Author's response to reviews

Title: Milk is not just food but most likely a genetic transfection system activating mTORC1 signaling for postnatal growth

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Author's response to reviews:

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Dr Nagaraj Nagathihalli
Nutrition Journal Editorial Team

Re-submission of our revised Review article MS Number: 1584683256986837
Milk is not just food but most likely a genetic transfection system activating mTORC1 signaling for postnatal growth
Melnik BC, John SM, Schmitz G

Dear Dr Nagathihalli,

we would like to re-submit the revised version of our manuscript. We have considered all most helpful remarks and concerns raised by our reviewers. We would like to respond now point-by-point:

Response to Reviewer Dr Joe Millward

We completely agree with Dr Millward that breast milk is the appropriate food for the newborn infant as outlined by WHO recommendations. In comparison to artificial formula feeding breast-fed human infants have much lower plasma levels of leucine, insulin and IGF-1, thus exhibit lower mTORC1-signaling than formula-fed infants. This important endocrine difference, which may deviate appropriate metabolic programming with possible life-long adverse health effects has already been a matter of own concern (Melnik BC (2012) Excessive leucine-mTORC1-signalling of cow milk-based infant formula: the missing link to understand early childhood obesity. J Obes 2012:197653).
In the revised version this important issue has been addressed in the ABSTRACT and at the beginning of the INTRODUCTION. For further support of the protective effects of breast-feeding against subsequent overweight and obesity we refer to Ip S et al. (2007) Breastfeeding and maternal and infant health outcomes in developed countries. Evid Rep Technol Assess (Full Rep) 153:1-186.

As the major intention of our review is to show the potential pathways involved in milk signaling, we did not focus on already known adverse effects of milk consumption during adolescence like the induction and aggravation of acne (Di Landro A et al. (2012) Family history, body mass index, selected dietary factors, menstrual history, and risk of moderate to severe acne in adolescents and young adults. J Am Acad Dermatol 67:1129-1135) or advanced prostate cancer later in life by daily milk consumption during adolescence (Torfadottir JE et al. (2012) Milk intake in early life and risk of advanced prostate cancer. Am J Epidemiol 175:144-153).

We hope that Dr Millward is content with the changes made in the revised manuscript.

Response to Reviewer Dr Ernesto Bernal-Mizrachi

Major compulsory revisions:

Point 1

Dr Bernal-Mizrachi asks a very important question concerning the survival of milk exosomes by the process of pasteurization. Chen et al. (2010) Identification and characterization of microRNAs in raw milk during different periods of lactation, commercial fluid, and powdered milk products. Cell Res 20:1128-1137 provided evidence that in fact milk-miRs survive common procedures of milk processing like pasteurization. MiRs have also been detected in milk powder. Izumi H et al. (2012) Bovine milk contains microRNA and messenger RNA that are stable under degradative conditions. J Dairy Sci 95:49831-4841 analyzed raw milk as well as processed infant formula. They found that milk RNA survives heat treatment, RNase treatment and acidic conditions and concluded in their paper that milk RNA could resist the industrial manufacturing process. This is the major reason why the Chinese chose milk RNA as a marker for the detection of milk manipulation (former melamin scandal). To our knowledge, studies comparing different treatment modalities of milk like ultra-heat versus pasteurization as well as homogenization have not yet been performed. These studies will be important for the dairy industry. There is good reason to assume that the exosome character of milk´s miRs and RNAs makes them very stable and resistant during various procedures of milk processing.

Point 2

The Reviewer asks to highlight additional effects of milk in comparison to other protein sources enriched in branched-chain amino acids (BCAAs).

As meat is the major “competitor” of milk providing high amounts of BCAAs, we
emphasized important functional differences in the kinetics of meat BCAAs intestinal absorption and milk proteins. Beef is a structural muscle protein, which has a texture and releases BCAAs more slowly than soluble, small molecular weight whey proteins like alpha-lactalbumin. In the revised version we point out that milk proteins exhibit a high insulinemic index which is twice as high than that of beef. We referred to Holt S et al. (1997) An insulin index of foods: the insulin demand generated by 1000-kK portions of common foods. Am J Clin Nutr 66:1264-1276 and Hoyt G et al. (2005) Dissociation of the glycaemic and insulinaemic responses to whole and skimmed milk. Br J Nutr 93:175-177. Furthermore, and most striking, milk contains the system of exosomal miRs, which are not present in muscle protein like beef or soybean products. Thus, milk differs in the kinetic of released BCAAs and in its unique content of exosomal miRs, whose potential functions are outlined in the manuscript.

Point 3
We agree that milk is undoubtedly the right system for a growing neonate. Milk is not only food, but a sophisticated signaling system. That is what we would like to communicate. Milk-miR-155 may switch off BAT thermogenesis and thereby “drives” excess calories into the WAT pool (as a potential energy reserve for extra-uterine life). The appropriate mTORC1-signaling mediated by breast-feeding is the evolutionarily system metabolic programming strictly controlled by the human lactation genome. Species-specific milk-driven mTORC1 signaling is terminated by all other mammals except Neolithic humans. The authors are very concerned about the negative effects of increased and persistent milk signaling by continued consumption of the milk of a faster growing species (Bos taurus). Persistent proliferation is the first hallmark of cancer. We agree with Zoncu et al. that all our diseases of civilization are indeed mTORC1-driven diseases (Zonco R et al. (2011) mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol 12:21-35).

Point 4
Dr Bernal-Mizrachi requested more information regarding the effects of BCAAs on the liver as another major metabolic organ.


Minor essential revisions:

Point 1
The reviewer asks to provide evidence that mTORC1 regulates insulin secretion and enhances beta-cell mass expansion.

We extended this section of our manuscript and provide further evidence that mTORC1 stimulates insulin secretion as well as beta-cell mass expansion. Rapamycin treatment is known to induce diabetes. The following requested references have been considered in the revised manuscript: Sener A et al. (1980) L-leucine and a nonmetabolized analogue activate pancreatic islet glutamate dehydrogenase. Nature 288:187-189; Malaisse WJ (1986) Branched-chain amino and keto acid metabolism in pancreatic islets. Adv Enzyme Regul 25:203-217; Fahien LA et al. (1988) Regulation of insulin release by factors that also modify glutamate dehydrogenase. J Biol Chem 263:13610-13614; Xu G et al. (2001) Metabolic regulation by leucine of translation initiation through the mTOR-signaling pathway by pancreatic #,-cells. Diabetes 50:353-360.

Point 2
The reviewer asks to be more specific about the meaning “peripheral”.

In the revised version we avoided the term “peripheral” cells. It was our intention to show that the important endocrine organs like the pancreatic islet and the liver respond to increased mTORC1 signaling. Their secretion products, insulin and IGF-1, drive cells in the whole organism (the periphery compared to the primary endocrine glands) in an mTORC1-dependent manner.

We hope that the changes made in the revised manuscript, which are marked in yellow for your convenience, satisfy the most helpful and justified remarks made by our reviewers.

We hope that the revised version of our manuscript will be suitable for publication in Nutrition Journal.

With best regards and many thanks for all support.

Prof Bodo C. Melnik, MD
(Corresponding author)