Low serum cholesterol, serotonin metabolism, and violent death

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LOW SERUM CHOLESTEROL, SEROTONIN METABOLISM, AND VIOLENT DEATH

Laag serum cholesterol, serotonine metabolisme en gewelddadige dood

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Les gens bien portants
sont des malades qui s'ignorent.
("Knock", Jules Romain)

Sokrates, der alte Greis,
Sagte oft in tiefen Sorgen:
"Ach, wie viel ist doch verborgen,
Was man immer noch nicht weiß."
("Tobias Knopp", Wilhelm Busch)

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Manuscripts based on the studies described in this thesis:

Chapter 1:

Steegmans PHA, Bak AAA, Does E van der, Grobbee DE, Hoes AW. Serum cholesterol, serotonin metabolism and violent death: A review. Cardiovasc Risk Factors, in press.

Chapter 2:

Steegmans PHA, Hoes AW, Bak AAA, Does E van der, Grobbee DE. Correlates of chronically low cholesterol levels in healthy middle-aged men. Submitted.

Chapter 3:

Steegmans PHA, Hoes AW, Bak AAA, Fekkes D, Does E van der, Grobbee DE. Lower levels of tryptophan and other large neutral amino acids in men with low cholesterol levels. Submitted.

Chapter 4:

Steegmans PHA, Fekkes D, Hoes AW, Bak AAA, Does E van der, Grobbee DE. Low cholesterol and serotonin metabolism in men. Br Med J, in press.

Chapter 5:

Steegmans PHA, Hoes AW, Bak AAA, Meesters CMG, Does E van der, Grobbee DE. Depression, hostility and impulsivity in middle-aged men with low serum cholesterol levels. Submitted.

Chapter 6:

Steegmans PHA, Hoes AW, Bak AAA, Does E van der, Bruijn AM de, Valkenburg HA, Grobbee DE. Low serum cholesterol concentrations and the risk of violent death: a prospective study in the Netherlands. Submitted.

Chapter 1

Serum cholesterol, serotonin metabolism, and violent death: A review

Introduction

A high serum cholesterol level is a well documented risk factor for atherosclerotic cardiovascular disease.[1,2,3,4] Consequently, a low serum cholesterol has in general been viewed as beneficial. However, since the early 70s, results from several cohort studies and randomized trials have suggested that low or lowered cholesterol may increase the risk of dying from non-atherosclerotic causes. A number of reviews discussing the putative association between low serum cholesterol levels and increased mortality has been published.[5,6,7,8,9] These reviews focused mainly on the possibility of a causal relation between low cholesterol and mortality from cancer. Although cancer mortality remains an important issue, the scope of recent studies on the association between low or lowered cholesterol and mortality has broadened towards all non-cardiovascular causes of death including accidents, violence and suicide.[10,11,12,13,14]

In the present paper the evidence is reviewed for an association between low or lowered cholesterol levels and aggression, impulsivity and (mortality from) suicide. Both studies addressing 'naturally' occurring low cholesterol levels and studies in which a low cholesterol was the result of (drug) intervention are discussed. This distinction is important from an etiological point of view, notably because a 'naturally' occurring low cholesterol reflects the lower end of the cholesterol distribution while lowered cholesterol levels of patients with hypercholesterolemia are still in the upper part of the distribution. Moreover, the duration of being 'exposed' to lower cholesterol levels is longer in those with 'naturally' occurring low cholesterol levels compared to those with 'lowered' cholesterol concentrations.

The currently most important hypothesis to explain an association between naturally occurring low cholesterol and violent death, involving dietary induced serotonin changes as a mediating factor, is discussed in detail.

RESULTS FROM STUDIES ON LOW SERUM CHOLESTEROL AND VIOLENT DEATH

* *Nonexperimental studies*

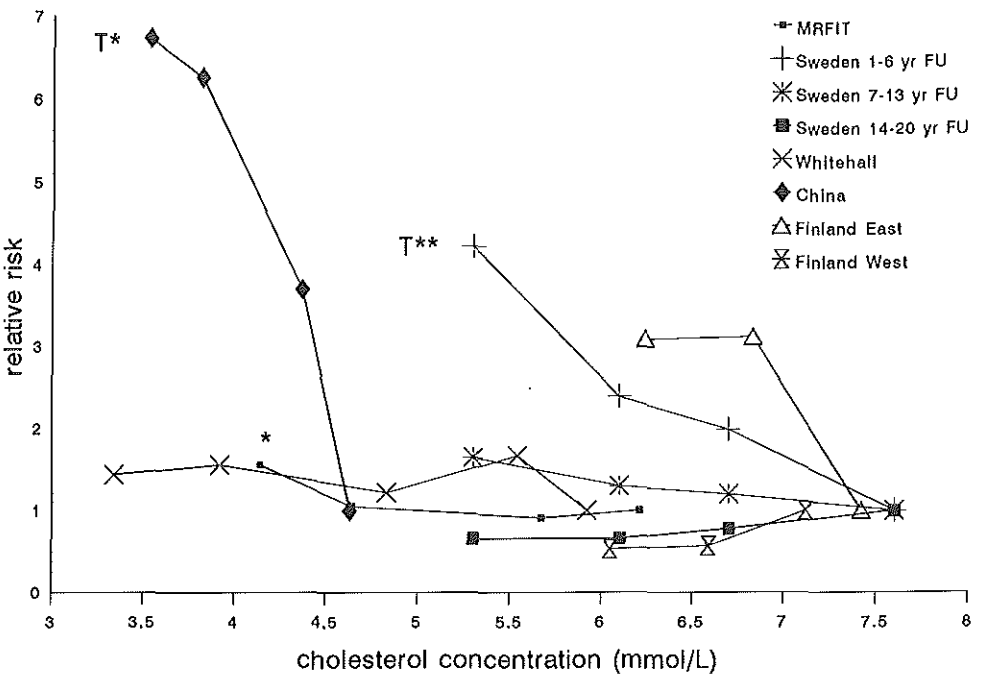
In nonexperimental, e.g. observational, studies participants/patients with different cholesterol levels are followed to compare subsequent mortality risk (table I, figure 1). In these studies participants are included with cholesterol levels as they occur naturally, and no predetermined interventions take place.

Table I. Cohort studies with separate mortality categories 'suicide', 'non-medical deaths' or 'unnatural deaths' (only males considered, unless otherwise indicated).

Study	Particip. (deaths)	FU (yrs)	Chol. level	Effect measure	Mort. category	p-value
MRFIT[18]	350,977(21,499)	12		RR	suicide	
			< 4.14 (ref.)	1.0		
			4.14 - 5.15	0.67		
			5.17-6.18	0.58		
			≥ 6.21	0.64		
			≥ 4.14	0.62		p < 0.05
Sweden[19]	26,693(n.a.)	20.5	(quart. means)	RR	suicide	
		1 - 6	5.3	4.22		
			6.1	2.39		
			6.7	1.99		
			7.6 (ref.)	1.00		p (trend) = 0.001
		7 - 13	5.3	1.65		
			6.1	1.30		
			6.7	1.20		
			7.6	1.00		NS
		14 - 20	5.3	0.66		
			6.1	0.66		
			6.7	0.78		
			7.6	1.00		NS
Whitehall[15]	17,718(4,022)	18	(quintiles)	mort./1000 py	suicide	
			< 3.34	0.13		
			3.34 - 4.50	0.14		
			4.51 - 5.14	0.11		
			5.15 - 5.92	0.15		
			> 5.92	0.09		NS
China[17]	9,021 (595) (♀+♂)	8 - 13	(quartiles)	obs./exp.	non-medical	
			< 3.53	1.55		
			3.54 - 4.10	1.44		
			4.11 - 4.62	0.85		
			≥ 4.63	0.23		p (trend) < 0.05
Finland[16]	1,580(n.a.)	25	(tertiles)	mort./100,000py	acc./violence	
(Eastern)			< 6.24	175.9		
			6.25 - 7.41	178.1		
			≥ 7.42	57.0		NS
(Western)			< 6.05	128.4		
			6.06 - 7.11	136.9		
			> 7.12	240.7		NS

acc. : accidents
chol. : cholesterol
FU : follow-up
n.a. : not available
mort. : mortality
NS : not significant
obs./exp. : observed/expected
particip. : participants
py : person-years
quart. : quartile
ref. : reference category

Figure 1. Cholesterol and violent death (cohort studies)



* p < 0,05 FU: follow-up period
T* p (trend) < 0,05
T** p (trend) = 0,001

In the Whitehall Study, plasma cholesterol levels were measured in 17,700 London civil servants.[15] During a follow-up period of over 20 years, noassociation was observed between cholesterol levels and death from suicide or all violent deaths. In two Finnish cohorts of the Seven Countries Study conflicting results were obtained.[16] A statistically non-significant, inverse association between serum cholesterol and mortality from suicide was observed in one cohort and a statistically significant, positive association in the other cohort. In a Chinese study with average cholesterol values far below those generally seen in Western populations a marginally significant inverse relationship between serum cholesterol concentrations and deaths attributed to non-medical causes was observed.[17] However, the total number of deaths in this category was small and after taking confounding variables into account the association became weaker. A recent 12

year follow-up analysis of 350,000 men screened in the Multiple Risk Factor Intervention Trial showed that the death rate from accidents, suicides and homicides was significantly higher for men with cholesterol levels below 4.14 mmol/l (about 6% of the participants) compared to men with higher cholesterol levels.[18] Of the three categories of violent death, only suicide was significantly associated with serum cholesterol level. In a Swedish cohort with a follow-up period of 20.5 years, a strong inverse relationship between cholesterol levels and mortality from injuries was observed in men during the first seven years of follow-up only.[19]

Recently, in a report of a National Heart, Lung, and Blood Institute (NHBLI) sponsored conference on Low Blood Cholesterol and Mortality Associations, 19 cohort studies were considered in a formal quantitative review.[12,13] Results from the individual studies were adjusted for differences in age, diastolic blood pressure, cigarette smoking, body mass index, and alcohol intake, when available. The risk for combined non-cardiovascular non-cancer causes of death decreased steadily across the range of total cholesterol. The risk ratio among men with serum cholesterol levels below 4.14 mmol/l was 1.32 compared to those with a cholesterol level between 4.14 and 5.15 mmol/l (no confidence interval given). This mortality category included deaths due to respiratory and digestive diseases, trauma and residual causes of death. The latter two categories showed risk ratios of 1.40 and 1.43, respectively. Exclusion of early deaths from the analysis (to account for the potential effect of preexisting illness on the entry cholesterol level and on subsequent disease relations) did not materially change the results.

In the NHANES I Epidemiologic Follow-up Study (NHEFS) the relative risk of non-cardiovascular non-cancer mortality in men with a cholesterol level below 4.1 mmol/l compared to those with a cholesterol of 4.1 to 5.1 mmol/l was 2.1 (95% CI 1.3 - 3.2) in the first 10 years of follow-up.[20] After 10 years of follow-up the relative risk decreased to 1.2 (95% CI 0.6 - 2.5).

In the Renfrew and Paisley survey total mortality was not related to cholesterol level.[21] There was a highly significant positive association between cholesterol level and mortality from coronary heart disease. However, this association was counterbalanced by inverse relations between cholesterol levels and cancer and 'other causes of death' (non-coronary heart disease non-cancer mortality). In women the association between cholesterol and 'other causes of death' was more pronounced ($p = 0.02$) than in men ($p = 0.07$).

A study among 3,000 Dutch male and female civil servants which was initiated in 1953-54 also showed an inverse relationship between serum cholesterol and 'death from external causes'.[22] In the first 15 years of follow-up a rate ratio of

3.7 (95% CI 0.8-17.2) was observed in the lowest cholesterol tertile (≤ 6.3 mmol/l) compared to the highest cholesterol tertile (≥ 7.3 mmol/l). The rate ratio decreased to 2.4 (95% CI 1.0-5.4) after 28 years of follow-up.

In summary, several non-experimental studies reported an association between 'naturally' occurring low cholesterol levels and consequent (long-term) risk of violent death. A potential hypothesis underlying this association involving dietary induced serotonin changes (figure 2) will be discussed in detail later in this review.

* *Experimental studies*

Muldoon et al. performed a meta-analysis on the effect of cholesterol lowering on total and cause-specific mortality in trials of primary prevention of coronary heart diseases.[11] Some characteristics of these trials are shown in table II.[23,24, 25,26,27,28,29] In total 24,847 men with an average age of 47.5 years were included in the meta-analysis amounting to 119,000 person years of follow-up and 1,147 deaths. The decrease in mortality from cardiovascular diseases in men receiving cholesterol-lowering intervention compared to control subjects was of borderline statistical significance (odds ratio (OR) 0.85, 95% confidence interval (CI) 0.69-1.05). Total mortality appeared not to be affected by treatment (OR 1.07, 95% CI 0.94-1.21). This was mainly due to an increase in deaths from unnatural causes in the intervention groups (OR 1.76, 95% CI 1.19-2.58). This higher proportion of mortality from accidents, suicide or violence was demonstrated in all individual studies included in the analysis. Separate analyses of dietary and drug intervention trials showed similar results.

In a subsequent pooled analysis, Davey Smith and Pekkanen [30] added the Finnish Mental Hospital study [31], a large cross-over trial of dietary intervention in hypercholesterolemic patients and results from the Expanded Clinical Evaluation of Lovastatin (EXCEL) study.[32] Moreover, they used a different method of meta-analysis. From this analysis, the estimated risk of violent death associated with dietary intervention was not significantly increased (OR 1.20, 95% CI 0.75-1.93). In the cholesterol lowering drug trials, however, an increased risk of death from injury was demonstrated in the treatment groups compared to the control groups (OR 1.75, 95% CI 1.07-2.85). This finding suggests that lowering cholesterol by drugs rather than lowering cholesterol level per se may be related to an increased risk of violent death.

The individual deaths due to accidents, violence and suicide in two primary prevention trials, the Lipid Research Clinics Coronary Primary Prevention Trial and

Table II. Trials (primary and secondary prevention) with 'unnatural' or 'nonmedical' mortality categories reported separately (only males considered).

Study	Intervention	Duration of follow-up (yrs)	Number of participants		Total number of deaths		No. of deaths from unnatural causes		p-value	Mortality category of interest	Remarks
			interv.	contr.	interv.	contr.	interv.	contr.			
PRIMARY PREVENTION											
LAVA[23]	diet	8	424	422	174	177	4	0	$p < 0.05$	trauma	> 6 yrs in trial
Minnesota[24]	diet	1.1 - 2	2197	2196	158	153	21	14	NS	external causes	
Finnish MH[30]	diet	6	902	928	188	217	13	18	NS	accidents / poisoning / violence	cross-over trial
WHO[25]	clofibrate	5.3	5331	5296	128	87	18	15	NS	accidents / violence	
		13.2			720	650	47	41	NS		post-trial included
Upjohn[26]	colestipol	1.9	548	546	17	27	2	0	NS	accidents	
LRC-CPPT[27]	cholestyramine	7.4	1906	1900	68	71	11	4	$p < 0.05$	accidents / suicide / homicide	
LRC-CPPT[28]		13.4			143	156	11	8	NS		post-trial included
Helsinki HS[29]	gemfibrozil	5	2051	2030	45	42	10	4	$0.05 < p < 0.10$	accidents / violence	
SECONDARY PREVENTION											
CDP[34]	niacin	6.2	1119	2789	277	723	8	15	NS	non-medical	
		15			582	1623	12	24	NS		post-trial included
	clofibrate	6.2	1103	2789	288	723	5	15	NS	non-medical	
		15			637	1623	11	24	NS		post-trial included
Stockholm[35]	clofibrate / nicotinic acid	5	279	276	61	82	3	1	NS	non-CVD non-cancer	

LAVA: Los Angeles Veterans Administration
Finnish MH: Finnish Mental Hospital Study
LRC-CPPT: Lipid Research Clinics - Coronary Primary Prevention Trial
Helsinki HS: Helsinki Heart Study
CDP: Coronary Drug Project

NS: non-significant ($p > 0.10$) interv.: intervention group contr.: control group

the Helsinki Heart Study, were examined in detail.[33] After considering dropouts and known risk factors such as alcohol intoxication and psychiatric histories, the authors conclude that little evidence remains to support the hypothesis that cholesterol-lowering drugs are causally related to deaths due to homicides, suicides, and accidents.

For most secondary prevention trials (trials in participants with manifest cardiovascular disease), deaths due to accidents, suicide or violence have not been reported separately. In the Coronary Drug Project, conducted between 1966 and 1975 to assess long-term efficacy and safety of lipid-influencing drugs, notably clofibrate and niacin, the 'nonmedical mortality' (including accidents, homicide and suicide) in the niacin treated group was 25 to 30% higher than in the placebo treated group both during and after the treatment phase (8/1119 vs 15/2789 and 12/1119 vs 24/2789 respectively) (table II).[34] The same tendency may have been present in the Stockholm Study among hypercholesterolemic patients with prior myocardial infarction (3/279 vs 1/276).[35] In this study, however, accidental and violent death was not distinguished from other non-cardiovascular and non-cancer deaths.

