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Risk assessment of "other substances" – L-leucine, L-isoleucine and L-valine, the branched chain amino acids (BCAA)

**Opinion of the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of
the Norwegian Scientific Committee for Food Safety**

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2016: 33
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Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has, at the request of the Norwegian Food Safety Authority (Mattilsynet; NFSA), assessed the risk of "other substances" in food supplements and energy drinks sold in Norway. VKM has assessed the risk of doses given by NFSA. The risk assessments are the scientific basis for NFSA in its efforts to regulate the use of "other substances" to food supplements.

"Other substances" are described in the food supplement directive 2002/46/EC *as substances other than vitamins or minerals that have a nutritional and/or physiological effect*. It is added mainly to food supplements, but also to energy drinks and other foods. VKM has not in this series of risk assessments of "other substances" evaluated any claimed beneficial effects from these substances, only possible adverse effects.

The present report is a risk assessment of L-leucine, L-isoleucine and L-valine and it is based on previous risk assessments and articles retrieved from a literature search. In this report L-leucine, L-isoleucine and L-valine may occasionally be termed merely leucine, isoleucine or valine.

L-leucine, L-isoleucine and L-valine are essential amino acids. L-leucine, L-isoleucine and L-valine are commonly known as Branched Chain Amino Acids (BCAAs), and are found in food items containing proteins and in particular, in protein-rich foods such as dairy products, meats, eggs, nuts, whole grains, seeds, avocados and edible seaweed.

According to information from NFSA, L-leucine, L-isoleucine and L-valine are ingredients in food supplements sold in Norway. NFSA has requested a risk assessment of the following doses of L-leucine, L-isoleucine and L-valine in food supplements for adults, adolescents and children 10 years and above: L-leucine: 2500, 3000, 4000, 5000 and 5250 mg/day, L-isoleucine: 1500, 1750, 2000 and 2500 mg/day and L-valine: 1500, 1750, 2000, 2250 and 2500 mg/day. Usual dietary intakes of these amino acids in Norway are not known. Based on data from the 1988–1994 NHANES III, mean daily intakes in adults of leucine, isoleucine and valine from food and supplements are 6.1, 3.6 and 4.0 g/day, respectively (IOM, 2005).

Most studies on BCAAs have focused on the three amino acids taken as single amino acids or together combined in food supplements. It has been shown that BCAAs are not metabolized in the liver as is common for most other amino acids but taken up by most peripheral tissues (in particular muscle) where they are either used in protein synthesis or as precursors for nitrogen and/or a number of carbon containing molecules.

There is a lack of relevant well-designed supplementation studies with L-leucine, L-isoleucine and L-valine in humans designed to address adverse effects and dose-response relationships as primary outcome.

However, daily doses of as much as 30 g BCAA per day given to athletes have been investigated and reported to improve performance. In these reports adverse effects were not addressed and not reported. L-leucine has been administered orally in single doses for one day of up to 50 g without showing any adverse effects. There are no published studies on the effects of longitudinal supplementation with either L-isoleucine or L-valine.

Thus, there are no published studies that can be used for suggesting a "value for comparison", and there is no scientific data in the literature suitable for assessing the specific doses in the terms of reference.

WHO (2007) recommendations for BCAAs are: Leucine 2730 mg/day, isoleucine 1400 mg/day and valine 1820 mg/day. For a 70 kg person, this corresponds to 39 mg leucine/kg body weight (bw) per day, 20 mg isoleucine/kg bw per day and 26 mg valine/kg bw per day.

The acute upper tolerable metabolic limit of L-leucine for men between 20 and 35 years was determined by administration of single doses of 550-700 mg/kg bw over one day. This corresponded to 39 to 50 g/day for a person of 70 kg. Furthermore, based on several studies investigating L-leucine, L-isoleucine and L-valine supplemented as single doses ranging from 10 to 30 g/day without any reported adverse effects. The uncertainties for this consideration are described in chapter 5.

VKM concludes that:

Due to lack of studies addressing adverse effects for the specified doses 2500, 3000, 4000, 5000 and 5250 mg/day L-leucine, 1500, 1750, 2000 and 2500 mg/day L-isoleucine and 1500, 1750, 2000, 2250 and 2500 mg/day L-valine in food supplements, no conclusions can be made for adults (≥ 18 years), adolescents (≥ 10 and < 18 years) or children (< 10 years).

Short summary

The Norwegian Scientific Committee for Food Safety (VKM) has, at the request of the Norwegian Food Safety Authority, assessed the risk of specified doses of L-leucine, L-isoleucine and L-valine in food supplements.

VKM concludes that:

Due to lack studies addressing adverse effects of the specified doses 2500, 3000, 4000, 5000 and 5250 mg/day L-leucine, 1500, 1750, 2000 and 2500 mg/day L-isoleucine and 1500, 1750, 2000, 2250 and 2500 mg/day L-valine in food supplements, no conclusions can be made for adults (≥ 18 years), adolescents (≥ 10 and < 18 years) or children (< 10 years).

Key words: Adverse health effect, BCAA, branched chain amino acids, food supplement, L-isoleucine, L-leucine, negative health effect, Norwegian Scientific Committee for Food Safety, other substances, risk assessment, L-valine, VKM.

Sammendrag på norsk

På oppdrag for Mattilsynet har Vitenskapskomiteen for mattrygghet (VKM) vurdert risiko ved tilsetning av "andre stoffer" i kosttilskudd og energidrikk som selges i Norge. VKM har risikovurdert ulike doser brukt av kosttilskudd og konsentrasjoner i energidrikker oppgitt fra Mattilsynet. Disse risikovurderingene vil gi Mattilsynet vitenskapelig grunnlag for å regulere andre stoffer.

«Andre stoffer» er beskrevet i kosttilskuddsdirektivet 2002/46/EC som *stoffer som har en ernæringsmessig og/eller fysiologisk effekt, og som ikke er vitaminer og mineraler*. De tilsettes i hovedsak til kosttilskudd, men også til energidrikker og andre næringsmidler. I disse risikovurderingene har VKM ikke sett på påståtte gunstige helseeffekter, men kun vurdert mulige negative helseeffekter.

