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Diurnal patterns of the concentrations of cholesterol, triglycerides, glucose, nonprotein nitrogen and urea in the serum of veal calves fed a milk replacer supplemented with cholesterol

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Summary

Veal calves (aged about 9 weeks; three animals per group) were fed twice a day liquid diets containing 60 % skim milk powder and 20 % crude fat (w/w) for 7.5 weeks. Addition of 1 % cholesterol to the diet, at the expense of fat, caused a 70 % increase in the level of serum cholesterol. Both in the control and cholesterol-fed calves, no diurnal rhythm in the concentration of cholesterol in the serum was observed. Serum triglycerides were not clearly elevated after feeding, but a steady increase during the day was observed, the increase being similar in both dietary groups. Cholesterol feeding seemed to induce lower postprandial levels of urea and higher levels of amino acid nitrogen, but this was only observed after feeding in the morning (8.00 a.m.), but not after feeding in the evening (8.00 p.m.). Dietary cholesterol significantly elevated postprandial glucose levels in veal calves. It is suggested that hypercholesterolemia effects an impaired glucose tolerance, possibly also in man.

Zusammenfassung

Mastkälber (ca. 9 Wochen alt; 3 Tiere pro Gruppe) wurden zweimal pro Tag während einer Periode von 7,5 Wochen mit einem Milchersatzmittel, das 60 % Magermilchpulver und 20 % Fett (Gewichtsprozente auf trockener Stoffbasis) enthielt, gefüttert.

Beim Ersatz von 1 % Fett durch Cholesterin in der Diät stieg der Serumcholesteringehalt um 70 %. Sowohl bei den Kontrolltieren als auch bei den cholesteringefütterten Kälbern wurde kein Tagesrhythmus der Serumcholesterinkonzentration beobachtet. Die Serumtriglyceriden waren nach dem Füttern nicht deutlich erhöht, es wurde jedoch bei beiden Diätgruppen während des Tages eine ähnliche, gleichmäßige Erhöhung beobachtet.

Cholesterin in der Diät verursachte nach dem Füttern um 8 Uhr eine Senkung der Harnstoffkonzentration im Serum und eine Erhöhung des Aminosäurestickstoffgehaltes. Dies wurde jedoch nach dem Füttern um 20 Uhr nicht beobachtet. Nahrungscholesterin erhöhte signifikant den postprandialen Glukosespiegel in den Mastkälbern. Es wird vorgebracht, daß Hypercholesterinämie eine verminderte Glukosetoleranz verursacht, möglicherweise auch beim Menschen.

Key words: dietary cholesterol, calves, serum metabolites, glucose tolerance

Introduction

Several studies have shown that the young bovine is an excellent model animal for study of human atherosclerosis and regulation of serum cholesterol concentration (9, 10, 19, 20). Calves fed high levels of cholesterol develop hypercholesterolemia and high liver cholesterol. Furthermore, atherosclerotic laesions are readily introduced in the arterial tissues (9, 10, 19, 20).

Cholesterol enrichment of cell membranes has been shown to occur in an experimental model of atherosclerosis in which guinea pigs were fed cholesterol (12, 17). Similar changes in membranes can be observed in some patients with liver cirrhosis (4). An increased cholesterol content of erythrocyte membranes decreases membrane fluidity and alters the permeability of several blood constituents such as glucose, glycerol and propionate (6, 13).

The present study was carried out to see whether cholesterol feeding of calves influences, possibly through altered membrane function, the postprandial levels of cholesterol, triglycerides, glucose, nonprotein nitrogen and urea in the serum. At the same time, this study may teach us something about possible metabolic disorders associated with hypercholesterolemia.

Materials and methods

Animals and diets

In this experiment, male Dutch Friesian-Holstein calves were used. The calves were purchased at a market at the age of about one week. The initial body weight of the calves was 51.1 ± 1.5 kg (± 1 SD). The calves were housed individually in wooden boxes with slatted floors. The calves were pail-fed twice a day (at 7.00 a.m. and 5.00 p.m.) a reconstituted milk replacer.

On arrival in the calf house, the animals were raised for 8 weeks on a commercial milk replacer (Trouw & Co., N.V., Putten, The Netherlands) containing 60 % skim milk powder and 17 % crude fat. The milk replacer was reconstituted in hot water to a concentration of 125 g/l. As is common practice, on arrival the animals received 1.5 l of this mixture, this amount being gradually increased to 7.5 l at 8 weeks. Then, at the end of this pre-experimental period, on the basis of their body weights and serum cholesterol concentrations, three animals were allocated to the control diet and three other animals to the cholesterol containing diet. The composition of the diets is given in table 1. The body weight of the calves at the end of the pre-experimental period was 95.7 ± 2.0 and 95.8 ± 3.7 kg (± SD) of the control and cholesterol-fed groups, respectively. The serum cholesterol values were 4.20 ± 0.46 and 4.68 ± 0.37 mmol/l, respectively.

