CIRCULATING BRANCHED CHAIN AMINO ACIDS ARE ASSOCIATED WITH LOW GRADE INFLAMMATION IN TYPE 2 DIABETES

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INTRODUCTION AND OBJECTIVES

In obese patients, branched chain amino acids (BCAA) have been identified as predictors of insulin resistance, type 2 diabetes and atherosclerotic complications, which are all conditions underlying a pro-oxidant and a pro-inflammatory status. Whether BCAA may act not only as predictors but also as effectors in the development of these conditions remains largely unknown. This study investigated the capacity of BCAA to directly trigger inflammation in human PBMCs in vitro and to identify potentially responsible signaling pathways.

METHODS

Peripheral blood mononuclear cells (PBMCs) were obtained from healthy donors and stimulated with BCAA. The signaling pathway were tested using specific inhibitors and measured protein expression and phosphorylation (western blot and confocal microscopy); DNA binding assay and gene expression (qPCR); ROS production (Mitosox, and NADPH assay), mitochondrial permeability transition pore (mPTP) formation and cell migration.

RESULTS

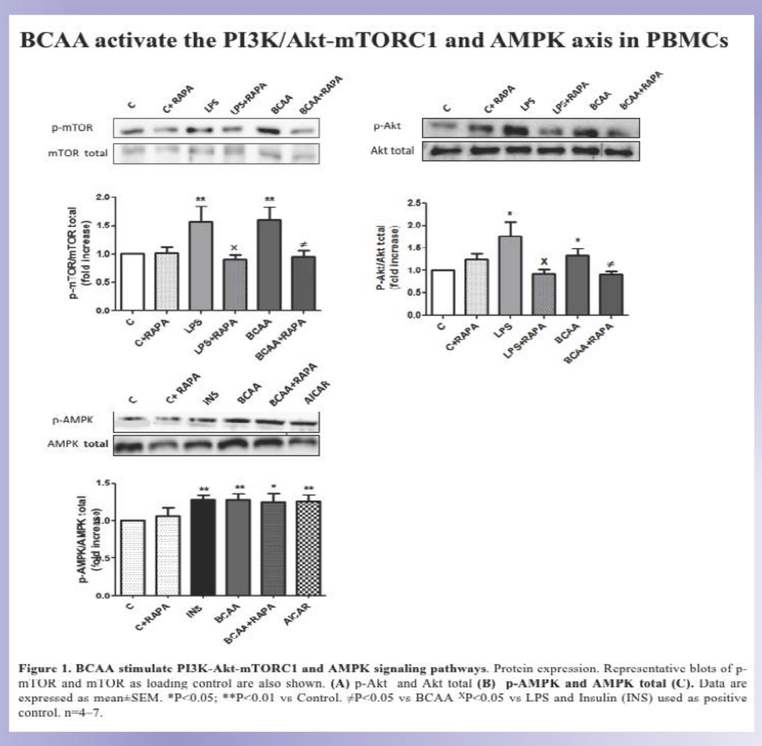

Figure 1. BCAA stimulate PI3K-Akt-mTORC1 and AMPK signaling pathways. Protein expression. Representative blots of p-m1OR and m1OR as loading control are also shown. **(A)** p-Akt and Akt total **(B) p-AMPK and AMPK total (C).** Data are expressed as mean±SEM. *P<0.05; **P<0.01 vs Control. ≠P<0.05 vs BCAA ^{X}P<0.05 vs LPS and Insulin (INS) used as positive control. n=4–7.

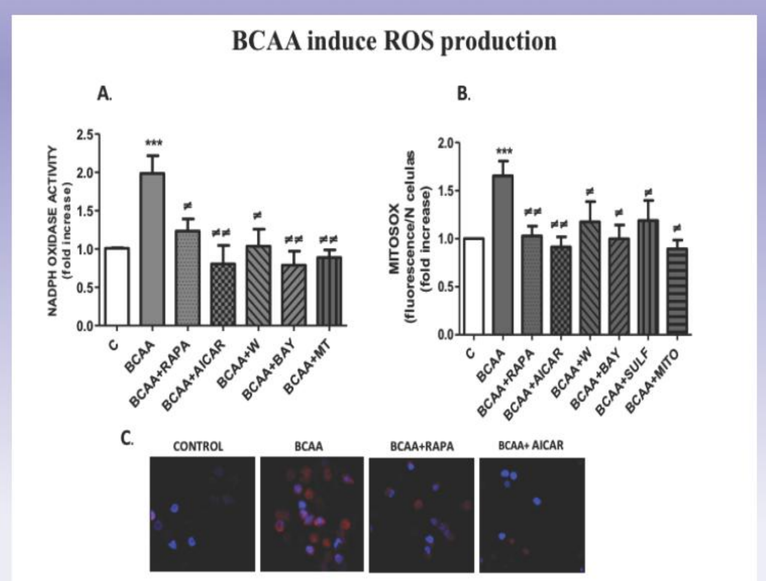

Figure 2. BCAA induce ROS production via mTOR and AMPK activation. Effect of BCAA, rapamicyne (mTORC1 inhibitor), AICAR (AMPK inducer), wortmannin (PI3K/Akt inhibitor), BAY-11-7082 (NF-kB inhibitor), diphenyliodonium chloride (DPI, NADPH oxidase activity inhibitor), sulforaphane (Nrf2 inducer) and mito-tempo (scavenger of mitochondrial superoxide) on NADPH activity **(A)** mitochondrial O_2^- was measured by MitoSOX **(B)** and confocal microscopy in PBMCs. Data represent average Z-stack of fluorescent images **(C)**. Data are expressed as mean±SEM. *P<0.05 **P<0.01 ***P<0.0005 vs Control; ≠P<0.05 ≠≠P<0.005 vs BCAA. n= 5–7.

