Human cardiometabolic health

SUMMARY

A balance between endogenous cholesterol biosynthesis, intestinal absorption of dietary and biliary cholesterol, and bile acid synthesis and excretion is essential to maintain healthy serum low-density lipoprotein cholesterol (LDL-C) concentrations and hence for cardiovascular disease risk prevention. Previous research has reported a large between-person variation in endogenous cholesterol synthesis and intestinal cholesterol absorption, which may be due to differences in genetic background. Furthermore, various biological processes follow a diurnal pattern, but little is known about the link between the circadian system and human cholesterol homeostasis, especially intestinal cholesterol absorption. In addition, individuals spend most of the day in the postprandial state, but data on the acute effects of macronutrient consumption on endogenous cholesterol synthesis and intestinal cholesterol absorption is limited. To maintain healthy serum LDL-C concentrations it is relevant to examine these abovementioned factors that may influence cholesterol homeostasis.

As mentioned above, the circadian system regulates daily oscillations of many physiological processes. Over the last few decades, the interest in chrononutrition, which studies the interplay between circadian biology, nutrition, and metabolism, has risen. The focus of nutrition research is no longer only on what, but also on when people eat. Common intermittent energy restriction (IER) protocols include time-restricted eating, alternate day fasting, and the 5:2 diet. Obesity, mainly abdominal obesity, is an important modifiable cardiovascular disease risk marker. To reduce the number of people living with (abdominal) obesity, it is important to study which weight loss approach is most beneficial to lower body weight and consequently improve cardiometabolic risk markers.

This thesis therefore aimed to study (1) the involvement of genetic variants, diurnal rhythms, and macronutrients in the regulation of endogenous cholesterol synthesis and intestinal cholesterol absorption, and (2) the effects of different IER diets with and without weight loss on body weight and cardiometabolic risk factors in apparently healthy individuals with and without overweight.

In **Chapter 2**, cross-sectional associations between single nucleotide polymorphisms (SNPs) in genes that encode for proteins involved in cholesterol metabolism with endogenous cholesterol synthesis and intestinal cholesterol absorption markers and LDL-C concentrations were studied. Pre-selected SNPs in *ABCG5, ABCG8, CYP51A1, DHCR7, DHCR24, HMGCR, HSD17B7, LBR, MSMO1,* and *NPC1L1* were studied in serum samples obtained from 456 Western European individuals. Two SNPs in *NPC1L1* (rs217429 and rs217416) were associated with the endogenous cholesterol synthesis marker lathosterol and two SNPs in *ABCG5* (rs4245786) and *ABCG8* (rs4245791) with intestinal cholesterol absorption markers. Furthermore, SNPs in *HMGCR* (rs12916) and *LBR* (rs12141732) were associated with serum LDL-C concentrations. Selected SNPs in *CYP51A1, DHCR24, HSD17B7,* and *MSMO1* were not associated with non-cholesterol sterols and LDL-C concentrations. In **Chapter 5**, comparable associations in the same population were studied, but now with selected SNPs located in circadian clock genes (*ARNTL, ARNTL2, CLOCK, CRY1, CRY2, PER1, PER2,* and *PER3*). One SNP in *ARNTL2* (rs1037924) was associated with cholesterol synthesis. Multiple SNPs in *ARNTL* (rs4146388, rs58901760, rs6486121), *ARNTL2* (rs73075788), *CLOCK* (rs13113518, rs35115774, rs6832769), and *CRY1* (rs2078074) were associated with campesterol or sitosterol levels, which reflect cholesterol absorption. Only *PER2* (rs11894491) was associated with serum LDL-C concentrations. SNPs in *CRY2*, *PER2*, and *PER3* were not related to non-cholesterol sterol levels. In both **Chapter 2** and **5**, associations

between SNPs and cholesterol synthesis or cholesterol absorption did not translate into significant associations with LDL-C concentrations. The findings suggest that genetic variants indeed partly explain the high inter-individual variation in intestinal cholesterol absorption and endogenous cholesterol synthesis.

The systematic review and single-arm study in **Chapter 3** studied the diurnal rhythms of bile acid synthesis, endogenous cholesterol synthesis, and intestinal cholesterol absorption markers. The systematic review indicated that the bile acid synthesis marker 7α-hydroxy-4-cholesten-3-one had a diurnal rhythm with peaks during the day and that cholesterol synthesis markers had a diurnal rhythm with a peak at night. The single-arm study confirmed the nocturnal endogenous cholesterol synthesis peak and found no significant diurnal rhythm for intestinal cholesterol absorption markers. Bile acid synthesis markers were not analyzed. A reciprocal relationship between endogenous cholesterol synthesis and intestinal cholesterol absorption, as frequently observed in the fasted situation, was not confirmed. We further hypothesized that the non-significant diurnal rhythm of cholesterol absorption may have been due to the consumption of low-fat meals during the study period.