Denne rapporten er en risikovurdering av, L-leucin, L-isoleucin og L-valin, basert på tidligere risikovurderinger og artikler identifisert gjennom nye litteratursøk. I denne rapporten kan L-leucin, L-isoleucin og L-valin bli omtalt som leucin, isoleucin eller valin.

L-leucin, L-isoleucin og L-valin er essensielle aminosyrer. Disse aminosyrene er kjent som Branched Chain Amino Acids (BCAAs) eller forgrenede aminosyrer. BCAA er en del av alle kjente proteinholdige matvarer som meieriprodukter, kjøtt, egg, nøtter, korn, frø, avokado, og spiselige sjøplanter.

I følge informasjon fra Mattilsynet er "Branched Chain Amino Acids" (BCAA), L-valin, L-isoleucin og L-leucin, sammen og hver for seg, ingredienser i kosttilskudd som selges i Norge. Oppdraget fra Mattilsynet var å risikovurdere inntak av L-leucin på 2500, 3000, 4000, 5000 og 5250 mg/dag, L-isoleucin på 1500, 1750, 2000 og 2500 mg/dag og, L-valin 1500, 1750, 2000, 2250 og 2500 mg/dag. Det normale inntaket av disse aminosyrene gjennom kosten er ikke kjent i Norge. Basert på data fra 1988–1994 NHANES III studien, er det anbefalt et daglig inntak av henholdsvis L-leucine, L-isoleucin and L-valin gjennom mat og kosttilskudd på 6,1, 3,6 og 4,0 g/dag (IOM, 2005).

De fleste studier som beskriver BCAAs som kosttilskudd har omfattet de tre aminosyrene sammen (BCAA) eller som enkelte aminosyrer. BCAA blir ikke metabolisert i leveren som andre kjente aminosyrer, men i perifere vev, spesielt i muskler hvor de enten blir benyttet til proteinsyntese eller brukt som forstadier for syntese av nitrogenholdige metabolitter og/eller karbonholdige molekyler.

Det foreligger ikke relevante kontrollerte humane studier av L-leucin, L-isoleucin og L-valin som kosttilskudd som dokumenterer skadelige effekter og ved hvilket dosenivå de evt. inntreer.

Til tross for dette er det rapportert at daglige doser av BCAA på 30 g gitt til idrettsutøvere resulterer i økt prestasjon. I disse studiene ble ikke skadelige effekter av BCAA målt eller

rapportert. L-leucin har blitt gitt som enkeltdose opp til 50/g per dag uten at skadelige effekter er rapportert. Ingen kjente studier av BCAA over lengre tid med skadelige effekter som endepunkter er kjent. Ingen studier av effekten av L-isoleucin og L-valin gitt separat over kort eller lang tid er kjent.

Ingen studier kan dermed brukes i "value for comparison", og det er ingen publiserte vitenskapelige data som kan brukes for å angi om dosene som etterspørres gir skadelige effekter.

WHO (2007) sine anbefalinger for BCAAs er: Leucin 2730 mg/dag, isoleucin 1400 mg/dag og valin 1820 mg/dag. For en person på 70 kg korresponderer dette til 39 mg leucin/kg kroppsvekt (kv) per dag, 20 mg isoleucin/kg kv per dag og 26 mg valin/kg kv per dag.

Den øvre tolerable metabolske grensen for L-leucin for menn mellom 20 og 35 år ble rapportert til å være mellom 550-700 mg/kg kv når aminosyren ble gitt i en enkeltdose og metabolske kapasiteten målt samme dag. Dette korresponderer til mellom 39 til 50 g/dag for en 70 kg person. Videre er det rapportert fra flere studier at tilskudd av L-leucin, L-isoleucin og L-valin gitt i enkeltdoser mellom 10 og 30 g per dag ikke er assosiert med rapporterte skadelige effekter. Usikkerheten i disse betraktningene er beskrevet i kapittel 5.

VKM konkluderer med:

På grunnlag av mangel på studier som undersøker skadelige effekter av L-leucin i doser på 2500, 3000, 4000, 5000 og 5250 mg/dag, L-isoleucin på 1500, 1750, 2000 og 2500 mg/dag og L-valin 1500, 1750, 2000, 2250 og 2500 mg/dag er det ikke mulig å konkludere om disse dosene er skadelige.

Kort sammendrag

Vitenskapskomiteen for mattrygghet (VKM) har på oppdrag fra Mattilsynet vurdert risikoen for spesifikke doser av L-leucin, L-isoleucin og L-valin som kosttilskudd.

På grunn av mangel på studier som undersøker skadelige effekter av L-leucin på 2500, 3000, 4000, 5000 og 5250 mg/dag, L-isoleucin på 1500, 1750, 2000 og 2500 mg/dag og L-valin 1500, 1750, 2000, 2250 og 2500 mg/dag er det ikke mulig å konkludere.

Abbreviations and glossary

Abbreviations

AAT	- aspartate amino transaminase
ATP	- adenosine triphosphate
AESAN	- Spanish Agency for Food Safety and Nutrition
AFSSA	- French Food Safety Agency
ANSES	- French Agency for Food, Environmental and Occupational Health and Safety
BCAA	- branched chained amino acid
BCAT	- branched chain aminotransferase
BCKDC	-branched chain keto acid dehydrogenase kompleks
bw	- body weight
CNS	- central nervous system
EFSA	- European Food Safety Authority
IOM	- Institute of Medicine, USA
KIC	- alpha-ketoisocaproate
LOAEL	- lowest observed adverse effect level
MOE	- margin of exposure
MSUD	- maple syrup urine disease
NADH	- reduced nicotinamide adenine dinucleotide
NFSA	- Norwegian Food Safety Authority [<i>Norw.</i> : Mattilsynet]
NOAEL	- no observed adverse effect level
OAA	- oxaloacetate
SAE	- serious adverse event
SCF	- Scientific Committee on Food
UL	- tolerable upper intake level
VKM	- Norwegian Scientific Committee for Food Safety [<i>Norw.</i> : Vitenskapskomiteen for Mattrygghet]
WHO	- World Health Organization

Glossary

"Other substances": a substance other than a vitamin or mineral that has a nutritional or physiological effect (European Regulation (EC) No. 1925/2006, Article 2; <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1925&from=en>).

"Negative health effect" and "adverse health effect" are broad terms. VKM uses the definition established by EFSA for "adverse effect": a change in morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (WHO, 1994).

