Changes in lipid metabolism in genetically different types of calves during chronic hyperthermia

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1. The effects of chronically raised body temperature on lipid metabolism were studied in groups of 3-month-old British (Shorthorn × Hereford) and Zebu (Africander × British) bull calves. Calves in control groups at ambient temperature were pair-fed to calves housed in a climate room.

2. In control groups, concentrations of plasma cholesterol and phospholipid were significantly higher and the excretion of fatty acids 14:0, 16:0 and 18:0 was lower in the Zebu than in the British calves.

3. In the climate room the constant daily environmental temperature required to maintain an increase in rectal temperature of 1·3° was 33° for British and 38° for Zebu calves. Changes in respiration rate, water metabolism and nitrogen metabolism associated with hyperthermia were similar in both breeds.

4. Hyperthermia in all animals was associated with lowered plasma concentrations of cholesterol and phospholipid and increases in the ratio of free to total cholesterol. It was without effect on the circulating concentrations of non-esterified fatty acids and about twice the amount of fat was excreted without change in its fatty acid composition.

5. The results showed that, when control of normal body temperature was lost, increased rectal temperature led to similar metabolic changes in animals of both breeds.

European-type cattle taken from temperate climates to tropical and subtropical areas tend to show impaired growth rates, reduced fertility and poor milk production (Rhoad, 1935; Bonsma, 1949; Findlay, 1950). From studies on the responses of temperate and tropical breeds of cattle, it is apparent that animals differ in their general tolerance of heat and their reactions to a particular environment (Rhoad, 1944; Robinson & Klemm, 1953; Brody, 1956; Schein, McDowell, Lee & Hyde, 1957). At high environmental temperatures cattle that are intolerant of heat show signs of heat stress, which include increased sweating (Allen, 1962), increased respiration rate (Bligh, 1957) and reduction in food consumption (Winchester, 1964). Disordered metabolism, in addition to a reduction in food intake, contributes to the depression in growth rate of cattle exposed to high ambient temperatures for prolonged periods.

Most of the work describing metabolic changes in cattle during heat stress has been concerned with alterations in hormonal status and nitrogen metabolism in adult animals. There is evidence that the concentrations of plasma lipids are decreased in steers during hyperthermia (O'Kelly, 1973), but there appears to be no published work on the effects of heat stress on lipid metabolism in young calves. It is possible that there may be a reduction in the rate of synthesis of lipids in young heat-susceptible calves exposed to high environmental temperatures and that this may be important in relation to their inability to grow normally in tropical areas.

As noted above, heat stress affects an animal's metabolism by causing a reduction in food intake (the anorectic effect) and by changes that result from increased body temperature alone, and which may be termed the 'specific effect'. This paper reports the changes in lipid metabolism caused by the specific effect of heat stress in genetically different types of calves. Other aspects of metabolism were also studied to obtain information on biochemical processes that may influence lipid metabolism at raised body temperature.

EXPERIMENTAL

Animals and treatments. Sixteen bull calves of two breeds, each about 3 months old, were used and the experiment was divided into two treatment periods of 10 d each. The British calves were eight Shorthorn × Hereford crosses; the animals referred to subsequently as Zebu were eight Africander × British crosses.

In each period one group of four animals (the stressed group) was housed in a controlled-climate room and another group of four animals (the control group) was kept in metabolism cages in a shed at ambient temperature.

British calves only were used in the first period and the mean daily rectal temperatures of the calves in the climate room were maintained about 1·3° higher than those of the control animals. This was achieved by exposing the calves in the climate room to a constant environmental temperature of 33° and 50% relative humidity. At the end of the 10 d period the calves in the climate room were returned to the shed at ambient temperature and all eight animals were fasted for 24 h. The same procedure was followed in the second period with the Zebu calves. However, to achieve an increase in rectal temperature of about 1·3° in the Zebu calves in the climate room a constant environmental temperature of 38° and 50% relative humidity were required.

The calves in the control groups were pair-fed to the calves in the climate room. A diet consisting of 750 g standard calf meal and 250 g lucerne hay per kg was offered in portions of 1 kg in the morning and afternoon to each calf in the climate room, and the controls were then given the amount eaten by their 'pairs'. Water was freely available throughout. The calf meal was composed of bran, pollard, wheatmeal, linseed, maize, bonemeal, minerals and vitamins; the protein content was 197 g/kg.

