Branched-chain amino acids alter neurobehavioral function in rats

Anna Coppola,1,2 Brett R. Wenner,1 Olga Ilkayeva,1 Robert D. Stevens,1,3 Mauro Maggioni,4 Theodore A. Slotkin,2 Edward D. Levin,3 and Christopher B. Newgard1,2,3

1Sarah W. Stedman Nutrition and Metabolism Center, 2Department of Pharmacology and Cancer Biology, 3Department of Medicine, Division of Endocrinology, 4Department of Mathematics, and 5Department of Psychiatry and Behavioral Sciences, Duke University, Durham, North Carolina

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Branched-chain amino acids alter neurobehavioral function in rats. Am J Physiol Endocrinol Metab 304: E405–E413, 2013. First published December 18, 2012; doi:10.1152/ajpendo.00373.2012.—Recently, we have described a strong association of branched-chain amino acids (BCAA) and aromatic amino acids (AAA) with obesity and insulin resistance. In the current study, we have investigated the potential impact of BCAA on behavioral functions. We demonstrate that supplementation of either a high-sucrose or a high-fat diet with BCAA induces anxiety-like behavior in rats compared with control groups fed on unsupplemented diets. These behavioral changes are associated with a significant decrease in the concentration of tryptophan (Trp) in brain tissues and a consequent decrease in serotonin but no difference in indices of serotonin synaptic function. The anxiety-like behaviors and decreased levels of Trp in the brain of BCAA-fed rats were reversed by supplementation of Trp in the drinking water but not by administration of fluoxetine, a selective serotonin reuptake inhibitor, suggesting that the behavioral changes are independent of the serotonergic pathway of Trp metabolism. Instead, BCAA supplementation lowers the brain levels of another Trp-derived metabolite, kynurenic acid, and these levels are normalized by Trp supplementation. We conclude that supplementation of high-energy diets with BCAA causes neurobehavioral impairment. Since BCAA are elevated spontaneously in human obesity, our studies suggest a potential mechanism for explaining the strong association of obesity and mood disorders.

IN THE PAST FEW DECADES, changes in food consumption and sedentary living conditions have contributed to a worldwide obesity epidemic, including in the US, where more than 34% of adults are obese (44, 45). Given the high prevalence of obesity and mood disorders, there is an increasing interest in the relationship of these two conditions (2, 17, 25, 33, 42, 58, 62) and in the concept that overnutrition may have a direct causal link to impairment of mental function.

Recent studies have revealed that a cluster of metabolites comprised of the branched-chain amino acids (BCAA) leucine (Leu), isoleucine (Ile), and valine (Val) and their metabolites, as well as the aromatic amino acids (AAA) tyrosine (Tyr) and phenylalanine (Phe), are strongly associated with obesity and insulin resistance in humans (1, 10, 29, 43, 67, 78). Moreover, a very similar metabolite cluster predicts incident type 2 diabetes in longitudinal studies in humans (64) and, when measured at baseline in obese subjects, also predicts improvement in insulin sensitivity in response to a dietary/behavioral weight loss intervention (63). These findings suggest the possibility that elevated BCAA could be linked to behavioral disorders.

BCAA are transported from the blood into the central nervous system (CNS) through the blood-brain barrier (BBB) by the large neutral amino acid transporter 1 (LAT1) (47). LAT1 transports all of the large neutral amino acids (LNAA), including the BCAA and AAA, Tyr, Phe, and tryptophan (Trp). This system is saturated at physiological amino acid concentrations, and uptake of BCAA is competitive with respect to uptake of AAA (46). Therefore, an increase in circulating BCAA is predicted to decrease uptake of AAA into the CNS (19). Tyr is the precursor of norepinephrine (NE) and dopamine (DA) (37), whereas Trp is the precursor of serotonin (5-HT) (38). Thus, the rate of production of important neurotransmitters may be affected by changes in amino acid concentrations in the brain (18). This could potentially include serotonin, which regulates a variety of behavioral functions, including mood and appetite regulation (16). Recent studies have indicated that other metabolic fates of Trp in brain could also modulate behavior (reviewed in Ref. 39), most notably its conversion to the neuroactive metabolite kynurenic acid (KYNA) (30, 34, 36, 39, 81).

Several studies provide evidence that different food components have a direct impact on brain processes at many levels (reviewed in Refs. 26, 40, and 79). Given the emergent and tight association of BCAA and AAA with obesity and insulin resistance, and in light of the potential behavioral impact of AAA via their role as precursors of key neurotransmitters, we adapted a dietary BCAA supplementation strategy as used previously for studying the relationship of BCAA and insulin resistance in rats (43) for studies of the impact of BCAA on behavior. This allowed us to test the hypothesis that long-term supplementation of BCAA leads to behavioral changes.

EXPERIMENTAL PROCEDURES

Animal studies. All procedures described in this article were approved by the Duke University Institutional Animal Care and Use Committee. Each study involved the use of male Wistar rats (150–175 g; Charles River Laboratories, Durham, NC) single-caged throughout the experiment. At the end of the experiments, rats were euthanized by decapitation and brain tissues rapidly dissected and snap-frozen in liquid nitrogen. Blood was collected from the body trunk at the time of euthanization.

Diets. All diets were formulated and custom made by Research Diets. Rats were fed for 9 wk with four different diets: low-fat, high-sucrose diet (LF); low-fat, high-sucrose diet supplemented with Leu, Val, and Ile by 150% (LF/BCAA); high-fat diet (HF); or high-fat diet supplemented with Leu, Val, and Ile by 150% (HF/BCAA). Composition of each of the diets is reported in Table 1.

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The data were acquired using a Waters Acquity UPLC system equipped with a triple quadrupole detector and a data system controlled by MassLynx 4.1 MS software platform (Waters, Milford, MA).

**LC-MS/MS analysis of Trp and KYNA.** Tissue homogenate or serum was filtered through Millipore Amicon Ultra-0.5 ml 3k MWCO centrifugal filters (UCP500396). Ten microtiter plates of isotope-labeled internal standards containing 10 μM l-tryptophan-d5 (DLM-1092-0.5; Cambridge Isotope Laboratories) and 0.04 μM kynurenic-3,5,6,7,8-d5 acid (D-4391; CDN Isotopes) was added to 70 μl of the filtrate and to a series of calibrators. An l-tryptophan (T0254; Sigma) calibration curve was prepared by diluting the stock solution to 0.4, 1, 2, 4, 10, and 20 μM, and a KYNA (K3375; Sigma) calibration curve was prepared by diluting the stock solution to 0.0008, 0.0020, 0.0040, 0.0080, 0.0200, and 0.0400 μM. Tryptophan and KYNA were analyzed on a Waters Acuity UPLC system coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer. The analytical column (Waters Acuity UPLC HSS T3 Column, 1.8 μm, 2.1 × 100 mm) equipped with a guard column (Agilent Rapid Resolution cartridge, ZORBAX SB-C8, 3.5 μm, 2.1 × 30 mm) was used at 30°C, and 7.5 μl of the sample was injected onto the column and eluted at a flow rate of 0.3 ml/min. The gradient began with 100% eluent A (0.1% formic acid in water) and was then programmed as follows: 0- to 2-min 0% eluent B (95:5 acetonitrile-water, 0.1% formic acid); 2- to 10-min gradient to 40% eluent B; 10- to 11-min gradient to 100% eluent B; 11- to 13-min hold at 100% eluent B; 13.0- to 13.5-min gradient to 0% eluent B; 13.5- to 15.5-min hold at 100% eluent A to reequilibrate the column. Mass transitions of m/z 205 → 146 for Trp, 210 → 150 for d5-Trp, 190 → 116 for KYNA, and 195 → 121 for d5-KYNA were monitored in positive ion electrospray ionization mode with the following parameters: capillary voltage 2,000 V, cone voltage 22 V for Trp and 2 V for KYNA, collision energy 16 V for Trp and 28 V for KYNA. Quantitation of Trp and KYNA from raw multiple reaction monitoring data was performed using Waters TargetLynx Quantitative Analysis.

**Monoamine analysis.** The levels of monoamines and their major metabolites were assayed using tissue homogenization and standard HPLC-EC methods, as reported previously (52). The HPLC system consisted of an isocratic pump (model LC1120; GBC Separations), a Rheodyne injector (model 7725i) with a 20-μl PEEK loop, and an INTRO amperometric detector (Antec Leyden, Warwick, RI). The signal was integrated using the EZChrom elite chromatography software (Scientific Software, Pleasanton, CA). The mobile phase was 50 mM H₃PO₄, 50 mM citric acid, 100 mg/l 1-octanesulfonic acid (sodium salt), 40 mg/l EDTA (disodium salt dihydrate), 2 mM KCl, and 3% methanol, corrected to pH 3.0 with NaOH. The mobile phase was continually degassed with a Degasys Populaire on-line degasser.
(Sanwa Tsusho, Tokyo, Japan) and delivered at a flow rate of 0.26 ml/min.

**Binding capacity assay.** The assay methodologies used in this study have appeared previously (65), so only brief descriptions will be provided here. 5-HT1A receptors, 5HT2 receptors, and the 5-HT transporter were studied. Frontal cortex tissue was homogenized (Polytron; Brinkmann Instruments, Westbury, NY) in ice-cold 50 mM Tris (pH 7.4), and the homogenates were sedimented at 40,000 g for 15 min. The pellets were washed, resedimented, and dispersed. Two radioligands were used to determine 5-HT receptor binding: 1 nM [3H]8-hydroxy-2-(di-n-propylamino)tetralin (specific activity, 170.2 Ci/mmol; Perkin-Elmer Life Sciences, Boston, MA;) for 5-HT1A receptors and 0.4 nM [3H]ketanserin (specific activity, 67 Ci/mmol; Perkin-Elmer) for 5-HT2 receptors. For 5-HT1A receptors, incubations lasted 15 min at 37°C in 50 mM Tris (pH 7.4), and the homogenates were sedimented at 40,000 g for 15 min. The pellets were washed, resedimented, and dispersed. Two radioligands were used to determine 5-HT receptor binding: 1 nM [3H]8-hydroxy-2-(di-n-propylamino)tetralin (specific activity, 170.2 Ci/mmol; Perkin-Elmer Life Sciences, Boston, MA;) for 5-HT1A receptors and 0.4 nM [3H]ketanserin (specific activity, 67 Ci/mmol; Perkin-Elmer) for 5-HT2 receptors. For 5-HT1A receptors, incubations lasted for 30 min at 25°C in a buffer consisting of 50 mM Tris (pH 8), 2 mM MgCl2, and 2 mM sodium ascorbate; 100 μM 5-HT (Sigma) was used to displace specific binding. For 5-HT2 receptors, incubations lasted 15 min at 37°C in 50 mM Tris (pH 7.4), and specific binding was displaced with 10 μM methylsergide (Sandoz Pharmaceuticals, East Hanover, NJ). Incubations were stopped by the addition of a large excess of ice-cold buffer, and the labeled membranes were trapped by rapid vacuum filtration onto glass fiber filters that were presoaked in 0.15% polyethyleneimine (Sigma), and following several washes, radiolabel retained on the filter was measured by scintillation counting. For binding to the presynaptic 5-HT transporter, the membrane suspension was incubated with 85 pM [3H]-paroxetine (Perkin-Elmer; specific activity 24.4 Ci/mmol) with or without addition of 100 μM 5-HT to displace specific binding, and incubations lasted 120 min at 20°C. Binding was calculated relative to membrane protein.

**Statistical analysis.** Data were assessed using multivariate ANOVA with factors of diet (LF vs. HF) and BCAA supplementation. When the rats were treated with fluoxetine or Trp, we included these additional factors. Significance was assumed at P < 0.05.

**RESULTS**

**Long-term consumption of diets supplemented with BCAA affects behavior.** To investigate the consequence of overnutrition and meal-based supplementation of BCAA on stress and behavior, we fed young Wistar rats with four different high-energy diets for a period of 9 wk: LF, LF/BCAA, HF, or HF/BCAA (see Table 1 for diet composition). Food intake by weight of food consumed and in terms of caloric intake was higher in rats fed the HF-based compared with the LF-based diets (P < 0.0001), but no significant differences were observed in food intake in groups fed with HF compared with HF/BCAA or LF compared with LF/BCAA diets (Fig. 1B). This suggests that supplementation of the diets with BCAA did not affect palatability of the food or cause a taste aversion response. There was a trend to increase body weight more rapidly in rats fed with HF diet compared with LF, but the differences were not statistically significant (Fig. 1A). Similarly, supplementation of either diet with BCAA caused a trend for a lower rate of body weight gain relative to the unsupplemented diets, but these effects also did not reach statistical significance. Note that we have shown previously that feeding of either the HF or LF diets increases weight gain relative to a low-fat, low-sucrose, standard chow diet (43, 57).

We analyzed the impact of 9 wk of feeding of the various diets on behavior of the rats using the EPM test. We found that BCAA supplementation (both LF/BCAA and HF/BCAA) induced a significant reduction of the time spent in the open arms, which was suggestive of an anxiogenic effect of BCAA supplementation leading to less exploratory behavior (Fig. 2A). The behavior of the rats fed the LF diet was indistinguishable from the behavior of rats fed the HF diet, and in fact, a comparison of all diets identified only a main effect of BCAA (P < 0.002) with no diet (LF vs. HF) × BCAA interaction.

Because differences in general motor activity among groups could have influenced the exploratory behavior in the EPM, we measured the number of closed-arm entries as an index of general amulatory activity (12, 54). No diet-induced difference was found in overall amulatory activity of the rats (Fig. 2B). We found that the effect of dietary supplementation of BCAA on behavior required long-term consumption of the diets, as overfeeding for only 2 wk did not induce significant changes (data not shown). We also found no significant differences in the levels of the stress hormone corticosterone in serum samples...
collected from the different dietary groups at the time of euthanization (data not shown).

AAA and serotonin levels in the CNS are reduced by BCAA supplementation. To determine whether the increase of BCAA in the diet may be responsible for disrupted transport of AAA across the BBB, we measured amino acid levels in plasma and prefrontal cortex using tandem mass spectrometry. We found a clear increase in the ratio of BCAA/LNAA (molar sum of LNAA) in plasma of LF/BCAA- and HF/BCAA-fed rats relative to LF and HF groups, respectively (Fig. 3). This change in ratio predicts an increase in the central uptake of BCAA through the BBB. Conversely, we found a decrease in the ratio of AAA/LNAA in plasma that predicts reduction in AAA uptake into the CNS (18). Consistent with these predictions, we observed a decreased concentration of Tyr (Fig. 4A) and Trp (Fig. 4B) in the brain tissues of rats in both the HF/BCAA and LF/BCAA groups compared with their control groups with no BCAA supplementation. Concerning both the serum and brain amino acid ratio values, a main effect of BCAA supplementation was seen, with no diet × BCAA supplementation interaction.

To test whether dietary supplementation of BCAA perturbs brain monoamine pathways, we measured 5-HT, NE, and DA levels as well as the major metabolites of 5-HT and DA (5-hydroxyindolacetic acid, 3,4-dihydroxyphenylglycol, and 3,4-dihydroxynylacetic acid, respectively) in prefrontal cortex. We found a significant decrease in 5-HT concentration in BCAA-fed rats compared with their control groups. Nevertheless, the concentration of 5-HIAA, which reflects the amount of 5-HT released into the synapse, was unchanged. Consequently, the turnover rate of serotonin, measured as the ratio of 5-HT and 5-HIAA (ANOVA: not significant). C: 5-HT turnover rate calculated as the ratio of 5-HT and 5-HIAA (ANOVA: BCAA, P < 0.004). Data are presented as means ± SE (n = 5/group).

BCAA-supplemented diets affect 5-HT transport in the CNS but not binding capacity of 5-HT receptors. Our results for 5-HT levels and turnover suggested that release of 5-HT into the synapse was maintained despite reductions in 5-HT levels caused by BCAA supplementation. To assess the functional measures that would verify this interpretation, we measured the binding capacity of the 5-HT1A and 5-HT2 receptors as well as the 5-HT transporter. Reduced 5-HT synaptic communication would be expected to upregulate 5-HT receptors, but we found no effect of BCAA supplementation on the binding capacity of either of the receptor subtypes. In contrast, we did find a significant decrease in the binding capacity of the 5-HT transporter in rats fed the BCAA-supplemented diet compared with control (Fig. 6); reduced presynaptic recapture would explain the maintenance of 5-HIAA levels in the face of reduced 5-HT.

Lack of effect of the antidepressant fluoxetine on BCAA-induced behavioral changes. We tested the impact of a 5-HT reuptake inhibitor, fluoxetine, on the BCAA-induced changes in behavior in the EPM test. Rats were fed the four different diets for 5 wk and then continued on those diets for an additional 4 wk with or without fluoxetine treatment. Whereas rats fed the BCAA-supplemented diets spent less time in the open arms in the EPM, confirming the findings of Fig. 1, fluoxetine treatment did not improve performance in rats fed the BCAA-supplemented diets (Fig. 7). Taken together, data in Figs. 6 and 7 suggest that, although BCAA supplementation alters presynaptic 5-HT levels, functional activity is maintained as a consequence of increased release and reduced recapture of the transmitter so that the anxiogenic effect of BCAA is not dependent on the changes in 5-HT function.

Tryptophan supplementation rescues the stressed behavior in rats fed BCAA-supplemented diets and lowers brain KYNA levels. In an attempt to modify the concentration of LNAA in the circulation and hence, to restore the uptake of Trp through the BBB, we supplemented rats fed the various diets with Trp dissolved in drinking water. Trp supplementation increased the level of Trp in the circulation in all groups of animals (main effect of Fig. 5. The effect of BCAA-supplemented diets on levels of serotonin (5-HT) and its metabolites in the central nervous system. A: levels of 5-HT measured in prefrontal cortex (ANOVA: BCAA, P < 0.003). B: 5-HT major metabolite 5-HIAA in prefrontal cortex (ANOVA: not significant). C: 5-HT turnover rate calculated as the ratio of 5-HT and 5-HIAA (ANOVA: BCAA, P < 0.004). Data are presented as means ± SE (n = 5/group).

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tryptophan (Fig. 8A), but the increase in Trp was smaller in BCAA-supplemented rats compared with their control groups (BCAA × Trp interaction, \( P < 0.03; P < 0.04 \) for Trp effect in the BCAA group, \( P < 0.001 \) for Trp effect in the control group). Nevertheless, Trp supplementation restored the Trp/LNAA ratio in LF/BCAA- and HF/BCAA-fed rats to the levels found in LF- and HF-fed controls (no significant BCAA × Trp interaction; Fig. 8B), most likely because the affinity of Trp for the transporter LAT1 is higher than its affinity for any of the BCAA (48, 66). As predicted by the Trp ratio, Trp levels in brain tissues were increased in rats fed BCAA-supplemented diets + Trp in the drinking water compared with the rats fed BCAA-supplemented diets and drinking regular tap water (Fig. 9A). The increases in both plasma (Fig. 8A) and brain Trp levels induced by Trp addition to the drinking water were smaller in BCAA-supplemented rats compared with their control groups, although the difference was at the margin of significance (\( P < 0.07 \) for the BCAA × Trp interaction). Considering that the measurements of Trp in the brain are dependent on the plasma levels, we conducted an analysis comparing treatment effects in plasma and brain and found an overall interaction for BCAA × Trp (\( P < 0.02 \)) that was indistinguishable between brain and plasma (no BCAA × Trp × tissue interaction), indicating that the effects in the brain do actually reflect the same BCAA × Trp interaction as that found in the plasma.