An adverse event is considered serious (SAE) if it results in death, is life-threatening, requires or prolongs hospitalisation, is a congenital anomaly or birth defect, is a persistent or significant disability/incapacity, or is another serious or important medical event.

Background as provided by the Norwegian Food Safety Authority

"Other substances" are substances other than vitamins and minerals, with a nutritional and/or physiological effect on the body. "Other substances" are mainly added to food supplements, but these may also be added to other foods and beverages, such as sports products and energy drinks. Ingestion of these substances in high amounts presents a potential risk for consumers.

In Norway, a former practice of classification of medicines had constituted an effective barrier against the sale of potentially harmful "other substances". Ever since this practice was changed in 2009, it has become challenging to regulate and supervise foods with added "other substances". Meanwhile, in the recent years, the Norwegian market has witnessed a marked growth in the sales of products containing "other substances". In 2011, food supplements containing "other substances" constituted more than 50% of the market share.

While within the European Economic Area, these substances fall under the scope of the European Regulation (EC) No. 1925/2006 on the addition of vitamins, minerals and certain other substances to foods and the European Regulation (EC) No 258/97 concerning novel foods and novel food ingredients, "other substances" remain largely unregulated. In order to ensure safe use of "other substances" many countries have regulated their use at a national level. For example, Denmark regulates these substances in a positive list i.e. a list of substances with maximal daily doses, permitted for use in food supplements and other foods (FVM, 2014).

NFSA is working on the establishment of a regulation on the addition of "other substances" to foods at a national level. The regulation will include a list of substances with permitted maximal doses, based on the substances and doses found in products on the Norwegian market. NFSA has therefore requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of "other substances" found on the Norwegian market. NFSA, in consultation with the industry, has compiled a list of "other substances" found in products marketed in Norway. Only substances with a purity of minimum 50% or concentrated 40 times or more have been included in the list. Substances regulated by other legislations like those for novel foods, food additives, aromas, foods for special medical purposes, etc. have been excluded from the list.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) has requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of L-leucine, L-isoleucine and L-valine in food supplements at the following doses:

L-leucine: 2500, 3000, 4000, 5000 and 5250 mg/day

L-isoleucine: 1500, 1750, 2000 and 2500 mg/day and

L-valine: 1500, 1750, 2000, 2250 and 2500 mg/day

NFSA requested VKM to assess the safety of "other substances" (in accordance to the guidance document developed in Phase 2) at the doses specified (Phase 3).

The safety assessments of "other substances" present in food supplements shall be carried out for a general population, ages 10 years and above.

Assessment

1 Introduction

"Other substances" are described in the food supplement directive 2002/46/EC *as substances other than vitamins or minerals that have a nutritional and/or physiological effect*, and may be added to food supplements or e.g. energy drinks. VKM has in this series of risk assessments of "other substances" not evaluated any potential beneficial effects from these substances, but merely possible adverse effects at specified doses sold in Norway.

This risk assessment regards the amino acids L-leucine, L-isoleucine and L-valine *per se*, and no specific products.

According to information from the Norwegian Food Safety Authority (NFSA), L-leucine, L-isoleucine and L-valine are ingredients in food supplements sold in Norway. NFSA has requested a risk assessment of the following doses of L-leucine, L-isoleucine and L-valine in food supplements for adults, adolescents and children 10 years and above: L-leucine: 2500, 3000, 4000, 5000 and 5250 mg/day, L-isoleucine: 1500, 1750, 2000 and 2500 mg/day and L-valine: 1500, 1750, 2000, 2250 and 2500 mg/day.

The intake of L-leucine, L-isoleucine or L-valine from other sources than food supplements is not included in this risk assessment.

L-leucine, L-isoleucine and L-valine are essential amino acids. L-leucine, L-isoleucine and L-valine are commonly known as Branched Chain Amino Acids (BCAAs). They are found in protein containing food items such as dairy products, protein-rich foods such as dairy products, meats, eggs, nuts, whole grains, seeds, avocados and edible seaweed. BCAA supplementation is commonly used in sports nutrition.

The BCAAs are released and absorbed in the intestine followed by transport to the liver through the portal vein. Most of the BCAAs ingested (50 to 70%) pass through the liver and are delivered unmetabolised to systemic circulation.

Most studies on BCAAs have focused on the three amino acids taken together as a food supplement. It has been shown that BCAAs are taken up by most peripheral tissues, particularly muscle (>50%) where they are either incorporated into proteins or metabolised as precursors for nitrogen and/or various carbon containing molecules. Hence, of particular importance is the role of BCAA in transporting nitrogen in the body. They may also be degraded to yield energy in the form of ATP and are known to play a key role in adipocyte metabolism and differentiation. As a single amino acid L-valine is only known for its role in protein synthesis while L-isoleucine is known to contribute to protein synthesis and to regulate glucose uptake into cells. L-leucine is known to have a number of additional

regulatory functions in addition to contributing to protein synthesis. These include anabolic effects on muscle protein synthesis and hypothalamic mediated regulation of hunger and satiety. Moreover, BCAA through the action of L-leucine are thought to prevent fatigue as they compete for the same amino acid transporter as L-tryptophan which is a precursor for serotonin that induces central nervous system (CNS) fatigue. L-leucine is also crucial for the regulation of glutamate levels in CNS, a regulation which is abrogated in patients with maple syrup urine disease (MSUD). MSUD, also called branched-chain ketoaciduria, is an autosomal recessive metabolic disorder caused by a deficiency of the branched-chain alpha-keto acid dehydrogenase complex (BCKDC), leading to a build-up of BCAAs in the blood and urine. Accumulation is toxic to the CNS and may cause death due to brain swelling.

The WHO (2007) recommends an intake of: leucine 2730 mg/day, isoleucine 1400 mg/day and valine 1820 mg/day (in a 70 kg person). The IOM report from 2005 recommends for adults: leucine 2380 mg/day, isoleucine 1050 mg/day and valine 1330 mg/day.

2 Hazard identification and characterisation

The present risk assessment is based on previous risk assessments of BCAA, L-leucine, L-isoleucine and L-valine, and articles retrieved from literature searches.

2.1 Literature

2.1.1 Recommendations from WHO, 2007

WHO (2007) recommendations: Leucine 2730 mg/day, isoleucine 1400 mg/day and valine 1820 mg/day (in a 70 kg person). This is equal to 39 mg leucine/kg bw per day, 20 mg isoleucine/ kg bw per day and 26 mg valine/kg bw per day.

Based on data from the 1988–1994 NHANES III, mean daily intakes of leucine, isoleucine and valine from food and supplements are 6.1, 3.6 and 4.0 g, respectively (IOM, 2005).

2.1.2 Previous risk assessments

Risks related to L-leucine, L-isoleucine and L-valine have previously been evaluated by the Institute of Medicine (IOM) in USA in 2005, the European Food Safety Authority (EFSA) as flavouring substance in 2008, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and VKM in 2011 and the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (ASEAN) for use in food supplements in 2012. The opinion from EFSA in 2008 evaluated amino acids at low doses not relevant for the present evaluation.

Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients) Chapter 10 "Protein and Amino Acids." Institute of Medicine (IOM). USA, 2005

The IOM (2005) report states that there is no evidence that any amino acids including BCAA derived from usual to high intake of proteins from food will cause any adverse health effects. Many of these studies addressing use of BCAA as supplements address treatment of a variety of metabolic disorders and because they involved patients with significant and sometimes unusual diseases, they are not directly relevant to the problem of assessing risks to normal, healthy humans.

The IOM (2005) concludes that there is no evidence that any amino acids including BCAA derived from usual to high intake of proteins from food will cause any adverse health effects.

Opinion of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) on the assessment of the risks associated with substances with nutritional or physiological effects with a view to restricting or prohibiting their use in foodstuffs. France, 2011

The opinion from ANSES (2011) mainly evaluated amino acids taken as protein. The report refers to previous reports on L-valine, L-isoleucine and L-leucine which describe adverse neurotoxic effects and mental retardation if these amino acids are taken in high doses (three times normal flux or 6 times higher than average dietary intake which is between 6 and 9 g/day for a 70 kg person), (reviewed in Matthews 2005). However, the ANSES (2011) report elaborates on the topic of amino acid metabolism and states that "on a nutritional and metabolic level, consumption of an amino acid at levels much higher than other amino acids, and much higher than the corresponding quantitative requirements for protein synthesis, induces changes in circulating pools, changes in functions directly controlled by the amino acids, the substantial entry of these amino acids into catabolic pathways (sometimes in "secondary" metabolic pathways) and the activation of excretion pathways. "Specific for L-leucine, L-isoleucine or L-valine, and based on animal experiments, the ANSES (2011) report lists hypoglycaemia, mental retardation, hypovalinemia and hypoisoleucineamia as possible adverse effects of high intakes of L-leucine, and mental retardation as possible adverse effects of high intakes of L-isoleucine or L-valine. However, doses and reliable experimental data are lacking. Hence, the claims made by ANSES (2011) are questioned. In general it is reported that the complexity of amino acid metabolism, the scarcity of toxicological data, and the many reasons for the insufficiently characterised risks, lead to the conclusion that it is impossible to conduct a proper risk assessment. The ANSES (2011) report therefore concludes the urgent need for a complete set of detailed and high-quality metabolic, physiological and toxicological data, with which to determine the benefits and risks associated with the addition of amino acids to foods for the general population.

VKM report on risk categorisation of amino acids. Norway, 2011

In 2011, VKM conducted a risk categorisation of about 30 amino acids and amino acid compounds. It was emphasised that the VKM report from 2011 has several limitations and can only be regarded as an initial screening and not as risk assessment of the many amino acids. Leucine and isoleucine were suggested categorised as substances with possible to moderate risk (VKM, 2011). This categorisation was based on no scientific documentation other than the general knowledge that amino acids in general are bioactive compounds. No studies reporting adverse effects were identified.

Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the use conditions for certain substances other than vitamins, minerals and plants in food supplements – 1. Spain, 2012

The AESAN (2012) report handled the BCAAs individually. L-leucine, L-isoleucine and L-valine were assessed mainly focusing on the assessment published in the WHO (2007) technical

report. In the AESAN report they conclude that it has not been possible to establish a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) for BCAA or L-leucine, L-isoleucine and L-valine from available studies.

AESAN concluded that, "although no toxicity studies in humans are available, the toxicity studies in other models of animals used for experimental purposes, and those carried out in humans with the administration of a single dose, indicate that intake levels of up to three times more than the intake recommendations are well-tolerated by healthy adult subjects. Therefore, the Scientific Committee concludes that, based on the information available to date and taking into account the considerations reflected in this report, a maximum daily amount of 5 g of the sum of L-isoleucine, L-leucine and L-valine is acceptable from a safety viewpoint for use as a food supplement. "

Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the use conditions for certain substances other than vitamins, minerals and plants in food supplements – 2. Spain, 2013

The AESAN (2013) evaluated a maximum daily quantity of 5 g of the sum of L-isoleucine, L-leucine and L-valine and concluded that this was acceptable from a safety viewpoint for use as a food supplement, provided that these amino acids are not consumed by pregnant women, children or for prolonged periods of time without medical supervision. The Committee also stated that, according to several studies, intake levels of up to three times more than the intake recommendations are well-tolerated by healthy adult subjects.

The Scientific Committee concluded that, based on the information available to date and taking into account the considerations indicated in its report dated 28 November 2012, the proposal of a maximum daily quantity of the sum of L-isoleucine, L-leucine and L-valine of 5.45 g (an increase from 5 g without justification) is acceptable from a safety viewpoint for use as a food supplement, with the warning that these amino acids are not consumed by pregnant women, children or for prolonged periods of time without medical supervision".

2.1.3 Literature search

Literature searches were performed in MEDLINE and EMBASE with no restriction on publication date in order to retrieve publications on adverse effects caused by L-leucine, L-isoleucine, L-valine and BCAA. These databases were chosen to ensure comprehensive study retrieval. One search was restricted to clinical human studies, and one search was specified for systematic reviews and meta- analyses. The literature searches were performed 16 November 2015. The search strategies are included in Appendix 1.

2.1.3.1 Publication selection and data extraction

The study types for inclusion in this opinion have been solely studies on healthy humans. Animal studies were not included as they do not reflect human physiology. Despite this

animal studies were critically scrutinized when identified in the previous risk assessments. The criteria for inclusion from human studies were:

- L-leucine, L-isoleucine, L-valine and BCAA in relation to adverse effect must be addressed in the abstracts of the paper.
- Outcome not affected by other substances than L-leucine, L-isoleucine, L-valine and BCAA.
- Oral route of exposure to L-leucine, L-isoleucine, L-valine and BCAA in human studies.
- Human studies were performed in apparently healthy individuals (not patient groups) who are assumed to have abnormal L-leucine, L-isoleucine, L-valine and BCAA absorption and/or metabolism.

In vitro studies were not included. Also papers in languages other than English, Norwegian, Danish or Swedish were excluded.

The inclusion criteria checklist was developed by members of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics and the Panel on Nutrition, Dietetic Products, Novel Food and Allergy. Articles that did not appear to meet the inclusion criteria were excluded from further analysis. In situations where it was unclear whether the publication was of relevance to the study, it was retained for further screening. The primary screening was performed independently by two persons.

The full text of articles that passed the primary screening was retrieved for secondary screening. In this screening, the full text articles were reviewed and compared against the inclusion criteria checklist. The secondary screening was performed by one person and resulted in 0 full text articles.

2.2 General information

2.2.1 Chemistry

L-leucine, L-isoleucine and L-valine are essential branched chained amino acids. They are equipped with a hydrophobic bulky side chain. Figures 2.2.1-1, 2.2.1-2 and 2.2.1-3 show the structural formulas for these amino acids.

L-leucine $C_6H_{13}NO_2$ ($(CH_3)_2CHCH_2CH(NH_2)CO_2H$) has CAS number 61-90-5.

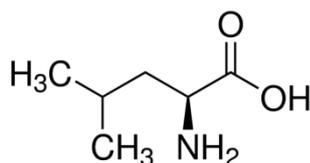


Figure 2.2.1-1 The structural formula of L-leucine.

L-isoleucine $C_6H_{13}NO_2$ ($C_2H_5CH(CH_3)CH(NH_2)CO_2H$) has CAS number 73-32-5.

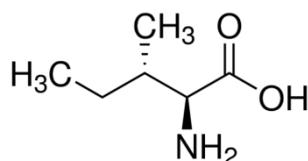


Figure 2.2.1-2 The structural formula of L-isoleucine.

L-valine $C_5H_{11}NO_2$ ($(CH_3)_2CHCH(NH_2)CO_2H$) has CAS number 72-18-4.

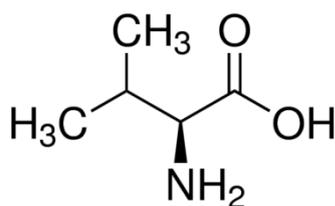


Figure 2.2.1-3 The structural formula of L-valine.

2.2.2 Occurrence

In the normal diet, the amino acids are ingested as components of food proteins and not as free amino acids. They are found in protein containing food items such as protein-rich foods such as dairy products, meats, eggs, nuts, whole grains, seeds, avocados and edible seaweed. In addition L-leucine, L-isoleucine and L-valine are available in food supplements either separately or together.

2.3 Absorption, distribution, metabolism and excretion

2.3.1 In humans

Because of the low hepatic expression of mitochondrial branched chain aminotransferase (BCAT2 or BCATm), BCAAs (L-leucine, L-isoleucine and L-valine) from dietary protein bypass the metabolism in the liver. This contributes to an elevation of L-leucine, L-isoleucine and L-valine in plasma after a meal (Matthews, 2005). Non-metabolised L-leucine, L-isoleucine and L-valine (in particular L-leucine) play a signaling role in the peripheral tissues by stimulating protein synthesis, particularly in muscle (Hutson et al., 2005). Furthermore, in humans L-leucine, but not L-isoleucine and L-valine, has been shown to prevent muscle protein degradation (Matthews, 2005). Furthermore, L-leucine, but not L-isoleucine and L-valine, may also act as a hormone in the hypothalamus for the regulation of hunger and satiety. In particular, L-leucine has been shown to stimulate satiety.

L-leucine, L-isoleucine and L-valine, and particularly L-leucine, act as nutrient signals and regulate protein degradation and synthesis, the latter through stimulating insulin release.

This also indicates that L-leucine in food improves muscle glucose uptake and whole-body glucose metabolism. L-leucine, L-isoleucine and L-valine also increase and contribute directly to adipocyte metabolism and differentiation (Green et al., 2016) and L-leucine stimulates postprandial leptin increase. Together L-leucine and leptin decreases food intake and body weight via stimulation of central mammalian target of rapamycin signaling in the hypothalamus. These effects of L-leucine, L-isoleucine and L-valine, and particularly L-leucine, are unique among the amino acids.

Finally, L-leucine is pivotal in glutamate metabolism in the brain (Fernstrom, 2005). Glutamate is excluded from the brain by the blood brain barrier. L-leucine in brain capillaries enters astrocytes via a neutral amino acid transporter. In capillaries L-leucine is transaminated via BCAT. In this process glutamate and KIC (the alpha keto acid of L-leucine) are formed. Glia cells release KIC to neurons where KIC is formed into L-leucine by transamination of glutamate into alpha-ketoglutarate. This is called the L-leucine–glutamate cycle and is important for the brain to keep glutamate concentrations at a very tight level and compartmentalized to avoid adverse effects (Yudkoff et al., 2005).

2.4 Toxicological data/Adverse effects

2.4.1 Human studies

BCAAs together or L-leucine, L-isoleucine or L-valine alone have been administered acutely (in single doses) and the effects monitored from 1 hour up to 1 day. The amounts of the BCAAs administered have typically been higher than the normal turnover of the BCAAs (75-260 $\mu\text{mol/kg/hour}$) and therefore several-fold greater than a normal daily intake. None of these studies reported any adverse effects of either BCAAs administered together or administration of L-leucine, L-isoleucine or L-valine alone. Neither BCAA nor L-leucine, L-isoleucine or L-valine administration significantly altered concentrations of circulating hormones such as insulin and glucagon. However, both BCAA and e.g. L-leucine administration significantly reduced the plasma concentration of several indispensable amino acids (phenylalanine, tyrosine, and methionine). The reduction was not caused by a direct alteration of amino acid transport across cells, but by a reduction of protein breakdown and reduced release of amino acids from cells. This effect has been identified predominantly in muscle.