The mean body-weights (kg ± standard errors) at the beginning of treatment were: British control group, 104 ± 4; British stressed group, 108 ± 8; Zebu control group, 113 ± 4; Zebu stressed group, 107 ± 2.

Daily water and feed intakes, respiration rate, rectal temperature and excretions of faeces and urine were measured for each animal in its treatment period. The urine was collected in a copper sulphate–sulphuric acid preservative. Daily samples of urine and faeces from each animal were bulked over each collection period in proportion to the amounts daily excreted. Blood samples were obtained by jugular venepuncture and each animal in the treatment groups and its pair-fed partner were bled at the same time after feeding.

Analytical procedures. For the determination of dry matter, the samples of feed and faeces were dried at 105° to constant weight. Total nitrogen in urine, wet faeces and

feed was estimated by measuring the amount of ammonia formed in a macro-Kjeldahl digestion with a Technicon AutoAnalyzer (Technicon Equipment Pty. Ltd, Lane Cove, NSW, Australia).

The fat contents of feed and faeces were measured by the method of Kamer, Huinink & Weyers (1949) as modified by Braddock, Fleisher & Barbero (1968), and the results were expressed as g stearic acid excreted/d. Methyl esters of the fatty acids were prepared with boron trifluoride in methanol and analysed by gas–liquid chromotography in a Shimadzu (Kyoto, Japan) model GC-IC apparatus with a dual-flame ionization detector. A 0·2 m stainless-steel column of 3 mm internal diameter was used packed with Chromosorb W (80–100 mesh) coated with 20% diethylene glycol succinate. The column temperature was 185° and nitrogen was the carrier gas. Methyl esters were identified by comparison with authentic samples.

Methods for the analysis of plasma lipids were those of O'Kelly (1968). Total protein was determined by the biuret method (King & Wootton, 1956) and plasma amino-N was estimated with 2,4-dinitrofluorobenzene (Goodwin, 1968). Plasma glucose was estimated with an *o*-toluidine reagent (Braun & Hofmann, 1965). Alkaline phosphatase was assayed with disodium phenylphosphate as substrate by determining the liberated phenol with Folin and Ciocalteu's reagent by the method of King & Wootton (1956). Haematocrit percentages of the packed cells were determined with a microcapillary centrifuge (15000 *g* for 4 min (International Equipment Co., Boston, Massachusetts, USA)). The results were analysed by analysis of variance.

RESULTS

The results are given in Tables 1–7, which show mean values with standard errors and significance of differences between various means.

The constant daily environmental temperatures which increased rectal temperatures by 1·1° in the British and 1·4° in the Zebu calves are shown in Table 1. In both breeds, respiration rate, water intake, urine volume and the apparent water balances significantly increased with raised rectal temperature (Table 1). There were no significant breed differences or interactions between breed type (Zebu *v.* British) and rectal temperatures for water metabolism.

Urinary total N excretion increased in all calves at raised rectal temperatures with a resultant significant decrease in N balance (Table 2). Heat-treatment was without effect on both faecal water and N and on apparent N digestibility. Hyperthermia significantly increased faecal dry matter and decreased dry-matter digestibility in both breeds (Table 3).

The quantities of fat ingested and excreted are given in Table 3. Within the control animals the amount of faecal fat excreted was significantly higher ($P < 0{\cdot}05$) in the British than in the Zebu calves. During hyperthermia the amount of fat excreted about doubled in all calves, but the breed difference persisted.

The fatty-acid compositions of the feed and faeces are given in Table 4. The raised rectal temperature did not alter the fatty-acid composition of the faeces in either breed, but between breeds the proportion of 18:0 was lower ($P < 0{\cdot}05$) and that of 18:1

Table 1. *Daily mean rectal temperature, respiration rate and water balance of calves at ambient temperature (control) and at constant high environmental temperature (stressed)*

(Mean values with their standard errors for four calves)

	Shorthorn × Hereford				Africander cross			
	Control		Stressed		Control		Stressed	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Environmental temperature (°C)	23	—	33	—	23	—	38	—
Rectal temperature (°C)	38·4	0·1	39·5**	0·2	38·1	0·1	39·5*	0·4
Respiration rate/min	42	2	117**	15	38	2	114**	13
Water intake (l)	12·4	1·3	35·8**	5·8	10·4	2·3	32·3**	3·9
Urine volume (l)	8·2	1·6	28·8*	5·6	6·8	2·0	25·4**	3·6
Faecal water (l)	1·2	0·2	1·4	0·2	1·5	0·2	1·4	0·2
Apparent water balance (l)	3·0	0·3	5·6**	0·4	2·2	0·2	5·6**	0·2