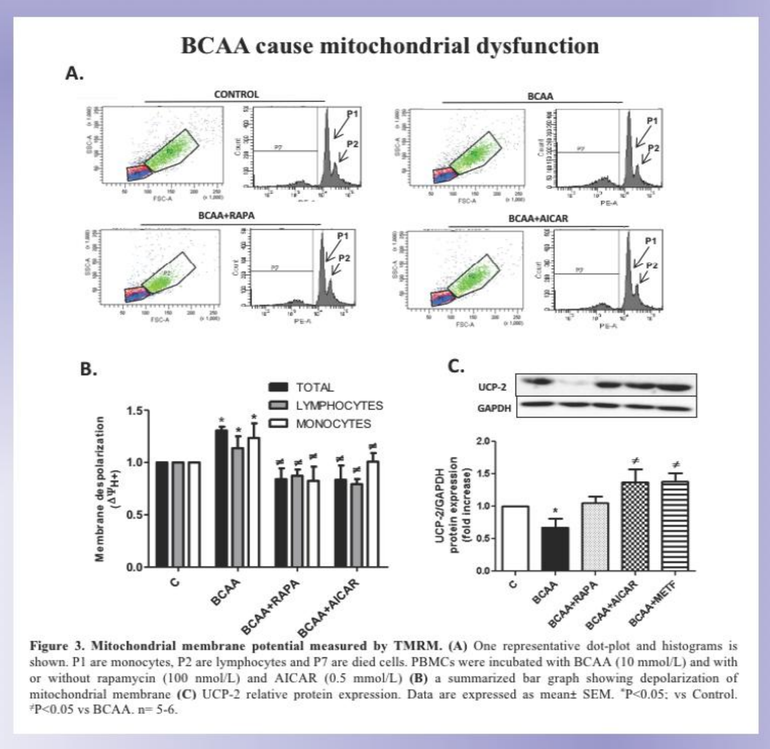

Figure 3. Mitochondrial membrane potential measured by TMRM. (A) One representative dot-plot and histograms is shown. P1 are monocytes, P2 are lymphocytes and P7 are died cells. PBMCs were incubated with BCAA (10 mmol/L) and with or without rapamycin (100 nmol/L) and AICAR (0.5 mmol/L) **(B)** a summarized bar graph showing depolarization of mitochondrial membrane **(C)** UCP-2 relative protein expression. Data are expressed as mean± SEM. *P<0.05; vs Control. ≠P<0.05 vs BCAA. n= 5-6.

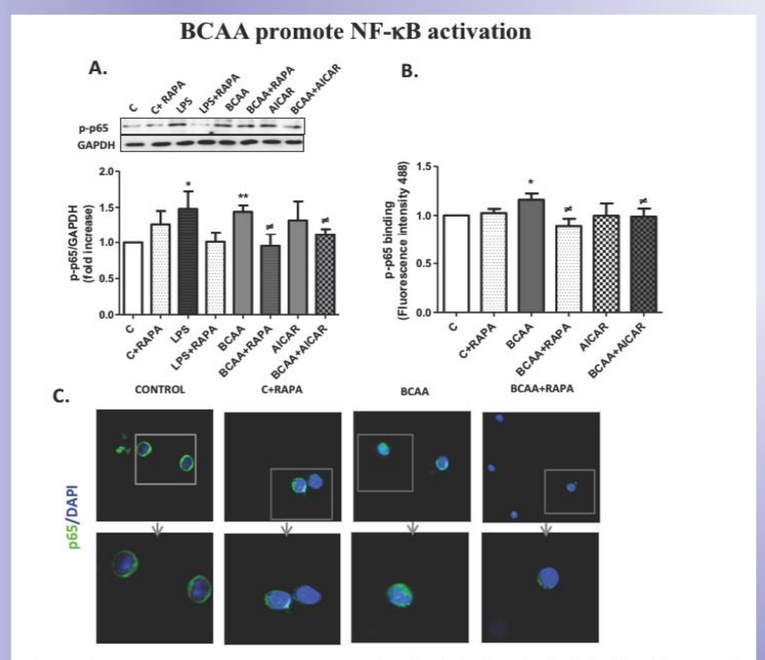

Figure 4. BCAA induces p-p65 NF-κB in PBMCs via mTORC1 axis. Effect of BCAA in p65 phosphorylation with or without rapamycin and AICAR **(B)** BAY-11-7082 , DPI and mito-tempo by Western blot. Representative blots are shown. **(A)** DNA-binding assay results.**(B)** Immunocytochemical analysis revealed localization of p65 in nucleus **(B).** Data are expressed as mean± SEM. *P<0.05; **P<0.01 vs Control. ≠P<0.05 vs BCAA ^{X}P<0.05 vs LPS used as positive control. n=4–7.

