een opinion-paper over dit onderzoek beschrijven Raff en Findling dat zij speekselcortison echter niet superieur vinden aan speekselcortisol (6). Een experimentele onderbouwing wordt niet gegeven, maar de studies die zij zelf met speekselcortisol hebben gepubliceerd zijn uitgevoerd met een immunologische RIA. Om hun mening te kunnen toetsen is het dus belangrijk om te weten in welke mate hun RIA kruisreageert met cortison. Van de RIA die wij hebben gebruikt is bekend dat de antistof sterk kruisreageert met cortison. Het is dan ook verklaarbaar waarom onze cortisol-RIA qua scheidend vermogen precies tussen de speekselcortisol en speekselcortison zit die zijn bepaald met onze LC-MS/MS techniek.

Conclusie

Non invasieve speekseldiagnostiek is een belangrijke additioneel hulpmiddel om hypercortisolisme te kunnen diagnostiseren. Hierbij is het wel essentieel om onderscheid te maken tussen speekselcortisoN en speekselcortisoL. Een massaspectrometrische techniek die onderscheid maakt tussen cortisol en cortison is derhalve superieur aan de huidige immunologische speeksel cortisolbepalingen.

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Measuring LDL-cholesterol: are we doing it wrong?

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High levels of low density lipoprotein cholesterol (LDL) are correlated with atherosclerosis and coronary heart disease (CHD) (1). Therapy is based upon decreasing LDL levels < 2.5 mmol/L in patients with CHD. Statin therapy reduces LDL and is associated with a statistically significant reduction in the risk of primary and secondary cardiovascular events (2-7). LDL can be measured with several methods i.e. by utracentrifugation, by direct enzymatic measurement and by Friedewald calculation (8). According to most literature studies, LDL cannot accurately be estimated from the Friedewald equation at triglyceride concentration exceeding 4.52 mmol/L (400 mg/100 ml). Important to realize is, that these studies are based upon the comparison of the Friedewald equation to the reference method of ultracentrifugation. Most Dutch laboratories, however, estimate LDL by Friedewald equation using cut-off levels of triglycerides differing from 2.0 to even 9.0 mmol/L. In this short communication, we discuss the limitations of the triglyceride concentrations currently used in our laboratory to calculate LDL with the Friedewald equation.

Methods

We anonymously screened 362 patients who were routinely checked for LDL. Patient samples were categorized in 3 groups having triglyceride concentrations <2 mmol/L (n=100), triglycerides between 2 and 7 mmol/L (n=234) and triglycerides >7 mmol/L (n=28). Fasting blood samples were taken from patients visiting the department of clinical chemistry of the Albert Schweitzer hospital. In our routine practice, LDL is estimated from total cholesterol, HDL and triglycerides up to 4.5 mmol/L using the Friedewald formula. For this study we measured direct LDL using enzymatic methods and reagents (Olympus LDL cholesterol OSR6183, Beckman Coulter) and estimated LDL in patient samples with triglycerides up till 15 mmol/L. All measurements were performed on an Olympus AU2700 automatic analyzer (Beckman Coulter) and were calibrated using matching standards

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from Beckman Coulter (Calibrator Beckman Coulter-cholesterol OD0012). The between-day CVs were 2.0 %, 2.1 % for LDL-c at concentrations of 3.4 and 6.0 mmol/l, respectively. To calculate LDL by Friedewald the following formula was used: LDL = CHOL – HDL – (0.45 x triglycerides), with all analytes in mmol/L.

Results
234 Patient samples were measured and divided in categories of triglyceride concentrations <2.0, 2.0, 3.0, 4.0, 5.0, 6.0 and >7.0 mmol/L. Figure 1 illustrates the average LDL value in each group both estimated by Friedewald and direct measurement. Surprisingly, we found that in our population LDL derived from Friedewald is significantly underestimated at triglyceride concentrations >2.0 mmol/L. In category 2.0, the average LDL calculated by Friedwald (2.19 mmol/L) was already 28% lower (-0.87 mmol/l) than direct LDL (3.06mmol/L). The higher the triglyceride concentration, the higher the negative bias in the Friedewald formula.

Discussion
The determination of LDL is essential to the assessment of risk of cardiovascular disease, and the treatment of dyslipidemias mostly is based on strategies reducing LDL concentration. The concentration of LDL that is determined from direct measurement or estimation is of crucial importance, as international guidelines use a LDL < 2,5 mmol/L as decision point for optimal lipid-lowering therapy. So, how reliable is our LDL at (mild) hypertriglyceridemia? In fact, as Dutch laboratories use cut-off levels of triglycerides ranging from 2.0 to 9.0 mmol/L, LDL is definitely not a standardized method throughout the country. Here, we show that the estimated LDL by Friedewald is already significantly underestimated at triglyceride concentration of ≥ 2.0 mmol/L. This a serious problem, as patients with (mild) hypertriglyceridemia are currently undertreated and are probably at higher risk of developing coronary heart disease. Furthermore, a complicating issue is the fact that the method of LDL analysis cannot always be derived from literature studies underlying internationally accepted guidelines, although it seems that most studies are based on direct measurement of LDL. In practice, we see many patients on statin therapy having triglycerides of 2,0-4,0 mmol/L. As we show thatcalculated LDL by Friedewald is already significantly underestimated at triglyceride concentration of 2.0 mmol/L, it is our opinion that this is unacceptable as it has direct implications for patient treatment. Currently, we investigate the used LDL methods underlying international guidelines in more detail. Furthermore, we are performing a multicentre study to investigate performance on other platforms of both direct LDL measurement and the Friedewald formula in patients with (mild) hypertriglyceridemia. It is our intention to publish these results soon.
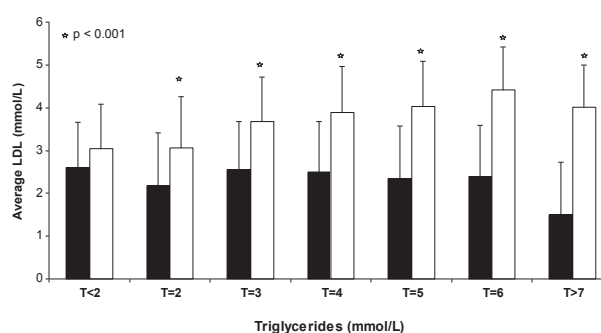

Figure 1. Comparison of LDL-c (average) directly measured or estimated by the Friedewald formula. Differences between direct LDL and estimated LDL by Friedewald are already significant at T>2.0 (P<0.001). ■ LDL Friedewald; □ LDL gemeten.

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