For each breed, the values marked with asterisks are significantly different from control values: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

Table 2. *Daily mean nitrogen balance and digestibility in calves at ambient temperature (control) and at constant high environmental temperature (stressed)*

(Mean values with their standard errors for four calves)

	Shorthorn × Hereford				Africander cross			
	Control		Stressed		Control		Stressed	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
N intake (g)	61·6	1·5	61·6	1·5	57·2	4·0	57·2	4·0
Urinary N (g)	36·0	1·0	43·1*	2·0	35·0	1·0	45·2**	2·1
Faecal N (g)	15·8	2·2	18·0	1·1	15·8	1·7	16·1	1·7
N balance (g)	9·8	0·4	0·5*	2·0	6·4	2·2	−4·2**	4·2
N digestibility ratio	0·744	0·034	0·702	0·015	0·725	0·014	0·720	0·011

For each breed, the values marked with asterisks are significantly different from control values: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

was higher ($P < 0{\cdot}05$) in the Zebu than in the British animals. The increased fat excretion with no change in percentage composition during hyperthermia resulted in an increased excretion of the amounts of all the identified individual fatty acids (Table 5).

There were breed differences in the amounts of individual fatty acids excreted (Table 5). Within the control animals the amounts excreted were significantly higher in the British than in the Zebu calves for 14:0 ($P < 0{\cdot}01$), 16:0 ($P < 0{\cdot}01$) and 18:0 ($P < 0{\cdot}01$). The same breed differences were found in the calves at raised rectal temperature, but, in addition, the amount of unidentified fatty acids excreted was higher ($P < 0{\cdot}01$) in the Zebu than in the British calves.

The concentrations of components in the plasma are given in Tables 6 and 7. Increasing rectal temperature decreased total cholesterol, free cholesterol, phospholipid and alkaline phosphatase and raised the ratio of free to total cholesterol and the

Table 3. *Daily mean dry-matter digestibility, fat intake and excretion of calves at ambient temperature (control) and at constant high environmental temperature (stressed)*

(Mean values with their standard errors for four calves)

	Shorthorn × Hereford				Africander cross			
	Control		Stressed		Control		Stressed	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Dry-matter intake (kg)	1·66	0·04	1·66	0·04	1·61	0·10	1·61	0·11
Faecal dry matter (kg)	0·41	0·04	0·48*	0·02	0·42	0·03	0·46*	0·04
Dry-matter digestibility ratio	0·758	0·022	0·709*	0·015	0·740	0·002	0·715**	0·004
Fat intake (g)	41·5	1·1	41·5	1·0	40·5	2·6	40·5	2·6
Faecal fat (g)	9·5	0·4	19·7**	1·5	6·3	0·3	11·9**	0·5

For each breed, the values marked with asterisks are significantly different from control values: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

Table 4. *Daily mean fatty acid composition (weight percentage of the total) of feed and of the faeces of calves at ambient temperature (control) and at constant high environmental temperature (stressed)*

(Mean values with their standard errors for four calves)

		Faeces							
		Shorthorn × Hereford				Africander cross			
		Control		Stressed		Control		Stressed	
Fatty acid	Feed	Mean	SE	Mean	SE	Mean	SE	Mean	SE
12:0	0·6	1·0	0·2	1·0	0·1	1·3	0·1	1·3	0·1
14:0	2·7	4·0	0·1	3·8	0·3	3·7	0·1	4·0	0·2
16:0	22·6	20·6	0·4	19·7	0·9	20·1	0·7	18·8	0·3
18:0	11·2	52·8	1·4	51·9	0·9	45·6	0·3	45·6	0·5
18:1	35·7	17·1	1·0	16·9	1·2	22·2	1·4	21·6	1·4
18:2 ($n-6$)	19·7	—		—		—		—	
18:3 ($n-3$)	4·1	—		—		—		—	
Unidentified	3·4	4·5	1·1	6·7	1·8	7·0	0·8	8·7	1·0

Table 5. *Daily mean faecal excretion of fatty acid (g) of calves at ambient temperature (control) and at constant high environmental temperature (stressed)*

(Mean values with their standard errors for four calves)

