# Effects of antibiotics, short-chain fatty acids and amino acids on Apoliprotein A-I transcription and synthesis in normal and inflamed HepG2 and Caco-2 cells

# Impact paragraph

## Social and economic relevance

In this section, the potential impact of our scientific findings for society and economy will be described. This mostly concerns the possible future applications of our findings in specific target groups, i.e. subjects with risk factors for cardiovascular diseases (CVD) such as dyslipidemia. Despite the fact that treatment has improved tremendously over the past decades, CVD remains the leading cause of morbidity and mortality worldwide (1). The most recent world health organization (WHO) report indicates that more than 17.9 million persons die from CVD each year. Currently, the number of newly diagnosed CVD cases in Europe is more than 11 million yearly, which results in a high economic burden and concomitant health care related costs of around €210 billion per year (1). Clearly, there is a need to discover and use additional therapies on top of already existing portfolios to further reduce the risk of CVD development and the high costs of CVD-related medical care. Although some studies showed conflicting results about the link between elevating circulating high-density lipoprotein (HDL) cholesterol concentrations and CVD (2), recent experimental studies have shown that CVD can be reduced by the enhancement of HDL functionality (3). The main function of HDL is to transport the excess cholesterol from peripheral tissues to the liver for excretion, a process called a reverse cholesterol transport (RCT) (4). Recent evidence suggested that increasing RCT process protects against CVD and atherosclerosis (5). Therefore, targeting factors known to stimulate RCT, such as increasing apolipoprotein A-I (ApoA-I) synthesis (6), is a promising strategy to reduce the progression of atherosclerosis development and consequent CVD. Although there are already some pharmaceutical options to increase ApoA-I production in specific high risk patient populations (7), dietary recommendations that aim to increase ApoA-I and HDL functionality may be needed to prevent CVD. Therefore, in our studies we aimed to discover the potential contribution of some (dietary natural) compounds on ApoA-I production with the ultimate aim to lower CVD risk and progression, and the financial costs of current CVD treatment. Thus, the main goal of this thesis was to explore the effects of several external stimuli on ApoA-I transcription and secretion. We focused on the effects of SCFAs, antibiotics and amino acids to understand underlying mechanisms and to generate leads for future CVD treatment and prevention.

## Scientific gain of this thesis

The research in this thesis supported our previous observation from a placebo-controlled double blind human intervention trial showing that antibiotic treatment was linked to alterations in metabolic biomarkers, amongst others a reduction in serum HDL cholesterol

concentrations (8). Our in vitro studies described in this thesis were the first showing that antibiotic treatment could directly influence ApoA-I transcription and secretion in human liver and intestinal cells. Moreover, in line with several human and animal studies (9-12), we revealed favorable effects of SCFAs (linking to potential indirect effects of antibiotic treatment) as well as specific amino acids on hepatic ApoA-I transcription. Our findings further provide a mechanistic insight underlying the effects of these compounds on ApoA-I transcription. Sharing these research findings within the scientific community helps to evolve our understanding of the underlying processes and the regulatory factors involved in ApoA-I transcription and to develop strategies targeting ApoA-I transcription via dietary approaches. Therefore, it is important that the data presented in this thesis is available to the (scientific) public through publications in international scientific journals. Also, our preliminary results were presented at national and international conferences that support the development of fundamental knowledge and created the possibility to discuss the findings with several experts from the field. Altogether, this stimulates that the leads we presented here can also be further developed by others. This thesis was funded by a scholarship granted by university of Jeddah, a leading governmental institution of higher education in Saudi Arabia. This implies that the collaboration between the university of Jeddah and Maastricht university contributes to obtain new international scientific perspectives, transfer knowledge between research institutions and to gain new insights into CVD treatment and prevention.

## Translation into clinical application

The studies described in this thesis are in vitro experiments using different cell lines, which means that our observations cannot be directly used as clinical applications. First, our findings need to be confirmed in additional (in vivo) experiments. In other words, this research alone is not enough to adapt the dietary guidelines for fiber or protein intake or to recommend any dietary adjustments for the food industry. If our findings could be confirmed in human interventions, i.e. elevating the uptake of specific SCFAs and/or amino acids effectively increased ApoA-I and HDL functionality, this will be of high relevance for nutritional interventions targeting ApoA-I. However, more studies are needed to identify the most effective dietary fibers (that need to be processed into the optimal SCFAs by our microbiota) or proteins (that need to contain the optimal amino acids composition), thereby stimulating similar effect as reported in our in vitro experiments. Into more detail, after the confirmation of these positive findings in human studies, the intake of SCFAs or amino acids could be used by health care professionals as a dietary recommendation to

prevent the development of CVD in general, or for example as adjunct therapy for patients treated with antibiotics or by the food industries for the development of novel functional foods.

To conclude, this thesis provides a first insight into the potential role of SCFAs and amino acids on ApoA-I transcription and secretion in human cell line models. Our finding can be used to design future human interventions to investigate the effects of SCFAs and amino acids in clinical applications. Finally, these positive effects might contribute to the development of new nutritional strategies to improve the HDL functionality and subsequently to reduce CVD risk.

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