A meta-analysis of both primary and secondary prevention studies could not demonstrate a beneficial effect of cholesterol lowering on overall mortality.[36] Trials for which figures on mortality from non-medical causes were available showed an increased risk of violent death in those treated with cholesterol-lowering drugs (OR 1.55, 95% CI 1.11-2.16).

A recent meta-analysis of cholesterol lowering trials (both primary and secondary prevention trials) revealed another interesting phenomenon.[37] The trials performed in 'low-risk' populations, defined by a low risk of dying from coronary heart disease in the control group of the trial, showed an increased mortality risk in the treated compared to the control group (OR 1.22, 95% CI 1.06-1.42). In the drug and dietary trials among high risk patients cholesterol lowering significantly reduced mortality (OR 0.74, 95% CI 0.60-0.92). Mortality from causes other than coronary heart disease was increased in the drug trials (OR 1.21, 95% CI 1.05-1.39), but not in non-drug trials (OR 1.02, 95% CI 0.88-1.19). In the drug trials among lower risk patients an increased non-coronary heart disease-mortality was observed (OR 1.27, 95% CI 1.05-1.53), whereas the trials in higher risk groups did not show a significant association (OR 1.14, 95% CI 0.92-1.41). This effect modification by baseline risk is reflected in the higher death rates in the non-cardiovascular non-cancer categories in the trials reviewed by Muldoon[11], as these trials mainly comprised low risk participants.

The inconsistent results from drug and dietary intervention studies with respect to the effect on mortality from non-natural causes make it difficult to conceive a single explanation for the possible association between lowered cholesterol levels and violent death. Potentially adverse drug reactions rather than cholesterol lowering per se are implicated. Moreover, any attempt to propose a common explanation for findings in trials and observational studies should consider the different parts of the cholesterol distribution that participants in these different studies are in.

STUDIES ON LOW SERUM CHOLESTEROL LEVELS AND BEHAVIOUR

* *Animal studies*

So far, only one study addressed the association of cholesterol levels and behaviour in animals. Kaplan et al. assigned 30 male monkeys to one of two dietary conditions: a relatively high fat, high-cholesterol diet or a relatively low fat, low-cholesterol diet, both diets having the same energy content.[38] After a 22 months intervention period the serum cholesterol levels were 3.8 and 12.1 mmol/l respectively. The occurrence of 21 behavioural acts frequently exhibited by monkeys in captivity was monitored. Only contact aggression differed between the two groups, with those on the low cholesterol diet exhibiting more aggression than the animals on the high cholesterol diet. The interpretation of this study, however, is hampered by a lack of baseline data concerning behaviour of the monkeys. Moreover, it is not clear whether this study should be classified as a study on the influence of low cholesterol or of lowering of cholesterol on behaviour. In addition, it is difficult to deduce which part of the diet was responsible for the observed discrepancies, since the diets differed in more aspects than the percentage of dietary fat and cholesterol.

Some animal experiments have been conducted to study the effects of specific changes in fat composition on behaviour.[39,40,41] Effects on rat behaviour, including physical activity and learning abilities were observed and changes in fluidity and cholesterol content of cell membranes within the central nervous system were reported.

* *Human studies*

Until now, few studies have addressed the relationship between serum cholesterol levels and behaviour in humans. Although some of these studies were conducted

long ago and some others apply to specific groups only, they consistently demonstrated a tendency of a higher prevalence of aggression with lower cholesterol levels. In one of the first studies higher cholesterol levels were observed among men who were particularly adherent to social norms, who placed high value on being dependable and conscientious, and who controlled their impulses compared with men without these characteristics.[42] Virkkunen measured serum cholesterol fasting concentrations in 274 subjects with personality disorders, of whom 139 were found to have an antisocial personality.[43] The group of subjects with an antisocial personality had a considerably lower mean level of serum cholesterol compared to a group with other personality disorders and a group of normal subjects. In another study by the same author the relationship between serum cholesterol and behaviour in 280 male homicidal offenders was assessed.[44] Of these, 73 had an antisocial personality and 100 had an intermittent explosive disorder (according to criteria laid out in the Diagnostic and Statistical Manual III). Both groups had a habitual tendency to violence under the influence of alcohol. A third group did not show this tendency to violence. Under the age of 30, the first two groups showed a markedly lower level of serum cholesterol compared to the third group. In another study among 47 boys with an attention deficit disorder of whom 22 also had an aggressive conduct disorder the cholesterol level in the 22 boys with aggressive conduct disorder was clearly lower (mean 3.75 ± 0.51 mmol/l) than in the other 25 boys (mean 4.90 ± 0.61 mmol/l; $p < 0.001$).[45]

It should be noted that in most of the studies mentioned above, important potential confounders such as physical activity, and drug and alcohol use, were not taken into account. Recently, in a sample of 1,592 men and women aged 55-74 years from the Edinburgh Artery Study fasting lipid levels were related to personality characteristics as measured by the Bedford Foulds Personality Deviance Scales.[46] In this study, serum cholesterol levels were not significantly associated with aggression in men. However, serum triglyceride levels were positively related to hostile acts and a domineering attitude, especially in men ($p < 0.001$). In women, a weaker association with domineering attitude was present ($p < 0.05$).

In a study by Morgan et al., plasma cholesterol and Beck Depression Inventories (BDI) were obtained from 1,020 participants in a cohort of white men aged 50 - 89 years.[47] Among the men aged 70 years and older, categorically defined depression (BDI score $\geq$ 13) was three times more common in those with low cholesterol levels than in those with higher concentrations (5/31 vs 22/363, p =

0.03). The inverse relationship between BDI scores and plasma cholesterol persisted after adjustment for several possible confounders such as age, health status and number of chronic illnesses.

In the Whitehall II Study hostility was measured by means of the Cook-Medley hostility scale.[48] No association between cholesterol concentration and hostility scores was demonstrated, either in male or in female participants. Other behavioural characteristics were also studied in this cohort by means of the General Health Questionnaire (GHQ).[49] Cases (defined by what the authors described as a "chronic scoring method") and non-cases showed a negligible difference as to their cholesterol level.

In a recent study the relationship between low cholesterol levels and depressive symptoms in elderly people (aged 71 and over) was assessed.[50] In both men and women severe depressive symptoms (as defined by Centers for Epidemiologic Studies' depression scale) were clearly associated with low cholesterol levels (< 4.14 mmol/l). After adjustment for several potential confounders (weight loss, drug use, physical function) however, the association weakened markedly and lost statistical significance.

Most of the studies mentioned above indicate that low cholesterol levels are associated with a tendency towards aggressive behaviour and depression. It is not clear, however, whether this association is causal. A low cholesterol level could merely act as an indicator of conditions associated with aggressive behaviour and depression.

In general, no specific attention has been paid to (changes in) behaviour in experimental studies in humans. Several cases of deterioration of pre-existent psychiatric problems and cases of a sudden occurrence of depression after the initiation of using cholesterol lowering drugs (mainly HMG-CoA-reductase inhibitors) have been reported.[51,52] The Family Heart Study included an evaluation of the behavioural changes following a 5-year dietary intervention program. They measured changes in negative emotions (including depression and aggressive hostility) by means of the Hopkins Symptom Checklist (SCL-90).[53] Participants who changed their dietary habits to a less fat-containing diet showed a significant decrease in their cholesterol levels ($p = 0.024$) compared with those who remained on a high-fat diet. In participants with a low-fat diet a significant decrease in the SCL-90 score was observed, indicating *lower* tendency towards depression ($p < 0.05$) and aggressive hostility ($p < 0.05$).

HYPOTHESES EXPLAINING THE RELATIONSHIP BETWEEN LOW/ LOWERED CHOLESTEROL LEVELS AND BEHAVIOUR

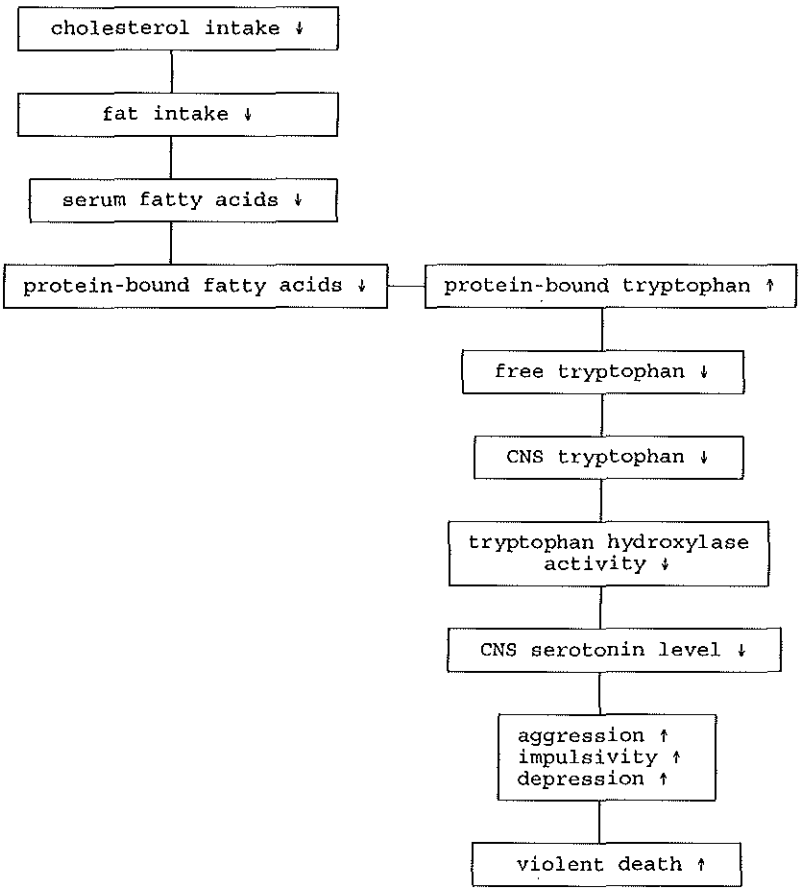
Several studies on the potentially deleterious effects of hypocholesterolemia have been conducted, mainly addressing the potential association between low cholesterol and cancer. These studies may also provide an explanation for a relationship between low cholesterol and behaviour. It has been assumed that changes in cholesterol level influence the stability and permeability of cell membranes. Marenah et al. tested this hypothesis by studying the lipid content and fluidity of blood mononuclear cells in healthy male volunteers with a wide range of cholesterol levels (3.2-10.0 mmol/l).[54] No significant differences in the cholesterol:phospholipid molar ratio of the cell membrane and relative microviscosity could be observed between subjects with high or low cholesterol levels. The investigators concluded that changes in cell membranes are unlikely to occur at serum cholesterol levels attainable by dietary or drug treatment of hyperlipidemia. In earlier studies, the influence of various cholesterol levels on the permeability of cell membranes, especially erythrocyte membranes, has been assessed in vitro.[55,56] The results of these studies suggest that changes in the cholesterol content of erythrocyte membranes in some way can influence the stability and permeability. Similar evidence is observed in recent in-vitro studies on nucleated cells, in which changes of cholesterol level are induced by using HMG-CoA-reductase inhibitors [57,58,59] or by cholesterol-rich liposomes.[60,61]

Changes in cholesterol level in these studies lead to a wide range of effects, mainly on membrane protein function and thus, in general, on membrane permeability. However, it is questionable whether the cholesterol changes in these experiments are comparable to changes expected in man. The clinical implications of the investigated changes are, therefore, unclear. Besides, it can not be excluded that the effects observed after lowering of the cholesterol level may in fact be attributable to concomitant drug-induced changes in other metabolic processes[62].

Although one could speculate that changes in membrane permeability could induce changes in tryptophan transport over the blood-brain barrier, it is not obvious that membrane permeability is strongly influenced by low serum cholesterol levels. Moreover, this 'changing membrane properties' hypothesis does not provide an easy explanation for the putative association between low cholesterol and higher risk of violent death.

On the basis of the results in the meta-analysis on low cholesterol and mortality from suicide by Muldoon[11], Engelberg proposed a mechanism for

Figure 2. Possible hypothesis underlying the relationship between low cholesterol and violent death [64]



decreased central nervous system (CNS) serotonin metabolism in subjects with decreased cholesterol levels.[63] In a reply on the original article, Salter reformulated the hypothesis as follows (figure 2).[64] A reduction in dietary cholesterol intake may reflect a reduction in overall fat intake. This results in a decrease in serum fatty acids. Since fatty acids and tryptophan (the serotonin precursor) compete for a binding site on serum albumin, more tryptophan will be bound. As the enzyme catalyzing the synthesis of serotonin (tryptophan

hydroxylase) is unsaturated with tryptophan, a decrease in free serum tryptophan will lead to a corresponding decrease in brain serotonin synthesis. Central nervous system serotonin is mainly thought of as inhibitor of depression, aggression and impulsivity. A decrease in brain serotonin levels could induce depression, aggression and impulsivity. This could eventually lead to violent death.

It should be noted that this hypothesis is mainly applicable to cholesterol that is lowered through diet. This could also provide an explanation for the putative association between a 'naturally' occurring low cholesterol levels and violent death, when supposing that the low cholesterol concentration is attained by long-term low-fat diet.

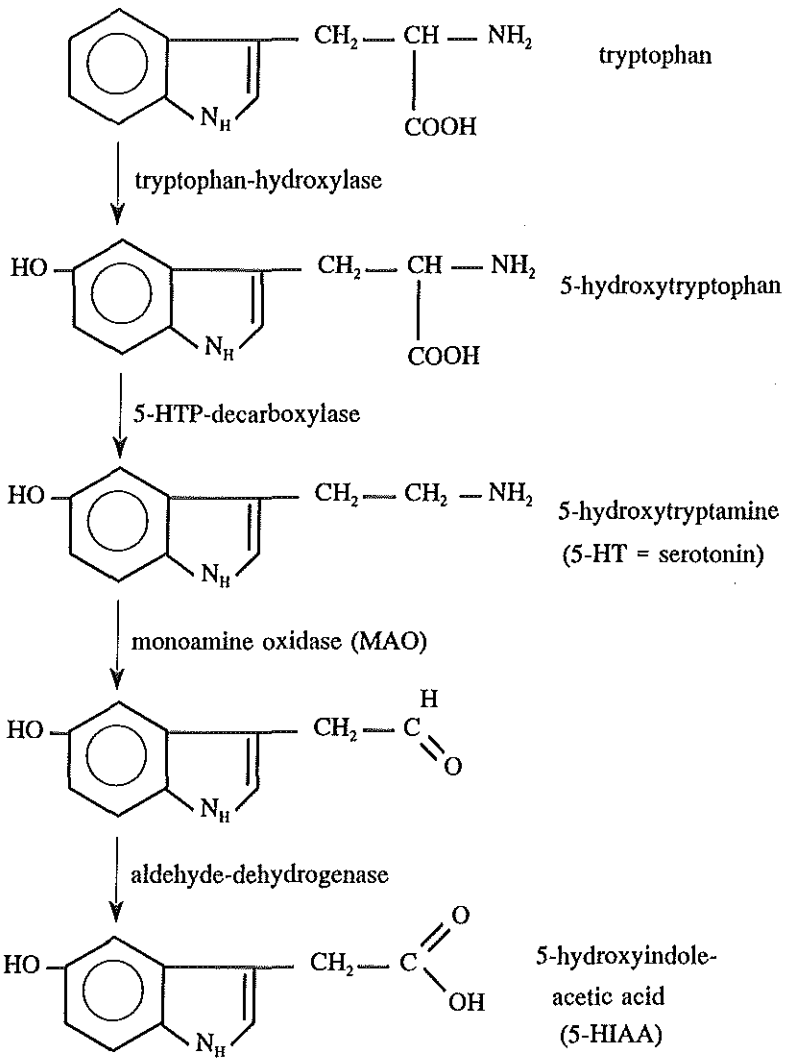
It remains to be established whether this hypothesis is applicable to the association between low cholesterol levels and violent death observed in some drug intervention studies. For drugs like cholestyramine and colestipol the bile acid binding capacity leads to less uptake of fat from the intestinal lumen. Serum triglycerides remain the same or increase. Clofibrate, gemfibrozil and the HMG-CoA-reductase inhibitors lower serum triglyceride levels. However, their influence on free fatty acids is unclear. Moreover, the way they interact with cholesterol uptake, synthesis or excretion makes it improbable that those drugs exhibit similar effects on other metabolic pathways.

