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Review article

# Sensing and signaling mechanisms linking dietary methionine restriction to the behavioral and physiological components of the response

Laura A. Forney[a], Kirsten P. Stone[a], Desiree Wanders[b], Thomas W. Gettys[a,*]

[a] Laboratory of Nutrient Sensing and Adipocyte Signaling, Pennington Biomedical Research Center, Baton Rouge, LA 70808, United States
[b] Department of Nutrition, Georgia State University, Atlanta, GA 30302, United States

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ABSTRACT

Dietary methionine restriction (MR) is implemented using a semi-purified diet that reduces methionine by ~80% and eliminates dietary cysteine. Within hours of its introduction, dietary MR initiates coordinated series of transcriptional programs and physiological responses that include increased energy intake and expenditure, decreased adiposity, enhanced insulin sensitivity, and reduction in circulating and tissue lipids. Significant progress has been made in cataloguing the physiological responses to MR in males but not females, but identities of the sensing and communication networks that orchestrate these responses remain poorly understood. Recent work has implicated hepatic FGF21 as an important mediator of MR, but it is clear that other mechanisms are also involved. The goal of this review is to explore the temporal and spatial organization of the responses to dietary MR as a model for understanding how nutrient sensing systems function to integrate complex transcriptional, physiological, and behavioral responses to changes in dietary composition.

## 1. Introduction

All life forms sense and respond to environmental cues by engaging coordinated homeostatic responses that function to enhance survival of the organism. In higher organisms, variation in the macronutrient makeup (e.g., carbohydrate, lipid, protein) of the diet requires tissue-specific adaptations to ongoing changes in the fuel sources and molecular building blocks that make up each meal. Simpson and Raubenheimer (1997) used an integrative modeling approach to develop a Geometric Framework to evaluate the effects of dietary macronutrients on response variables such as nutrient selection, body composition, and longevity. The authors originally tested the Geometric Framework in experiments where lifespan or fecundity were endpoints in insect species given ad libitum access to 28 different diets varying in their protein to carbohydrate ratio. The responses to all 28 diets were summarized by plotting the protein consumed per day on the X axis, the carbohydrate consumed per day on the Y axis, and the biological response (e.g., longevity, fecundity, etc.) to each nutritional combination as a heat map in the Z-plane (Raubenheimer and Simpson, 1997; Piper et al., 2011). The effectiveness of this approach in describing biological responses to nutritional complexity led to its application in examining how varying dietary macronutrient composition affected ingestive behavior, and how animals prioritize macronutrient intake when given a choice (Simpson and Raubenheimer, 1997, 2005; Piper et al., 2011; Wilder et al., 2012). It was found that lowering the percentage of protein in the diet causes a concomitant increase in energy intake to maintain constant protein intake. This leveraging of carbohydrate and fat intake that occurs with dilution of dietary protein represents the conceptual basis for the Protein Leverage hypothesis (Simpson and Raubenheimer, 2005). Recent studies of dietary protein dilution show that in addition to the hyperphagia predicted by the Protein Leverage hypothesis, low protein diets also produce an increase in EE of sufficient magnitude to limit fat deposition (Laeger et al., 2016, 2014b; Morrison and Berthoud, 2007; Solon-Biet et al., 2015).

These findings support an emerging consensus that dietary protein intake is monitored through nutrient sensing mechanisms that detect changes in the essential amino acid (EAA) composition of the consumed protein (Simpson and Raubenheimer, 1997; Morrison et al., 2012; Gosby et al., 2011; Bosse and Dixon, 2012). Studies with semi-purified diets that restrict single EAAs within a defined narrow range show that such diets mirror the effects of low protein diets on ingestive behavior, energy expenditure, insulin sensitivity, and lipid metabolism (Hasek et al., 2010, 2013; Plaisance et al., 2010; Stone et al., 2014; Forney et al., 2017; Wanders et al., 2015b). Collectively, these studies provide compelling support for the existence of real-time EAA sensing systems linked to translational mechanisms that function together to detect changes in dietary EAAs and affect a highly integrated and beneficial set of adaptive responses. The goal of the present review is to explore

* Corresponding author at: Laboratory of Nutrient Sensing & Adipocyte Signaling, 6400 Perkins Road, Pennington Biomedical Research Center, Baton Rouge, LA 70808, United States.
*E-mail address:* gettystw@pbrc.edu (T.W. Gettys).

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recent findings on how these sensing and effector systems are organized, with special emphasis on how endocrine and neuroendocrine mechanisms may function to provide the communication networks that integrate the overall response.

It should be noted that our work, and to our knowledge all work reported to date on dietary methionine restriction, has been conducted in male rodents. However, recent work with low protein diets reported sex differences in the hormonal and metabolic responses to dietary protein dilution (Larson et al., 2017). Given the many similar responses to methionine restriction and protein dilution, it will be important in future preclinical studies to extend work on dietary methionine restriction to female subjects and identify any gender-specific responses in this model.

## 2. The role of amino acid sensing in maintenance of protein nutrition

### 2.1. Essential amino acids are the measured indices of protein nutrition

A subgroup of amino acids that make up proteins cannot be synthesized endogenously and must be provided in the diet (e.g. EAAs). Therefore, the ability to detect and respond to dietary EAA deficiencies is an indispensable survival mechanism. Although a number of molecular and cellular EAA sensing mechanisms have been identified (Anthony et al., 2001, 2004; Kimball et al., 2004; Wek et al., 2006; Zhang et al., 2002; Hao et al., 2005; Deval et al., 2009; Maurin et al., 2005), recent studies suggest that multiple sensing mechanisms are involved in responding to low protein or EAA-restricted diets (Laeger et al., 2016; Wanders et al., 2016). The well documented *in vivo* responses to perturbation of dietary EAA composition make a compelling case that EAAs play the dominant role as mediators of the effects of dietary protein restriction on metabolism and energy balance (Guo and Cavener, 2007; Hasek et al., 2010; Plaisance et al., 2010). Thus, the primary experimental model examined in this review involves the responses of rats or mice to ad libitum consumption of semi-purified diets with defined restrictions of specific EAAs, with special emphasis on methionine.