Many studies on the effects of BCAA supplementation involving healthy individuals have been directed at their potential for improving physical or mental performance as they may reduce muscle catabolism and CNS fatigue, respectively (Gleeson, 2005; Matthews, 2005). This has been done despite the fact that no alteration or stimulation of protein synthesis with either BCAA or L-leucine, L-isoleucine or L-valine administration has been demonstrated in humans. The numbers of studies in athletes to investigate sports performance are many. A few examples show: Schena et al. (1992) that BCAA supplementation (11.5/day; 2:1:1, leucine:isoleucine:valine) tested on 16 individuals for 21 days during trekking at high altitude (3255 m above sea level) may prevent muscle loss during chronic hypobaric hypoxia. In a

RCT, Coombes and McNaughton (2000) examined the effects of BCAA supplementation in sixteen male subjects that were assigned to two groups (8 + 8): the supplemental group (consuming 12 g per day of BCAA for 14 d in addition to their normal diet and the control group consumed normal diet only. Baseline serum creatine kinase (CK) and lactate dehydrogenase (LDH) which are assumed to be accurate indicators of muscle damage, were determined during the week before the exercise test. The exercise test was administered on day seven and required the subjects to cycle for 120 min on an ergometer at approximately 70% VO_2 max. Blood samples were taken prior to and immediately following exercise and at 1 hr, 2 hrs, 3 hrs, 4 hrs, 1 d, 3 d, 5 d and 7 d post exercise. All subjects had their diets analysed daily during the 14 days and demonstrated that all subjects consumed the recommended daily intake of BCAA (0.64 g x per kg) in their normal diets. Baseline serum values for CK and LDH were shown not to differ between groups in the 7 d prior to the test ($p > 0.05$). Significant increases ($p < 0.05$) between the pre-exercise and post exercise values for LDH and CK until 5 d post exercise test. BCAA supplementation significantly reduced this change in LDH from 2hrs to 5 d post-test, and CK from 4 hrs to 5 d post-test ($p < 0.05$). It should be noted that none of these studies addressed or reported on adverse effects on intake of BCAA as high as 11.5 g per day for 21 days. Furthermore, of the individual BCAA, leucine has received the most study because of its relatively greater rate of oxidation and because it is associated with the rapid release of glucogenic precursors from muscle. There have been several reports of clinical trials in which groups of healthy humans, in most cases trained athletes, were given high doses of leucine by intravenous infusion. Most of the studies involved a single dose of the amino acid. These trials measured physical and mental performance and the impact on blood levels of other amino acids. None of these studies provides evidence of an adverse effect of leucine as they were not addressed. Thus, this collection of studies is considered to be of limited value in assessing health risks. To understand why humans appears to tolerate several grams of BCAA without experiencing adverse effects it may be useful to address the amount of BCAA in normal food alternatives ingested on a daily basis. For example, a single chicken breast (100 g) contains ~470 mg valine, 375 mg isoleucine, and 656 mg leucine. A typical BCAA supplement sold in tablet form contains 100 mg valine, 50 mg isoleucine, and 100 mg leucine implying that 10 tablets are needed to level what is ingested in 100 g of chicken breast. Moreover, 60 g (a regular bag) of unsalted peanuts contains 1030 mg leucine, 589 mg isoleucine and 740 mg valine (<http://www.matvaretabellen.no/?language=en>). Together this reveals that the relative content of BCAAs in regular food items is high and it is assumed that recommended intake is easily met (IOM, 2005; WHO, 2007). The intake of BCAAs in regular food item may therefore easily be exceeded through a regular diet. This may in part provide an explanation that high intake of BCAAs is well tolerated.

To this end, there is one report testing the acute tolerable upper limit for L-leucine in humans (Pencharz et al., 2012) reviewed in Kimura et al. (2012) and Elango et al. (2015). Five healthy men between 20 and 35 years of age were challenged with single oral doses of 50, 150, 250, 750, 1000 and 1250 mg L-leucine /kg bw. The acute upper tolerable metabolic limit of L-leucine was determined to be 550 to 700 mg/kg bw per day, or between 39 and 50 g/day for a person of 70 kg. To determine this, a range of biological markers were

measured, including glucose, insulin, alanine aminotransferase, and ammonia. Plasma leucine concentrations significantly increased beyond an intake of 550 mg/kg per day. An increase in blood ammonia concentrations was observed at leucine intakes >550 mg/kg per day. Glucose concentrations fell below 5.5 mmol/L but remained within the normal range and without any change in insulin. However, it is not known whether chronic administration of L-leucine at this level may be associated with adverse effects.

2.4.1.1 Interactions

There was no information concerning interactions in the literature reviewed in the present risk assessment. The absence of information in the literature does not document an absence of interactions.

2.4.1.2 Allergic sensitisation (including adjuvant effects)

There was no information concerning allergic sensitisation or allergy adjuvant effects in the literature reviewed in the present risk assessment. The absence of information in the literature does not document an absence of allergic sensitisation or allergy adjuvant effects.

2.4.2 Animal studies

Animal studies on BCAA, L-valine, L-isoleucine and L-leucine have been performed, but not included in the present evaluation. This has been decided for three reasons:

1. BCAA metabolism differs between animals such as rats (commonly used experimental animal model), monkeys (higher primate) and humans. The main issue is the concentration of BCAA which on average is 10 fold higher in major tissues such as muscle in rats compared to humans.
2. The oxidative capacity (turnover per min) of BCAA in rats, monkeys and humans, based on the expression of BCKD, are 7.11, to 0.32 and 0.7, respectively. Together this makes extrapolation of results of feeding experiments from animals to humans inappropriate (Hutson et al., 2005).
3. Administration of L-leucine resulted in comparable effects on muscle mass in animals and humans, but through different mechanisms: L-leucine increases muscle protein synthesis in rats whereas in humans administration of L-leucine prevented muscular breakdown (Matthews, 2005).

2.4.3 Mode of action for adverse effects

No mode of action for adverse effects has been identified.