To further support our hypothesis that BCAA-supplemented diets disrupt Trp uptake and metabolism, we measured KYNA, an endogenous neuroactive metabolite of Trp, in the frontal cortex. We found a decrease in KYNA levels after supplementation of BCAA in both the LF and HF diet backgrounds (\( P < 0.0001 \) for BCAA effect). Mirroring our findings with brain Trp levels, supplementation of Trp in the drinking water restored KYNA levels in LF/BCAA and HF/BCAA-fed rats to the levels found in LF- and HF-fed controls (\( P < 0.0001 \) for Trp effect; Fig. 9B).

Importantly, Trp addition to the drinking water increased the time spent in open arms (main effect of Trp), regardless of whether animals received BCAA supplementation or not. The positive effect of Trp offset the reduction caused by BCAA supplementation so that BCAA-treated animals given Trp showed a behavioral pattern similar to that of the non-BCAA-supplemented groups without Trp (compare LF-water with LF/BCAA-Trp; compare HF-water with HF/BCAA-Trp; Fig. 10).

**Discussion**

As the prevalence of obesity has grown, a clear relationship between obesity and mental illness has emerged (17, 55). Both obesity and mood disorders are associated with diabetes (4), cardiovascular diseases (50), and increased risk of mortality (11, 60). Treatment of obesity often leads to a decrease in depression (74), the most striking example being weight loss after gastric bypass surgery (15). Taken together, these studies support the idea of a common pathophysiology between obesity and mental illness, but much remains unknown about underlying mechanisms.

In the current study, we have built upon recent findings of strong associations between the levels of BCAA and AAA, obesity, and obesity-related metabolic dysregulation (10, 43, 63) and have begun to explore the possible connection between BCAA, AAA, and behavioral modifications. We have found that dietary supplementation of BCAA in either of two energy-dense diets, a low-fat, high-sucrose diet or a high-fat diet, has a clear effect to decrease exploratory behavior in rats subjected to an EPM test, indicating increased stress/anxiety (49, 53). Importantly, whereas this effect was evident in response to BCAA supplementation, changing the diet from low fat, high sucrose to high fat, low sucrose had no comparable effect. This provides important evidence that specific dietary components, rather than fat or caloric content, can contribute to diet-induced changes in behavior.

One likely mechanism for the BCAA effect is the impact of increased BCAA on transport of competing amino acids across the BBB into the CNS. Synthesis of several neurotransmitters is dependent upon the transport of AAA precursors to maintain synaptic levels and activity, notably 5-HT (synthesized from...
Trp, NE, and DA (synthesized from Tyr). Both BCAA and AAA engage with the large neutral amino acid transporter (18, 19), so a rise in BCAA would be predicted to cause a decrease in transport of the competing AAA. Consistent with this prediction, we found a decrease in Trp and Tyr concentrations in the brain in BCAA-supplemented rats.

A key question was whether the reduced AAA availability was sufficient to have an impact on transmitter levels or synaptic function. The measured decrease in brain Tyr levels did not result in a corresponding change in either NE or DA, likely because tyrosine hydroxylase, the rate-limiting enzyme in the generation of NE and DA, is close to full saturation with the tyrosine substrate, so changes in availability do not alter the net biosynthetic capability (31). Indeed, prior studies show only a limited sensitivity of NE and DA to circulating Tyr levels (8, 20). In contrast, tryptophan hydroxylase, the rate-limiting enzyme in the synthetic pathway of 5-HT, is subsaturated at normal levels of Trp, the AAA precursor for this transmitter (9, 77). Therefore, diminished substrate availability does have the potential to decrease 5-HT synthesis and affect 5-HT-related behaviors (67, 72, 80). Here, we found a decrease in CNS 5-HT levels in animals fed on the BCAA-supplemented diets. However, the levels of 5-HIAA, which reflect the amount of 5-HT released into the synapse, were unchanged, indicating that compensatory mechanisms were activated to offset the reduction in 5-HT availability. In keeping with this interpretation, 5-HT receptor binding was unchanged, whereas if presynaptic function had been impaired, binding sites would have been upregulated. We identified the underlying compensatory mechanisms: increased presynaptic activity (higher neurotransmitter turnover) and reduced synaptic recapture (decreased 5-HT transporter binding). These results suggest that, although the amount of 5-HT is reduced by BCAA, net serotonergic function was maintained by compensatory increases in release and reduction in recapture. We confirmed this interpretation by showing that the behavioral effects of BCAA were not reversed by fluoxetine, a serotonin-specific reuptake inhibitor that boosts 5-HT levels in the synapse.

