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In vitro evaluation of branched chain amino acids-mediated cellular lipid content alterations using endometrial stem cells

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Previously it has been shown by research groups that higher concentrations of branched chain amino acids (BCAA) such as Leu, Ile and Val can alter the cellular lipid content by targeting the leptin receptor and the downstream mTOR pathways leading to obesity. However, many of the experiments were performed using adipocytes and other human cell lines but not stem cells. In this study we evaluated the effect of BCAA, either individually or in various combinations, on cellular lipid content using the endometrial stem cells isolated from the menstrual waste. Our results suggest that high concentrations of BCAA were non-toxic to the cells and did not affect their ability to proliferate based on the MTT and scratch assays, respectively. Moreover, the cellular lipid content was analyzed using the standard sudanIII dye test in which Val showed the highest stain followed by Ile, combinations and Leu. In conclusion, the endometrial stem cells exhibited a similar behavior compared to the other human cell lines.

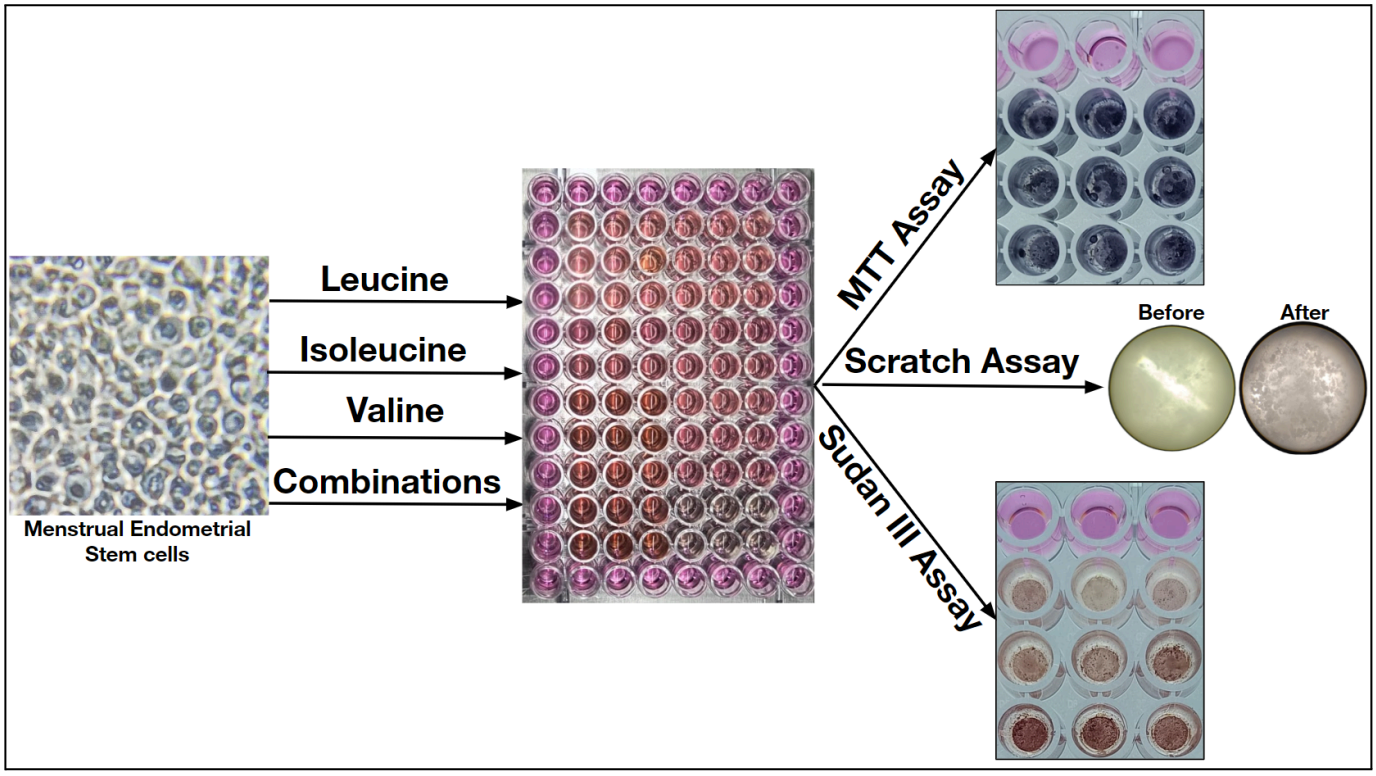

Figure 1. Overall process of BCAA evaluation in the current study.