2.4.4 No specific or definite mechanism for any adverse effects have been described in vulnerable groups

L-leucine, L-isoleucine and L-valine are the only amino acids to share common metabolism and share enzymes for the first 2 degradative steps: transamination (transfer of an amino group to an alpha-keto acid) and subsequent decarboxylation of the resulting BCKAs. The BCAAs are also the only indispensable amino acids to have degradative metabolic pathways active in muscle and not liver. In muscle BCAAs are metabolised to L-alanine and L-glutamine that are released to circulation and returned to gut and liver for metabolism.

This system is very efficient as the metabolic capacity of a single dose of e.g. L-leucine is (550-700 mg/kg bw per day) or 39-50 g per day for an adult of 70 kg (Pencharz et al., 2012).

A special case of concern is MSUD, which is defined as defect BCAA degradation. MSUD influences the malate–aspartate shuttle which transports reduced nicotinamide adenine dinucleotide (NADH) produced by the glycolysis from cytosol into the mitochondrion. It involves cytosolic production of oxaloacetate (OAA) from aspartate and alpha-ketoglutarate using the enzyme aspartate amino transaminase (AAT). OAA is reduced to malate in the cytoplasm by the use of NADH followed by transport of malate into the mitochondrion where it is oxidised to OAA. This mitochondrial reaction forms and hence restores NADH for oxidation in the electron transport chain. Under conditions such as MSUD where BCAAs are accumulating, KIC also accumulates. This leads to increased consumption of glutamate and increased production of both L-leucine and alpha-ketoglutarate via BCAT. The net result is diminished intracellular glutamate, increased alpha-ketoglutarate and enhanced combustion of aspartate via AAT. As a result, overall flux through AAT is disrupted and the malate-aspartate shuttle can no longer effectively transfer reducing equivalents from cytosol to mitochondrion. The net result is decreased mitochondrial ATP synthesis which has physiological consequences. Low ATP is associated with fatigue and reduced muscular strength contributing to fatigue.

2.5 Summary of hazard identification and characterisation

Previous risk assessments conducted by IOM (2005) and ANSES (2011) did not conclude regarding safe doses of L-leucine, L-isoleucine, L-valine or BCAAs due to lack of adequate scientific documentation.

The AESAN reports from 2012 and 2013 stated that it was not possible to give a NOAEL or LOAEL for L-leucine, L-isoleucine or L-valine. In the 2012 report, AESAN concluded that up to 5 g/day of the sum of L-isoleucine, L-leucine and L-valine was safe for human consumption, this dose was increased to 5.45 g/day in the 2013-report (no reason was given for this increase). Moreover, it was emphasised that children and pregnant women should refrain from consuming supplements containing L-isoleucine, L-leucine and L-valine.

VKM has not identified any studies in healthy humans addressing adverse effects of consumption of L-isoleucine, L-leucine, L-valine or BCAA in combination. L-leucine has been administered orally at acute doses of up to 50 g/day for a day without showing any adverse effects. There have been several reports of trained athletes given various doses of BCAA ranging from 10 to 30 g per days up to 3 weeks. These trials measured physical and mental performance including muscle strength and endurance and did not address nor report on adverse effects of BCAA supplementation. It should be noted that this collection of studies do not provide evidence of adverse effects of BCAA supplementation. However, they are of highly limited value in assessing health risks, as they do not address adverse effects of longitudinal administration.

Furthermore, due large differences in metabolism of BCAA, L-leucine, L-isoleucine and L-valine between animal and humans, VKM considers that animal studies are not relevant to assess adverse effects of BCAA, L-leucine, L-isoleucine and L-valine in humans (see also paragraph 2.3.2. and Hudson 2000).

Based on available data it is not possible to establish any "value for comparison" for a risk characterisation of BCAA together or L-leucine, L-isoleucine or L-valine.

Due to lack of studies addressing adverse effects of the specified doses 2500, 3000, 4000, 5000 and 5250 mg/day of L-leucine; 1500, 1750, 2000 and 2500 mg/day of L-isoleucine and 1500, 1750, 2000, 2250 and 2500 mg/day of L-valine in food supplements, no conclusions can be made for adults (≥ 18 years), adolescents (≥ 10 and < 18 years) and children (< 10 years).

3 Exposure / Intake

Exposure of L-leucine, L-isoleucine and L-valine was estimated from the intake of food supplements for the age groups children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years).

3.1 Food supplements

The NFSA has requested a risk assessment of the intake of 2500, 3000, 4000, 5000 and 5250 mg L-leucine per day, 1500, 1750, 2000, 2500 mg L-isoleucine per day and 1500, 1750, 2000, 2250, 2500 mg L-valine per day in food supplement for children, adolescents and adults. The default bw for these groups as determined by EFSA were used: 10 to <14 years; 43.4 kg, 14 to <18 years; 61.3 kg and adults; 70.0 kg (EFSA, 2012). The intakes per kg bw are given in (Table 3.1-1)

Table 3.1-1: Estimated exposures of L-leucine, L-isoleucine and L-valine from specified doses in food supplements in children, adolescents and adults.

Groups	Daily doses (mg)	Body weight (kg)	Exposures (mg/kg bw per day)
Children (10 to <14 years)	L-leucine: 2500, 3000, 4000, 5000, 5250	43.4	58, 69, 92, 115, 121
	L-isoleucine: 1500, 1750, 2000, 2500		35, 40, 46, 58
	L-valine: 1500, 1750, 2000, 2250, 2500		35, 40, 46, 52, 58
Adolescent (14 to <18 years)	L-leucine: 2500, 3000, 4000, 5000, 5250	61.3	41, 49, 65, 82, 86
	L-isoleucine: 1500, 1750, 2000, 2500		25, 29, 33, 41
	L-valine: 1500, 1750, 2000, 2250, 2500		25, 29, 33, 37, 41
Adults (≥18 years)	L-leucine: 2500, 3000, 4000, 5000, 5250	70.0	36, 43, 57, 71, 75
	L-isoleucine: 1500, 1750, 2000, 2500		21, 25, 29, 36
	L-valine: 1500, 1750, 2000, 2250, 2500		21, 25, 29, 32, 36

Exposure of L-leucine, L-isoleucine and L-valine were estimated from the intake of food supplements alone.

