Application of Key Events Dose Response Framework to Defining the Upper Intake Level of Leucine in Young Men1–4

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Abstract

Leucine is sold in large doses in health food stores and is ingested by weight-training athletes. The safety of ingestion of large doses of leucine is unknown. Before designing chronic high-dose leucine supplementation experiments, we decided to determine the effect of graded doses of leucine in healthy participants. The Key Events Dose Response Framework is an organizational and analytical framework that dissects the various biologic steps (key events) that occur between exposure to a substance and an eventual adverse effect. Each biologic event is looked at for its unique dose-response characteristics. For nutrients, there are a number of biologic homeostatic mechanisms that work to keep circulating/tissue levels in a safe, nontoxic range. If a response mechanism at a particular key event is especially vulnerable and easily overwhelmed, this is known as a determining event, because this event drives the overall slope or shape of the dose-response relationship. In this paper, the Key Events Dose Framework has been applied to the problem of leucine toxicity and leucine’s tolerable upper level.

The establishment of tolerable upper intake levels (UL) for nutrients began in the US with the most recent edition of the Dietary Reference Intakes (1). The UL is defined as the highest level of intake of a nutrient when taken on a daily basis that poses no risk of toxicity for almost all individuals in a population. The risk assessment framework under which the UL for nutrients have been established consists of the following:

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secondary analysis, and lack of dose-response data. Further, the uncertainty factor proved to be highly subjective and inconsistently applied.

Recently, a different analytical framework was proposed for establishing safe UL for nutrients as well as for other categories of agents: environmental chemicals, allergens, and microbes (2). The framework is known as the Key Events Dose Response Framework, which systematically considers each individual biologic event (key event), which then combine to determine whether or not an organism will respond adversely after being exposed to a particular dose of a substance (e.g., a nutrient). The deconstructed biologic chain of key events after nutrient exposure can include nutrient uptake by the intestinal cell, nutrient metabolism by the intestinal cell, nutrient transport in the circulation, initial uptake of the nutrient by the liver and other tissues, nutrient storage, nutrient metabolism by target tissues, nutrient excretion, etc.

Nutrient intake levels typically vary day by day and homeostatic controls are in place to ensure that proper blood and tissue levels are maintained. These controls may include one or more kinetic events and one or more dynamic events and can operate at one or more steps or events (e.g., the intestinal uptake step, the nutrient storage step, etc.) in the biologic chain of events that determines whether an adverse effect will happen or not. The questions then become: 1) Are there control points in the biologic chain of events that are readily overwhelmed by dose or other factors? 2) And is there a control point, which due to its vulnerability, disproportionately affects the probability of an adverse (toxic) outcome occurring? 3) In other words, does a particular key event appear to drive the shape of the overall response relationship and at what threshold does this occur?

The Key Events Dose Framework could prove to be very useful in the setting of UL for nutrients. This approach will complement (not replace) empirical, computational, and modeling techniques that are already in use. For many nutrients, there will not be enough known about each key event to do a complete analysis. The Key Events Dose Framework nevertheless can highlight focused research questions. An application of the framework to the setting of a UL for vitamin A was recently published (3). In this current paper, the Key Events Dose Framework is applied to the determination of a UL for the amino acid leucine.

In a previous paper in this workshop, the first author described his group’s results of a study in which the effects of acute, 1-d ingestion of graded doses of leucine on a variety of metabolic variables was observed (4). This approach was used rather than chronic ingestion of high doses of leucine, because the literature provided no information regarding what dose to use. It was reasoned that the acute study would provide data upon which a chronic ingestion study of high doses of leucine could be rationally based. All participants were started at the mean requirement level of leucine [50 mg/(kg · d)] and the highest leucine intake was 1250 mg/(kg · d), which is 25 times the mean requirement. No gut intolerance was seen. Blood glucose fell progressively but remained within normal values without any changes in plasma insulin. Maximal leucine oxidation levels occurred at an intake of 350 mg leucine/ (kg · d), after which plasma leucine progressively increased and plasma ammonia also increased in response to leucine intakes >500 mg/( kg · d). Thus, the key determining event appears to be when the participants reach their maximal leucine oxidation level, after which the risk of metabolic adverse effects progressively increased. The increase in ammonia appeared not to be the result of liver cell damage in that liver enzymes levels did not change (4). The possible metabolic mechanisms that explain the increase in plasma ammonia may be found in our earlier paper from this workshop (4).

Finally, it is important to note that this study was of an acute ingestion of leucine. If such high levels were ingested over a period of time, although adaptation might occur, it is also possible that adverse effects might be seen at lower levels.

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Literature Cited