### 2.2. Experimental models – dietary methionine restriction

The focus of this review is an experimental diet that restricts dietary methionine from normal levels of 0.86% to 0.17% while also eliminating dietary cysteine. The diet was originally described by Orentreich and colleagues (Orentreich et al., 1993; Richie Jr. et al., 1994), who documented its ability to increase mean and maximal life span in rats. Their findings have been extended to other species (Lee et al., 2014; Sun et al., 2009; Johnson and Johnson, 2014; Miller et al., 2005), and a unique but common feature of these studies is that the life extending properties of the MR diet do not require food restriction (Orentreich et al., 1993; Richie Jr. et al., 1994; Malloy et al., 2006). The short-term physiological responses to dietary MR have come into sharper focus over the last two decades because the diet produces improvements in essentially all biomarkers of metabolic health and extends healthspan (Malloy et al., 2006, 2013; Perrone et al., 2012b, 2012a, 2008, 2009; Hasek et al., 2010; Plaisance et al., 2010; Zimmerman et al., 2003; Lee et al., 2014; Sun et al., 2009; Miller et al., 2005; Lees et al., 2014; Ghosh et al., 2017; Wanders et al., 2013). The most prominent physiological responses to MR are increased insulin sensitivity and coordinated increases in energy intake and expenditure (Hasek et al., 2010; Stone et al., 2014; Wanders et al., 2015a). The proportionately larger effect of the diet on EE limits ongoing fat deposition by increasing the proportion of energy intake required for maintenance of existing tissue (Hasek et al., 2010; Wanders et al., 2015a). When the MR effect on EE is integrated over time, it effectively limits the normal age-associated expansion of adipose tissue. Dietary MR also initiates a transcriptional program in liver that coordinately down-regulates lipogenic gene expression and produces a corresponding reduction in the capacity of the liver to synthesize and export lipid (Hasek et al., 2013). In adipose tissue, dietary MR induces a depot-specific increase in lipogenic and oxidative genes that increase the capacity of these fat depots to synthesize and oxidize fatty acids (Hasek et al., 2013; Patil et al., 2015).

The initial emphasis of many studies has been to document the effects of the MR diet on specific metabolic endpoints, while more recent studies have been directed towards understanding how the reductions in methionine are being sensed and how the sensing systems are linked to specific physiological responses. Most work on EAA restriction has focused on methionine, but it will be important in future studies to extend this work to other EAAs and determine whether the beneficial responses are specific to methionine or can be reproduced to varying degrees by restricting other EAAs.

### 2.3. Experimental models – dietary EAA deprivation

Diets devoid of single EAAs such as leucine or threonine have also received significant attention (Blais et al., 2003; Gietzen et al., 2004; Leung and Rogers, 1971; Koehnle et al., 2003) because they produce a well-documented, albeit counterproductive series of responses including food aversion, increased EE, rapid loss of body weight (BW) and adiposity, and ultimately death (Maurin et al., 2005; Hao et al., 2005; Guo and Cavener, 2007; Anthony et al., 2004; Cheng et al., 2011, 2010). In contrast, the responses to dietary methionine or leucine restriction (Wanders et al., 2015b) are fundamentally different from EAA deprivation because they do not produce harmful health effects. The primary basis for this difference is the opposing effects of the two diets on energy intake. Whereas EAA restriction produces hyperphagia (Wanders et al., 2015b; Hasek et al., 2010; Malloy et al., 2006), leucine or threonine deprivation produce food aversion and a cumulative decrease in energy intake (Guo and Cavener, 2007; Anthony et al., 2004). Interestingly, both EAA restriction and EAA deprivation produce significant increases in EE (Hasek et al., 2010; Plaisance et al., 2010; Cheng et al., 2010, 2011). However, the strong anorexigenic response to EAA deprivation, combined with increased EE, produces a profound negative energy balance and unsustainable weight loss (Cheng et al., 2010, 2011; Guo and Cavener, 2007; Ross-Inta et al., 2009). The mechanistic basis for this difference between EAA restriction and EAA deprivation represents a critical gap in our understanding of how the requisite sensing systems detect and mediate these opposing responses.

### 2.4. EAA-specific versus concentration-dependent effects of limiting dietary EAAs

The differential responses to EAA restriction versus EAA deprivation may stem from their respective impacts on circulating levels of the limited EAA, and this difference may dictate recruitment of the responses that are unique to each diet. For example, leucine deprivation produces a rapid 7-fold decrease in circulating leucine while threonine deprivation produces a 5-fold decrease in plasma threonine (Maurin et al., 2005). Studies with the 0.17% MR diet showed that it reduced plasma methionine by ~3-fold in rats (Perrone et al., 2012b; Elshorbagy et al., 2013, 2010). Given that restricting methionine to 0.17% produces hyperphagia while leucine deprivation produces food aversion, an important question is whether these opposing responses are EAA-specific or a function of the degree of EAA restriction. Support for the latter can be found in recent work showing that restricting dietary leucine to 0.17% increased food intake in a manner that paralleled the increase in energy intake produced by dietary MR (Wanders et al., 2015b). Thus, with leucine, the data suggest that it is the degree of restriction rather than the EAA being restricted that determines the effect on food intake. In contrast, when dietary methionine deprivation was compared to methionine restriction, we found that methionine deprivation reversed the hyperphagia produced by dietary MR, but did