Several studies have shown an association of low serotonin levels with the increase in anxious-like behavior (7, 14, 68). Most of these previous studies assessed the effect of short-term intervention as opposed to our long-term (9 wk) feeding regimen. Our dietary manipulation affected the performance of the rats only when tested with the EPM, but not when behavior was evaluated with open-field or sucrose preference tests (data not shown). Most of the prior studies did not use EPM, and different tests may reveal different elements of anxious or depressed behaviors (22, 51).

We demonstrate that 4 wk of Trp supplementation is sufficient to reverse cautious behavior in rats fed BCAA-supplemented diets. We show that Trp supplemented into the drinking water increased the level of this amino acid in the circulation of rats fed BCAA-supplemented diets and increased uptake of Trp through the BBB. This is consistent with several clinical studies showing the positive effect of Trp supplementation on mood disorders (reviewed in Ref. 71). Our data in aggregate demonstrate that the behavioral effects by BCAA supplementation of high-energy diets are not explained by modification of the serotonergic pathway.

Further studies will be needed to define the mechanism of the anxiogenic effects of chronic BCAA supplementation in rats. One possible mechanism is dysregulation of the kynurenine (KYN) pathway secondary to reduced Trp levels. Only 5% of the Trp in the brain is converted to serotonin, with most of the remainder being catabolized through the KYN pathway (24). KYN can either be metabolized to quinolinic acid (QUIN) or undergo transamination to KYNA. The kynurenine metabolites have a central effect, and recently, there has been
increasing interest in their involvement in the etiology of mood disorders (35, 36, 39, 70) and as targets for new drugs (56, 61). In particular, KYNA is an antagonist of the glycine-binding site of the NMDA receptor (6, 21) and may serve as an endogenous antie excitotoxic agent by modulating glutamnergic neurotransmission to achieve neuroprotective effects (5, 28). Whereas KYN and QUIN exerted an endogenous anxiogenic effect in experimental models (35, 73), KYNA has an anxiolytic pharmacological profile when tested in the EPM model (34, 35). Therefore, our findings of a decrease in KYNA and Trp in response to BCAA supplementation are consistent with a model in which the fall in KYNA decreases the level of an anxiolytic agent. Moreover, our findings of reversal of BCAA-induced behavioral abnormalities by Trp supplementation, coupled with normalization of KYNA and Trp levels and the absence of an effect of fluoxetine, are consistent with a serotonin-independent mechanism of BCAA-induced behavioral change that could involve KYNA pathway metabolites.

Mood disorders are complex and likely involve an intricate interplay between a variety of factors. The contribution of monoamines to the pathophysiology of mood disorders was uncovered in the 1950s with the empirical discovery of drugs that were able to induce or to cure depression by altering the levels of NE and 5-HT (23, 59). Despite the progress in our understanding of the neurobiology of the brain, we still have an incomplete picture of the pathophysiology of depressive disorders, and monoamine-based drugs are still the most commonly prescribed therapeutic agents. Emerging insights from neurobiological studies suggest that the etiology of mood disorders is likely to involve dysregulation of neural plasticity (13, 32, 69). This hypothesis is consistent with our data showing changes in animal behavior only after a long-term dietary manipulation.

To summarize, the long-term consumption of a diet rich in BCAA disrupted the transport of Trp across the BBB in rats, leading to reduced exploratory behavior of rats in EPM testing, a sign of increased anxiety. Recent studies demonstrating a strong association between BCAA levels, obesity, and obesity-related metabolic disorders (27, 29, 43, 62, 63, 74), when linked to the findings reported here, may help to explain the strong association between obesity and behavioral abnormalities, including depression and anxiety.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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