Current In Vitro Testing of Bioactive Peptides Is Not Valuable\textsuperscript{1,2}

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Abstract
Dietary peptides have been suggested to possess biological activity in vivo and could affect cardiovascular disease parameters, based on data derived from in vitro experiments. Isolated peptides are often tested in in vitro cellular assays or on heterologously expressed molecular target proteins. The stimulatory or inhibitory effect on target proteins in vitro has often been used as the justification to test these compounds directly in vivo. Unfortunately, this research approach has an inherent flaw. It neglects the poor absorption, distribution, metabolism, and excretion (ADME) properties of peptides resulting in low peptide bioavailability. Because peptides are prone to extensive hydrolysis in the gastrointestinal tract by stomach, small intestinal, and brush border peptidases, most of them do not reach the absorption stage in the duodenum and jejunum. Therefore, a valid research approach should include the demonstration of stability of the peptide toward luminal and brush border peptidases and evaluate its ADME properties. Surprisingly, only very few animal and human studies determined absolute concentrations and kinetics of bioactive peptides. These studies have shown the presence of selected peptides in plasma samples at pico- and nanomolar concentrations with fast elimination kinetics in the minute range. For the correct interpretation of results, it is advised that researchers refer to the data currently available concerning bioavailability and ADME properties in humans. Two mandatory criteria for future in vitro studies investigating potential biological activities of peptides should be using physiologically relevant concentrations and times. J. Nutr. 140: 117–118, 2010.

Small endogenous peptides, such as peptide hormones and signaling peptides, present in the systemic circulation have strong effects on human physiology. It is hence a very appealing idea to develop tailor-made small peptides targeted at specific receptors and enzymes. This concept has been successfully applied in pharmacological approaches where, e.g., synthetic insulin and gonadotropin-releasing hormone 1 are first lines of treatment for diabetes mellitus and hypogonadism, respectively. The same concept has gained increasing interest from academia and food industries where it is reasoned that certain dietary peptides could also be potentially used as bioactive ingredients in functional foods. Dietary proteins have been shown to contain sequences of peptides, partially similar to those found in endogenous peptides, with hormonal or neuronal functions. These exogenous dietary peptides are proposed to exert physiological effects by acting either agonistically or antagonistically on the same targets as their endogenous counterparts. Based on these observations, many peptides derived from common food proteins have been proposed to influence several different physiological parameters, e.g., blood pressure, insulin and glucose homeostasis, plasma cholesterol concentrations, and enzymatic activity, among which those with potential antihypertensive activity have been studied the most (1). Data from observational studies supported this hypothesis by showing an inverse association between dairy consumption and blood pressure independent of calcium (2,3), suggesting that milk protein may play a critical role. Ideally, one would aim to confirm the findings from observational data in human intervention studies. However, because these studies are expensive and tedious to conduct, initial in vitro experiments are often performed to identify potential bioactive peptides before committing to full-blown human trials. For example, a huge variety of peptides derived from various dietary protein sources have been examined in cellular assays for their inhibitory properties on angiotensin-I converting enzyme (ACE) activity (4), one of the key enzymes in regulating blood pressure, and several food-grade peptides such as the tripeptides Ile-Pro-Pro and Val-Pro-Pro have been found to decrease, in vitro, the conversion of angiotensin I to the vasoconstrictor angiotensin II. These peptides displayed moderate, reversible ACE inhibitory activity in the micromolar range (5). The hypothesis to use ACE inhibitory peptides for modulating blood pressure is very appealing, because a similar mechanism of action has been shown to be effective in lowering blood pressure using potent pharmaceutical ACE inhibitors, although those exhibit strong inhibition constants for ACE in the low

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norous and functional food approaches. A food-derived peptide is transiently synthesized and absorbed from the gastrointestinal tract, often as a result of proteolytic digestion. Peptide absorption can be influenced by factors such as gastric pH and enzyme activity, as well as the presence of dietary fibers and other compounds that can influence peptide stability. Once absorbed, the peptide can be transported across the intestinal epithelium and enter the bloodstream, where it can be modified by circulating enzymes. The stability of a peptide in the bloodstream is critical for its biological activity, as peptides are susceptible to degradation by proteolytic enzymes. This degradation can occur in the gut, liver, or other tissues, and can be influenced by factors such as the presence of other peptides and proteins in the circulation. The ADME properties of a peptide can be evaluated using in vitro and in vivo assays, such as plasma clearance profiles and bioavailability studies. These studies can help identify peptides with desirable ADME properties, such as long plasma half-lives and high bioavailability, which are important for the development of therapeutic peptides. However, the in vivo efficacy of a peptide cannot be determined at this stage, as it requires testing in disease models to assess its therapeutic potential. The lack of in vivo efficacy can be due to a number of factors, such as the presence of inhibitors that can interfere with the peptide's biological activity, or the lack of a suitable target in the disease model. Therefore, in vivo efficacy studies are crucial for the development of therapeutic peptides, and should be performed early in the development process to identify potential issues and optimize the peptide's properties.