During the experimental period, which lasted 7.5 weeks, the feeding level was increased from 940 g feed per meal in the 1st week to 1200 g in the 7th week. From three days before blood sampling, the calves were fed at 8.00 a.m. and 8.00 p.m. in order to allow them to become accustomed to feeding times 12 h apart. This feeding schedule was expected to cause a symmetrical pattern of the levels of serum components during the day. All calves consumed all their feed within 10 min.

Table 1. Composition of the experimental diets[1]).

	Control diet	Cholesterol-enriched diet
Ingredient	(g/kg)	
Fat blend	194.7	185.3
Skim milk powder	605.2	605.2
Whey powder	160.6	160.6
Vitamin/mineral premix	39.5	39.5
Cholesterol[2])	–	9.4
Chemical analysis	(weight %)	
Moisture	3.1	3.3
Ash	6.2	6.2
Crude protein	22.8	22.7
Crude fat	19.6	20.0
Carbohydrates[3])	48.3	47.8
Cholesterol	0.051	0.953

[1]) Supplied by Trouw & Co., N.V., Putten, The Netherlands.
[2]) Purchased from Van Schuppen-Chemicals, Veenendaal, The Netherlands.
[3]) Calculated values.

Chemical analyses

At the end of the experimental period, blood samples were taken at the indicated times (fig. 2–7) from the jugular vein into vacuum tubes without anticoagulant and were allowed to clot at room temperature for 1.5 h. The serum was collected by low-speed centrifugation.

Serum total cholesterol was determined by the method of Röschlau et al. (15), using the kit (CHOD-PAP method) supplied by Boehringer Mannheim GmbH, FRG. As cholesterol standards, sera calibrated by the method of Abell et al. (1) were used.

Triglycerides in serum were determined enzymatically, using the kit (lipase method) supplied by Boehringer Mannheim GmbH, FRG. Glucose was assayed in deproteinized serum by the GOD-Perid method (Boehringer Mannheim GmbH, FRG).

Nonprotein nitrogen was determined in the supernatant of trichloric acetic acid treated serum as described by Rizvi and Josephson (15). Urea was analysed by the urease-phenol-hypochloric acid method (Boehringer Mannheim GmbH, FRG).

The difference between nonprotein nitrogen and urea nitrogen was assumed to represent amino-acid nitrogen.

Cholesterol in the diets was determined by gas-liquid chromatography (14) of the non-saponifiable fraction.

Results

Feeding cholesterol to calves significantly elevated serum cholesterol levels (fig. 1). After 7.5 weeks, when a steady state was established (fig. 1), the concentration of serum cholesterol was 4.58 ± 0.67 and 7.81 ± 1.55 mmol/l (± 1 SD) for the control and cholesterol-fed calves, respectively (P

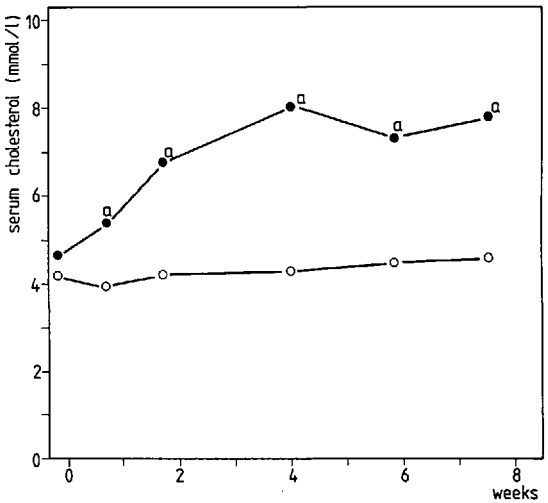

Fig. 1. Time course of the concentration of serum cholesterol in control (O) and cholesterol-fed (●) veal calves. Blood samples were taken at 10.00 a.m. (a) Significantly different from control animals at the $P < 0.05$ level.

< 0.025, Student's t test). These values were measured in fasting serum, but as figure 2 shows, a single intake of about 11 g cholesterol did not cause fluctuations in the concentration of cholesterol in the serum throughout the day. Likewise, the control calves did not appear to show a diurnal rhythm in the absolute level of cholesterol in serum.

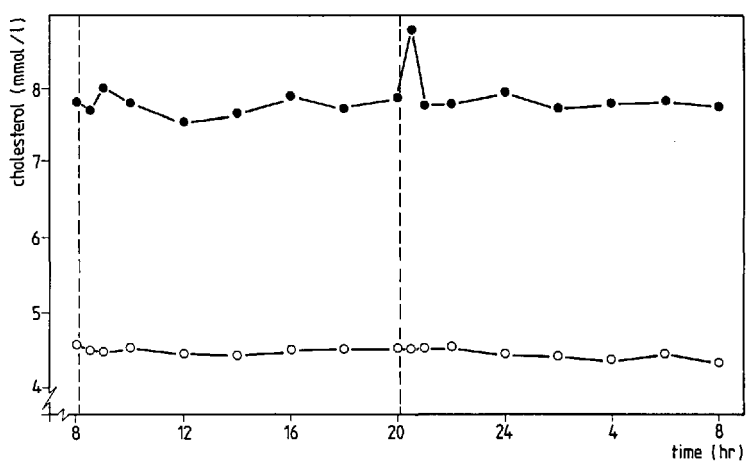

Fig. 2. Diurnal pattern of the concentration of cholesterol in the serum of veal calves. Control calves, O; cholesterol-fed calves, ●. Broken lines indicate the time of feeding. Significantly different from control animals (Student's t test): $P < 0.05$, a; $P < 0.10$, b.

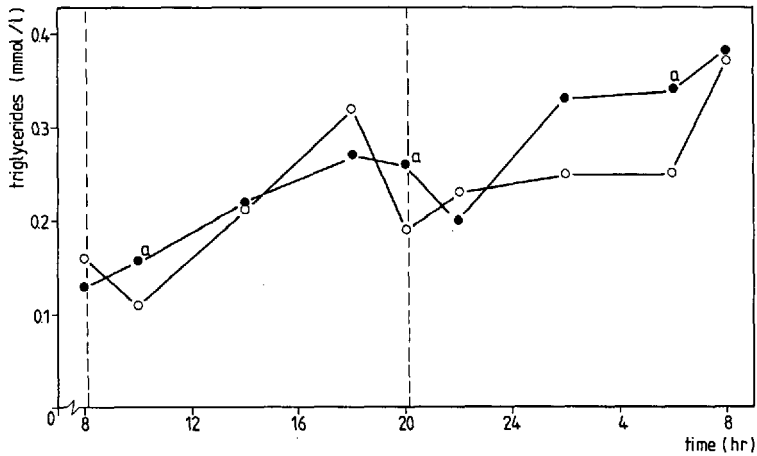

Fig. 3. Diurnal pattern of the concentration of triglycerides in the serum of veal calves. For explanation of symbols, see figure 2.

Cholesterol feeding did not significantly affect growth of the calves; at the end of the experimental period, body weights were 174.4 ± 4.4 kg (± 1 SD).

Figure 3 illustrates that no clear effect of feeding on serum triglyceride concentrations was observed. Although the cholesterol-fed calves had significantly elevated triglyceride levels at some times during the day, no consistent pattern could be detected. Cholesterol feeding did not affect fasting triglyceride concentrations in the serum. Figure 3 suggests a steady increase in serum triglycerides during the day in both dietary groups.

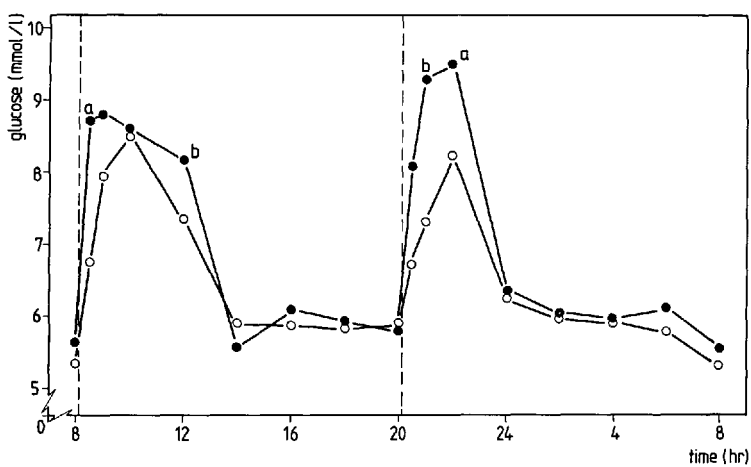

Fig. 4. Diurnal pattern of the concentration of glucose in the serum of veal calves. For explanation of symbols, see figure 2.

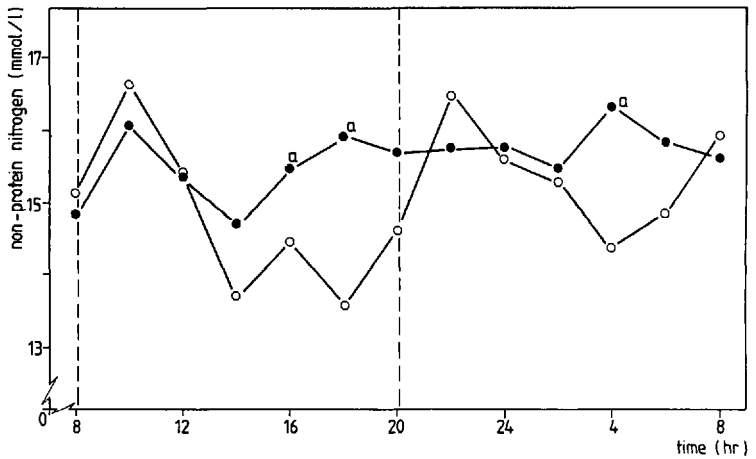

Fig. 5. Diurnal pattern of the concentration of nonprotein nitrogen in the serum of veal calves. For explanation of symbols, see figure 2.

There was a pronounced diurnal rhythm in the concentration of glucose in serum. A transient postprandial increase was seen (fig. 4), the maximum increase at about 2 h after feeding being approximately 60 %. Although cholesterol feeding did not affect fasting glucose levels, the postprandial increase was significantly higher in the calves fed cholesterol (fig. 4). This phenomenon was observed after feeding in the morning as well as after the evening meal.

The level of nonprotein nitrogen in the serum of the control calves displayed a distinct pattern during the day; an increase at about 2 h after feeding, which had disappeared by 4 h after feeding, followed by a continuous decrease until about 10 h after feeding, and then a slight rise

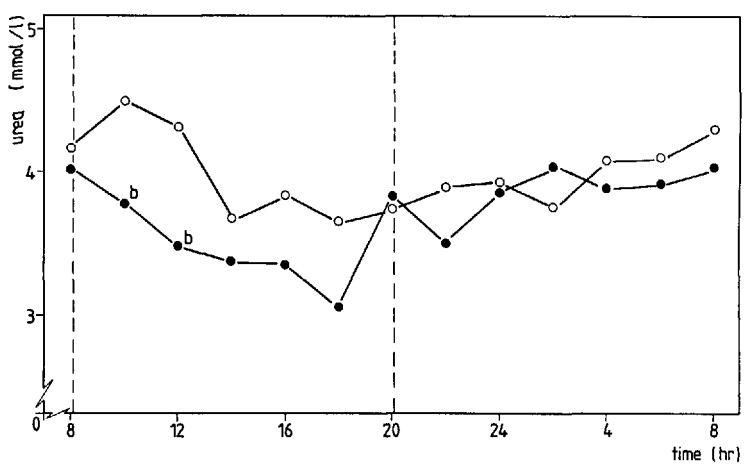

Fig. 6. Diurnal pattern of the concentration of urea in the serum of veal calves. For explanation of symbols, see figure 2.

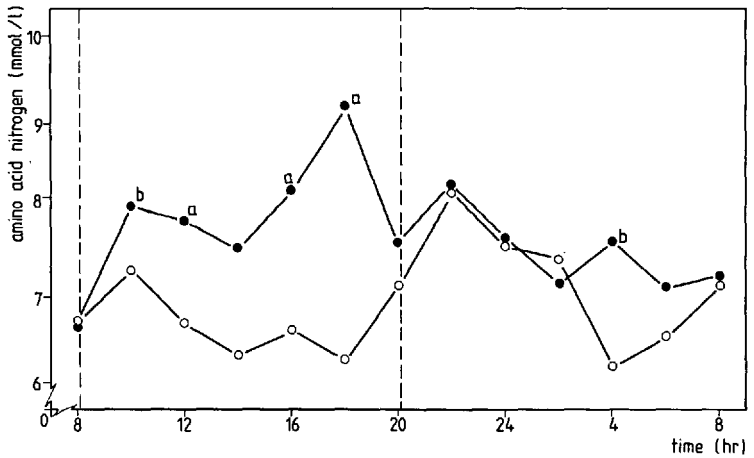

Fig. 7. Diurnal pattern of the concentration of amino acid nitrogen in the serum of veal calves. For explanation of symbols, see figure 2.

before feeding time (Fig. 5). Cholesterol feeding did not influence nonprotein nitrogen levels in serum obtained just before feeding, but it seemed to prevent the decrease between 4 and 10 h after feeding (fig. 5).