SEROTONIN

* *Metabolism of serotonin*

Serotonin, or 5-hydroxy-tryptamin (5-HT), can be isolated from platelets and gastro-intestinal mucosal cells. About 85% of the body serotonin content is found in the gastro-intestinal tract, where most of the serotonin is synthesized. Another 10% is stored in platelets, which do not synthesize, but actively take up 5-HT from the blood. A small amount of 5-HT can be found in the central nervous system (CNS), where it exerts its principal functions. CNS serotonin is synthesized in the brain itself from the amino-acid tryptophan (Figure 3). Tryptophan has to pass the blood-brain barrier and the presynaptic membrane before being synthesized to serotonin. Under normal circumstances, the enzyme tryptophan-hydroxylase, which catalyzes the first step in the serotonin synthesis, is not saturated, and the synthesis rate varies according to the serum level of tryptophan. Until now, four 5-HT-receptors have been distinguished in different tissue types. The 5-HT_1 receptor is mainly observed in the CNS, the 5-HT_2 receptor in the gastro-intestinal tract and on blood platelets. Catabolism of CNS 5-HT to 5-hydroxyindoleacetic acid (5-HIAA) mainly takes place

Figure 3. Serotonin metabolism



via two enzymatic reactions mediated by monoamine-oxidase (MAO) and aldehyde-dehydrogenase. The 5-HIAA level in central spinal fluid (CSF) is frequently used as an indicator for CNS 5-HT activity. A low 5-HIAA level indicates a low level of central nervous system serotonin activity, which could result in an impaired suppression of aggression, violence and impulsivity. A frequently used indirect measure for CNS serotonin activity is the fenfluramine stimulation test. Oral fenfluramine administration causes prolactin to be excreted in the blood. Its level is considered to reflect CNS serotonin activity.

* *Studies on behaviour and serotonin or its metabolites*

The influence of central nervous system serotonin on behaviour has not been fully elucidated yet. The main function of CNS serotonin is considered to be the control of aggression and impulsivity. Since the mid seventies, the association of central nervous system serotonin activity and behaviour has received considerable attention.[65,66] Especially the relationship between serotonin and depression has been extensively investigated, as well as the association between serotonin and aggressive and impulsive behaviour. In general, a decreased CNS serotonin activity is associated with depression and impaired suppression of aggression and impulsivity.[67] Suicide is often considered as inwardly directed aggression.[68] There is growing evidence that decreased CNS serotonin activity leads to a tendency towards aggressive behaviour, rather than that it directly induces aggressive behaviour.[69] Recent findings suggest that reduced 5-HT activity is not associated with aggression in the absence of sufficient arousal.[70] This raises the possibility that a decreased 5-HT activity changes the threshold for aggressive responses to noxious stimuli rather than that it provokes aggressive behaviour.

In a study among healthy men, the influence of three amino acid mixtures on mood was assessed: a tryptophan-free (T-), a balanced (B) and a tryptophan-supplemented (T+) mixture.[71,72,73] Depression scale scores were significantly higher in the T- group, but no effect was observed on aggression.

The relationship between psychobiological variables and recidivism in criminals was determined by Virkkunen.[74] Recidivists had significantly lower CSF 5-HIAA and HVA (hydroxyvanillic acid, a dopamine neurotransmitter metabolite) concentrations compared to non-recidivists. Unfortunately, levels of cholesterol were not measured.

In normal volunteers the relationship between CSF 5-HIAA and scores on the Hostility and Direction of Hostility Questionnaire was examined.[75] A statistically significant inverse association between scores on an 'urge to act out hostility' subscale and CSF 5-HIAA levels was observed. Again, however, cholesterol levels were not measured.

In summary, the available studies on serotonin and behaviour suggest that in subjects with a tendency towards criminal behaviour or suicide, a decreased CSF level of 5-HIAA is present.

* *Studies on cholesterol and serotonin levels*

Until now only two studies have reported on the association between serum cholesterol and (indirect measures for) CNS serotonin metabolism. In a study by Muldoon et al., 9 male monkeys on a low-fat diet were compared with 10 male monkeys put on a high-fat diet.[76] After a 7 months intervention period the serum cholesterol levels were 4.0 and 10.9 mmol/l respectively. Body weight was similar in both groups. The hypothesis that a cholesterol-lowering diet reduces CNS serotonergic activity was tested by using the fenfluramine stimulation test. Prolactin response levels were significantly lower in the monkeys on a low-fat low-cholesterol diet, compared to those on the high-fat high-cholesterol diet. The frequency of aggressive behavioural acts was not assessed. The investigators concluded that among monkeys on a low-cholesterol, low-fat diet the CNS serotonergic activity and responsiveness is lower compared to monkeys on a high-fat, high-cholesterol diet. Although similar in tryptophan content, the diets differed in more aspects than cholesterol only. It is, therefore, possible that the observed differences are attributable to other dissimilarities in the diet.

In another study, the effect of cholesterol-lowering drugs on several platelet functions was evaluated.[77] This study was performed in 12 hypercholesterolemic patients treated during 12 weeks with simvastatin which reduces cholesterol synthesis through the inhibition of HMG-CoA-reductase activity. In particular, intraplatelet serotonin concentration increased towards normal values following the initiation of the drug treatment. It is not clear, however, whether platelet serotonin changes are related to CNS serotonin changes.[78]

CONCLUSIONS AND IMPLICATIONS FOR FUTURE RESEARCH

A low serum cholesterol level has been associated with elevated risks of unnatural and violent death. Some studies address the influence of 'naturally' occurring low cholesterol levels on behaviour and violent death, others address the influence of the lowering of cholesterol levels on behaviour. From an etiological point of view this is a crucial difference. The widespread use of cholesterol lowering drugs and reports of an association between low cholesterol levels and violent death justify a thorough and detailed study of this issue. The limited number of investigations performed up to date and the fact that they differ widely in scope and methods, make it difficult to draw firm conclusions. The association between low cholesterol concentrations and serotonin metabolism is still nebulous, as is the association between low cholesterol levels and behaviour. The results from lipid-

lowering trials are often viewed as evidence of increased risk of violent death associated with low cholesterol.

Cohort studies have provided important evidence of associations between low cholesterol and violent death. However, there are several disadvantages of these studies. A major limitation is that in general results are not based on a series of cholesterol measurements over time. A low cholesterol level in a subject may reflect a final stage of a decreasing cholesterol level. Thus, the population of these studies may actually consist of two different subpopulations: one with a naturally occurring, continuously low cholesterol level and one with a low cholesterol level because of a decrease of a higher cholesterol level, for example due to the development of cancer.[79] This is the main reason that in many studies the deaths occurring within the first 5 or 10 years of follow-up are excluded from the analysis.[15,20,21] In Lindberg's study the association of low cholesterol and death from suicide attenuated after the first seven years.[19] Another study, however, still observed an association after exclusion of the deaths occurring during the first 5 or 10 years.[18] It therefore remains unclear whether a chronically low cholesterol level in itself is a hazard to health. One could even doubt whether the procedure of excluding the deaths occurring in the first years after the baseline measurement is helpful in revealing associations between low cholesterol and violent death. Cancer is considered a long term process, and it is questionable whether this is a valid approach for violent death.

Assessment of the influence of low cholesterol levels on violent death in trials, however, almost always suffers from insufficient numbers of deaths in the categories of interest (suicide, accidents). A careful verification of these causes of death, however, may be very helpful in unravelling some of the mysteries in this field. Studying other endpoints, such as behaviour, in experimental studies is of clear interest. Therefore, more attention should be paid to changes in behaviour in experimental studies, which implies a baseline and follow-up assessment of tendencies towards aggression and impulsivity. Consequently, questionnaires should be validated and made suitable for use in (large-scale) clinical trials.

That the effects of a 'low' and a 'lowered' cholesterol level may be different is not hypothetical. From the point of view of homeostasis, it is more likely to have a imbalance in a certain metabolism (e.g. serotonin) in people who have their cholesterol lowered than in those with a long lasting, naturally occurring low cholesterol. The latter probably reflects a 'steady state' model. It is therefore of crucial importance to distinguish between patients with low or lowered cholesterol levels in future nonexperimental studies by using results from multiple cholesterol

levels, measured during the follow-up period. Moreover, the cholesterol levels in experimental and nonexperimental studies differ widely, the latter ones dealing with participants at the low end of the cholesterol level distribution, the former with those at the high end of the distribution. It cannot be excluded that the mechanism underlying the association between low cholesterol and behaviour or violent death may be different for the participants in these two types of study.

As for possible clues in the association between low cholesterol and violent death, it is necessary to measure those characteristics that may be related to both the cholesterol level and the occurrence of violent death. For example, in two studies a larger proportion of the participants with low cholesterol levels had diabetes compared to those with higher cholesterol levels.[80,81] In addition, a possible link of low cholesterol with hepatic synthetic function has been suggested, because of a positive relationship between cholesterol levels and albumin and factor VII levels.[82] As for these measurements, it is possible that pre- or co-existent illnesses (one of which is depression) play an important or even crucial role in the occurrence of low cholesterol. This was hypothesized by a recent analysis by Wald et al., who found no excess mortality from non-natural causes when restricting to cohorts with employed men, i.e. to healthier populations.[14]

In the only animal study until now addressing the relationship between low cholesterol levels and behaviour baseline assessment of behavioural characteristics is lacking. This, however, is necessary to interpret behaviour after intervention has taken place. Further, the dietary differences within one experiment were not restricted to the cholesterol content, and therefore other nutrients could be responsible for observed differences in behaviour. A study which combines all characteristics of previously conducted (animal) studies could yield more convincing results, as well as create a more complete picture of metabolic changes caused by intervention.

There have been many studies of the influence of cholesterol lowering drugs on metabolic processes in animals and in men. Unfortunately, similar measurements have not been reported from large-scale trials.

In view of the possible role of serotonin on the association between low cholesterol and violent death, baseline and follow-up measurements of serotonin metabolism and behaviour should preferably be included in future experimental and nonexperimental studies. This could possibly even reveal the sequence of events: Is there already a pre-existent violent death related behaviour, or is this behaviour induced by a decrease of the cholesterol level?

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Chapter 2

Correlates of chronically low cholesterol levels in healthy middle-aged men

Introduction

A chronically elevated serum cholesterol concentration is an established independent risk factor for the development of coronary heart disease, and various measures have been advocated to lower high cholesterol levels in individuals or populations in order to prevent premature cardiac death.[1,2,3,4] Consequently, chronically *low* cholesterol levels are commonly considered healthy and desirable. However, in several observational studies subjects with very low cholesterol levels appeared to be at an increased mortality risk compared to those with cholesterol levels in the normal range. This excess risk was mainly attributable to a higher incidence of violent death (suicides, accidents) in the low cholesterol group. [5,6,7,8]

Several hypotheses have been proposed to account for the findings in these studies.[9,10,11] Although studies aiming at identifying determinants of chronically low cholesterol levels could provide additional insight in this issue, only few such studies have been performed.[12,13] We compared men with low serum total cholesterol levels ($\leq$ 4.5 mmol/L) with men with cholesterol levels between 6 and 7 mmol/L to assess correlates of chronically low cholesterol concentrations. Potential determinants studied included dietary habits, drug use, chronic diseases and anthropometric measurements.

Methods

In 1990 - 1991 a large cholesterol screening study among men aged between 40 and 70 years was conducted in the Rotterdam metropolitan area. In total, 30,359 men were screened. Non-fasting blood samples were obtained and cholesterol was measured enzymatically.[14] Those men with a serum cholesterol level below the fifth percentile of the cholesterol distribution (4.5 mmol/L or lower) were invited in 1993-1994 to have a second cholesterol measurement, unless they used anti-diabetic medication or cholesterol-lowering drugs, had kidney- or thyroidal diseases, or were known to have cancer. After an overnight fast, blood samples were taken. If the cholesterol level was again 4.5 mmol/L or lower, they were selected in the study. The reference group consisted of men with a cholesterol level between the 35th and 75th percentile of the cholesterol distribution (between 6 and 7 mmol/L) in the cholesterol screening study in 1990-1991, whose cholesterol level was again between 6 and 7 mmol/L in 1993-1994 after an overnight fast. Again, those using antidiabetic medication or cholesterol lowering drugs, and those with kidney- or thyroidal diseases or cancer were excluded. Subjects from the reference group

were selected from the same age category (± 5 years) and socio-economic background, as indicated by the postal code, as those in the low cholesterol group.

Of those with a low cholesterol level during the screening survey in 1990-1991, 75% visited the research centre for a second measurement in 1993-1994. The response in the reference group was 71%. After remeasurement of cholesterol levels, 47% of the participants with cholesterol concentrations between 6 and 7 mmol/L during the screening in 1990-1991 was excluded because their cholesterol levels were lower than 6 mmol/L. Of the participants with initially low cholesterol levels, 33% was excluded after remeasurement because their cholesterol level exceeded 4.5 mmol/L.

Before a second visit to the research centre in 1993-1994, participants from both groups completed various questionnaires including questions on physician prescribed medication use and chronic diseases, and filled out a semi-quantitative food frequency questionnaire.[15] At the research centre all questionnaires were checked, a medical history was taken and a physical examination was performed by a physician.

Medication use was coded according to ATC-categories.[16] Specific chronic diseases assessed included psychiatric, pulmonary, gastro-intestinal and cardiovascular disorders. A history of psychiatric disease was defined as either a self-reported history of depression, treatment by a psychiatrist or suicide attempt. A 49 item semi-quantitative food frequency questionnaire was used. Information on frequency and mean quantity of main food categories, such as vegetables, fish, meat, soup, bread, dairy products, fruits, alcohol use, snacks, and the use of specific fats and oils, was obtained.[15] On the basis of these data mean daily intake of nutrients could be calculated. Nutrient intake was adjusted for differences in energy intake by linear regression analysis.

The physical examination consisted of measurements of height, weight, blood pressure, heart rate, skinfold thickness[17], and waist and hip circumference.

Laboratory measurements included serum albumin, HDL-cholesterol and triglyceride levels. Serum LDL-cholesterol level was calculated using the Friedewald formula.[18]

Participants were asked whether they smoked or had smoked in the past, and if so, how many cigarettes per day, the age at which they started smoking and the estimated amount of cigarettes per day in the period they smoked. Pack years were calculated as the years smoked times the mean number of cigarettes per day. One pack year was defined as 20 cigarettes per day during one year.

Differences in means were tested with two group t-tests, unless otherwise stated. Chi-square tests were used to test differences in categorical variables.

Table 1. Medication use in subjects with low cholesterol levels (≤ 4.5 mmol/L) (n=130) and those with cholesterol levels between 6 and 7 mmol/L (n=130).

	low cholesterol group	reference group	two-sided p-value
	n (%)	n (%)	
any medication	41 (32)	38 (29)	0.79
1 drug	21 (16)	16 (12)	0.48
2 drugs	10 (8)	13 (10)	0.66
3 or more drugs	10 (8)	9 (7)	1.00
cardiovascular (ATC-C)	13 (10)	12 (9)	1.00
alimentary tract (ATC-A)	8 (6)	11 (9)	0.63
respiratory system (ATC-R)	9 (7)	6 (5)	0.59
nervous system (ATC-N)	9 (7)	10 (8)	1.00
psycholeptics (ATC-N05)	6 (5)	2 (2)	0.28

psycholeptics = antipsychotics, anxiolytics, hypnotics and sedatives.
ATC = anatomical therapeutic chemical classification index

Table 2. Prevalence of chronic diseases in subjects with low cholesterol levels (≤ 4.5 mmol/L) (n=130) and those with cholesterol levels between 6 and 7 mmol/L (n=130).

	low cholesterol group	reference group	two-sided p-value
	n (%)	n (%)	
history of psychiatric diseases	7 (5)	8 (6)	1.00
depression	2 (2)	2 (2)	1.00
treated by psychiatrist	6 (5)	7 (5)	1.00
suicide attempt	1 (1)	0 (0)	1.00
pulmonary diseases	7 (5)	2 (2)	0.17
gastro-intestinal disorders	13 (10)	17 (13)	0.56
gastric disorders	9 (7)	17 (13)	0.15
intestinal disorders	4 (3)	0 (0)	0.12
cardiovascular diseases	12 (9)	5 (4)	0.13
hypertension	2 (2)	8 (6)	0.11

Results

The mean age of the 130 participants in each group was 55.1 years (SD 9.4) in the low cholesterol and 55.6 years (SD 9.1) in the reference group. 32% of the men with low cholesterol levels and 29% of those in the reference group used drugs (table 1). For most of the medication groups the differences between the two groups were negligible, but psycholeptics, notably anxiolytics (i.e. diazepam and chlordiazepoxide) were more frequently used by men with low cholesterol levels (5% vs 2%; p = 0.28).

Pulmonary, intestinal and cardiovascular diseases were somewhat more prevalent in those with low cholesterol levels, while gastric disorders and hypertension were more frequent in the reference group (table 2). However, no statistically significant differences in the presence of these chronic diseases were observed.