To study the possible role of the low fat meals as explanation for the results of **Chapter 3**, the randomized cross-over trial in **Chapter 4** compared the effects of three isoenergetic meals high in fat (fat [f], carbohydrates [c], protein [p]: 55.2 g/52.3 EN% f, 93.5 g/39.2 EN% c, 19.2 g/8.0 EN% p), carbohydrates (10.2 g/9.6 EN% f, 194.3 g/81.5 EN% c, 20.4 g/8.6 EN% p), and proteins (11.3 g/10.6 EN% f, 122.7 g/51.5 EN% c, 87.9 g/36.9 EN% p) on intestinal cholesterol absorption and endogenous cholesterol synthesis markers over a four-hour period in overweight/obese men. Acute meal consumption did not change serum total cholesterol concentrations and cholesterol-standardized campesterol, sitosterol, and cholestanol levels. This suggests that meal consumption did not explain the absence of a diurnal rhythm of cholesterol absorption. The cholesterol synthesis intermediates 7-dehydrocholesterol, lanosterol, lathosterol, zymostenol, and zymosterol all decreased significantly over time, but no significant differences between the meals were found for these intermediates.

The systematic review and meta-analysis in **Chapter 6** compared IER diets with continuous energy restriction (CER) in healthy individuals. No different changes in anthropometrics (body weight, body mass index, and fat mass) and cardiometabolic risk markers (fasting total cholesterol, high-density lipoprotein cholesterol [HDL-C], LDL-C, triacylglycerol, glucose and insulin concentrations, homeostatic model assessment for insulin resistance [HOMA-IR] and blood pressure) were found for IER compared to CER. However, larger reductions in fat free mass (weighted mean difference [WMD]: -0.20 kg; 95% CI: -0.39 to -0.01; p=0.044) and waist circumference (WMD: -0.91 cm; 95% CI -1.76 to -0.06; p=0.036) were observed for IER diets. Further, body weight, fat mass, and fat free mass were more reduced in time-restricted eating, HOMA-IR decreased more in alternate-day fasting, and body mass index was more decreased after CER compared with the 5:2 diet. It remains uncertain whether the findings of the meta-analysis were completely due to the type of diet or also to differences in energy intake between groups within the studies. The findings further suggest that weight loss may be more important than the type of diet to improve cardiometabolic risk markers.

To further study the benefit of IER for cardiometabolic health in absence of weight loss, we compared a 4-week alternating energy intake schedule to a regular energy intake schedule in individuals with abdominal obesity (**Chapter 7**). No differences between the two dietary patterns were reported for

anthropometrics and fasting glucose, insulin, total cholesterol, HDL-C, LDL-C, triacylglycerol, and high-sensitivity C-reactive protein concentrations. A high-fat mixed meal was consumed at the end of both four-week periods, and no significant between-group differences in postprandial triacylglycerol, glucose, and insulin concentrations were found. Overall, the results of this study suggest that an IER approach without weight loss was not superior in improving anthropometrics and cardiovascular risk markers compared with a regular energy intake schedule. It may therefore be suggested that beneficial effects of intermittent energy restriction diets on cardiometabolic health are primarily due to the loss in body weight instead of to the eating pattern.

In conclusion, the major findings of the studies included in this these were:

1. Genetic variants in endogenous cholesterol synthesis, intestinal cholesterol absorption, and circadian clock genes are partly responsible for differences in endogenous cholesterol synthesis and intestinal cholesterol absorption among individuals.
2. Endogenous cholesterol synthesis depicts a clear diurnal pattern with a nocturnal peak, whereas no diurnal rhythm was found for intestinal cholesterol absorption markers.
3. Acute high-fat, high-carbohydrate or high-protein consumption had no effect on postprandial intestinal cholesterol markers and thereby also did not explain the absence of a diurnal rhythm in intestinal cholesterol absorption. Various intermediates in the endogenous cholesterol synthesis pathway decreased after meal consumption.
4. In apparently healthy individuals, the effects of IER diets on weight loss and cardiovascular risk markers were not significantly better compared to the CER diet.
5. An alternating energy intake approach without a net reduction in energy intake did not improve anthropometrics and cardiometabolic risk markers compared to a regular energy intake schedule.