The diurnal pattern of the serum urea level is given in figure 6. After feeding in the morning, an increase in serum urea was observed in the control group and a decrease in the cholesterol-fed calves (fig. 6). However, this observation should be interpreted with caution, since this difference between the dietary groups was not seen after feeding at 8.00 p.m. Cholesterol in the diet of the calves did not affect urea levels in fasting serum.

Figure 7 shows the levels of amino acid nitrogen in the serum of veal calves during a 24-h period. Amino acid nitrogen values were found by calculating the difference between nonprotein nitrogen and urea nitrogen. After feeding in the morning, significantly higher levels of amino acid nitrogen were measured in the cholesterol-fed calves when compared with the control animals. This difference was not observed after feeding in the evening.

Discussion

In agreement with previous studies, we have shown that veal calves fed liquid diets supplemented with cholesterol become hypercholesterolemic. When fed a diet containing 1 % (on the basis of dry matter) cholesterol, the new hypercholesterolemic steady state was achieved after about 5 weeks (fig. 1). In this steady-state condition, the intake of about 11 g of cholesterol with a single meal did not affect the postprandial level of total cholesterol in the serum (fig. 2). Since cholesterol absorption in nonruminant calves on high fat diets is a very efficient process (20), this points to a well-balanced coordinated control of synthesis, absorption and excretion of cholesterol.

An interesting observation in the present study was the steady increase in the level of serum triglycerides during the day (fig. 3). This was possibly

related to stress of the calves, induced by the sampling of blood and presence of the investigators. Stress will activate adipose tissue lipolysis through elevated plasma levels of adrenalin and glucagon, which will result in increased concentrations of free fatty acids in the circulation. Although stress hormones inhibit fatty acid esterification in the liver (5), high levels of free fatty acids may override this inhibitory action of the hormones and cause an enhanced output of triglycerides by the liver (7).

Figures 6 and 7, at least the values measured between 8.00 a.m. and 8.00 p.m., suggest that protein metabolism is altered in cholesterol-fed calves. Dietary cholesterol seems to lower urea levels and to increase amino acid nitrogen levels. This would indicate that in veal calves fed cholesterol, amino acid absorption is enhanced and/or that amino acid removal from the blood is depressed. The latter possibility is attractive since urea levels in the serum are lowered by cholesterol in the diet, which suggests that amino acid breakdown is inhibited in hypercholesterolemic calves. So far, we can only speculate on this point, and it should be emphasized that the postprandial patterns of serum urea and amino acid nitrogen observed in the interval 8.00 a.m. to 8.00 p.m. were not seen after feeding at 8.00 p.m.

A convincing observation in the present work was that cholesterol-fed calves had significantly higher postprandial glucose levels (fig. 4), indicating an impaired glucose tolerance. In another experiment we have shown that glucose administered intravenously was removed more slowly by cholesterol-fed calves than by control animals (2). This suggests that the impaired glucose tolerance in cholesterol-fed calves is due to a decreased clearance of blood glucose rather than an increased intestinal absorption.

Studies of high carbohydrate diets in adult-type diabetics have shown improvement of glucose tolerance, which was associated with a decrease in serum cholesterol levels (3, 11, 18). Houtsmuller and colleagues (8) have recently demonstrated that a linoleic acid-rich diet improved glucose tolerance in diabetic women, but not in men. In the women, unlike the men, serum cholesterol was significantly lowered upon dietary treatment (8). These studies in man support our observations in calves, namely that glucose tolerance decreases with increased serum cholesterol levels. Possibly, high concentrations of serum cholesterol induce an increased cholesterol-phospholipid ratio of membranes, as has been shown in cholesterol-fed guinea pigs (17), which in turn may result in a diminished sensitivity to insulin and/or impaired glucose transport across membranes.

In conclusion, the present study has shown that dietary cholesterol may affect the dynamics of protein and glucose metabolism in veal calves. It is suggested that hypercholesterolemia causes deterioration of glucose tolerance, an effect that may also occur in man. This would explain the fact that the risk factors in the development of coronary heart disease, elevated serum cholesterol levels and impaired glucose tolerance, are generally strongly associated. This study may contribute to unravel the cause-and-effect relationship between both risk factors for atherosclerotic disease.

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