Table 3. Results of physical examination in subjects with low cholesterol levels (≤ 4.5 mmol/L) (n=130) and those with cholesterol levels between 6 and 7 mmol/L (n=130).

	low cholesterol group	reference group	two-sided p-value
	mean (SEM)	mean (SEM)	
height (cm)	176.7 (0.7)	176.2 (0.6)	0.57
weight (kg)	76.1 (1.1)	77.4 (0.9)	0.34
BMI (kg/m^2)	24.3 (0.3)	24.9 (0.3)	0.10
waist circumference (cm)	91.6 (0.9)	94.6 (0.9)	0.02
hip circumference (cm)	99.5 (0.5)	100.9 (0.5)	0.04
waist/hip ratio	0.92 (0.01)	0.94 (0.01)	0.04
total skinfold thickness (mm)	52.4 (1.5)	63.1 (1.5)	< 0.001
biceps (mm)	5.6 (0.2)	7.0 (0.2)	< 0.001
triceps (mm)	9.4 (0.3)	11.2 (0.3)	< 0.001
subscapular (mm)	16.1 (0.6)	19.3 (0.6)	< 0.001
suprailiacal (mm)	21.3 (0.7)	25.6 (0.7)	< 0.001
DBP (mm Hg)	84.5 (1.0)	85.5 (1.0)	0.48
SBP (mm Hg)	134.5 (1.7)	136.2 (1.5)	0.45
heart rate (min^{-1})	67 (1)	68 (1)	0.41

SEM = standard error of the mean
BMI = body mass index
DBP = diastolic blood pressure
SBP = systolic blood pressure

The results of the physical examination are shown in table 3. The most marked differences between the low cholesterol and the reference group were found for the skinfold thicknesses. In addition, both waist and hip circumference and the ratio of these two measurements were lower in the low cholesterol group. Mean body mass index was 24.3 kg/m^2 in the low cholesterol group and 24.9 kg/m^2 in the reference

Table 4. Intake of various nutrients per day in subjects with low cholesterol levels (≤ 4.5 mmol/L) (n=130) and those with cholesterol levels between 6 and 7 mmol/L (n=130).

	low cholesterol group	reference group	two-sided p-value	two-sided p-value adjusted*
	mean (SEM)	mean (SEM)		
energy intake (kJ)	11450 (300)	10970 (280)	0.24	
total protein (g)	106 (3)	104 (3)	0.64	0.37
vegetable protein (g)	39.1 (1.2)	35.0 (0.9)	0.008	0.01
total fat (g)	111.9 (3.9)	105.4 (3.5)	0.21	0.65
saturated fat (g)	43.0 (1.7)	41.1 (1.6)	0.43	0.74
monounsaturated fats (g)	39.0 (1.4)	37.4 (1.4)	0.42	0.78
polyunsaturated fats (g)	21.9 (1.0)	19.4 (0.8)	0.04	0.10
P/S ratio	0.55 (0.02)	0.50 (0.02)	0.06	
linoleic acid (g)	18.3 (0.9)	15.9 (0.7)	0.04	0.09
cholesterol (mg)	296 (12)	305 (11)	0.58	0.04
total carbohydrates (g)	301 (9)	274 (8)	0.02	0.01
mono-disaccharides (g)	152 (6)	139 (6)	0.12	0.30
polysaccharides (g)	148 (4)	134 (3)	0.007	0.01
dietary fiber (g)	21.0 (0.6)	18.7 (0.5)	0.004	0.01
water (L)	2.68 (0.07)	2.88 (0.09)	0.07	< 0.01
alcohol (g)	14.4 (1.7)	23.4 (2.3)	0.002	< 0.01
percentage of energy				
protein (%)	15.5 (0.2)	16.0 (0.2)	0.17	
fat (%)	36.4 (0.7)	35.8 (0.5)	0.47	
carbohydrates (%)	44.3 (0.7)	42.0 (0.6)	0.01	

* = adjusted for total energy intake
SEM = standard error of the mean
P/S ratio = ratio of polyunsaturated fats / saturated fats intake

Table 5. Serum lipid and albumin levels in subjects with low cholesterol levels (≤ 4.5 mmol/L) (n=130) and those with cholesterol levels between 6 and 7 mmol/L (n=130).

	low cholesterol group	reference group	two-sided p-value
	mean (SEM)	mean (SEM)	
total cholesterol (mmol/L)	3.8 (0.04)	6.6 (0.04)	*
HDL-cholesterol (mmol/L)	1.14 (0.03)	1.24 (0.03)	0.008
triglycerides (mmol/L)	1.13 (0.06)	1.84 (0.07)	< 0.001
LDL-cholesterol (mmol/L)	2.47 (0.04)	4.99 (0.05)	< 0.001
albumin (g/L)	42.8 (0.2)	43.6 (0.2)	0.004
total cholesterol/HDL-cholesterol ratio	3.56 (0.08)	5.68 (0.13)	< 0.001
LDL-cholesterol/HDL-cholesterol ratio	2.34 (0.07)	4.34 (0.12)	< 0.001

* selection of groups based on cholesterol level
HDL = high density lipoprotein
LDL = low density lipoprotein

group (p = 0.10). Although both diastolic and systolic blood pressure were lower in the low cholesterol group, the differences were not statistically significant.

Both the individual lipid values and all composite measures to estimate risk for cardiovascular disease (such as total cholesterol - HDL ratio and the HDL - LDL ratio) were considerably lower in the low cholesterol group (table 5). Albumin level was also found to be lower in subjects with low cholesterol levels.

A comparison between mean intake of several nutrients is shown in table 4. The ratio of the dietary polyunsaturated and saturated fats (P/S ratio) which is regularly used as indication for the effect of the diet on serum cholesterol levels, was higher in the low cholesterol group (mean P/S-ratio 0.55) compared to the reference group (mean P/S ratio 0.50; p = 0.06). Daily intake of vegetable proteins, carbohydrates (mainly polysaccharide intake) and dietary fiber were all higher in the low cholesterol group, while in this group a lower daily intake of water and alcohol was observed. The percentage of energy derived from carbohydrates was significantly higher in men with low cholesterol levels. Cholesterol intake was lower in the low cholesterol group than in the reference group.

Except for the proportion of never-smokers, which was considerably higher in men with low cholesterol levels (24.5%, vs 12.5% in the reference group; p = 0.02), no obvious differences in smoking habits were present between both groups.

Discussion

The most marked differences between two groups of men selected on the basis of contrasting cholesterol levels (low cholesterol group ≤ 4.5 mmol/L, and reference group between 6 and 7 mmol/L) were observed for dietary habits, notably intake of vegetable proteins, total carbohydrates, polysaccharides and dietary fiber, which were all higher in men with low cholesterol levels, and cholesterol, alcohol and water intake, which were lower in the low cholesterol group. In addition, skinfold thickness was clearly lower in the low cholesterol group.

To our knowledge, only two other studies reported on characteristics of subjects with low cholesterol levels. Franzblau and Criqui compared the lowest to the middle decile (46th to 55th percentile) of the cholesterol distribution in two age categories (30-54 and 55-79 years old). In the oldest age category, the mean cholesterol levels were 4.0 mmol/L in the lowest decile (n = 149) and 5.3 mmol/L in the middle decile (n = 140).[12] Manolio et al. investigated correlates of low cholesterol levels in the Cardiovascular Health Study by comparing men and women, aged 65 to 100 years, with cholesterol levels of 4.14 mmol/L and lower with those with a higher cholesterol levels.[13] Results of these studies will be discussed later on.

Because of the higher risk of death from violent causes observed in several studies,[5,6,7,8] we specifically examined the use of psychotropic drugs. Although no difference in the prevalence of psychiatric history was observed between the groups, the use of psycholeptic drugs was somewhat more prevalent in the low cholesterol group. This could be of importance in view of the supposedly higher risk of violent death in those with low cholesterol levels (see chapter 1).

The proportion of energy delivered by carbohydrates was higher in the group with low cholesterol levels, as was the poly-unsaturated - saturated fat intake ratio, which may both be partly responsible for the difference in cholesterol levels between the two groups.[19,20] Another possible explanation is the difference in the vegetable protein intake, which was considerably higher in the low cholesterol group. It is known that increased vegetable protein intake leads to decreased cholesterol levels.[21,22,23] In addition, fiber intake was higher in the low cholesterol group, and this may further shed light on the mechanisms resulting in lower serum cholesterol levels.[24,25,26] Alcohol intake was markedly higher in the reference group, which is reflected by the higher HDL-cholesterol levels in this group.[27,28,29] Our finding of a lower cholesterol intake in those with low serum cholesterol concentrations is of particular importance in view of the serotonin mechanism proposed to be involved in the low cholesterol-associated increased risk

of depression and violent death, where low cholesterol intake is the initial step in the causal pathway (see chapter 1).[9,10] However, energy adjusted fat intake, another step in this hypothesis, was similar in the two groups.

In a cross-sectional sample of Framingham males, aged 37 to 70, dietary determinants of serum cholesterol were investigated.[30] Positive associations with serum cholesterol levels of fat, cholesterol and protein intake were observed. Inverse associations with serum cholesterol levels were found for total and simple carbohydrate (= mono- and disaccharides) intake. Complex carbohydrates (= polysaccharides) showed a marginal correlation with serum cholesterol levels. Except for the higher protein intake in subjects with higher cholesterol levels, these results are quite similar to those observed in our study.

As expected, serum triglyceride, HDL-, LDL-cholesterol and the total cholesterol / HDL-cholesterol and LDL-cholesterol / HDL-cholesterol ratios were all significantly lower in the group of men with low cholesterol levels. These results are in accordance with earlier studies. Manolio et al.[13] obtained similar results for the lipid levels, and Franzblau and Criqui[12] found a large difference in (12 hour fasting) triglyceride levels (1.16 mmol/L in those in the lowest and 1.47 mmol/L in the middle decile; $p < 0.01$).

Manolio et al. reported low cholesterol to be associated with lower levels of hemoglobin and factor VII, and they suggest that this may be linked with hepatic synthetic dysfunction. Although we did not measure these two parameters, the lower plasma amino acid levels we observed in the low cholesterol group (see chapter 3) is in conflict with their findings, because hepatic dysfunction would raise amino acid levels. A continuous inverse relation between total cholesterol and factor VII activity in elderly subjects was recently reported from the Rotterdam Study.[31]

In conclusion, there are indications that men with low cholesterol levels differ in several aspects from those with a cholesterol level between 6 and 7 mmol/L, notably with respect to dietary habits. These factors may play a role in the way these low cholesterol levels are achieved. Whether the observed differences play a causative role in the reported relationship between low cholesterol levels and the risk of depression or violent death remains to be established.

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Chapter 3

Lower levels of tryptophan and other large neutral amino acid levels in men with low cholesterol levels

Introduction

From several large follow-up studies associations have been reported between chronic low cholesterol levels and mortality caused by violence, in particular suicide.[1,2,3] A low serum cholesterol and this specific mortality category are not necessarily causally related. However, it has been suggested that low cholesterol levels may be mechanistically involved in the increased mortality from violent causes. Engelberg postulated a role for the neurotransmitter serotonin in this relationship and Salter elaborated on this.[4,5] According to these views, low cholesterol levels may be accompanied by decreased fatty acid levels in serum. Fatty acids are bound to serum albumin, as is most of the serotonin precursor tryptophan, a large neutral amino acid. When fatty acid levels decrease, more tryptophan will be bound to albumin, and serum free tryptophan levels will decrease. As a consequence, there is less supply of this amino acid to the brain, where it is used to synthesize serotonin. Low brain serotonin levels have been observed in depression and suicide[6,7], and in impulsive and aggressive behavior.[8]

Tryptophan transport across the blood-brain barrier is mediated by a carrier within the brain capillary wall, which is also used by the other large neutral amino acids (tyrosine, phenylalanine, valine, leucine and isoleucine). The availability of tryptophan to the brain is in general thought to be reflected by the tryptophan-ratio, i.e. the ratio of the total tryptophan level to the sum of the other large neutral amino acids.[9,10]

To our knowledge, no studies on tryptophan levels and its relationship with cholesterol levels in humans are available. We compared levels of tryptophan and other large neutral amino acids and the calculated tryptophan-ratio in healthy middle-aged men with low serum cholesterol levels (4.5 mmol/L or lower) and a reference group of men with cholesterol levels between 6 and 7 mmol/L.

Methods

A cholesterol screening study among 30,359 men, aged 40 - 70 years, was conducted in Rotterdam in 1990-1991. Non-fasting blood samples were obtained and cholesterol was measured enzymatically.[11] Those men with a serum cholesterol level below the fifth percentile of the cholesterol distribution (4.5 mmol/L or lower) were invited to have a second cholesterol measurement, unless they used antidiabetic medication or cholesterol-lowering drugs, or had kidney- or thyroidal diseases or prevalent cancer. After an overnight fasting, blood samples were taken. If the cholesterol level was again 4.5 mmol/L or lower, they were selected in the

study. The reference group consisted of men with a cholesterol level between the 35th and 75th percentile of the cholesterol distribution (between 6 and 7 mmol/L) at the moment of the cholesterol screening study, whose cholesterol level remained between 6 and 7 mmol/L after an overnight fasting. Again, those using antidiabetic medication or cholesterol lowering drugs, and those with kidney- or thyroidal diseases or prevalent cancer were excluded.

Subjects from the reference group were selected from the same age category (± 5 years) and socio-economic background, indicated by postal code, as those in the low cholesterol group. Participants from both groups completed a questionnaire including information on medication use and chronic diseases and a self-administered, semi-quantitative food frequency questionnaire [12], and were invited for an additional visit to the research center. At the center, all questionnaires were checked, a medical history was taken and a physical examination was performed by a physician.

Plasma amino acid concentrations were determined by an HPLC method using automated precolumn derivatization with o-phthaldialdehyde.[13] The tryptophan-ratio was calculated by dividing the total tryptophan level by the sum of the levels of the other large neutral amino acids. The plasma 'total' tryptophan level was used, because the affinity of the blood-brain-barrier transport molecule for tryptophan is much greater than the affinity of albumin, and thus transport to the brain of the portion of plasma tryptophan bound to albumin is almost as efficient as that of free tryptophan.[9,10]

The means of the tryptophan level, the other amino acid levels and the tryptophan-ratio in the two cholesterol groups were compared by way of two-sample t-tests or, in case of a non-normal distribution, the Mann-Whitney-U test. Multivariate linear regression analyses with amino acid levels as dependent variables were performed to adjust for potential confounders. Results of all analyses were expressed as means with standard errors of the mean (SEM). Two-sided p-values were used.

To assess whether the possibly low amino acid levels in subjects with low cholesterol concentrations could be attributed to hemodilution, serum albumin levels were measured and a multivariate linear regression analysis was performed including both albumin level and an indicator for cholesterol group (low vs reference) as independent and amino acid levels as dependent variables in the model.

Results

Table 1 shows selected general characteristics of the study population. In total, 106 men were included in each group. The mean tryptophan level in the low cholesterol group was 44.0 (SEM 0.6) μmol/L, compared to 50.0 (SEM 0.6) μmol/L in the reference group ($p < 0.001$). Reduced levels were also found for the other large neutral amino acids (table 2). Compared to the reference group, the distribution of tryptophan levels in the low cholesterol group was shifted to the left (figure 1). The other large neutral amino acids showed similar shifts in their distributions. The results of the measurements of the remaining plasma amino acids are shown in table 3. Plasma amino acid levels in the men with low cholesterol levels were lower than in the reference group. Figure 2 shows the tryptophan-ratio

Table 1. General characteristics of the study population (n=212).

	low cholesterol group (n = 106)	reference group (n = 106)	two-sided p-value
	mean (SEM)	mean (SEM)	
Age (yr)	55.1 (0.9)	55.6 (0.9)	0.75
Height (m)	176.6 (0.7)	176.0 (0.6)	0.51
Weight (kg)	76.2 (1.2)	76.6 (1.0)	0.78
Body mass index (kg/m^2)	24.4 (0.3)	24.7 (0.3)	0.38
Diastolic blood pressure (mm Hg)	84.5 (1.1)	85.8 (1.1)	0.38
Systolic blood pressure (mm Hg)	134.5 (1.9)	136.8 (1.8)	0.36
Cholesterol (mmol/L)	3.8 (0.05)	6.6 (0.05)	*
Albumin (g/L)	42.7 (0.2)	43.8 (0.2)	0.001
Smoking (%)	43	40	0.68
Energy intake (kJ/day)	11450 (340)	10880 (330)	0.23
Protein intake (g/day)	105.6 (3.6)	102.4 (3.3)	0.51
Carbohydrate intake (g/day)	300 (10)	275 (9)	0.05
Alcohol intake (g/day)	12.9 (1.7)	21.9 (2.4)	0.002
Fat intake (g/day)	113.3 (4.4)	104.2 (4.1)	0.13

* selection of groups based on cholesterol level

Figure 1. Distribution of plasma tryptophan concentration in men with low cholesterol levels (≤ 4.5 mmol/L) (n=106) and in men in the reference group (cholesterol levels between 6 and 7 mmol/L) (n=106).

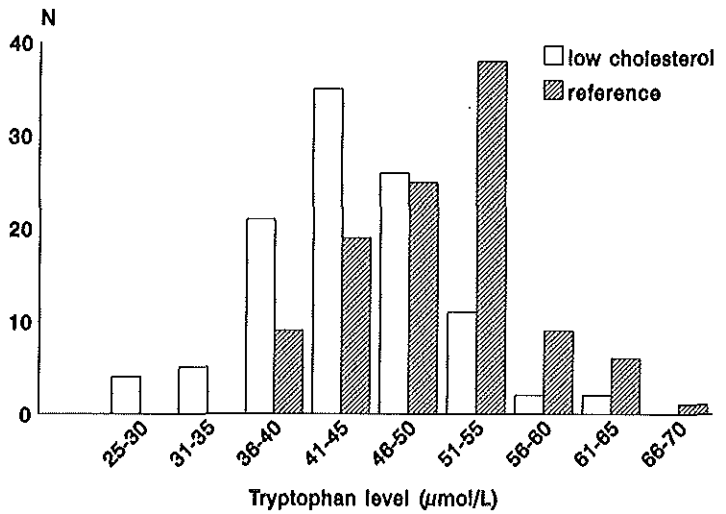

Figure 2. Distribution of the tryptophan-ratio in men with low cholesterol levels (≤ 4.5 mmol/L) (n=106) and in men in the reference group (cholesterol levels between 6 and 7 mmol/L) (n=106).

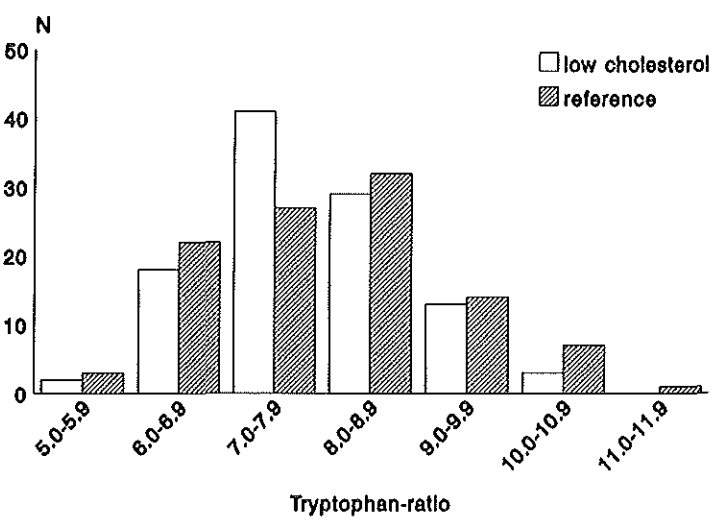

Table 2. Large neutral amino acid plasma levels and tryptophan-ratio in men with low cholesterol levels (≤ 4.5 mmol/L) (n=106) and in men in the reference group (cholesterol levels between 6 and 7 mmol/L) (n=106).

	low cholesterol group	reference group	two-sided p-value
	mean (SEM)	mean (SEM)	
Tryptophan (μmol/L)	44.0 (0.6)	50.0 (0.6)	< 0.001
Phenylalanine (μmol/L)	54.5 (0.6)	60.9 (0.6)	< 0.001
Isoleucine (μmol/L)	75.1 (1.2)	81.3 (1.6)	0.002
Leucine (μmol/L)	120.8 (1.6)	142.3 (2.2)	< 0.001
Tyrosine (μmol/L)	59.3 (1.1)	66.6 (1.0)	< 0.001
Valine (μmol/L)	260.2 (3.7)	282.5 (4.7)	< 0.001
Tryptophan-ratio*	7.76 (0.10)	7.99 (0.12)	0.12

* ratio of tryptophan and the sum of tyrosine, valine, isoleucine, leucine, and phenylalanine

in the two groups. The mean tryptophan-ratio in the low cholesterol group was 7.76(SEM 0.10), compared to 7.99 (SEM 0.12) in the reference group. Although this difference was not statistically significant (p = 0.12), a shift towards the left is observed here as well. Adjustment for serum albumin levels in a multivariate linear regression analysis did not materially change the results.

Discussion

We observed lower levels of tryptophan and other amino acid levels in a group of healthy men with low cholesterol levels (4.5 mmol/L or lower) compared to a reference group of men with cholesterol levels between 6 and 7 mmol/L. The tryptophan-ratio did not differ significantly between the two groups, although a tendency to a lower ratio in men with low cholesterol levels was present.

To our knowledge, no other studies investigating the relationship between cholesterol levels and tryptophan or other amino acid levels in humans are available. Importantly, the mean amino acid levels in our reference group were comparable to levels reported for the general population.[13] Several explanations may account for the lower levels of tryptophan and tryptophan-ratio observed in the low cholesterol group.

Table 3. Other plasma amino acid levels in men with low cholesterol levels (≤ 4.5 mmol/L) (n=106) and in men in the reference group (cholesterol levels between 6 and 7 mmol/L) (n=106).

	low cholesterol group	reference group	two-sided p-value
	mean (SEM)	mean (SEM)	
asparagine (Asn)	51.0 (0.8)	54.7 (0.8)	0.001
serine (Ser)	96.7 (2.0)	100.4 (1.4)	0.14
glycine (Gly)	185.5 (3.6)	197.5 (4.2)	0.03
threonine (Thr)	134.7 (2.6)	137.5 (2.3)	0.39
citrulline (Cit)	37.9 (0.9)	42.7 (0.8)	< 0.001
arginine (Arg)	74.0 (1.6)	81.6 (1.4)	< 0.001
alanine (Ala)	324.5 (7.4)	374.8 (7.4)	< 0.001
taurine (Tau)	40.5 (0.7)	46.0 (0.7)	< 0.001
methionine (Met)	27.5 (0.4)	29.0 (0.4)	0.003
lysine (Lys)	149.1 (2.5)	180.2 (2.6)	< 0.001
ornithine (Orn)	62.9 (1.3)	72.8 (1.5)	< 0.001
aspartic acid (Asp)	4.5 (0.5) (n = 105)	4.3 (0.2) (n = 106)	0.76
glutamic acid (Glu)	49.2 (1.6) (n = 105)	59.1 (2.2) (n = 106)	< 0.001
glutamine (Gln)	541.6 (6.2) (n = 103)	603.7 (9.8) (n = 47)	< 0.001
histidine (His)	69.5 (0.9) (n = 102)	83.3 (2.0) (n = 25)	< 0.001

The results are compatible with the hypothesis proposed by Engelberg and Salter.[4,5] According to their view lower cholesterol levels are accompanied by a reduced tryptophan availability to the brain and thus may lead to a lower brain serotonin level. The lower plasma level of tryptophan and the, although less pronounced, decreased availability of tryptophan to the brain (reflected by the tryptophan-ratio) in the low cholesterol group are important features, that form the essence of the hypothesis.

Alternatively, the observed lower plasma tryptophan levels among men in the low cholesterol group could reflect a hemodilution effect, especially as the levels of

the other amino acids were lower as well. Adjustment for possible dilution by including albumin levels in the analysis did, however, not change the results.

Another possible explanation for the association between low cholesterol and tryptophan observed in our cross-sectional study may be that plasma amino acid levels (including tryptophan) affect cholesterol levels. This could potentially involve mediating factors such as thyroxine[14,15], insulin/glucagon ratio[16] and (inhibition of) hepatic cholesterol synthesis[17,18]. Several animal studies and one study in humans investigated the short-term effects of test meals, containing proteins from various sources (casein, soy bean, whey) or different amounts of protein on lipid levels.[14,15,16,17,18,19,20,21] Casein was shown to increase cholesterol levels, while soy bean and whey decreased cholesterol levels. Until now, specific cholesterol-lowering amino acids have not been identified, and only short-term effects of ingested proteins have been studied with a maximum follow-up period of 6 weeks.[15] In our study, the mean protein intake in the low cholesterol and reference groups was comparable, and adjustment for this intake did not influence the findings. Hence, it seems unlikely that cholesterol changes following low amino acid levels account for the results, although measurements of intake of specific amino acids were not obtained in our study.

Finally, the cholesterol-tryptophan association could be caused by impaired or suboptimal intestinal uptake of amino acids and cholesterol, leading to both low amino acid and low cholesterol levels. The number of men with intestinal diseases (e.g. Crohn's disease), however, was small (n=4) and they were all in the low cholesterol group. Thus, this can not explain the large differences in plasma amino acid levels between the two groups. In addition, no difference in defaecation frequency, a possible indicator of intestinal diseases or problems, between the two groups was present.

In conclusion, we observed lower plasma levels of tryptophan and all other large neutral amino acids, and a tendency to lower values of an index of the tryptophan availability to the brain in men with low cholesterol levels compared to a reference group of men with cholesterol levels between 6 and 7 mmol/L. These results merit further investigation, and provide support for the hypothesis that changes in central serotonin metabolism may underlie the reported association between low cholesterol levels and violent death.

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Chapter 4

Low cholesterol and serotonin metabolism in men

Introduction

Recent findings in a number of studies have suggested a relationship between low serum cholesterol levels and risk of death from violent causes, notably suicide.[1,2] To explain a possible association between low cholesterol and violent death a role for serotonin metabolism was suggested by Engelberg, and subsequently elaborated by Salter.[3,4] According to these views low cholesterol levels may be accompanied by a decrease in serum free tryptophan levels. As a consequence there is less supply of this amino acid to the brain, where it is used to synthesize serotonin. Low serotonin levels have been observed in depression and suicide. To our knowledge, no studies have been reporting on serotonin metabolism and its relationship with cholesterol levels in humans.

We compared indices of serotonin metabolism in healthy middle-aged men with low serum cholesterol levels (4.5 mmol/L or lower) with a reference group with cholesterol levels between 6.0 and 7.0 mmol/L.

Methods

A cholesterol screening study among 30,359 men, age 40 - 70 years, was conducted in Rotterdam in 1990-1991. Non-fasting blood samples were obtained and cholesterol was measured enzymatically. Those men with a serum cholesterol level below the fifth percentile of the cholesterol distribution (4.5 mmol/L or lower) were invited to have a second cholesterol measurement in 1993-1994, unless they used anti-diabetic medication or cholesterol-lowering drugs, or had kidney- or thyroidal diseases or cancer. If the overnight fasting cholesterol level was again 4.5 mmol/L or lower, they were selected in the study. The reference group consisted of men with a cholesterol levels between the 35th and 75th percentile of the cholesterol distribution (between 6 and 7 mmol/L) at both occasions. Subjects from the reference group were selected from the same age category (± 5 years) and socio-economic background, indicated by postal code, as those in the low cholesterol group.

Plasma and platelet serotonin levels were measured using a reversed phase HPLC method with electrochemical detection. In addition, Kd (dissociation constant, in nmol/L) and Bmax (maximal number of binding sites for serotonin on platelets, in fmol/mg protein) were assessed.

Plasma and platelet serotonin levels were measured in 100 subjects in each group, and serotonin binding parameters were measured in a random subsample of 50 subjects of each group. Differences in means were analysed with two-sample t-

tests, except for skewed distributions, where non-parametric tests (Mann-Whitney) were used.

Results

Table 1 provides general characteristics and serotonin measures of the two groups of men. The plasma serotonin distribution in the men with low cholesterol levels was shifted towards the left, with a mean difference compared to the reference group of 1.8 nmol/L (p = 0.01). No apparent differences were present for the other parameters of serotonin metabolism.

Table 1. General characteristics and serotonin measures of the study population (n=200).

	low cholesterol (n = 100)	reference (n = 100)	two-sided p-value
	mean (SEM)	mean (SEM)	
Age (yrs)	55.0 (1.0)	54.9 (1.0)	0.97
Body mass index (kg/m^2)	24.5 (0.3)	24.6 (0.3)	0.77
Diastolic blood pressure (mm Hg)	85.7 (1.1)	85.5 (1.1)	0.89
Systolic blood pressure (mm Hg)	134.8 (1.9)	136.1 (1.8)	0.63
Cholesterol (mmol/L)	3.8 (0.05)	6.5 (0.05)	*
Smoking (%)	44	41	0.72
Energy intake (kJ/day)	11375 (350)	10850 (340)	0.29
Alcohol intake (g/day)	13.1 (1.8)	21.9 (2.5)	0.01
Fat intake (g/day)	112.6 (4.6)	104.4 (4.3)	0.20
Family history of depression or suicide (%)	8.0	8.0	1.0
Parameters of serotonin metabolism			
Plasma serotonin (nmol/L)	9.31 (0.77)	11.10 (0.72)	0.01
Platelet serotonin (nmol/10^9 platelets)	2.94 (0.10)	3.01 (0.12)	0.84
Kd (nmol/L)	0.12 (0.01)	0.12 (0.01)	0.78
Bmax (fmol/mg protein)	2103 (81)	2148 (103)	0.68

* selection of groups based on cholesterol level

Kd = dissociation constant
Bmax = platelet serotonin uptake capacity

Discussion

The results of our study suggest that plasma serotonin levels are lower in untreated men with persistently low serum cholesterol levels compared to a reference group drawn from the same non-hospitalized population.

To our knowledge, this is the first study in humans investigating a potential relationship between cholesterol levels and (indirect) parameters for central nervous system serotonin metabolism. One study in monkeys [5] showed the animals with low cholesterol levels to have lower central nervous system serotonin activity. These animals, however, had their cholesterol concentration lowered or increased during dietary intervention. The implications of their findings for persistently low cholesterol levels are, therefore, unclear.

In conclusion, our results indicate that serotonin metabolism may be different in men with low cholesterol levels. This finding lends support to the hypothesis that serotonin metabolism may be implicated in the observed association between low cholesterol levels, behavioral changes and violent death.

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Chapter 5

Depression, hostility and impulsivity in middle-aged men with low serum cholesterol levels

Introduction

Recently, a number of studies reported on the inverse relationship between cholesterol levels and death from violent causes, notably suicide.[1,2,3,4,5] It has been suggested that low cholesterol levels could lead to depression or increased risk of suicide, possibly mediated by changes in serotonin metabolism.[6,7,8] The proposed mechanism would also be responsible for the association between cholesterol level and increased aggression observed in several studies.[9,10, 11,12,13] Impulsivity has also been associated with suicide and aggression, but its relationship with low cholesterol levels has not been addressed previously.[14,15,16] In addition, serum cholesterol level has been shown to be positively associated with 'vital exhaustion'.[17,18]

So far, two cross-sectional studies have addressed the association between cholesterol level and depression in older men and in both a higher prevalence of depression was found among those with lower cholesterol levels.[19,20] In a number of short-term experimental studies among hypercholesterolemic patients, in which cholesterol levels were lowered by drugs or diet, a higher mortality from violent causes was observed in the intervention group.[21,22,23,24] It has been suggested that depression or hostility induced by lowering of cholesterol could be involved in this unexpected phenomenon.[25,26] In only one experimental (dietary intervention) study behavioral characteristics were measured and a statistically significant positive association between lowered cholesterol and depression or hostility was observed.[27]

To study the relationship between cholesterol level and depressive symptoms, aggression, impulsivity and 'vital exhaustion', we compared two groups of middle-aged non-hospitalized men: one group with a low cholesterol level (4.5 mmol/L or lower) and one group with a cholesterol level between 6 and 7 mmol/L.

Methods

A cholesterol screening study among 30,359 men, aged 40 - 70 years, was conducted in the Rotterdam metropolitan area in 1990-1991. Non-fasting blood samples were obtained and cholesterol was measured enzymatically.[28] Those men with a serum cholesterol level below the fifth centile of the cholesterol distribution (4.5 mmol/L or lower) were invited to have a second cholesterol measurement in 1993-1994, unless they used anti-diabetic medication or

cholesterol-lowering drugs, or had kidney- or thyroidal diseases or cancer. If this overnight fasting cholesterol level was again 4.5 mmol/L or lower, they were selected for the study. The reference group consisted of men with a cholesterol level between the 35th and 75th centile of the cholesterol distribution (between 6 and 7 mmol/L) at both occasions. Triglyceride levels were also measured in the fasting blood sample. Subjects from the reference group were selected from the same age category (± 5 years) and socio-economic background, indicated by postal code, as those in the low cholesterol group. Participants from both groups completed a questionnaire including information on medication use and chronic diseases and a semi-quantitative food frequency questionnaire [29], and were invited to visit the research centre. At the centre all questionnaires were checked, a medical history was taken and a physical examination was performed by a physician. In addition, questions were asked on change of appetite over the last year and on weight loss (>5% of usual body weight) in the previous six months. Alcohol use was assessed (in the food frequency questionnaire) by asking the daily number of consumptions and multiplied by a standard mean alcohol content per consumption. Specific questions were asked about the prevalence of chronic diseases, notably pulmonary, gastro-intestinal and cardiovascular disorders.