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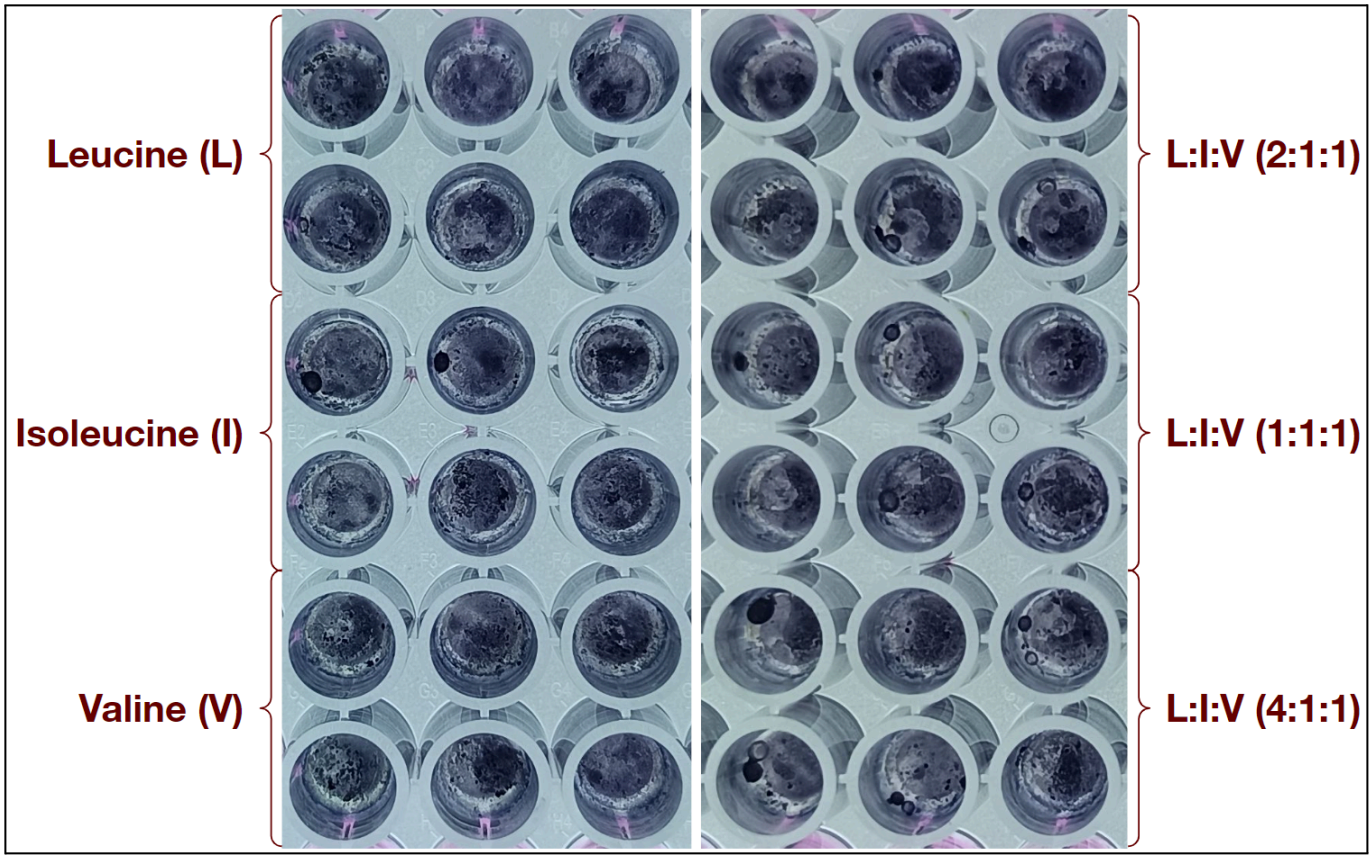

Figure 2. MTT assay results showing the formation of farmazan crystals in all wells suggesting that high quantities of BCAAs are non-toxic to the MnSc either tested individually or in combinations. Each test was performed in triplicates for reproducibility.

Previously we have shown that the menstrual endometrial stem cells can be used as a highly economic resource for standard laboratory tests such as the MTT assays [1]. In this study we used the endometrial stem cells isolated from the menstrual waste blood (MnSC) to evaluate the effect of the branched chain amino acids (BCAA), leucine (L), isoleucine (I) and valine (V). BCAA are known to be a part of all high protein foods such as meat, fish, tofu, etc. However, BCAA from plant sources were shown to be less risky compared to the ones from the animal sources due to slow release [2]. BCAA not only are involved in obesity [3] and other metabolic disorders causation [4-7] but also can control the food intake by interacting with ghrelin pathway [8]. Here we performed 3 *in vitro* assays using the MnSCs, MTT assay to test the toxicity associated with high amounts of BCAA, scratch assay to test the effect of BCAA on the proliferation MnSCs and SudanIII dye test to evaluate any changes in the cellular lipid contents (Figure 1). All assays were performed in the standard 96-well plates by seeding the MnSCs @ 10,000-15,000 cells per well per assay. All wells contained the same cell count on average.

Three 96-well plates were used each dedicated to one assay out of the three. The MTT assay was performed as reported previously [1]. As shown in Figure 2 the 3 BCAAs either individually taken or in combination did not exhibit any toxicity resulting in multiple farmazan crystals. Cells were incubated with BCAA overnight before adding the MTT dye. The SudanIII dye test was also performed similarly. As shown in Figure 3 the presence of valine exhibits the highest lipid content compared to isoleucine and leucine while leucine exhibits the least amount of lipid content. These results suggest that the presence of valine has a significant effect on enhancing the cellular lipid content either used solely or in combination with other BCAAs. The scratch assay was performed using a standard protocol where the cells are incubated either with a scratch or without a scratch (control). All 3 BCAAs were tested either alone or in combination in different ratios. Figure 4 shows a representative image of all the scratch assays performed in this study in triplicates. Evidently, the BCAAs did not affect the proliferative capability of the MnSCs suggesting that high BCAA concentrations are pro-cellular proliferation.

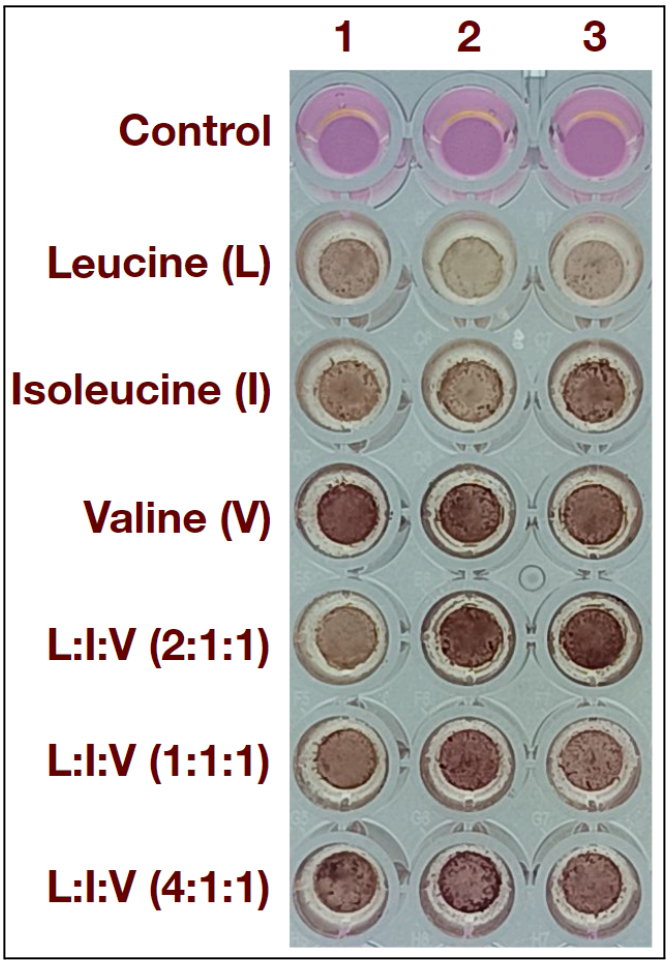

Figure 3. SudanIII dye test results. Leu, Ile and Val exhibited increasing order of brown color suggesting Val shows the most effect. Combinations gave similar results irrespective of the Leu content. Each test was performed in triplicates for reproducibility.

In conclusion the results from the present study suggest that high amounts of BCAAs are non-toxic to the MnSCs (MTT assay results) and have no effect on the cell proliferation (scratch assay) but alter (increase) the overall cellular lipid content (SudanIII test). These *in vitro* results if translated to the clinical settings, it is safe to say that BCAAs indeed contribute to obesity problems as reported earlier by other research groups [9]. In future a systematic study of even higher concentrations of BCAA should be evaluated.

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Conflict of interest: The authors declare no conflict of interest in this study.

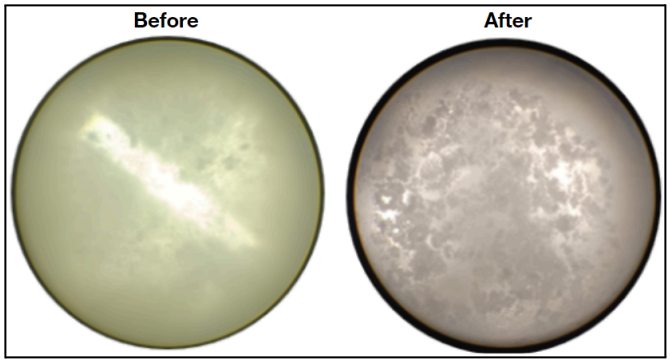

Figure 4. Scratch assay results suggest that BCAAs have no effect on MnSC proliferation.

Author contributions: S.A. performed all the wet lab experiments along with G.Ch. & G.V.M.Ch. R.S.Y., principal investigator, designed the project, trained S.A., G.Ch. & G.V.M.Ch., secured required material for the project, provided laboratory space/facilities needed. R.S.Y. wrote, edited, finalized the manuscript approved by all authors.

References

1. Goddu *et al*. (2026). *In vitro* evaluation of menstrual blood-derived endometrial stem cells for standard laboratory cell-based assays, an economic approach. *TCABSE-J*, Vol. 2, Issue 2:9-11.
2. Wang *et al*. (2026). Branched-chain amino acids from plants and the metabolic syndrome: pathways and pharmacological applications. *Frontiers in nutrition*, *13*, 1805807.
3. Yang *et al*. (2025). Branched- Chain Fatty Acids and Obesity: A Narrative Review. *Nutrition reviews*, *83*(7), 1314–1326.
4. Siddik, M. A. B., & Shin, A. C. (2019). Recent Progress on Branched-Chain Amino Acids in Obesity, Diabetes, and Beyond. *Endocrinology and metabolism (Seoul, Korea)*, *34*(3), 234–246.
5. Mansoori *et al*. (2025). Branched-chain amino acid metabolism: Pathophysiological mechanism and therapeutic intervention in metabolic diseases. *Obesity reviews : an official journal of the International Association for the Study of Obesity*, *26*(2), e13856.
6. Li *et al*. (2020). Branched chain amino acids exacerbate myocardial ischemia/reperfusion vulnerability via enhancing GCN2/ATF6/PPAR-α pathway-dependent fatty acid oxidation. *Theranostics*, *10*(12), 5623–5640.
7. Huang *et al*. (2025). Branched-Chain Amino Acids in Parkinson's Disease: Molecular Mechanisms and Therapeutic Potential. *International journal of molecular sciences*, *26*(14), 6992.
8. Lueders *et al*. (2022) Effect of branched-chain amino acids on food intake and indicators of hunger and satiety- a narrative summary. *Human Nutrition & Metabolism*, *30*, 200168.
9. Yu *et al*. (2021). The adverse metabolic effects of branched-chain amino acids are mediated by isoleucine and valine. *Cell metabolism*, *33*(5), 905–922.e6.