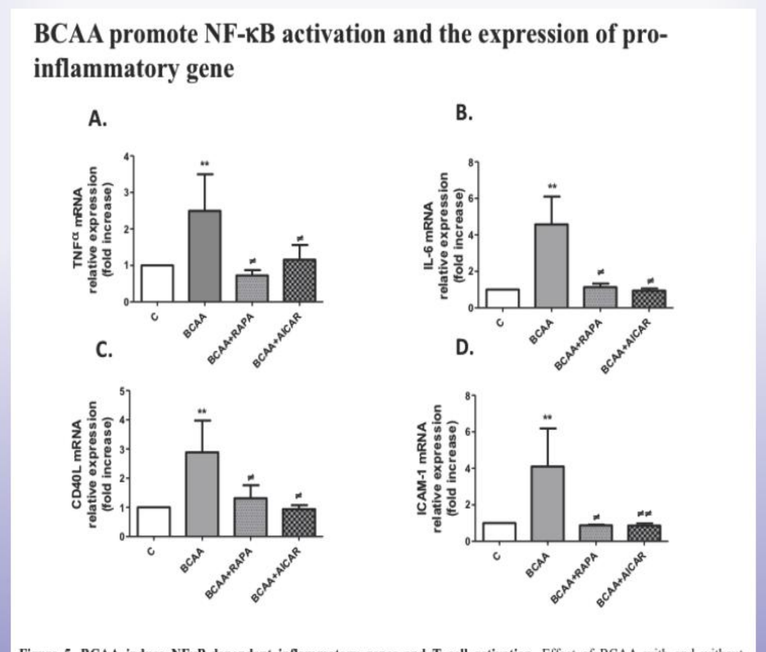

Figure 5. BCAA induce NFκB-dependent inflammatory genes and T cell activation. Effect of BCAA with and without rapamycin and AICAR measured by RNAm levels in human PBMCs. **(A)** TNFα **(B)** IL-6 **(C)** CD40L and **(D)** ICAM-1. Data are expressed as mean ±SEM. *P<0.05; **P<0.01 vs Control. ≠P<0.05 ≠≠P<0.005 vs BCAA. n= 5–7.

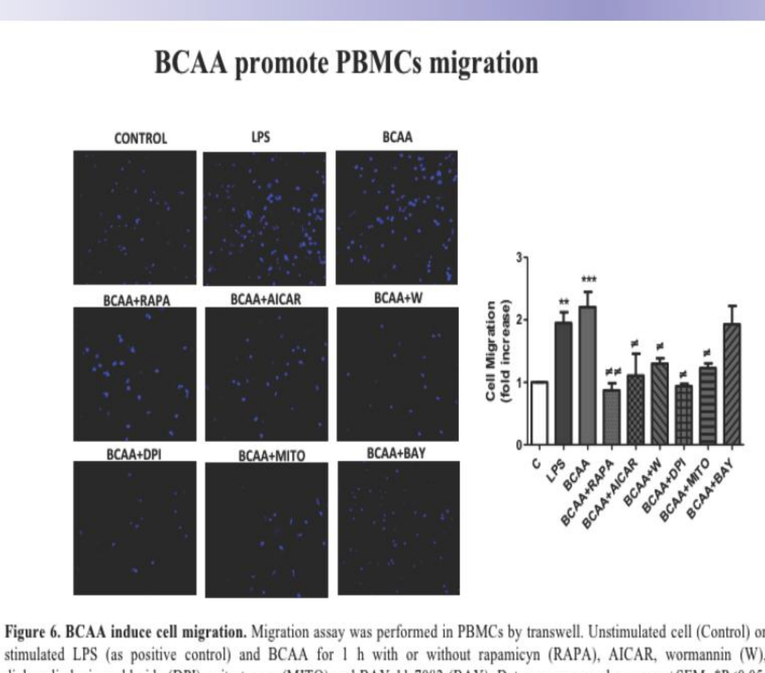

Figure 6. BCAA induce cell migration. Migration assay was performed in PBMCs by transwell. Unstimulated cell (Control) or stimulated LPS (as positive control) and BCAA for 1 h with or without rapamicyn (RAPA), AICAR, wormannin (W), diphenyliodonium chloride (DPI), mito-tempo (MITO) and BAY-11-7082 (BAY). Data are expressed as mean ±SEM. *P<0.05; **P<0.01 or ***P<0.005 vs Control. ≠P<0.05 ≠≠P<0.005 vs BCAA. n= 5–7.

CONCLUSION

The present study shows that BCAA can promote the activation of circulating immune cells, through a mechanism centered on mTORC1 activation. Thus, besides their role as biomarkers in the context of T2DM, BCAA may directly contribute to creating the oxidative and inflammatory environment, which is at the basis of different complications associated to this disease.

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