Furthermore, participants filled out six psychological questionnaires (table 2), comprising a 25-item state-trait impulsivity questionnaire (IMP, based on Eysenck) [30], a 40-item Self Expression and Control Questionnaire (SECQ, in Dutch 'Zelf-Expressie en Controle Vragenlijst'[31], based on the Spielberger Anger Expression Scale[32]), the 75-item Buss-Durkee Hostility Inventory (BDHI) [33], the 21-item Beck Depression Inventory (BDI) [34], the 23-item Maastricht Questionnaire (MQ, on vital exhaustion, in Dutch 'Maastrichtse vragenlijst') [35], and the 20-item Self Analysis Questionnaire (SAQ, in Dutch 'Zelf Analyse Vragenlijst', based on the Spielberger State-Trait Anger Scale).[36] For the Beck Depression Inventory two cut-points were used to define depression: 15 or above and 17 or above.[37] All questionnaires were in Dutch. Subjects with a total score on the vital exhaustion part of the Maastricht questionnaire of 17 or higher were considered vitally exhausted.[35] The BDHI was studied on two distinct factors, the experiential (resentment and suspicion subscales) and the expressive (assault, verbal and indirect hostility, and irritability subscales) factor.[38,39,40]

Data analysis

The mean scores on the psychological questionnaires in the two groups were compared applying the Mann Whitney U test, because of their non-normal distribution. Differences of categorical variables between the cholesterol groups were tested with the chi-square test. For those questionnaire scores with established clinical cut-points, a dichotomous variable was created. A multivariate logistic regression model was used, with the dichotomized questionnaire score as the dependent variable, and adjustment for potential confounders, such as weight loss, alcohol use and chronic diseases.

Associations of serum triglyceride levels with scores on psychological questionnaires were also investigated. In the analysis, the logarithm of triglyceride levels was used to obtain a normal distribution.

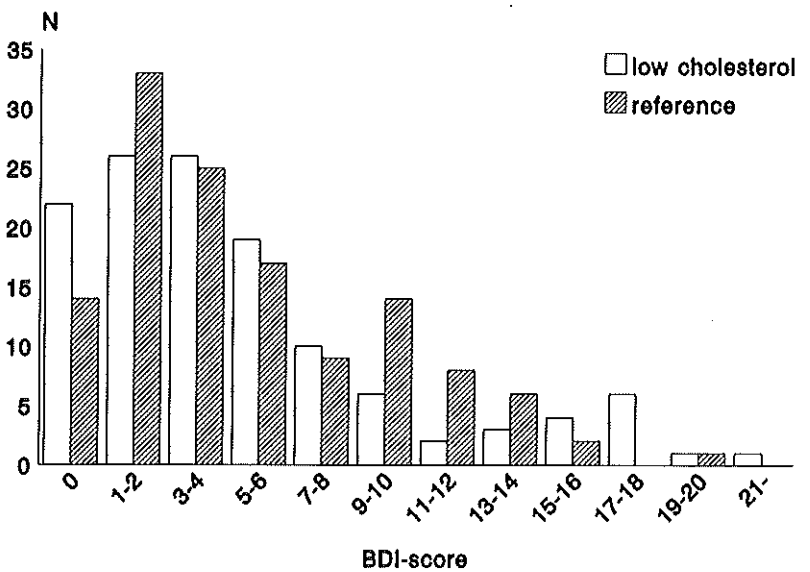
Results of all analyses are expressed as means or odds ratios (as approximations of relative risks) with 95% confidence intervals (95%CI).

Table 1. General characteristics of the study population (n=255).

	low cholesterol group (n = 126)	reference group (n = 129)	two-sided p-value
	mean (SEM)	mean (SEM)	
Age (yrs)	55.1 (0.8)	55.6 (0.8)	0.69
Height (m)	176.8 (0.6)	176.2 (0.6)	0.52
Weight (kg)	76.4 (1.1)	77.4 (0.9)	0.46
Body mass index (kg/m^2)	24.4 (0.3)	24.9 (0.3)	0.15
Diastolic blood pressure (mm Hg)	84.7 (1.0)	85.7 (1.0)	0.47
Systolic blood pressure (mm Hg)	134.9 (1.7)	136.5 (1.5)	0.48
Cholesterol (mmol/L)	3.8 (0.04)	6.6 (0.04)	*
Smoking (%)	37	45	0.22
Energy intake (kJ/day)	11450 (300)	10970 (280)	0.23
Alcohol intake (g/day)	14.7 (1.7)	23.4 (2.3)	0.003
Fat intake (g/day)	112.1 (3.9)	105.4 (3.5)	0.20
Family history of depression or suicide (%)	7.1	8.5	0.68

* selection of groups based on cholesterol level

Figure 1. Distribution of scores on the Beck Depression Inventory in men with low cholesterol levels (≤ 4.5 mmol/L) and a reference group of men with cholesterol levels between 6 and 7 mmol/L.



Results

Table 1 shows relevant general characteristics of the study population. In total, 130 men were included in each group. Data from psychological questionnaires were not available from four participants in the low cholesterol group and from one in the reference group, mainly because of language problems.

The distribution of the Beck Depression Inventory (BDI) scores in the low cholesterol and in the reference group is shown in figure 1. Twelve men in the low cholesterol group and three in the reference group had scores of 15 or higher, and eight and one, respectively, had scores of 17 or higher. When applying these cut-points of 15 or 17 to classify symptoms of depression, the relative risks of being depressed for those in the low cholesterol group compared with the reference group were 4.4 (95% CI 1.2 - 16.1) and 8.7 (95% CI 1.1 - 70.3), respectively. After adjustment for differences in age, recent weight loss, alcohol use, total energy intake, body mass index and presence of chronic diseases, these relative risks were 6.1 (95% CI 1.5 - 24.7) and 15.9 (95% CI 1.3 - 199.9).

Table 2. Scores on the psychological questionnaires included in the study

Questionnaire	score range	median low chol. group	median reference group	mean low chol. group	mean reference group	p-value (npar.)
IMP (impulsivity) [21]						
trait	11-44	17	17	17.8	17.5	0.30
state	14-56	17	17	19.0	18.5	0.38
SECQ (anger) [22]						
control anger-in	10-40	31	33	30.9	31.4	0.27
control anger-out	10-40	33	34	31.7	32.6	0.28
anger-in	10-40	20	19	20.1	19.9	0.60
anger-out	10-40	16	17	17.0	18.0	0.19
BDHI (hostility) [23]						
assault	0-20	5	6	5.4	6.0	0.43
indirect hostility	0-18	4	4	4.2	3.9	0.46
irritability	0-22	8	8	7.9	7.7	0.79
negativism	0-10	5	5	5.1	5.2	0.52
resentment	0-16	2	2	3.6	3.2	0.58
suspicion	0-20	6	6	6.2	5.9	0.57
verbal hostility	0-26	12.5	13	12.3	13.2	0.20
guilt	0-18	9	8	9.1	8.7	0.29
BDI (depression) [24]	0-63	4	4	5.0	5.1	0.37
MQ (vital exhaustion) [25]	0-42	4	4	6.3	6.7	0.58
SAQ (anger) [26]						
anger-state	10-40	10	10	10.9	10.6	0.27
anger-trait	10-40	14	13	14.7	14.8	0.87

IMP = Impulsivity questionnaire
SECQ = self expression and control questionnaire
(in Dutch: Zelf Expressie en Controle Vragenlijst (ZECV))
BDHI = Buss-Durkee hostility inventory
BDI = Beck depression inventory
MQ = Maastricht questionnaire
(in Dutch: Maastrichtse Vragenlijst (MV))
SAQ = Self analysis questionnaire
(in Dutch: Zelf Analyse Vragenlijst (ZAV))
chol. = cholesterol
npar. = non-parametric

In this multivariate analysis both weight loss (more than 5% in the previous 6 months) (RR = 6.9 and 18.7, for BDI cut-points 15 and 17, respectively), and lower energy intake (per 1000 kJ) (RR = 1.3 and 2.1, for BDI cut-points 15 and 17, respectively) were associated with an increased risk of depression.

The mean and median scores on the subscales of the Buss-Durkee Hostility Inventory (BDHI), the impulsivity questionnaire (IMP), the Self Expression and Control Questionnaire (SECQ), the Maastricht Questionnaire (MQ) and the Self Analysis Questionnaire (SAQ) in the two groups are shown in table 2. Results for these scales were similar in the low cholesterol and the reference group, as was the distribution of the scores. In addition, categorization of the scores on these questionnaires did not reveal any relevant differences between the low cholesterol and the reference group.

Neither the experiential nor the expressive hostility factors of the Buss-Durkee Hostility Inventory differed between the two groups. The anger-in and anger-out subscales of the Self Expression and Control Questionnaire showed a positive association with serum triglyceride levels (both subscales $p = 0.003$). No associations were observed between triglyceride levels and the scores on the other psychological questionnaires.

Discussion

In our study among men aged 40 to 70 years we observed a six-fold increased risk of depression (defined as a score of 15 or higher on the Beck Depression Inventory) among men selected on the basis of a low cholesterol level compared to a reference group with a cholesterol level between 6 and 7 mmol/L. No clear differences were found in measures of hostility, anger, impulsivity, and vital exhaustion.

In a similar study, Morgan et al. found a significantly higher risk of severe depressive symptoms (BDI score $\geq$ 13) among elderly men (70 years or over) with a low cholesterol level ($<$ 4.14 mmol/L, mean not reported) compared to those with higher cholesterol levels ($\geq$ 4.14 mmol/L, mean not reported).[19] Brown et al. used the Centers for Epidemiologic Studies' depression scale in men and women aged 71 and over.[20] In subjects of 80 years and over only, depressive symptoms were more common in those with low cholesterol levels ($<$ 4.14 mmol/L, mean not reported) compared to those with a cholesterol level of 4.14 mmol/L or over (mean not reported). However, after multivariate adjustment for self reported health, physical function and number of drugs used, this relationship weakened markedly. The Beck Depression Inventory and the Centers for Epidemiologic Studies' depression scale may differ in their ability to identify depression, although Zich et al.

in their report, indicate that the CES-D and the BDI perform similarly as screening instruments for depression.[41]

In contrast to Morgan's and Brown's studies, we neither included a self rated health measure, nor did we explicitly ask participants about their physical functioning. Adjustment for these factors in a multivariate analysis can be criticized as these factors can act as intermediates in the relationship between cholesterol and depression, and could, therefore, dilute a true existing association. In our analysis, adjustment for presence of chronic diseases (mainly gastro-intestinal disorders) did not materially change the findings.

Associations between low cholesterol and depressive symptoms were observed in the higher age categories and not in the younger age categories in the studies by Brown and Morgan. We, however, observed the same association in younger age categories. Food pattern (or changes in food pattern) as potential confounders in the relationship between a low cholesterol and depression was not investigated in the two previous studies. Adjustment for energy intake in our analyses did not change the association between low cholesterol on depression. Moreover, none of the participants stated major changes in food pattern over the last 5 years.

No clear differences in hostility between the low cholesterol and the reference group were observed in our study. In one other study various types of hostility, among which were subscales of the BDHI [12] and expressive hostility (i.e. the sum score of the assault, verbal and indirect hostility subscales), showed a significant positive association with both total cholesterol and LDL-cholesterol levels. We could not confirm this finding, which may be partly attributable to the lack of participants with high cholesterol levels in our study.

Fowkes et al. examined the relationship between serum cholesterol, triglycerides and aggression in the general population.[9] In this study the Bedford Foulds Personality Deviance Questionnaire was used. Serum triglycerides levels were positively associated with hostile acts and domineering attitudes in men, independent of age, cholesterol (total and HDL), cigarette smoking and alcohol consumption. Our findings of a positive association between serum triglyceride levels and the anger-in and anger-out scores of the Self Expression and Control Questionnaire appear to confirm the findings by Fowkes et al., although the anger-in and anger-out subscales we used may not be completely equivalent to the

'domineering attitude' and 'hostile acts' subscales of the Bedford Foulds Personality Deviance Questionnaire. The anger-trait subscale of the Self Analysis Questionnaire did not show any relationship with triglyceride levels. The mechanisms underlying the correlation between triglycerides and anger remain to be established, although some investigators have suggested that aggressive behaviour may lead to increased triglyceride levels, mediated by enhanced sympathetic drive.[42] In another study, investigating the relationship between lipid levels and anger in 18 to 30 year old young males, no significant associations between total serum cholesterol levels and anger-in and anger-out scales could be observed, but a statistically significant positive correlation between HDL-cholesterol and an anger-out subscale of the Spielberger Anger Expression Scale ($p = 0.02$) was present.[13] We could not reproduce this finding in our study (data not shown).

To our knowledge, one earlier study among 33 male subjects in their thirties investigated the relationship between cholesterol levels and vital exhaustion, showing a positive relationship between scores on the Maastricht questionnaire and cholesterol levels.[17] The different age category and distribution of cholesterol levels hamper the comparison of the two studies. We did not observe clear differences in scores on the Maastricht questionnaire between the low cholesterol and the reference group.

Although impulsivity is thought to be associated with aggression and depression, low cholesterol levels and impulsivity measured with the adapted version of Eysenck's questionnaire were not related in our study. We could not identify other studies assessing the association between cholesterol levels and impulsivity. This might be due to the lack of generally used, reliable questionnaires to assess impulsivity.

It is still unclear how cholesterol levels could influence the occurrence of depressive symptoms. It has been proposed that changes in the serotonin metabolism of the central nervous system, through decreased availability of the serotonin-precursor tryptophan is involved in this process.[6,7] The decreased availability of tryptophan to the brain may be induced by an increased binding of this amino acid to albumin, as a result of a low cholesterol-induced reduction in binding of fatty acids. Fatty acids compete with tryptophan for binding to albumin.

The association between low cholesterol and depression may have implications for the use of cholesterol lowering drugs, although one has to bear in

mind that this intervention takes place at levels at the other end of the cholesterol distribution than the cholesterol levels included in our study. In some cholesterol lowering trials mortality from suicide was significantly higher in the intervention group. If the proposed mechanism is the same for the drug-induced low cholesterol levels, suicide should be one of the causes of death which have to be examined carefully. Although the number of suicides has always been relatively small, the widespread use of cholesterol lowering drugs could quickly increase this number. Because of the different points of action of cholesterol lowering drugs it is, however, doubtful whether the mechanism through which they would increase the risk of suicide is similar. Assessment of baseline and follow-up levels of biochemical parameters with possible indicative value for depressive symptoms, such as serotonin, could shed light on this matter.

In conclusion, we observed an increased prevalence of depression among men with low serum cholesterol. This may partly explain the observed association between low cholesterol and death from violent causes. Future studies are needed to reveal the mechanisms to explain this increased risk and demonstrate its causal association with chronically low cholesterol levels.

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Chapter 6

Low serum cholesterol concentrations and the risk of violent death: a prospective study in the Netherlands

Introduction

A high serum cholesterol concentration is an established risk factor for cardiovascular diseases.[1,2,3,4,5] More recently, it has been suggested that low cholesterol levels may also be disadvantageous. Especially the possibility that low or lowered cholesterol levels are related to violent death (i.e. suicide, fatal accidents, homicide) has received much attention.[6,7,8]

The objective of our study was to determine the relationship between cholesterol levels and violent death in a 9 - 12 year follow-up study in the Netherlands.

Methods

In 1975 a follow-up study (EPOZ; Epidemiological Preventive Study Zoetermeer) was initiated in Zoetermeer, a town in the western part of the Netherlands with at that time 60,000 inhabitants. The objective was to study cardiovascular and other chronic diseases and their determinants. All 13,462 inhabitants aged 5 years and over of two districts were invited and only those above 20 years of age were considered in this report (response 75.6% (n = 6,547)). Of this cohort, 490 members (7.5%) were lost to follow-up due to migration, and because the dates of migration were unknown they were excluded from the analysis. Serum cholesterol concentrations were not available in 134 participants. Thus, the present analysis includes 5,923 participants (2,769 men and 3,154 women). The mean follow-up time was 10 years.

A detailed report on the methods applied has been published previously.[9] Serum total cholesterol concentrations were measured at the participants' entry into the study, using an automated enzymatic method.[10]

Information on the vital status and migration of the participants has been provided by the municipal authorities since the start of the study. During the follow-up period the cause of death of participants was reported to the EPOZ research centre by the general practitioners working in the area. In case the cause of death was not reported by the general practitioner, it was provided by the Netherlands Central Bureau of Statistics (CBS). All causes of death were coded according to the International Classification of Diseases (ICD-9). The causes of death of participants that died before May 1st 1987 are available.

Cox' regression analysis was applied to assess the association between cholesterol and the risk of violent death (ICD-9 990-999), using cholesterol both as a continuous and as a categorical variable. A low cholesterol level was defined as a serum concentration of 4.5 mmol/L or lower. Adjustments were made for the potential confounding effects of age and gender.