3.2 Other sources

BCAAs are present in some quantity in almost all kinds of food, and more in protein-rich foods such as dairy products, meats, soy, gluten, eggs, nuts, whole grains, seeds,

avocadoes, and edible seaweed, beans and pulses. L-leucine, L-isoleucine and L-valine are sold as supplements in various concentrations and under various names.

The normal intake of an adult with a mixed protein diet is 5.4 g/100 g of mixed protein (IOM, 2005). Based on intake distribution data from the 1988–1994 NHANES III, mean daily intakes for all life stage and gender groups of L-leucine, L-isoleucine and L-valine from food and supplements are 6.1, 3.6 and 4.0 g, respectively. Men 51 through 70 years of age had the highest reported intake at the 99th percentile for L-leucine at 14.1 g/day, L-isoleucine at 8.2 g/day and L-valine at 9.1 g/day(IOM, 2005).

4 Risk characterisation

The doses received from NFSA are: 2500, 3000, 4000, 5000 and 5250 mg/day for L-leucine, 1500, 1750, 2000 and 2500 mg/day for L-isoleucine and 1500, 1750, 2000, 2250 and 2500 mg/day for L-valine in food supplements, and the exposures for adults, adolescents and children at or above 10 years are given in chapter 3.

No studies can be used for suggesting a "value for comparison", and there are no scientific data in the published literature suitable for assessing the specific doses in the terms of reference.

The acute upper tolerable metabolic limit of L-leucine was determined to be 550-700 mg/kg bw per day, or between 39 and 50 g per day for a person of 70 kg. As it has not been addressed, it is not possible to conclude on the long term metabolic capacity of these doses of L-leucine.

Several studies investigating L-leucine, L-isoleucine and L-valine in single doses ranging from 10 to 30 g per day did not address or report on adverse effects. The uncertainties for this consideration are described in chapter 5.

VKM considers that:

Due to lack of studies addressing adverse effects of the specified doses 2500, 3000, 4000, 5000 and 5250 mg/day of L-leucine; 1500, 1750, 2000 and 2500 mg/day of L-isoleucine and 1500, 1750, 2000, 2250 and 2500 mg/day of L-valine in food supplements, no conclusions can be made for adults (≥ 18 years), adolescents (≥ 10 and < 18 years) and children (< 10 years).

5 Uncertainties

- A major uncertainty is the lack of studies in adults, children and adolescents reporting on potential adverse health effects of BCAA and/or L-leucine, L-isoleucine and L-valine supplementation.

- Animal studies have not been included due to metabolic differences. Hence there is uncertainty about relevant safety factors for extrapolation from animals to humans from toxicological studies on BCAA and/or L-leucine, L-isoleucine and L-valine.

6 Conclusions with answers to the terms of reference

The Norwegian Food Safety Authority (NFSA) has requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of L-leucine, L-isoleucine and L-valine in food supplements at the following doses: L-leucine: 2500, 3000, 4000, 5000 and 5250 mg/day, L-isoleucine: 1500, 1750, 2000 and 2500 mg/day and L-valine: 1500, 1750, 2000, 2250 and 2500 mg/day for the general population, ages 10 years and above.

No data are available to suggest a "value for comparison" or make a risk characterisation for the specified doses of L-leucine, L-isoleucine or L-valine.

VKM concludes that:

Due to lack of studies addressing adverse effects of the specified doses 2500, 3000, 4000, 5000 and 5250 mg/day of L-leucine; 1500, 1750, 2000 and 2500 mg/day of L-isoleucine and 1500, 1750, 2000, 2250 and 2500 mg/day of L-valine in food supplements, no conclusions can be made for adults (≥ 18 years), adolescents (≥ 10 and < 18 years) or children (< 10 years).

Table 6.1 An overview of the conclusions on L-leucine, L-isoleucine and L-valine in food supplements.

Food supplement Age groups	L-leucine 2500, 3000, 4000, 5000 and 5250 mg/day	L-isoleucine 1500, 1750, 2000 and 2500 mg/day	L-valine 1500, 1750, 2000, 2250 and 2500 mg/day
Children (10 to <14 years)	No data available	No data available	No data available
Adolescents (14 to <18 years)	No data available	No data available	No data available
Adults (≥ 18 years)	No data available	No data available	No data available

7 Data gaps

- There are no reports on negative health effects related to BCAA, L-valine, L-isoleucine or L-leucine in adults (> 18 years) children and adolescents.
- No studies are found that include effects of these substances in lactating or pregnant women.
- There is lack of an acute reference dose or other acute data for BCAA, L-valine, L-isoleucine and L-leucine.

8 References

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Appendix 1

Search strategies for this risk assessment

Search strategy for human studies

Database: Embase <1974 to 2015 November 16>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) <1946 to Present>

1. leucine*.ti. (17478)
2. isoleucine*.ti. (2743)
3. valine*.ti. (4104)
4. branched chain amino acid*.ti. (3160)
5. branched-chain amino acid*.ti. (3160)
6. branched chained amino acid*.ti. (4)
7. branched-chained amino acid*.ti. (4)
8. 1 or 2 or 3 or 4 or 5 or 6 or 7 (25852)
9. leucine-rich.ti. (2433)
10. 8 not 9 (23419)
11. (risk* or safety or adverse or side-effect*1 or hazard* or harm* or negative or contraindicat* or contra-indicat* or interact* or toxicity or toxic).tw. (9612023)
12. 10 and 11 (3370)
13. (conference abstract* or letter* or editorial*).pt. (4808208)
14. 12 not 13 (3232)
15. limit 14 to (danish or english or norwegian or swedish) (3157)
16. remove duplicates from 15 (1724)
17. limit 16 to human (530)

Search strategy for systematic reviews and meta analyses

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1. leucine*.ti. (17478)
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6. branched chained amino acid*.ti. (4)
7. branched-chained amino acid*.ti. (4)

8. 1 or 2 or 3 or 4 or 5 or 6 or 7 (25852)
9. limit 8 to yr="2005 -Current" (7451)
10. limit 9 to ("review" or meta analysis or systematic reviews) [Limit not valid in Embase; records were retained] (381)
11. limit 10 to (danish or english or norwegian or swedish) (357)
12. remove duplicates from 11 (227)
13. leucine-rich.ti. (2433)
14. 12 not 13 (149)