

**Title: Intestinal microbiota in overweight men evaluated by Fluorescent *in situ* Hybridization technique**

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**Abstract:** There are scientific evidences of the increase in obesity worldwide and that diseases such as diabetes mellitus type 2 are related to the intestinal microbiota composition. We evaluated the microbial community structure of overweight men in submitted to caloric restriction (250 kcal/d) for consecutive 30 days. Microbiota of overweight men (BMI > 27), aged between 18 and 50 years was analyzed by Fluorescent *in situ* Hybridization technique (FISH). Fecal samples were collected in the first and last day of the study. Samples were weighed (0.5 g) and fixed with PFA (final concentration 2%), sonicated (three times with amplitude of 110.7  $\mu$ M for 60s) and centrifuged (three times 500x g for 5 min). Next, the samples were filtered (filter polycarbonate 0.2 $\mu$ M) and submitted to FISH protocol. Probes were used to identify and quantify the following bacterial groups: Archaea (Arc 915); Actinobacteria (HGC 236); Firmicutes (LGC354 A, B and C); Proteobacteria (class alpha, beta and gamma – Alf 968, Bet 42a, Gam 42a respectively), and Bacteroidacea \ Prevotellaceae \ Porphyromonadaceae (BAC 303). The total density of bacteria was quantified by staining with DAPI. There was no significant difference between microbial community before and after the caloric restriction. Mean density of total bacteria was  $17 \pm 8.7$  cels  $10^9$  g<sup>-1</sup>. Bacteria of the Firmicutes group were the most abundant ( $1.8 \pm 1.9$  cels  $10^9$  g<sup>-1</sup>) after 30 days. Conversely, bacteria of the families belonging to the phylum Bacteroidetes were less abundant ( $1.8 \pm 1.9$  cels  $10^9$  g<sup>-1</sup>). The standard deviation values were very close to the mean values, sometimes higher. These results show a wide variation in microbial community structure within the group of people with excess weight. The taxonomic levels of the probes used were above family, specific differences have not been evaluated and may occur. However, individual microbiota among overweight people should also be considered.

Keywords: obesity, microbial diversity, FISH

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