Results

At baseline the mean age of the members in the cohort was 45.1 years (SD 14.7) for men and 46.6 years (SD 16.0) for women. The mean cholesterol level was 5.8 mmol/L (SD 1.1) for both men and women. The total number of deaths up to May 1st 1987 was 576. The number of violent deaths was 22 (13 men and 9 women). The mean age at (violent) death was 44.6 years (SD 15.8); 45.1 years (SD 19.2) for men and 44.0 years (SD 9.9) for women.

Ten deaths were due to suicide (5 males, 5 females), 9 deaths caused by accidents (5 males, 4 females), 1 death due to homicide (male) and 1 death due to either suicide or an accident (male). For one violent death (male) the specific cause of death could not be retrieved.

After adjustment for differences in age and sex, the relative risk of violent death in those with a cholesterol concentration of 4.5 mmol/L or lower compared to those with a cholesterol level above 4.5 mmol/L was 2.85 (95% confidence interval (CI) 1.01 - 8.05). The relative risk for hypocholesterolemic men and women separately was 2.99 (95% CI 0.79 - 11.36) and 2.64 (95% CI 0.52 - 13.6), respectively (table 1). An increase in the cholesterol concentration of 1 mmol/L was associated with a 34% reduction in the risk of violent death (RR 0.66, 95% CI 0.43 - 1.02). The relationship between cholesterol levels and violent death was similar among the younger (20 to 45 years) and older (45 years and over) participants.

Separate analyses using death from suicide or accidents as the endpoint, yielded point estimates of the relative risks associated with low cholesterol levels that were similar to the estimates for all violent deaths combined.

Table 1. Low cholesterol concentrations and risk of violent death.

	number of violent deaths	Relative risk of violent death (95% Confidence Interval)	
		cholesterol ≤ 4.5 versus > 4.5 mmol/L	per 1 mmol/L increase in cholesterol level
men (n = 2,769)	13	2.99 (0.79 - 11.36)*	0.58 (0.29 - 1.18)*
women (n = 3,154)	9	2.64 (0.52 - 13.58)*	0.72 (0.42 - 1.24)*
total (n = 5,923)	22	2.85 (1.01 - 8.05)**	0.66 (0.43 - 1.02)**

* adjusted for differences in age
** adjusted for differences in age and gender

Discussion

In this 9 to 12 year prospective follow-up study of a cohort of 5,923 men and women we observed an increased risk of death from violent causes for those with a serum cholesterol concentration at baseline of 4.5 mmol/L or lower (RR 2.85, 95% CI 1.01 - 8.05). The risk of violent death decreased 34% per 1 mmol/L increase in the cholesterol level.

These results correspond with several earlier reports.[11,12] In the Multiple Risk Factor Intervention Trial a strong association between a low cholesterol level (4.14 mmol/L or lower) and risk of death from violent causes, notably suicide (RR = 1.62) was observed, persisting over the 12 year follow-up period. In a recent Swedish study, people with the lowest cholesterol levels (5.3 mmol/L or lower) had the highest risk of violent death (RR = 2.75). Again, the strongest association was found for death from suicide (RR = 4.22), although the relative risk was reduced after 7 years of follow-up (RR = 1.65). In our study, low cholesterol levels were not more strongly related to death from suicide than other categories of violent death. The number of deaths in these categories, however, was small and the statistical power decreased correspondingly. A recent analysis of several cohort studies and randomized trials did not show any significant relationship between low or (drug- or diet-induced) lowered cholesterol levels and death from accidents and suicide.[8]

Several hypotheses have been proposed to explain the association between low cholesterol levels and risk of violent death. In general, these are linked with drug-induced lowering of cholesterol levels. Recent meta-analyses show that dietary-induced low cholesterol levels are not clearly associated with violent deaths.[13]

In view of the relative lack of dietary or drug interventions in hyper-cholesterolemic patients at the time of initiation of our study (mid-seventies), our estimates are most likely to represent risks associated with 'naturally occurring' low cholesterol levels rather than lowered cholesterol levels. As to the hypothesis underlying an association between 'naturally occurring' low cholesterol levels and violent death, it has been postulated that low cholesterol is related to a reduced serotonin production in the central nervous system, which may induce depression and eventually lead to violent death (see chapter 1).[14,15]

In conclusion, the results from our 9 - 12 year follow-up study provide evidence that low cholesterol levels are associated with violent death.

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Chapter 7

General discussion

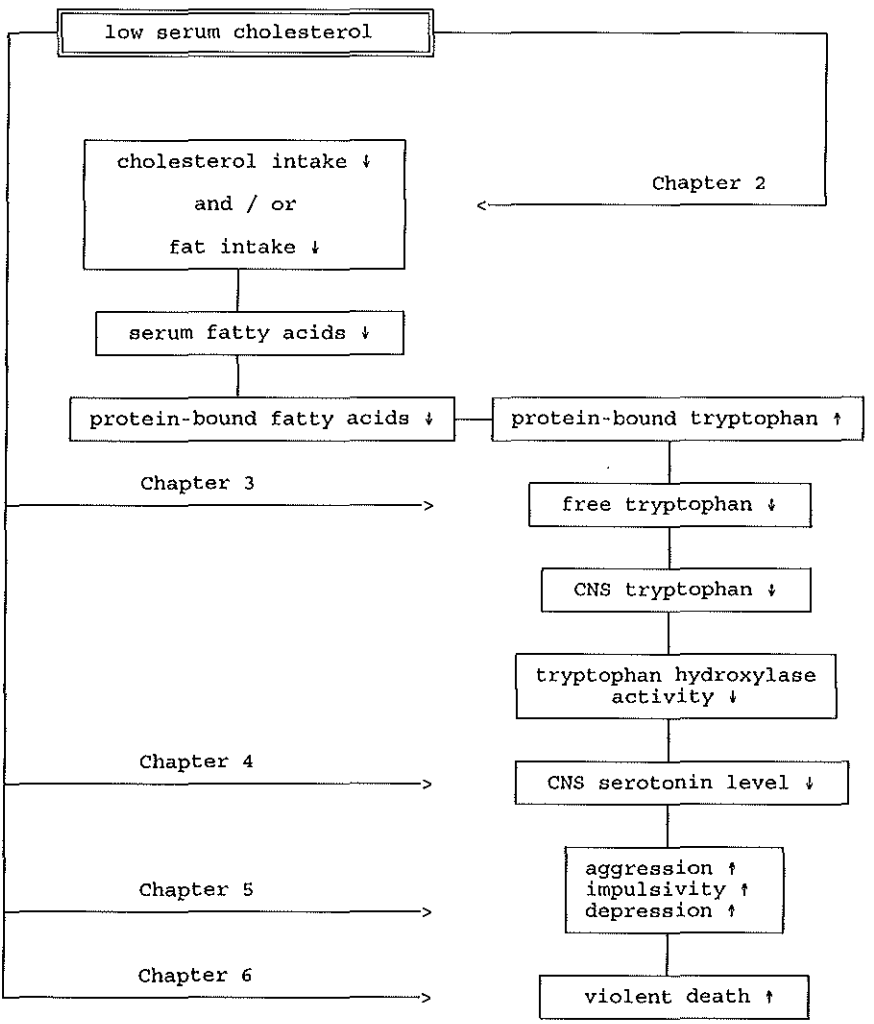
General discussion

In this thesis, studies on associations between chronically low cholesterol levels, depression and several parameters of serotonin metabolism are described in subjects who were not treated with cholesterol lowering drugs. The aim of these studies was to shed light on the mechanisms underlying the reported relationship between low cholesterol levels and the (increased) risk of mortality from violent causes (i.e. suicide and accidents). This inquiry was inspired by a hypothesis proposed by Engelberg and Salter (figure 1).[1,2] According to this view a lower cholesterol intake may be accompanied by a lower intake of fatty acids. This could lead to a drop in plasma tryptophan (the precursor of serotonin) levels, and subsequently lower central nervous system serotonin levels, which may increase the prevalence of depression, aggression and impulsivity, and eventually the risk of death from violent causes. The components of this mechanism studied in this thesis included cholesterol intake, tryptophan and other plasma amino acid levels and plasma serotonin concentrations (which were all lower in those with low cholesterol levels), measures of depression, aggression and impulsivity (higher prevalence of depression among those with low cholesterol levels), and mortality from violent causes (increased in those with low cholesterol levels). Although some of the parameters measured in our studies only indirectly address the underlying mechanism, the observed associations partially confirm the proposed serotonin hypothesis.

To our knowledge this is the first study relating various factors involved in the serotonin hypothesis (including tryptophan, plasma serotonin, depression, aggression, impulsivity) to cholesterol levels in humans. One animal study showed that lowering cholesterol levels resulted in lower central nervous system serotonergic activity and higher levels of aggression, but tryptophan levels were not measured.[3]

In a population-based cross-sectional study, we found plasma tryptophan levels to be markedly lower in a group of 130 men with low cholesterol levels ($\leq$ 4.5 mmol/L) compared to a reference group of 130 men with cholesterol levels between 6 and 7 mmol/L. The tryptophan-ratio, an indirect measure of the availability of tryptophan to the brain, was also lower in the low cholesterol group, without, however, reaching statistical significance. Interestingly, the serum levels of almost all measured amino acids were lower in the low cholesterol group, indicating that an impaired intestinal uptake in these men may be responsible for the findings.

Figure 1.


low serum cholesterol
cholesterol intake ↓
and / or
fat intake ↓
Chapter 2
serum fatty acids ↓
protein-bound fatty acids ↓
protein-bound tryptophan ↑
Chapter 3
free tryptophan ↓
CNS tryptophan ↓
tryptophan hydroxylase activity ↓
Chapter 4
CNS serotonin level ↓
aggression ↑
impulsivity ↑
depression ↑
Chapter 5
Chapter 6
violent death ↑

Preferably, central nervous system serotonin activity should be measured in spinal fluid through lumbar puncture. Since this would have greatly reduced the participation rate in these healthy, non-hospitalized men, selected plasma and platelet serotonin parameters were used to obtain estimates of the (potential) differences in serotonin activity between the low cholesterol group and the reference group. Of the four plasma and platelet serotonin parameters measured, plasma serotonin level was statistically significantly lower in the group with low cholesterol levels. One could question the use of plasma as a model for the synaptic cleft in the central nervous system. A study in psychiatric inpatients has raised doubt whether these plasma biochemical parameters provide a good representation of the processes in the brain, because there was little concordance between the central nervous system serotonin activity and plasma serotonin parameters.[4] Although it remains unclear whether the relationship between low cholesterol and plasma serotonin levels we observed is indeed a reflection of a lower brain serotonin activity, the findings are indicative for an altered serotonin metabolism in men with low cholesterol levels.

In our study of behavioral and psychological characteristics, the strongest association was observed between low cholesterol and presence of depression, as defined by the score on the Beck depression inventory, even after adjustment for potential confounders (figure 1). Scores on the other questionnaires (notably those regarding aggression and impulsivity) did not reveal clear associations with low cholesterol levels. Other studies have shown similar results, using the same or equivalent questionnaires.[5,6]

The cross-sectional design of our study limits the possibility to definitely assess the time-sequence, or time-dependence. The serotonin hypothesis assumes that a low serum cholesterol is resulting from a low cholesterol and low fat intake. We indeed observed a lower (energy-adjusted) cholesterol intake, but no difference in fat intake. Although questions were asked about dietary changes in the past (e.g. food pattern over the last 5 years, weight loss), it was impossible to assess whether, and if so how, the biochemical parameters, such as tryptophan and serotonin levels changed over time.

Although we observed differences in plasma tryptophan(-ratio) level, plasma serotonin level and depressive symptoms between the low cholesterol and the reference group, further analyses did not reveal clear relationships between these three parameters. This may be due to limitations in sample size or could indicate that only part of the hypothesis is correct. The observed associations for these biochemical measures were, however, in the expected direction.

Obviously, it is unlikely that changes in a single neurotransmitter can fully explain the putative association between low cholesterol and depressive symptoms or violent death. The complexity of the matter suggests that other, including compensatory, mechanisms are involved. Recently, a hypothesis was proposed, in which interleukin-2 (IL-2) plays an important role.[7] It is thought that oxidized low density lipoprotein induces IL-2 production, which causes both a decrease in serum cholesterol levels and a decrease in pineal gland melatonin production. The latter change would lead to depression and suicidal tendency.

In addition to a cross-sectional study, we performed an analysis of a population-based cohort study in the Netherlands with a follow-up period of 9 to 12 years and observed an increased risk for violent death of those with (untreated) low cholesterol levels. A limitation of this study is the use of a single (baseline) cholesterol measurement, although misclassification due to errors in individual cholesterol measurements is likely to bias the association towards the null. Also, no information on tryptophan or serotonin levels and depressive symptoms was available.

In summary, the findings of the studies described in this thesis suggest that serotonin may be involved in the reported association between low cholesterol levels and violent death, as hypothesized by Engelberg and Salter[1,2], although we could not confirm all parts of the suggested causal pathway (figure 1). Further investigations of components of this hypothesis and additional causal mechanisms might provide more insight in the mechanisms relating low cholesterol levels and violent death or depression.

Clinical implications

An elevated risk of depressive symptoms among men with cholesterol levels of 4.5 mmol/L or lower, compared to those with cholesterol levels between 6 and 7 mmol/L is a major finding of our study, and might have implications for clinical practice. In patients with known chronically low cholesterol levels physicians should be aware of potential depressive symptoms. It should be emphasized, however, that subjects with low cholesterol levels (i.e. $\leq$ 4.5 mmol/L) constitute only about 5% of the population and that, unless expensive large scale cholesterol screening surveys were to be implemented, the vast majority of those with low cholesterol levels remains undetected. The estimated prevalence of depression among those with low cholesterol levels ranges from 5 to 10%.

From a public health point of view, case finding and screening are usually aimed at identifying and treating patients with high cholesterol levels. If the serotonin mechanism (figure 1) also applies to those whose (high) cholesterol levels are being lowered, the potential health impact of cholesterol-related changes in serotonin metabolism may be greater than in those with chronically low cholesterol levels. Our studies do not provide data on this possibility. In the former group, two questions need to be answered. First, does lowering of (high) cholesterol levels cause shifts in central nervous system neurotransmitters? Second, if lowering of cholesterol levels in itself is without danger, what level of serum cholesterol can be considered optimal? Should the target level be as low as possible, or is a level of about 5.0 mmol/L to be preferred? The results of our study suggest that a cholesterol level below 4.5 mmol/L may have disadvantages. However, no randomized trials have been conducted to conclusively show the beneficial and adverse effects of lowering cholesterol levels to such extreme values.

Future research

We observed some distinct differences, most markedly in food pattern, between subjects in the low cholesterol and the reference groups, that could partly, though not fully, explain the difference in serum cholesterol concentrations. As for the influence of every-day dietary habits on cholesterol levels, the main obstacle is the lack of reliable dietary questionnaires. Studies in subjects on a standardized food intake may shed more light on the dietary mechanisms involved in the regulation of serum cholesterol levels. Combined food intake and -uptake studies could reveal whether cholesterol levels are affected by differences in uptake levels.

Investigations in family members of those with low (or high) cholesterol levels may give opportunities to assess to what extent genetic factors play a role in the determination of cholesterol levels.

Measurement of serum fatty acid levels might also unravel relationships with serum cholesterol levels. Biochemical assessment of the equilibrium of fatty acid and tryptophan transport on albumin is difficult, because of the strong dependence of the measurements of the relevant parameters on temperature and acidity. We, therefore, chose to assess the tryptophan ratio. Our finding that mean amino acid levels are nearly all consistently lower in those with low cholesterol levels justifies studies into the underlying mechanism. The general reduction in the amino acid levels suggests that intestinal uptake may be impaired in subjects with lower cholesterol levels. Another possibility is that a slight systemic imbalance (in e.g.

thyroid or glucocorticoid metabolism) may lead to both low cholesterol levels and lower amino acid levels. A low cholesterol level might then merely be a marker for such a minor deviation.[8,9] In addition, disturbances in these endocrine systems are known to influence human mood, although these minor changes are unlikely to lead to overt psychiatric or psychological pathology, except perhaps for depressive symptoms.

In several studies among psychiatric patients cholesterol levels have been measured, in particular of those patients with suicidal tendencies.[10,11] Multiple measurements of parameters of serotonin metabolism in these patients could further clarify the association between cholesterol levels and both depression and suicides.

To examine subsequent steps in the cascade, it is necessary to obtain central spinal fluid samples. Assessment of various substances in this fluid, such as central nervous system tryptophan, tryptophan hydroxylase, and serotonin can more easily be done in animals. Some serotonin metabolites have already been measured in monkeys[3], and these results confirm the serotonin hypothesis. Especially in these animal models, ascertainment of behavioral characteristics is difficult, but in man measurement of these characteristics is not without problems either. In particular, improvement of questionnaires measuring impulsivity and aggression would help to disentangle the postulated relationship with low cholesterol levels.