	Excretion in faeces							
	Shorthorn × Hereford				Africander cross			
	Control		Stressed		Control		Stressed	
Fatty acid	Mean	SE	Mean	SE	Mean	SE	Mean	SE
12:0	0·09	0·01	0·19**	0·01	0·08	0·01	0·16*	0·01
14:0	0·38	0·01	0·75**	0·07	0·24	0·01	0·48**	0·05
16:0	1·95	0·09	3·91*	0·40	1·27	0·04	2·23**	0·10
18:0	5·04	0·36	10·24**	0·97	2·89	0·12	5·40**	0·26
18:1	1·63	0·13	3·37*	0·44	1·41	0·14	2·54**	0·10
Others	0·42	0·08	0·22	0·07	0·44	0·04	0·85*	0·17

For each breed, the values marked with asterisks are significantly different from control values: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

Table 6. *Plasma lipid composition of calves at ambient temperature (control) and at constant high environmental temperature (stressed)*

(Mean values with their standard errors for four calves)

	Shorthorn × Hereford				Africander cross			
	Control		Stressed		Control		Stressed	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total cholesterol (mg/l)	743	46	437**	49	1021	17	682**	58
Free cholesterol (mg/l)	160	13	108**	12	217	7	164*	15
Ratio, free: total cholesterol	0·215	0·006	0·247**	0·002	0·212	0·004	0·240**	0·002
Phospholipid (mg/l)	1218	48	880**	26	1482	25	1221*	48
Non-esterified fatty acid (μequiv./l):								
Non-fasting	185	10	179	11	222	33	317	36
24 h fasting	631	71	640	113	1349	46	1197	70

For each breed, the values marked with asterisks are significantly different from control values: * $P < 0.05$; ** $P < 0.01$.

Table 7. *Haematocrit, plasma nitrogenous constituents, plasma alkaline phosphatase activity and glucose concentration of calves at ambient temperature (control) and at constant high environmental temperature (stressed)*

(Mean values with their standard errors for four calves)

	Shorthorn × Hereford				Africander cross			
	Control		Stressed		Control		Stressed	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Haematocrit	34·3	0·8	33·9	0·5	36·9	0·9	36·0	0·9
Total protein (g/l)	68·7	1·4	68·7	1·5	65·8	1·1	65·3	1·8
α-Amino-*N* (mg/l)	37·6	0·6	40·0*	0·4	38·2	0·7	40·0*	0·8
Alkaline phosphatase (King-Armstrong units/l)	60·7	4·9	38·2**	0·7	77·5	6·6	53·2**	4·1
Glucose (mg/l):								
Non-fasting	555	17	536	20	730	27	716	44
24 h fasting	406	8	496**	9	635	40	643	28

For each breed, the values marked with asterisks are significantly different from control values: * $P < 0.05$; ** $P < 0.01$.

α-amino N concentrations. Hyperthermia had no significant influence on the concentrations of non-esterified fatty acids (NEFA), haematocrit, total protein, or glucose. Within both control and treated animals the plasma concentrations of cholesterol, phospholipid and glucose were significantly lower in the British than in the Zebu animals.

When the calves were subjected to a 24 h fast the plasma NEFA concentrations significantly increased ($P < 0.001$) in all animals (Table 6). However, within breeds there were no significant differences between groups in response to fasting, but the NEFA concentrations during fasting were higher in the Zebu calves ($P < 0.01$) than in the British. In contrast, the 24 h fast reduced plasma glucose concentrations in all animals, but the differences from pre-fasting values only reached significance ($P < 0.05$) in control animals (Table 7). The breed differences in glucose values found in the non-fasting calves were maintained in the fasting state.

The mean body-weights (kg ± standard errors) at the end of the treatment periods were: British control group, 101 ± 4; British stressed group, 106 ± 7; Zebu control group, 112 ± 2; Zebu stressed group, 111 ± 3. There were no significant changes in body-weight during experimental periods.

DISCUSSION

The heat loads imposed upon the animals in the climate chamber were adjusted to raise their rectal temperatures by about 1·3°. At this raised body temperature, animals of both breeds showed signs of heat stress. However, the environmental temperature which caused stress in the British calves was 33°, whereas the same effects in the Zebu calves were only observed at 38°. These findings agree with the acknowledged superior heat-tolerance of Zebu cattle.

The alterations in water exchange with raised body temperature were similar in both breeds and consisted of an increase in water intake and urine volume. Although the increases in apparent water balances were similar, it is possible that there may have been quantitative breed differences in evaporative water loss and changes in body fluids. Fourie, Grey & Louw (1964) reported that, although similar quantities of water were consumed by Africander and Shorthorn steers at an ambient temperature of 34°, the excretion of moisture was quite different in the two breeds; the Africanders appeared to lose most moisture by evaporation from the skin; Shorthorns excreted it through the urine, faeces and respiratory surfaces. It is clear, however, that under the environmental conditions described by Fourie *et al.* (1964) only the Shorthorn steers showed increased rectal temperatures, whereas in the experiments described here Africander cross and British animals are compared at the same raised rectal temperature.

An increased N excretion is a well-established effect of stress, and the negative N balance and unaltered apparent N digestibility found in the calves during hyperthermia are in agreement with results observed in adult cattle (Vercoe, 1969). There is some evidence that cattle exposed to high ambient temperatures show depressed rumen activity (Attebery & Johnson, 1969) and decreased

production of total volatile fatty acids (Weldy, McDowell, Van Soest & Bond, 1964; Kelley, Martz & Johnson, 1967). The present experiment demonstrated a reduction in dry-matter digestibility in calves at raised rectal temperature.

If there were alterations in energy expenditure involving fat metabolism in the heat-stressed animals, these were not indicated by changes in plasma concentrations of NEFA. Since fat mobilization is under hormonal control, it seemed of interest to see if hyperthermia interfered with the normal metabolic responses to fasting. However, when the calves were subjected to a 24 h fast the changes in the plasma glucose and NEFA concentrations (Tables 6, 7) were similar in control and heat-stressed animals within breeds. The reasons for the breed difference in plasma glucose concentrations is not apparent, but the breed difference in plasma concentrations of NEFA during fasting has been reported previously (O'Kelly, 1972).

O'Kelly (1968) reported evidence of breed differences and genetic differences within breeds in the concentrations of lipid in the plasma of cattle. Decreased concentrations of plasma cholesterol and phospholipid have been reported in adult cattle exposed to heat in a climate chamber (Noble, O'Kelly & Moore, 1973; O'Kelly, 1973). In the present studies the plasma concentrations of cholesterol and phospholipid were significantly higher in the Zebu than in the British calves, but hyperthermia depressed the concentrations of these components similarly in both breeds. The unaltered haematocrits and serum protein concentrations indicate that the lowered plasma lipid concentrations were not due to change in hydration of the plasma volumes.

The changes in blood composition at raised rectal temperature provide little information about the effects of hyperthermia on lipid metabolism in general or the cause of the reduced concentrations of cholesterol and phospholipid in plasma. The reduced alkaline phosphatase activity in plasma suggests some liver dysfunction and might imply interference with normal lipid production and supply to the plasma. There is evidence to suggest that the relationships between plasma lecithin-cholesterol-acyl transferase (LCAT) (*EC* 2.3.1 group) activity and the polyunsaturated fatty acids of the circulatory lipids may be severely disturbed in cattle exposed to high environmental temperatures (Noble *et al.* 1973). In agreement with previous work (O'Kelly, 1973), the specific effect of heat was found to increase the ratio of free to total cholesterol. The increased ratio in the heat-stressed animals resulted from a differential decrease in the free and esterified cholesterol fractions and may have involved altered LCAT enzyme activity.

The present investigation clearly demonstrated that increased rectal temperature in calves was associated with a large increase in the excretion of faecal fatty acids. Because large amounts of endogenous lipids are added to the chyme in the intestines (Adams & Heath, 1963; Leat & Harrison, 1967), the apparent digestibilities determined in this study are of uncertain significance.

It is possible only to speculate on the origin of the increased faecal fat. After ingestion by ruminants, the unsaturated acids are readily hydrogenated by micro-organisms in the rumen to form a complex mixture of positional and geometrical isomers (Garton, 1967). Nearly all the lipids passing to the small intestine from the abomasum

are free fatty acids (Garton, 1960) and only very small amounts of monoglycerides are detectable in the small intestines of adult sheep (Leat & Harrison, 1967). Very little degradation of long-chain fatty acids takes place in the rumen and there is no evidence that acids of chain length C_{16} and greater are absorbed to any appreciable extent from this part of the alimentary tract (Garton, 1969). Although the heat-stressed calves excreted greater amounts of fat, its composition was not changed from the control value. This tends to suggest that hydrogenation was just as effective in the heat-stressed as in the control animals and that reduced alimentary absorption was the likely cause of the increased fat excretion.

There are many factors which might alter the intestinal absorption of fatty acids in the heat-stressed animal. The adrenocortical hormones play a part in fat absorption and the production of these hormones is altered during stress (Thompson, Johnston, Breidenstein, