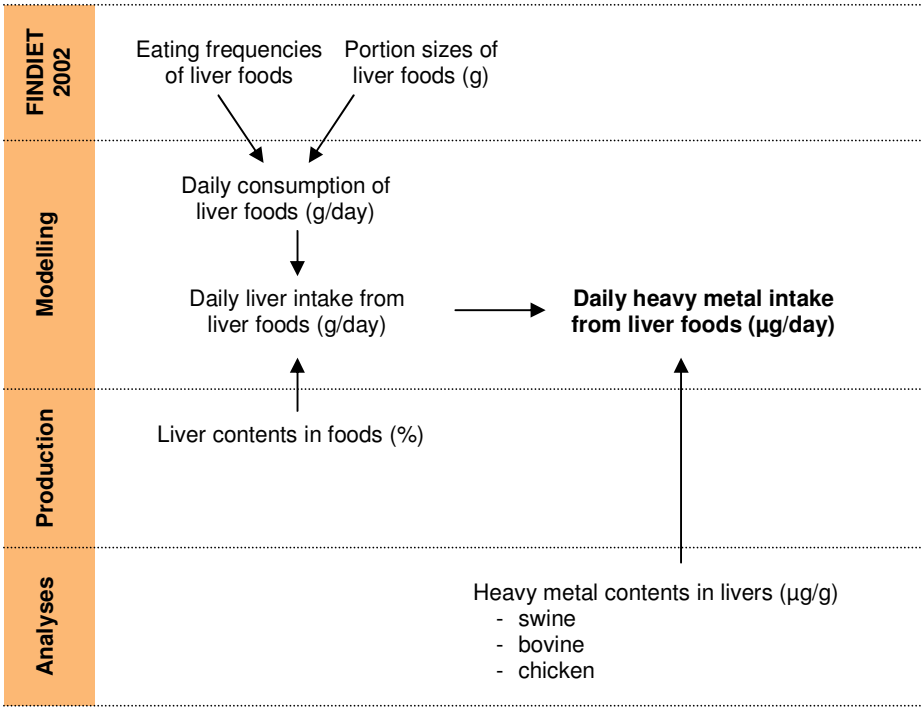


Figure 10. Basic structure of the simulation model for heavy metal exposure via liver foods. Model output is bolded.

6.7.5 Limitations of the simulation model

The limitations of the designed simulation model are mainly related to limitations and uncertainties in liver consumption data. The number of liver eaters in the data was quite low, especially in the case of liver patties and liver stew. Small amounts of data may result in an estimate bias for both portion sizes and eating frequencies. Consequently, estimated consumption distributions may not fully reflect true intakes.

It is likely that the data used contains some underreporting, i.e., reported intakes are lower than true intakes. When reported portion sizes are compared to with standard portion sizes (Sääksjärvi & Reinivuo 2004), the reported portion sizes for main course liver foods seems quite small, especially in the case of liver casserole (Table 21). For liver sausage and pâté, the reported portion sizes fits well to given standards. Due to potential underreporting in the consumption data, the true risk for of excess retinoid intake via main course liver foods may be higher than estimated by the simulation model.

The consumption data included no information about the proportion of liver eaters and non-liver eaters in the real population, i.e., the size of the sub-populations with possible liver consumption in the long run or no liver consumption at all. Therefore, no reliable calculations could be done to estimate the general intake of vitamin A from liver foods among liver eaters, and estimated liver consumption reflects only those eight-day periods when at least one liver food is eaten.

6.7.6 Scenarios in vitamin A model

Risk management scenarios

In addition to modelling present vitamin A intake with and without liver foods, simulations were done to estimate the effects of some risk management options to vitamin A intake. Risk management options may involve actions at the food processing level or in consumer level. Therefore, the following two risk management scenarios were studied:

1. Sow livers are not used as an ingredient in liver foods (action at the food processing level)

2. Pregnant women are allowed to eat liver sausage or pâté but not liver casserole, liver patties or liver stew

Reduction of liver retinoid levels

Retinoid concentrations in animal products are strongly correlated with the level of retinoids in animal feeds. It has been recently recommended

Table 21. Reported liver food portion sizes[1] used in risk assessment and the suggestive standard portion sizes[2]

	Reported portion size (g)[1]			Standard portion size (g)[2]		
	n	Median	Range (min-max)	Small	Medium	Large
Liver sausage	68	32	10-190	15	30	60
Liver casserole	15	164	70-300	250	300	350
Liver patties	4	130	85-130	100		
Liver stew	4	150	100-150	200	220	240

[1] FINDIET 2002 data (Männistö et al. 2003)
[2] Sääksjärvi & Reinivuo 2004

by the Scientific Committee for Animal Nutrition that the possibility of reduction in retinoid content of animal feeds and the effect of this action on retinoid intake among consumers should be explored (SACN 2005). On this basis, we simulated dietary retinoid intake in a situation where the retinoid content of the livestock livers were 10-90% of the present level. The scenario focused only on effects and no evaluation was done whether the reduction is possible in practice. An assumption was made that retinoid levels in other animal products than liver will not be reduced.

7 Risk characterisation

7.1 Vitamin and retioids

7.1.1 Retinoids in liver foods

Simulated mean retinoid contents of Finnish liver foods are presented in Table 22. The mean retinoid concentration (± sd) of liver sausage and pate is 35 ± 8 µg RE/g, of liver casserole 19 ± 4 µg RE/g, of liver patties 88 ± 45 µg RE/g, and of liver stew 59 ± 30 µg RE/g. However, if all the livers used in the product are from the highest decile of the retinoid concentration distribution, the mean retinoid contents of liver foods are around three times higher than in the average situation (Table 22).

7.1.2 Daily vitamin A intake

Daily intake of total vitamin A from different sources

Simulated total vitamin A intake distributions are shown in Figure 11. Among liver eaters the median intake of total vitamin A is 1,170 µg RE/day (5th-95th range 500-4,700 µg RE/day), and among non-liver eaters 490 µg RE/day (5th-95th range 240-970 µg RE/day), when both dietary and supple-mental intake is taken into account. Without dietary supplements the median intake would have been 1,070 µg RE/day (5th-95th range 490-4,600 µg RE/day) among liver eaters and 440 µg RE/day (5th-95th range 260-720 µg RE/day) among non-liver eaters.

Table 22. Simulated mean retinoid contents of Finnish liver foods. Mean retinoid contents are estimated in a situation where retinoid concentrations of livers are randomly sampled from concentration distribution (average liver retinoid content) and in a situation where retinoid concentrations of livers are sampled only from the upper decile of concentration distribution (high liver retinoid content).

	Retinoid content (µg RE/g product , mean ± sd)	
Liver food	Average [1]	High [2]
Liver sausage or pâté	35 ± 8	82 ± 9
Liver casserole	19 ± 3	47± 4
Liver patties	88 ± 45	23 ± 5
Liver stew	59 ± 30	153 ± 33

[1] Retinoid content of livers are randomly sampled from concentration distribution
[2] Retinoid content of livers are above 90th percentile

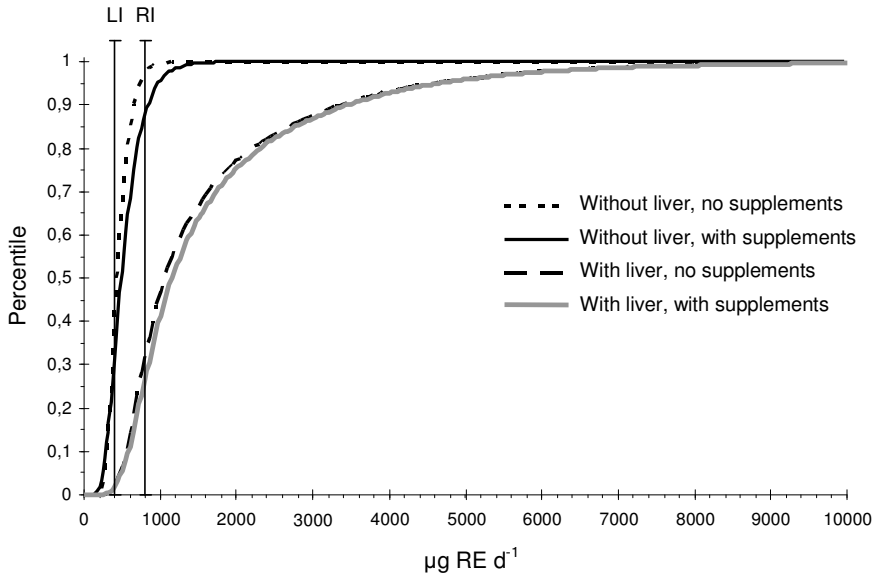


Figure 11. Average daily intakes of vitamin A from different dietary sources among Finnish women aged 25-44 years. Cumulative distributions based on simulation model results. The reference lines indicate the lower level of intake (LI) for women (400 µg RE d-1) and the recommended intake (RI) of pregnant women (800 µg RE d^{-1}) (Nordic Council of Ministers 2004).