New imaging techniques, such as the PET (positron emission tomography) scan, have opened new possibilities for more direct research, and are becoming used more often in studies of metabolic processes, in particular in the central nervous system. The potential of these methods for etiological research is increasingly being recognized. In the investigations to reveal the effect of (low or lowered) cholesterol levels on mood or violent death, detection of metabolic changes in the brain by this methodology in subjects selected on the basis of their cholesterol level could provide important causal information.

As for the final step in the cascade, relating low cholesterol levels to violent death, it is of importance that in future (population-based) cohort studies all potentially violent deaths are carefully recorded and validated. Because of the diversity within this death category (that includes suicides, accidents and homicides) there is a need to examine associations with each subcategory separately. Because of its most direct link to depression, the 'suicide'-subcategory seems most important.

In conclusion, several parts in the postulated serotonin pathway, relating chronically low cholesterol levels to depression or violent death, require thorough

investigation. Specific attention should be paid to the role of potential systemic metabolic imbalances.

Because associations between, notably drug-induced, lowering of (high) cholesterol levels and death from violent causes have also been observed[12], it is possible that the same serotonin-related mechanisms may be of importance in patients with lowered high cholesterol levels. It should be stressed, however, that, in contrast to those with chronically low cholesterol levels, these patients are in the upper part of the cholesterol distribution and that it seems unlikely that, in view of the different actions of the cholesterol-lowering drugs studied, all medications would exert their influence on the risk of violent death via the same mechanism. Nevertheless, it is of interest to measure pre- and post-treatment parameters of serotonin metabolism in future trials on cholesterol-lowering drugs.

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Chapter 8

Summary

Summary

High cholesterol levels are considered disadvantageous because of their association with an increased risk of coronary heart disease morbidity and mortality. Consequently, low cholesterol levels are often thought to be beneficial. However, evidence is growing that subjects with chronically low cholesterol levels may be at an increased mortality risk also. In particular, a higher incidence of death from violent causes (i.e. suicide, accidents and homicide) in these subjects has repeatedly been reported.

Chapter 1 reviews the available evidence pertaining to the association between low cholesterol levels and violent death. It is emphasized that, in case of *lowering* of cholesterol levels, this regards the upper end of the cholesterol distribution, because it concerns subjects with initially high cholesterol levels, whereas subjects with chronically low cholesterol levels are in the lower end of the cholesterol distribution.

An increased mortality from violent causes among those with (untreated) chronically low cholesterol concentrations compared to those with higher concentrations has been reported from several studies. Among those whose (high) cholesterol level was lowered (compared to those whose levels remained high) an increased risk of violent death has also been reported, although findings are conflicting.

Recently, a hypothesis has been formulated suggesting an important role for serotonin as an intermediate factor in the cascade relating chronically low cholesterol levels to violent death. In short, a low cholesterol level will, indirectly, cause increased binding of free amino acid tryptophan (a precursor in the production of serotonin) to albumin. Therefore, less free (unbound) tryptophan will be available to the brain, leading to a decrease in brain serotonin synthesis. A reduced brain serotonin level is thought to be related to depression, aggression and impulsivity. Depressions, aggression or impulsivity, might eventually lead to suicide or accidents.

Several indications for a role of serotonin in the development of depressions can be found in the literature. Mood affecting properties of tryptophan have also been observed. Experimental behavioral studies in animals are limited, but the results of those studies published are in accordance with the hypothesis. Similar studies in humans are not available.

In chapters 2 to 5 the results of epidemiologic research into the various steps of the proposed serotonin hypothesis are presented. In these studies 130 men with

chronically very low cholesterol levels (lower than or equal to 4.5 mmol/L, below 5th percentile of the cholesterol distribution) were studied together with a reference group of 130 men with cholesterol levels between 6 and 7 mmol/L (between 35th and 75th percentile). Subjects were eligible to be included in one of the two groups if their cholesterol concentration was in the same range both during a cholesterol screening survey in 1990-1991 and after remeasurement in 1993-1994. They were all between 40 and 70 years old, and living in the Rotterdam metropolitan area. Subjects from the reference group were selected from the same age category (± 5 years) and socio-economic background, indicated by postal code, as those in the low cholesterol group.

A comparison of the low cholesterol and the reference group presented in chapter 2 showed that differences in intake of a number of nutrients existed between the two groups. This provides insight in the way these low cholesterol levels may potentially be attained. In particular, a higher intake of dietary fiber, carbohydrates and poly-unsaturated fats (relative to saturated fats) was observed in men in the low cholesterol group, compared to the men in the reference group.

Chapter 3 deals with differences concerning the amino acid tryptophan levels between the two groups. Plasma tryptophan level was significantly lower in the low cholesterol group compared with the reference group (44.0 (SEM 0.6) μmol/L vs 50.0 (SEM 0.6) μmol/L; $p < 0.001$). The availability of tryptophan to the brain (calculated as the ratio of plasma tryptophan level to the plasma level of the five other large neutral amino acids competing for the same carrier across the blood brain barrier (valine, leucine, isoleucine, phenylalanine and tyrosine)) was somewhat lower in those with low cholesterol levels (7.76 (SEM 0.10) vs 7.99 (SEM 0.12); $p = 0.12$), which is in accordance with the proposed hypothesis. Unexpectedly, the mean level of almost all measured amino acids was lower in the low cholesterol group than in the reference group. Adjustment for albumin level to account for a possible dilution effect did not change the results.

Results as to serotonin measurements in the two groups are presented in chapter 4. Rather than using central spinal fluid (which would have required a lumbar puncture), a model with thrombocytes as its main component was used as an approximation of central nervous system presynaptic neurons. Our finding of a lower mean plasma serotonin level in the low cholesterol group compared to the reference group (9.31 nmol/L and 11.10 nmol/L, respectively; $p = 0.01$) illustrates that serotonin metabolism may be different in subjects with low cholesterol levels. Platelet serotonin binding parameters and platelet serotonin levels, however, were similar in the two groups.

We assessed presence of (symptoms of) depression, aggression, impulsivity and vital exhaustion by self-administered questionnaires. The results are presented in chapter 5. The prevalence of depression (defined as a score of 15 or higher on the Beck depression inventory) was markedly higher in the group with low cholesterol levels, even after adjustment for potential confounders (age, body mass index, weight loss, alcohol use, energy intake and chronic diseases) (relative risk = 6.1; 95% CI 1.5 to 24.7). No clear differences between the groups were observed for measures of aggression, impulsivity and vital exhaustion.

Based on cholesterol measurements at entry and on mortality causes in the EPOZ follow-up study, the risk of dying from violent causes was examined in those with low cholesterol levels (lower than or equal to 4.5 mmol/L) compared to those with cholesterol levels higher than 4.5 mmol/L (chapter 6). The risk of violent death during the 9 to 12 year follow-up period for those with low cholesterol levels was higher than in the reference group (relative risk = 2.85, 95% CI 1.01 to 8.05).

In the general discussion (chapter 7) inferences from our findings are discussed and suggestions for future research are given. Although a limitation of our study is its cross-sectional nature, implicating that it is not possible to definitely conclude whether low cholesterol levels are a primary cause of the observed differences, or a secondary feature of a yet unknown cause, our findings support the view that the serotonin hypothesis is involved in the reported association between chronically low cholesterol levels and depression or violent death. Potentially important future developments and additional research include the use of new imaging techniques, genetic investigations and endocrinological studies.

Chapter 9

Samenvatting

Samenvatting

In het algemeen wordt een hoog cholesterolgehalte als nadelig beschouwd, vanwege het verband met een verhoogd risico op coronaire hartziekte en -sterfte. Een laag cholesterolgehalte wordt daarom veelal als gunstig gezien. Uit de recente medische literatuur komen echter steeds meer signalen dat personen met een langdurig laag cholesterolgehalte mogelijk ook een verhoogde sterftekans hebben. Vooral een hogere incidentie van sterfte ten gevolge van geweld (suïcide, ongevallen en moord) bij deze personen is herhaaldelijk gerapporteerd.

In hoofdstuk 1 wordt een overzicht gegeven van de beschikbare gegevens met betrekking tot het verband tussen een laag cholesterolgehalte en gewelddadige dood. Benadrukt wordt dat, als het om een *verlaging* van het cholesterolgehalte gaat, zich dit in het bovenste gedeelte van de populatie cholesterolverdeling afspeelt, omdat het daarbij personen betreft die in eerste instantie een hoog cholesterolgehalte hebben, terwijl personen met langdurig lage cholesterolgehaltes zich in het onderste deel van de cholesterolverdeling bevinden.

In verschillende onderzoeken is een verhoogde sterfte ten gevolge van geweld gevonden bij degenen met een langdurig laag (onbehandeld) cholesterolgehalte, vergeleken met degenen met een chronisch hoger cholesterolgehalte. Bij degenen bij wie het (hoge) cholesterolniveau werd verlaagd (vergeleken met degenen bij wie het cholesterol hoog bleef) is ook een verhoogd risico op gewelddadige dood gevonden, hoewel de bevindingen tegenstrijdig zijn.

Recent werd een hypothese gepubliceerd, waarin een belangrijke rol wordt toegeschreven aan serotonine als tussenstap in de cascade die lage cholesterolgehaltes in verband brengt met gewelddadige dood. In het kort komt het erop neer dat een laag cholesterolgehalte indirect leidt tot een toename in de binding van het vrije aminozuur tryptofaan (noodzakelijk voor de vorming van serotonine) aan albumine. Daardoor zal er minder van het vrije (niet gebonden) tryptofaan beschikbaar zijn voor de hersenen, wat een vermindering van de serotonine-productie in de hersenen tot gevolg heeft. Een verlaagd serotonineniveau in de hersenen wordt in verband gebracht met depressies, agressie en impulsiviteit. Depressies, al of niet in combinatie met agressie en impulsiviteit, zouden uiteindelijk de aanleiding kunnen zijn tot zelfmoord of ongevallen.

In de literatuur zijn diverse aanwijzingen te vinden voor een rol van serotonine in het ontstaan van depressies. Stemmingsbeïnvloedende eigenschappen van tryptofaan zijn ook waargenomen. Dierexperimenteel gedragsonderzoek is schaars, maar de resultaten van deze studies zijn in overeenstemming met de hypothese. Vergelijkbare studies bij mensen zijn niet voorhanden.

In de hoofdstukken 2 tot en met 5 worden de resultaten van epidemiologisch onderzoek, dat zich richtte op de verschillende stappen van de voorgestelde serotonine hypothese, weergegeven. In deze onderzoeken werden 130 mannen met een langdurig laag cholesterolgehalte (lager dan of gelijk aan 4,5 mmol/l, beneden het 5e percentiel van de cholesterolverdeling) vergeleken met een referentiegroep van 130 mannen met een cholesterolniveau tussen de 6 en 7 mmol/l (tussen het 35e en 75e percentiel). Personen kwamen in aanmerking om in een van de twee groepen te worden opgenomen, indien hun cholesterolgehalte zich zowel bij een cholesterol-screening in 1990-1991, als bij een hermeting in 1993-1994 binnen dezelfde grenswaarden bevond. Alle deelnemers waren tussen de 40 en 70 jaar oud en woonachtig in of rond Rotterdam. De groepen waren vergelijkbaar voor wat betreft leeftijd en postcode, als indicator voor sociaal-economische status.

Een vergelijking van de laag-cholesterolgroep met de referentiegroep (hoofdstuk 2) liet zien dat er verschillen waren in de inname van een aantal voedings-bestanddelen. Dit geeft enig inzicht in de wijze waarop deze lage cholesterol-concentraties mogelijk bereikt zijn. Met name werden een hogere inname van voedingsvezel, koolhydraten en meervoudig onverzadigde vetzuren (ten opzichte van verzadigde vetzuren) waargenomen bij de mannen uit de groep met laag cholesterol-gehalte, vergeleken met de mannen uit de referentiegroep.

Hoofdstuk 3 beschrijft de verschillen tussen de twee groepen met betrekking tot de concentratie van het aminozuur tryptofaan. Het plasma tryptofaangehalte was significant lager in de laag-cholesterol groep vergeleken met de referentiegroep (44,0 (standard error of the mean (SEM) 0,6) μmol/l versus 50,0 (SEM 0,6) μmol/l; $p < 0,001$). Ook de beschikbaarheid van het tryptofaan voor de hersenen (berekend als de verhouding van het plasma tryptofaan niveau met de som van de vijf andere grote neutrale aminozuren, die van hetzelfde transportmechanisme over de bloed-hersen-barrière gebruik maken (valine, leucine, isoleucine, fenylalanine en tyrosine)) was wat lager bij degenen met een laag cholesterolgehalte (7,76 (SEM 0,10) versus 7,99 (SEM 0,12), $p = 0,12$), hetgeen in overeenstemming is met de hypothese. Opvallend was de bevinding dat het gemiddelde van vrijwel alle gemeten aminozuren lager was in de groep met een laag cholesterolgehalte dan bij degenen in de referentiegroep. Correctie voor het albuminegehalte, als indicator voor een mogelijk verdunnings-effect, gaf geen verandering in de resultaten.

In hoofdstuk 4 worden de resultaten beschreven ten aanzien van de serotonine-bepalingen in de twee groepen. In plaats van liquor (dat een lumbaal-punctie zou hebben vereist), werd een model gebruikt met bloedplaatjes, als benadering van presynaptische neuronen in het centrale zenuwstelsel, als belangrijkste component.

Onze bevinding van een lagere gemiddelde plasma serotonine concentratie bij degenen met een laag cholesterol vergeleken met de referentiegroep (respectievelijk 9,31 nmol/l en 11,10 nmol/l; p = 0,01) illustreert dat het serotonine metabolisme mogelijk anders is bij personen met een laag cholesterolgehalte. De serotonine bindingsparameters van plaatjes en serotonine concentratie in plaatjes waren vergelijkbaar in de twee groepen.

Door middel van vragenlijsten, die door de deelnemers zelf moesten worden ingevuld, werd de aanwezigheid van depressies, agressiviteit, impulsiviteit en vitale uitputting gemeten in de twee groepen. De resultaten worden in hoofdstuk 5 beschreven. Depressie (gedefinieerd als een score van 15 of hoger op de Beck depressie vragenlijst) kwam beduidend vaker voor in de groep met een laag cholesterolgehalte, ook na correctie voor potentiële verstorende factoren (o.a. leeftijd, Quetelet index, gewichtsverlies, alcoholgebruik, totale energie-inname en chronische ziekten) (relatief risico 6,1; 95% betrouwbaarheidsinterval (BI) 1,5 tot 24,7). Er werden geen duidelijke verschillen in agressiviteit, impulsiviteit en vitale uitputting gevonden tussen de beide groepen.

In het Epidemiologisch Preventief Onderzoek Zoetermeer (EPOZ), een follow-up onderzoek, werd het risico op een gewelddadige dood onderzocht bij degenen met een laag cholesterolgehalte (lager dan of gelijk aan 4,5 mmol/l) in vergelijking met degenen met een cholesterolgehalte boven de 4,5 mmol/l. (hoofdstuk 6). Degenen met een laag cholesterol hadden een hoger risico op gewelddadige dood gedurende de 9 tot 12 jaar durende follow-up periode dan degenen in de referentiegroep (relatief risico 2,85; 95% BI 1,01 tot 8,05).

In de algemene bespreking (hoofdstuk 7) worden de gevolgtrekkingen van onze bevindingen besproken en aanbevelingen voor toekomstig onderzoek gedaan. Een beperking van ons onderzoek is haar cross-sectionele opzet, hetgeen met zich meebrengt dat het niet mogelijk is na te gaan of lage cholesterolgehaltes een primaire oorzaak zijn van de gevonden verschillen, of secundair zijn aan een tot nog toe onbekende oorzaak. Toch geven onze bevindingen steun aan de serotonine hypothese als mogelijke verklaring voor het aangenomen verband tussen langdurig lage cholesterol niveaus en depressie of gewelddadige dood. Mogelijk belangrijke toekomstige ontwikkelingen en aanvullende onderzoekingen zijn ondermeer het gebruik van nieuwe beeldvormende technieken, genetisch onderzoek en endocrinologische studies.

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Curriculum vitae

The author of this thesis was born on May 11, 1959. He attended secondary school (Dominicus College) in Nijmegen, which he finished in 1978. In 1979 he spent half a year in Aix-en-Provence attending a French language course. After having studied biology for one year, he began his medical training in 1980 at the Catholic University of Nijmegen. He received his medical degree in 1988 and participated in a one-year-course in epidemiology at the EMGO-institute (Free University of Amsterdam). In 1990 he started working as a medical doctor and as an epidemiologist at the Public Health Services (Gemeenschappelijke Gezondheidsdienst) Noordwest-Veluwe in Nunspeet. He started to work on this thesis in 1992 at the department of Epidemiology & Biostatistics (head: Prof. dr. A. Hofman) and the department of General Practice (head: Prof. dr. E. van der Does, in 1992 succeeded by Prof. dr. A. Prins) of the Erasmus University Medical School. In 1995 he obtained a MSc-degree in Clinical Epidemiology.