Table 23. Daily intakes of total vitamin A in proportion to reference levels. Simulated results from different dietary sources among Finnish women aged 25-44 years.

Source		% of women below reference levels		
Food source	Supplement use	< LI [1]	< AR [2]	< RI [3]
Food with liver	With supplements	2	5	26
	Without supplements	2	6	32
Food without liver	With supplements	32	52	88
	Without supplements	39	67	98

[1] LI = Lower level of intake, 400 µg RE d^{-1} (Nordic Council of Ministers 2004).
[2] AR = Average requirement, 500 µg RE d^{-1} (Nordic Council of Ministers 2004).
[3] RI = Recommended intake for pregnant women, 800 µg RE d^{-1} (Nordic Council of Ministers 2004).

In Table 23, the intake estimates are compared with reference intakes of vitamin A. One of four liver eaters fall below the recommended vitamin A intake for pregnant women (800 µg RE/day), but the average requirement (500 µg RE/day) is achieved by almost everyone. However, the majority of non-liver eaters remain below the recommended intake, and one-third has the intake even below the lower intake limit (400 µg RE/day). Supplement use has a minor effect on vitamin A intake when compared with liver consumption.

Daily intake of retinoids from different sources

Simulated intake distributions for retinoids are shown in Figure 12. Among liver eaters the median retinoid intake is 880 µg RE/day (5th-95th range 310-4,430 µg RE/day), and among non-liver eaters 250 µg RE/day (5th-95th range 120-530 µg RE/day), when both dietary and supplemental intake is taken into account. Without dietary supplements the median intake would have been 880 µg RE/day (5th-95th range 310-4,430 µg RE/day) among liver eaters and 250 µg RE/day (5th-95th range 140-430 µg RE/day) among non-liver eaters.

In Table 24, the retinoid intake estimates are compared with upper tolerable intake level for pregnant women (3,000 µg RE/day). Eleven percent (11%) of liver eaters exceed the recommended upper intake limit. The result is independent of supplement use. Among non-liver eaters, there is no risk of exceeding the maximum intake limit (Table 24).

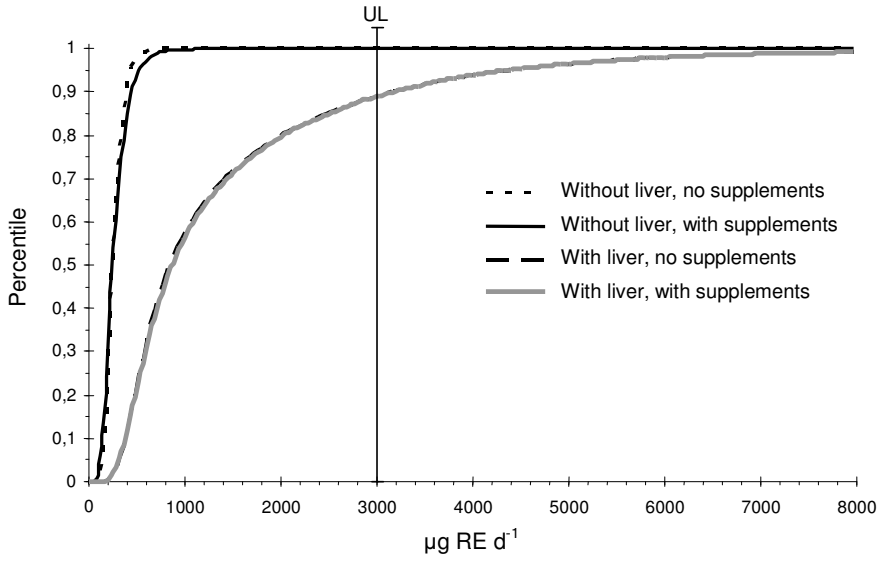


Figure 12. Average daily intakes of retinoids from different dietary sources among Finnish women aged 25-44 years. Cumulative distributions based on simulation model results. The reference line indicates the upper tolerable intake level (UL) for pregnant women (3,000 µg RE d^{-1}, Scientific Committee on Food 2002).

Table 24. Daily intakes of retinoids in proportion to upper tolerable intake level. Simulated results from different dietary sources among Finnish women aged 25-44 years.

Source		% of women above UL [1]
Food source	Supplement use	
Food with liver	With supplements	11
	Without supplements	11
Food without liver	With supplements	0
	Without supplements	0

[1] UL = Upper tolerable intake level for pregnant women, 3,000 µg RE d^{-1} (Scientific Committee on Food 2002).

Retinoid intake in proportion to total vitamin A intake

Based on simulation results, retinoids constitute 80% and 50% of total vitamin A intake among liver eaters and non-liver eaters, respectively. In the data used, 39% of women reported liver consumption. Thus it can be calculated that Finnish women aged 25-44 years gets an average 60% of their vitamin A intake in retinoid form (weighted mean).

The magnitude of liver as a vitamin A source is clearly seen in the simulation results: among liver eaters on average 60% of total vitamin A intake and 70% of retinoid intake comes from liver foods.

Effects of scenarios on daily vitamin A and retinoid intake among liver consumers

The effects of selected scenarios were estimated in a situation where intake of both foods and dietary supplements was taken into account. Since the scenarios assumed no effects on non-liver intake, simulations were focused to liver consumers only. Simulated effects on intake distributions of total vitamin A and retinoids are shown in Table 25 and Figure 13.

If sow livers were not used in liver foods, the effect on daily retinoid intake would be small. The proportion of women with retinoid intake more than the upper tolerable level (3,000 µg RE/day) would fall from 11% to 10% (Table 25). Median retinoid intake would be 810 µg RE/day (5th-95th range 300-4,260 µg RE/day). The exclusion of sow livers would not have significant effects on total vitamin A intake (median 1090 µg RE/day, 5th-95th range 470-4,540 µg RE/day).

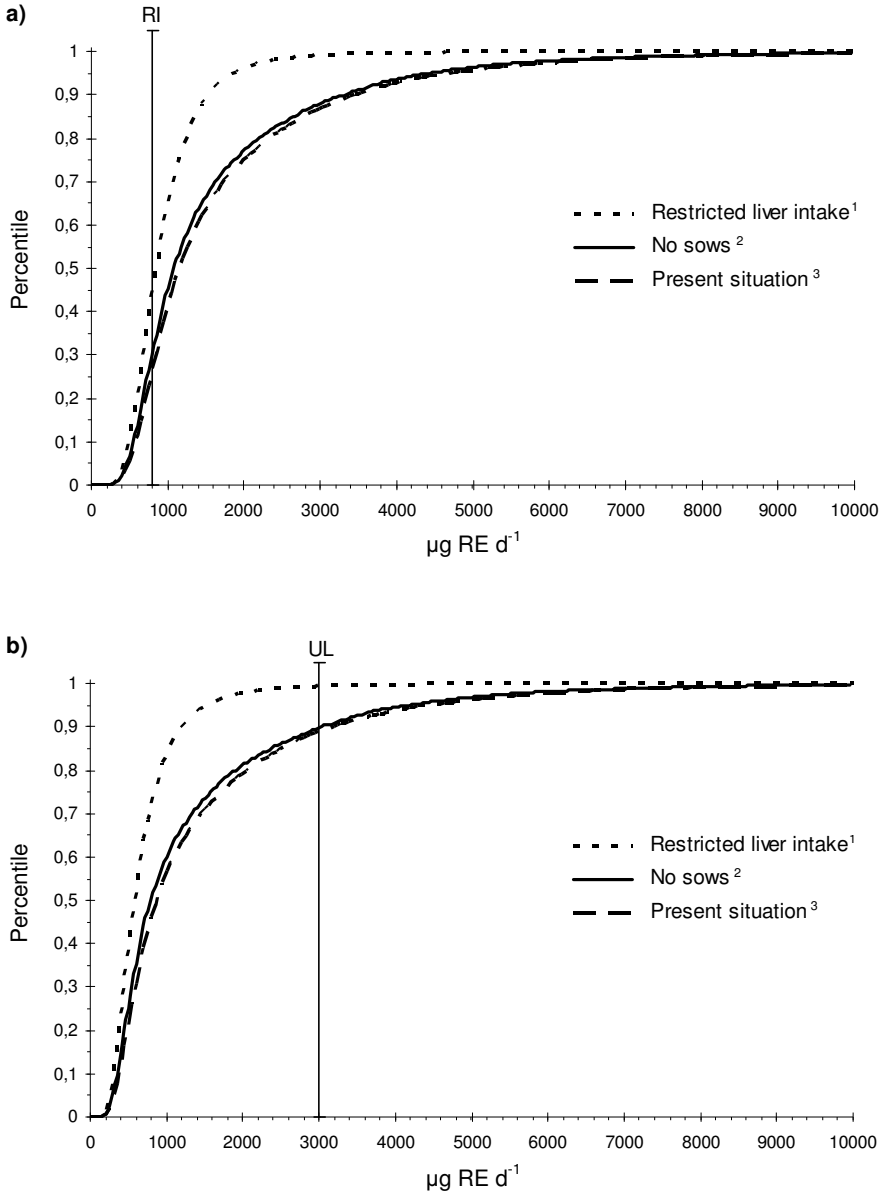
In the restricted consumption scenario, women were allowed to eat liver sausage/pâté but not main course liver foods. This eating restriction would have a more powerful effect on retinoid intake than the exclusion of sow livers from food production. As a resultant for eating restrictions, only less than 1% of women would exceed the upper tolerable level of 3,000 RE/day (Table 25). Median retinoid intake would be 580 µg RE/day with 5th-95th range 270-1,510 µg RE/day, i.e., significantly lower than in the sow exclusion scenario (Figure 13b). Intake of total vitamin A would be clearly reduced too (median 850 µg RE/day, 5th-95th range 420-1,850 µg RE/day). As a consequence, 45 % of women would receive less than the recommended 800 µg RE/day, and 4 % of women would remain below the minimum intake limit (400 µg RE/day) (Table 25).

Table 25. Effects of action scenarios on daily intakes of total vitamin A and retinoids among liver eaters in proportion to reference levels. Simulation model results based on intake of foods and dietary supplements among Finnish women aged 25-44 years with liver consumption.

Scenario	% of women below the reference levels for vitamin A			% of women above the UL [7] for retinoids
	< LI [4]	< AR [5]	< RI [6]	
Present situation [1]	2	5	26	11
No sows [2]	2	6	29	10
Restricted intake [3]	4	11	45	< 1

[1] Swine livers from finishing pigs (90 %) and from sows (10 %).
[2] All swine livers are finishing pig livers
[3] Liver derived intake only from liver sausage/pâté. (Intakes from liver casserole, liver patties and liver stew are excluded.)
[4] LI = Lower level of intake, 400 µg RE d^{-1} (Nordic Council of Ministers 2004)
[5] AR = Average requirement, 500 µg RE d^{-1} (Nordic Council of Ministers 2004)
[6] RI = Recommended intake for pregnant women, 800 µg RE d^{-1} (Nordic Council of Ministers 2004)
[7] UL = Upper tolerable intake level for pregnant women, 3,000 µg RE d^{-1} (Scientific Committee on Food 2002)

The effect of reduction of liver retinoid content on retinoid intake is shown in Figure 14. If effects similar to a restricted consumption scenario were tried to achieve by reducing liver retinoid contents, the retinoid content of livers should be less than 25% of the present level. If liver retinoid content was decreased to 50% of the present level, 2.5% of women would still have retinoid intake more than the recommended limit of 3,000 µg RE/day.



[1] Liver derived intake only from liver sausage or pâté. (Intakes from liver casserole, liver patties and liver stew are excluded.)
[2] All swine livers are finishing pig livers.
[3] Swine livers are from finishing pigs (90 %) and from sows (10 %).

Figure 13. Effects of action scenarios on daily intake of total vitamin A (a) and retinoids (b) from foods and dietary supplements among Finnish women aged 25-44 years with liver consumption. Cumulative distributions based on simulation model results. The reference lines indicate the recommended intake (RI) of pregnant women (800 µg RE d^{-1}, Nordic Council of Ministers 2004) and the upper tolerable intake level (UL) for pregnant women (3,000 µg RE d^{-1}, Scientific Committee on Food 2002).

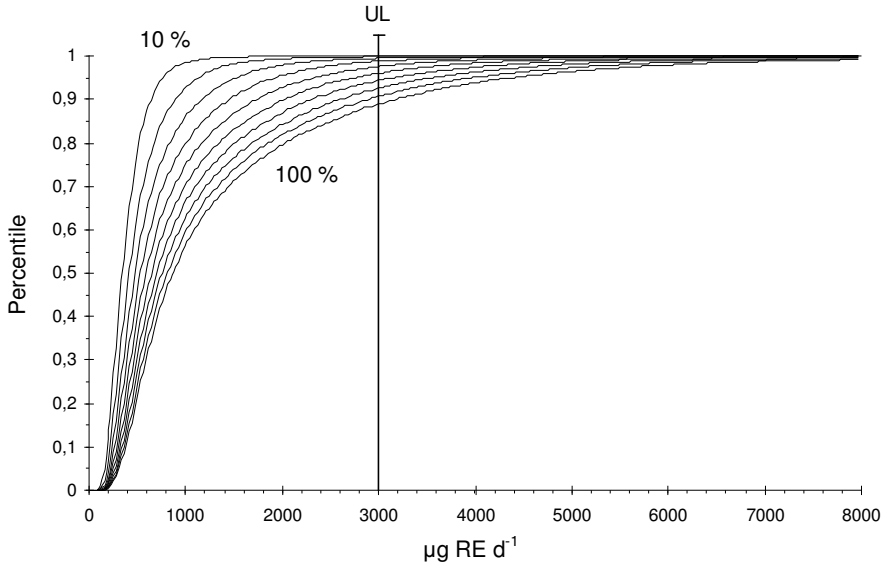


Figure 14. Effect of reduction of liver retinoid content on retinoid intake from liver foods among Finnish women aged 25-44 years. Cumulative distributions based on simulation model results. Curves represent the retinoid intake if liver retinoid content is 10-100 % of present level (on 10 % intervals). The reference line indicates the upper tolerable intake level (UL) for pregnant women (3,000 µg RE d^{-1}, Scientific Committee on food 2002).

7.1.3 Single meal retinoid intake

Single meal intake of retinoids from different liver foods

A single meal was defined as an amount of certain liver product eaten in one day. Intake estimates were calculated separately for every liver food studied. The simulated medians, 5th-95th percentile ranges and proportions of users with retinoid intake over 7,500 µg RE per portion are shown in Table 26. The cumulative intake distributions are shown in Figure 15.

On average, single portions of liver sausage or pâté does not seem to expose women to harmful doses of retinoids. However, intakes from liver stew and especially from liver patties clearly exceed the level considered safe during pregnancy. Liver casserole falls between these end points, the safety being dependent of the retinoid concentration of the liver used as an ingredient.

Effects of scenarios on intake of retinoids from single portion

The exclusion of sow livers would have only a minor effect on single-meal retinoid intake. The relative effect would be clearest on those products with liver largely from swine origin, i.e., liver sausage and pâté and liver casserole (Table 26).

The high retinoid intakes from single meals of main course liver foods are difficult to reduce also by decreasing liver retinoid contents. In an average situation, liver retinoid contents should be 70% lower than the present level so that eating liver patties or liver stew could be stated as free of risk of teratogenicity (97.5 percent of population remains below the intake of 7,500 µg RE per portion). If livers in the product are sampled from the highest decile of retinoid distribution, the reduction should be 85%.

Portion sizes needed to achieve retinoid intake limits

The portion sizes of different liver products needed to achieve the intake limits of 3,000 µg RE/day (upper daily dose for pregnant women) and 7,500 µg RE per portion (upper single dose for pregnant women) were also estimated (Table 27). The 95th percentile of non-liver retinoid intake (530 µg RE/day) was used as a background intake. Estimates are expressed as medians, meaning that the intake limit was met below the value shown in one half of the cases, and in the other half even larger doses could be eaten without grossing the given level of exposure.

If estimated results are compared with reported intakes in the FINDIET 2002 data (see Chapter 6.2), only liver sausage and pâté are generally eaten as amounts below the estimated upper limits. Average portions of liver casserole slightly exceed the median in high retinoid content situation. For liver patties and liver stew, the means of the reported portion sizes equals the estimated upper limit even in the average situation.

Table 26. Present retinoid intakes (µg RE) from single portions of different liver products and the effect of exclusion of sow livers. Simulation model results among Finnish women aged 25-44 years. Intakes are calculated from liver only. Intakes are estimated in a situation where retinoid concentrations of livers are randomly sampled from concentration distribution (average liver retinoid content) and in a situation where retinoid concentrations of livers are sampled only from the upper decile of concentration distribution (high liver retinoid content).

	Liver retinoid content					
	Average [2]			High [3]		
Sources and scenarios	Median	5th-95th range	% > 7,500 µg RE [4]	Median	5th-95th range	% > 7,500 µg RE [4]
Liver sausage or pâté						
Present situation	1,150	340-3,420	< 1	2,760	840-8,010	6
No sows [1]	1,010	310-2,920	< 1	2,260	690-6,480	3
Liver casserole						
Present situation	2,910	1,430-5,580	1	6,880	3,420-12,780	41
No sows [1]	2,640	1,310-4,860	1	5,950	2,980-10,850	26
Liver patties						
Present situation	10,430	5,550-21,440	81	26,370	22,240-39,950	100
No sows [1]	9,930	5,070-20,820	76	24,720	20,790-38,280	100
Liver stew						
Present situation	8,030	4,270-16,500	57	20,290	17,100-30,730	100
No sows [1]	7,640	3,900-16,010	52	19,020	16,000-29,450	100

[1] Sow livers are not used as ingredient
[2] Retinoid content of livers are randomly sampled from concentration distribution
[3] Retinoid content of livers are above 90th percentile of retinoid concentration distribution
[4] Upper tolerable single meal intake for pregnant women

When considering the results, it should be noted that if single portions up to 7,500 μg RE are consumed regularly, the maximum weekly intake may be exceeded and the risk of retinoid teratogenicity may increase. If product is eaten frequently, the single portion size should be accordant with portion size for daily consumption (3,000 μg RE) for safe intake.

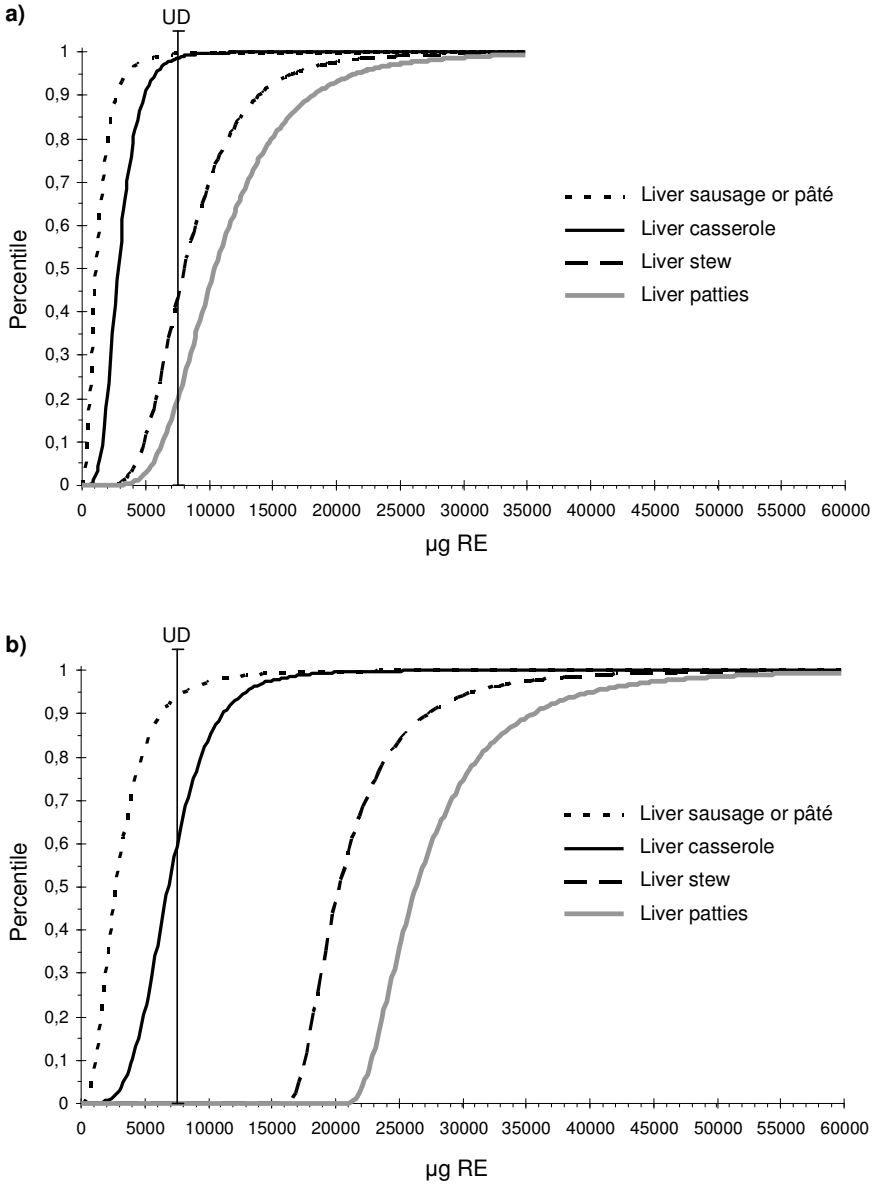


Figure 15. Retinoid intakes from single portions of liver foods among Finnish women aged 25-44 years with liver consumption. Cumulative distributions based on simulation model results. The intakes in situations of average liver retinoid content (a) and high liver retinoid content (b) are shown separately. In an average situation (a), the livers used as ingredient are randomly sampled from retinoid concentration distributions. In high retinoid content situation (b), the livers used as an ingredient are sampled from the highest decile of retinoid concentartion distributions. The reference line indicates the upper tolerable single dose (UD) for pregnant women (7,500 μg RE). Intakes are from liver only.

Table 27. The simulated median portion sizes of liver foods needed for pregnant women to achieve the upper limits of retinoid intakes (daily dose and single dose) when retinoid exposure from liver is additive to retinoid exposure from non-liver sources. As a background intake, the 95th percentile of the non-liver intake of Finnish women aged 25-44 years (530 µg RE d^{-1}) was used. Portion sizes are estimated in a situation where retinoid concentrations of livers are randomly sampled from concentration distribution (average liver retinoid content) and in a situation where retinoid concentrations of livers are sampled only from the upper decile of concentration distribution (high liver retinoid content).

	Intake (g) needed to gain					
	3,000 µg RE daily [1]					
	Daily dose		Weekly dose		7,500 µg RE in a single portion [2]	
	Liver retinoid content		Liver retinoid content		Liver retinoid content	
Product	Average [3]	High [4]	Average [3]	High [4]	Average [3]	High [4]
Liver sausage or pâté	70	30	490	210	210	90
Liver casserole	130	50	910	350	370	150
Liver patties	30	10	210	70	90	30
Liver stew	50	20	350	140	140	50

[1] Upper tolerable daily intake for pregnant women (Scientific committee on Food 2002)
[2] Upper tolerable single dose for pregnant women
[3] Retinoid concentrations of livers are randomly sampled from concentration distribution
[4] Retinoid concentrations of livers are above 90th percentile of concentration distribution

7.2 Cadmium and lead

Exposure to cadmium and lead via liver products was estimated in the present situation among liver consumers only. Cumulative intake distributions are shown in Figure 16. According to the simulation model, average intake of cadmium from liver is 0.17 μg/day (median 0.09 μg/day, 5th–95th range 0.01-0.64 μg/day) and average lead intake 0.32 μg/day (median 0.16 μg/day, 5th–95th range 0.03-1.14 μg/day). Estimated exposures reflect only intakes during those eight-day periods when at least one liver food is eaten. If liver is eaten less frequently in the long run, the average heavy metal exposure from liver foods is smaller. Cadmium and lead exposure from liver foods is relatively low when compared with the total dietary intakes of 10 μg Cd/day and 20 μg Pb/day (Mustaniemi & Hallikainen 1994; Mustaniemi et al. 1994).

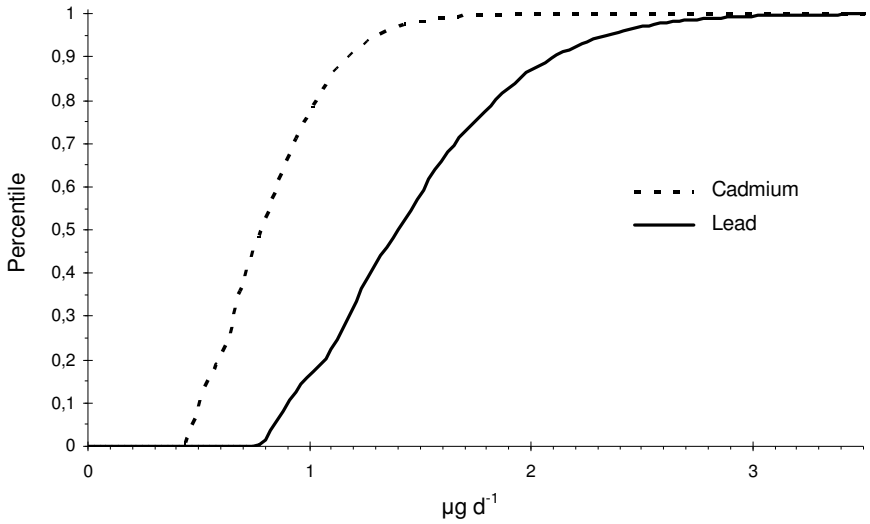


Figure 16. Simulated cadmium and lead exposures from liver foods among Finnish women aged 25-44 years (liver consumers only).

8 Discussion

Retinoids and total vitamin A

This risk assessment model indicates that liver consumption may predispose women to retinoid intakes higher than what is considered safe during pregnancy. However, the risk seems to pertain mainly to liver foods eaten as a main course (i.e., liver casserole, liver patties and liver stew), and not those products used as accompaniment (liver sausage and pâté). The most efficient way to manage the risk is for women to avoid main course liver foods during pregnancy. Exclusion of sow livers from food production would have only a minor effect on retinoid intakes from liver foods. If results similar to a restricted consumption scenario were tried to achieve by reducing liver retinoid contents, the retinoid content of livers should be less than 25% of the present level.

When considering these results, it has to be noticed that they are produced by mathematic model with uncertainties and hypotheses. The results should be reviewed in concurrence with the assumptions of the model.

The simulated retinoid intakes were compared with the limits set to represent safe retinoid exposure during pregnancy. For long-time daily intake, the commonly recommended maximum intake of 3,000 µg RE/day was used. For single high retinoid exposure there is no established safety threshold. Based on the available evidence the intake of 7,500 µg RE as a single dose was considered the maximum safe intake limit.

The simulation model results are based on liver food portion size data of 60 women and eating frequency data of 24 women. Only few women reported consumption of main course liver foods, especially liver patties and liver stew. Small data may lead to bias in the defined input distributions and thus skew the results. Anyhow, there is no reason to believe that the magnitude and profile of the simulated results are faulty. Liver consumption increases retinoid intake markedly compared with non-liver diet, and some liver consumers are at risk for possibly teratogenic daily retinoid intakes (> 3,000 µg RE/day). When reviewing retinoid intakes from single liver meals, liver eaten as a main course is probable to cause retinoid intake above the specified safe level of 7,500 µg RE. This safety threshold is not likely to be exceeded if only liver sausage or pâté is eaten in moderate amounts.

In the model, all eaten liver foods were assumed to be parts of larger manufacturing batch with several livers, and in many case also livers from different animal species. This simulates the consumption of industrially produced liver foods and liver foods served in canteens. The situation is slightly different in households where mainly bovine liver, probably originated from one animal, is used. However, magnitudes of simulated retinoid intakes are valid also for homemade liver foods. This is the case especially for liver patties and liver stew because their lot sizes were assumed to be small and their recipes consist mainly of bovine liver. These are also the types of liver foods most probably made in home, whereas liver sausage and pâté and liver casserole are often bought as prepared food.

When considering the total daily vitamin A intake from the nutritional point of view, liver consumption clearly has a positive effect. Without liver foods, one-third of the population studied had inadequate vitamin A intake (< 400 µg RE/day). If liver is eaten, daily vitamin A needs are commonly fulfilled. In addition to vitamin A, liver contains also other beneficial nutritional elements. Liver is rich in folic acid and contains iron in easily absorbed form. Both of these micronutrients are important during pregnancy. However, the benefits of eating liver can probably be substituted by a well-balanced diet with plenty of vegetables and a reasonable amount of meat without the risk of an excess intake of retinoids.

In the scientific literature, the foetal malformations due to dietary retinoid excess are mainly reported to be caused by high intakes of retinoid supplements. However, in this study the use of supplemental vitamin A did not contribute significantly on the risk of possibly teratogenic dietary retinoid exposure.

Cadmium and lead

Cadmium and lead exposure from liver foods is relatively low when compared with other dietary sources even during those weeks when liver is eaten. Since absorption of both cadmium and lead is increased if body iron stores are depleted, the high iron content of liver may further reduce the relative impact of cadmium and lead exposure from liver foods. However, the risk-benefit effect is difficult to establish. Pregnant women may be especially vulnerable to any additional heavy metal exposure due to increased physical need of iron and redistribution of body heavy metal burden.

Conclusions

Among Finnish liver consumers, dietary retinoid intakes may exceed the tolerable upper level for pregnant women. However, this risk assessment indicates that moderate consumption of liver sausage or pâté during pregnancy does not result in retinoid intake with potentially increased risk of retinoid teratogenicity. Moderate consumption of liver sausage or pâté may reduce the percentage of women with vitamin A intake below the recommended level during pregnancy.

9 References

Akase T, Yamashina S, Akase T, Onodera S, Okuda H, Tashiro S (2003). Effects of liver-supplemented food on the development of embryos in mice. Biol. Pharm. Bull. 26:553-556.

Alfthan G, Männistö S, Valsta L, Pietinen P, Valkonen S, Aitio A (1994). Veren lyijypitoisuus ja lyijyn saanti ravinnosta pääkaupunkiseudulla. (In Finnish). Elintarvikeviraston tutkimuksia 1/1994; Helsinki; Finland; 17 p.

Azaïs-Braesco V & Pascal G (2000). Vitamin A in pregnancy: requirements and safety limits. Am J Clin Nutr 71(suppl):1325S-1333S.

Barua AB & Olson J (1998). Reversed-phase gradient high-performance liquid chromatographic procedure for simultaneous analysis of very polar to nonpolar retinoids, carotenoids and tocopherols in animal and plant samples. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 707:69-79.

Bendich A (1988). The safety of beta-carotene. Nutr. Cancer. 11:207-14.

Bendich A & Langseth L (1989). Safety of vitamin A. Am. J. Clin. Nutr. 49:358-371.

Bergström L (1994). Nutrient losses and gains in the preparation of foods. Report 32/94; National Food Administration; Uppsala; Sweden; 223 p. http://www.slv.se/upload/dokument/Rapporter/Mat_och_nutrition/1994_32_Livsmedelsverket_nutrient_losses_and_gains.pdf

Biesalski HK (1989). Comparative assessment of the toxicology of vitamin A and retinoids in man. Toxicology 57:117-161.

Blomhoff R, Green MH, Green JB, Berg T, Norum KR (1991). Vitamin A metabolism: new perspectives on absorption, transport, and storage. Physiol. Rev. 71:951–90.

Booth SL, Johns T, Kuhnlein HV (1992). Natural food sources of vitamin A and provitamin A. Food Nutr. Bull. 14:6-19.

Borgejordet Å (2006). Unpublished data. Norwegian Food Safety Authority.

Burri BJ, Clifford J (2004). Carotenoid and retinoid metabolism: insights from isotope studies. Arch. Biochem. Biophys. 430:110-119.

Buss NE, Tembe EA, Prendergast BD, Renwick AG, George CF (1994). The teratogenic metabolites of vitamin A in women following supplements and liver. Hum Exp Toxicol 13:33-43.

Clagett-Dame M & DeLuca HF (2002). The role of vitamin A in mammalian reproduction and embryonic development. Annu. Rev. Nutr. 22:347-381.

CAC (2004). Working principles for risk analysis for application in the framework of the Codex Alimentarius. In: Codex Alimentarius Commission, Procedural Manual, 14th ed; WHO; Rome. ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_14e.pdf

Dawson MI (2000). The importance of vitamin A in nutrition. Curr. Pharm. Des. 6:311-325.

Debier C & Larondelle Y (2005). Vitamins A and E: metabolism, roles and transfer to offspring. Br. J. Nutr. 93:153-174.

EMEA (1998). Vitamin A. Summary report; European Agency for the Evaluation of Medicinal Products; Committee for veterinary medicinal products. http://www.emea.eu.int/pdfs/vet/mrls/036598en.pdf

Erkkola M, Karppinen M, Knip M, Virtanen S (2001). Raskaudenaikainen ravitsemus – kohtaavatko suositukset ja käytöntö? (In Finnish.) Duodecim 117:149-155.

Ervin RB, Wright JD, Wang CY, Kennedy-Stephenson J (2004). Dietary intake of selected vitamins for the United States population: 1999-2000. Advance data from vital and health statistics no 339; National Center of Health Statistics; Hyatsville, Maryland. http://www.cdc.gov/nchs/data/ad/ad339.pdf

EVI (2002) Riskiraportti – elintarvikkeiden ja talousveden kemialliset vaarat. (In Finnish.) Valvontaopas-sarja 2/2002. Elintarvikevirasto; Helsinki; p. 5-9. http://www.palvelu.fi/evi/files/55_519_255.pdf

EVM (2003). Safe upper levels for vitamins and minerals. Expert Group on Vitamins and Minerals; Food Standard Agency publications; UK; p. 110-126. http://www.food.gov.uk/multimedia/pdfs/vitmin2003.pdf

FAO/WHO (2002). Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation, Bangkok, Thailand. http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/004/Y2809E/y2809e00.htm

Finfood (2005). Official communication, Finnish Food Information Service, Finland.

FNB (2001). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Report of the Food and Nutrition Board; National Academy Press; Washington DC; p. 82-161.

Gerster H (1997). Vitamin A – functions, dietary requirements and safety in humans. Internat. J. Vit. Nutr. Res. 67:71-90.

Goodman DS (1984). Overview of current knowledge of metabolism of vitamin A and carotenoids. J. Natl. Cancer Inst. 73:1375-1379.

Gundersen TE & Blomhoff R (2001). Qualitative and quantitative liquid chromatographic determination of natural retinoids in biological samples. J. Chromatogr. A. 935:13-43.

Gulson BL, Jameson CW, Mahaffey KR, Mizon KJ, Korsch MJ, Vimpani G (1997). Pregnancy increases mobilization of lead from maternal skeleton. J. Lab. Clin. Med. 130:51-62.

Gulson BL, Mizon KJ, Palmer JM, Korsch MJ, Taylor AJ, Mahaffey KR (2004). Blood lead changes during pregnancy and postpartum with calcium supplementation. Environ. Health Perspect. 112:1499-1507.

Harrison EH (2005). Mechanisms of digestion and absorption of dietary vitamin A. Annu. Rev. Nutr. 25:87-103.

Hart RC, McCue PA, Ragland WL, Winn KJ, Unger ER (1990). Avian model for 13-cis-retinoic acid embryopathy: demonstration of neural crest related defects. Teratology 41:463-72.

Harville EW, Hertz-Picciotto I, Schramm M, Watt-Morse M, Chantala K, Osterloh J, Parsons PJ, Rogan W (2005). Factors influencing the difference between maternal and cord blood lead. Occup. Environ. Med. 62:263-269.

Hasunen K, Kalavainen M, Keinonen H, Lagström H, Lyytikäinen A, Nurttila N, Peltola T, Talvia S (2004). Lapsi perhe ja ruoka. Imeväis- ja leikki-ikäisten lasten, odottavien ja imettävien äitien ravitsemussuositus. (In Finnish) Sosiaali- ja terveysministeriön julkaisuja 2004:11; Helsinki; Finland; 254 p. http://www.stm.fi/Resource.phx/publishing/store/2004/09/pr1095673148360/passthru.pdf

Hathcock JN, Hattan DG, Jenkins MY, McDonald JT, Sundaresan PR, Wilkening VL (1990). Evaluation of vitamin A toxicity. Am. J. Clin. Nutr. 52:183-202.

Heinonen M (1990). Carotenoids and retinoids in Finnish foods and the average diet. University of Helsinki; Academic dissertation; 75 p.

Henson MC, Chedrese PJ (2004). Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. Exp. Biol. Med. 229:383-392.

Hirvi T, Lindfors E, Saari L, Hirn J (1992). Vitamin A i lever från finsk tamboskap och ren (In Swedish). Vår Föda 44:449-452.

Hirvonen T, Männistö S, Roos E, Pietinen P (1997). Increasing prevalence of underreporting does not necessarily distort dietary surveys. Eur. J. Clin. Nutr. 51:297-301.

Holson RR, Gazzara RA, Ferguson SA, Adams J (1997). Behavioral effects of low-dose gestational day 11-13 retinoic acid exposure. Neurotoxicology and Teratology 19:355-362.

Innis JW (1997). Role of Hox genes on human development. Curr. Opin. Pediatr. 9:617-22.

IPCS (1977). Environmental Health Criteria 3: Lead. International Programme on Chemical Safety; World Health Organisation; Geneva. http://www.inchem.org/documents/ehc/ehc/ehc003.htm

IPCS (1992). Environmental Health Criteria 134: Cadmium. International Programme on Chemical Safety; World Health Organisation; Geneva. http://www.inchem.org/documents/ehc/ehc/ehc134.htm

IPCS (1995). Environmental Health Criteria 165: Inorganic lead. International Programme on Chemical Safety; World Health Organisation; Geneva. http://www.inchem.org/documents/ehc/ehc/ehc165.htm

IUPAC-IUB (1981). Nomenclature of retinoids. Recommendations 1981. IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). http://www.chem.qmul.ac.uk/iupac/misc/ret.html

Julkunen P, Hasunen K, Idänpään-Heikkilä J (1990). Maksan A-vitamiini ja terveysongelmat. Suomen Lääkärilehti 45:261-264.

Järup L, Berglund M, Elinder CG, Nordberg G, Vahter M (1998). Health effects of cadmium exposure – a review of the literature and a risk estimate. Scand. J. Work Environ. Health 24 (Suppl 1): 1-51.

Kanai M, Raz A, Goodman DS (1968). Retinol-binding protein: the transport protein for vitamin A in human plasma. J. Clin. Invest. 1968 47:2025-44.

Korpela H, Loueniva R, Yrjänheikki E, Kauppila A (1986). Lead and cadmium concentrations in maternal and umbilical cord blood, amniotic fluid, placenta, and amniotic membranes. Am. J. Obstet. Gynecol. 155:1086-1089.

KTL (2005). Fineli. Finnish food composition database. Release 5. Helsinki 2005. http://www.ktl.fi/fineli. National Public Health Institute of Finland, Nutrition Unit.

KTL (2006). Official communication, National Public Health Institute of Finland.

Lammer E, Chen D, Hoar R, Agnish N, Benke P, Braun J, Curry C, Fernhoff P, Grix A, Lott I, Richard J, Sun S (1985). Retinoic acid embryopathy. N. Engl. J. Med. 313:837-841.

Larsen WJ (1997). Human embryology. 2nd ed. Churchill Livingstone; Hong Kong; 512 p.

Li J, Molkentin JD, Colbert MC (2001). Retinoid acid inhibits cardiac neural crest migration by blocking c-Jun N-terminal kinase activation. Dev. Biol. 232:351-361.

van Lieshout M, West CE, Muhilal, Permaesih D, Wang Y, Xu X, van Breemen RB, Creemers AF, Verhoeven MA, Lugtenburg J (2001). Bioefficacy of ß-carotene dissolved in oil studied in children in Indonesia. Am. J. Clin. Nutr. 73:949-58.

Livsmedelsverket (2006). Livsmedelsdatabas version 04.1.1. http://www.slv.se/templates/LDB_Search____6242.aspx

Louekari K, Uusitalo U, Pietinen P (1989). Variation and modifying factors of the exposure to lead and cadmium based on epidemiological study. Sci. Total Environ. 89:1-12.

Louekari K, Mäkelä-Kurtto R, Pasanen J, Virtanen V, Sippola J, Malm J (2000). Cadmium in fertilizers - risks to human health and the environment. Publications of Ministry of Agriculture and Forestry 4/2000; Helsinki; Finland; 119 p.

Maden M (2000). The role of retinoic acid in embryonic and post-embryonic development. Proc. Nutr. Soc. 59:65-73.

Majchrzak D, Fabian E, Elmadfa I (2006). Vitamin A content (retinol and retinyl esters) in livers of different animals. Food Chemistry 98:704-710.

Mallia AK, Smith JE, Goodman DS (1975). Metabolism of retinol-binding protein and vitamin A during hypervitaminosis A in the rat. J. Lipid Res. 16:180-188.

McLaren DS & Frigg M (2001). Sight and Life manual on vitamin A deficiency disorders (VADD). 2nd ed. Task Force, Sight and Life. http://www.sightandlife.org/booksAll/Manual2.html#6

van Merris V, Meyer E, de Wasch K, Burvenich C (2002). Simple quantification of endogenous retinoids in bovine serum by high-performance liquid chromatography - diode-array detection. Anal. Chim. Acta 468:237-244.

Miller DR & Hayes KC (1982). Vitamin excess and toxicity. Fat-soluble vitamins. Vitamin A. In: Nutritional toxicology, vol.1; Ed. Hathcock JN; Academic press; New York; p. 83-91.

Miller RK, Hendrickx AG, Mills JL, Hummler H, Wiegand U-W (1998). Periconceptional vitamin A use: how much is teratogenic. Reprod. Toxicol. 12:75-88.

Morriss-Kay G, Ruberte E, Fukiishi Y (1993). Mammalian neural crest and neural crest derivatives. Ann. Anat. 175:501-507.

Mustaniemi A & Hallikainen A (1994). Kadmiumin saanti ravinnosta. (In Finnish.) Elintarvikeviraston tutkimuksia 13/1993; Helsinki; Finland.

Mustaniemi A, Hallikainen A, Männistö S (1994). Elintarvikkeiden lyijypitoisuus ja lyijyn saanti ravinnosta. (In Finnish). Elintarvikeviraston tutkimuksia 1/1994; Helsinki; Finland.

Myhre AM, Carlsen MH, Bøhn SK, Wold HL, Laake P, Blomhoff R (2003). Water-miscible, emulsified, and solid forms of retinol supplements are more toxic than oil-based preparations. Am. J. Clin. Nutr. 78:1152-9.

Männistö S, Ovaskainen M, Valsta L, toim. (2003). Finravinto 2002 –tutkimus./The National FINNDIET 2002 Study. Publications of the National Public Health Institute B3/2003; Finland; 130 p.

Møller A, Saxholt E, Christensen AT, Hartkopp HB, Hess Ygil K (2005). Danish Food Composition Databank, revision 6.0. Food Informatics, Department of Nutrition, Danish Institute for Food and Veterinary Research. June 2005. http://www.foodcomp.dk/

Nagy NE, Holven KB, Roos N, Senoo H, Kojima N, Norum KR, Blomhoff R (1997). Storage of vitamin A in extrahepatic stellate cells in normal rats. J. Lipid Res. 38:645-658.

NCM (2003). Heath risks related to high intake of preformed retinol (vitamin A) in the Nordic countries. TemaNord 2003:502. Nordic Council of Ministers; Copenhagen; 113 p.

NCM (2004). Nordic Nutrition Recommendations 2004. 4th edition. Integrating nutrition and physical activity. Nord 2004:13. Nordic Council of Ministers; Copenhagen; 436 p.

Nickel R, Schummer A, Seiferle E (1977). The viscera of the domestic birds. Parey; Berlin; 202 p.

Nickel R, Schummer A, Seiferle E (1979). The viscera of the domestic mammals; 2nd ed. Parey; Berlin; 401 p.

Nishijo M, Nakagawa H, Honda R, Tanebe K, Saito S, Teranishi H, Tawara K (2002). Effects of maternal exposure to cadmium on pregnancy outcome and breast milk. Occup. Environ. Med. 59:394-397.

Nusser SM, Carriquiry AL, Dodd KW, Fuller WA (1996). A semiparametric transformation approach to estimating usual daily intake distributions. J. Am. Stat. Assoc. 91:1440-1449.

Olson JA (1984). Serum levels of vitamin A and carotenoids as reflectors of nutritional status. J. Natl. Cancer Inst. 73:1439-44.

Olson J (1987). Recommended dietary intakes (RDI) of vitamin A in humans. Am. J. Clin. Nutr. 45:704-716.

Olson JA (1996). Vitamin A. In: Ziegler EE & Filer LJ (eds.); Present knowledge in nutrition, 7th ed. ILSI Press; Washington DC; p. 109-119.

Papanikolaou NC, Hatzidaki EG, Belivanis S, Tzanakakis GN, Tsatsakis AM (2005). Lead toxicity update. A brief review. Med. Sci Monit. 11:RA329-336.

Penttinen R, Kallio-Mannila K, Nikander A (2002). Ravinnon tuotanto-olosuhteet ja turvallisuus – ympäristöongelmien vaikutukset Suomessa. (In Finnish). Suomen ympäristö 568. Publications of Finnish Environment Institute; Helsinki; p. 93-105.

Perrotta S, Nobili B, Rossi F, Di Pinto D, Cucciolla V, Borriello A, Oliva A, Della Ragione F (2003). Vitamin A and infancy. Biochemical, functional, and clinical aspects. Vitam. Horm. 2003:457-591.

Piersma AH, Bode W, Verhoef A, Olling M (1996). Teratogenicity of a single oral dose of retinyl palmitate in the rat, and the role of dietary vitamin A status. Pharmacol. Toxicol. 79:131-135.

Reinivuo H, Bingham C, Korhonen T, Pakkala H (2005). Elintarviketaulukko. Tiedot ravinto-koostumuksesta. Otava; Helsinki; 90 p.

Rosa FW, Wilk AL, Kelsey FO (1986). Teratogen update: vitamin A congeners. Teratology 33:355-364.

Ross C. 1981. Separation of long-chain fatty acid esters of retinol by high-performance liquid chromatography. Anal. Biochem. 115:324-330.

Ross SA, McCaffery, Drager UC, De Luca LM (2000). Retinoids in embryonal development. Physiol. Rev. 80:1021-1054.

Rothenberg SJ, Karchmer S, Schnaas L, Perroni E, Zea F, Fernandez Alba J (1994). Changes in serial blood lead levels during pregnancy. Environ Health Perspect. 102:876-80.

Rothman KJ, Moore LL, Singer MR, Uyen-Sa DTN, Mannino S, Milunsky A (1995). Teratogenicity of high vitamin A intake. N. Engl. J. Med. 333:1369-1373.

SACN (2005). Scientific Advisory Committee on Nutrition. Review of dietary advice on vitamin A. The Stationery Office; United Kingdom; 82 p. http://www.sacn.gov.uk/reports/#

Satarug S & Moore MR (2004). Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. Environ. Health Perspect. 112:1099-1103

SCF (1992). Report on the risks of hypervitaminosis A. In: Reports of the Scientific Committee for Food (Twenty-seventh series); p. 25-26. http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_27.pdf

SCF (1993). Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food (Thirty-first series). http://europa.eu.int/comm/food/fs/sc/scf/out89.pdf

SCF (2002). Opinion on Scientific Committee on Food on the tolerable upper intake level of preformed vitamin A (retinol and retinyl esters). http://europa.eu.int/comm/food/fs/sc/scf/out145_en.pdf

Schnaas L, Rothenberg SJ, Flores M-F, Martinez S, Hernandez C, Osorio E, Velasco SR, Perroni E (2006). Reduced intellectual development with prenatal lead exposure. Environ. Health Perspect. 114:791-797.

SCOOP 2004. Assessment of the dietary exposure to arsenic, cadmium, lead and mercury of the population of the EU Member States. Report of experts participating in Task 3.2.11. http://ec.europa.eu/food/food/chemicalsafety/contaminants/scoop_3-2-11_heavy_metals_report_en.pdf

Sklan D (1987). Vitamin A in human nutrition. Prog. Food Nutr. Sci. 11:39-55.

Soininen L, Mussalo-Rauhamaa H, Hyvönen S (2002). Ihmisen terveys ja lapin ympäristö (In Finnish). In: AMAP II - Lapin ympäristön tila ja ihmisen terveys (Suomen ympäristö: 581); Outi Mähönen (ed.); Lapin ympäristökeskus, Rovaniemi; p. 103-115.

Sommer A & Davidson FR (2002). Assessment and control of vitamin A deficiency: the annecy accords. J. Nutr. 132(suppl):2845S-2850S.

STAKES (2005). Parturients, births and newborns 2004. Officional Statistics of Finland, Statistical Summary 21/2005. National Research and Development Centre for Welfare and Health. http://www.stakes.info/files/pdf/Tilasto-tiedote2005/Tt21_05.pdf

Sääksjärvi K & Reinivuo H (2004). Ruokamittoja (In Finnish). Publications of the National Public Health Institute B15/2004; Finland; 46 p. http://www.ktl.fi/portal/suomi/osastot/eteo/yksikot/ravitsemusyksikko/julkaisut/ruokamittoja/

Thurnham DI, Northrop-Clewes CA (1999). Optimal nutrition: vitamin A and the carotenoids. Proc. Nutr. Soc. 58:449-457.

Tsutsumi C, Okuno M, Tannous L, Piantedosi R, Allan M, Goodman DS, Blaner WS (1992). Retinoids and retinoid-binding protein expression in rat adipocytes. J. Biol. Chem. 267:1805-1810.

Tzimas G & Nau H (2001). The role of metabolism and toxicokinetics in retinoid teratogenesis. Curr. Pharm. Des. 7:803-831.

Underwood B (1994). Maternal vitamin A status and its importance in infancy and early childhood. Am. J. Clin. Nutr. 59(suppl):517S-524S.

UNEP (1998). Global opportunities for reducing the use of leaded gasoline. UNEP Chemicals 9/98; Switzerland; p. 7-16.

Vahteristo L, Lyytikäinen T, venäläinen E-R, Eskola M, Lindfors E, Pohjanvirta R, Maijala R (2003). Cadmium intake of moose hunters in Finland from consumption of moose meat, liver and kidney. Food Addit. Contam. 20:453-463.

Vorhees CV (1994). Developmental neurotoxicity induced by therapeutic and illicit drugs. Environ. Health Perspect. 102(Suppl 2):145-153.

VRN (2005). Valtion ravitsemusneuvottelukunta. Suomalaiset ravitsemussuositukset – ravinto ja liikunta tasapainoon (In Finnish). Edita Publishing Oy; Helsinki.

West KP jr. (2002). Extent of vitamin A deficiency among preschool children and women of reproductive age. J. Nutr. 132(suppl):2857S-2866S.

WHO (1989a). Cadmium. In: Evaluation of certain food additives and contaminants: thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 776. World Health Organization; Geneva; p. 28-31. http://whqlibdoc.who.int/trs/WHO_TRS_776.pdf

WHO (1989b). Cadmium. In: Toxicological evaluation of certain food additives and contaminants. WHO Additives series 24. http://inchem.org/documents/jecfa/jecmono/v024je09.htm

WHO (1993). Lead. In: Evaluation of certain food additives and contaminants: forty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 837. World Health Organization; Geneva; p. 32-35. http://whqlibdoc.who.int/trs/WHO_TRS_837.pdf

WHO (1998). Safe vitamin A dosage during pregnancy and lactation. Recommendations and report of consultation. WHO/NUT/98.4. http://whqlibdoc.who.int/hq/1998/WHO_NUT_98.4.pdf

WHO (2000). Lead. In: Safety evaluation of certain food additives and contaminants. WHO Food additives series 44; p. 273-312.

WHO (2001). Cadmium. In: Safety evaluation of certain food additives and contaminants. WHO Food additives series 46; p. 247-305. http://www.inchem.org/documents/jecfa/jecmono/v46je11.htm

WHO (2003). Lead in drinking-water. Background document for development of WHO guidelines for drinking-water quality. World Health Organization; Geneva; 16 p. http://www.who.int/water_sanitation_health/dwq/chemicals/lead.pdf

WHO (2004). Cadmium. In: Evaluation of certain food additives and contaminants: sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 922. World Health Organization; Geneva; p. 127-132. http://whqlibdoc.who.int/trs/WHO_TRS_922.pdf

Williams SS, Mear JP, Liang H-C, Potter SS, Aronow BJ, Colbert MC (2004). Large-scale reprogramming of cranial neural crest gene expression by retinoic acid exposure. Physiol. Genomics 19:184-197.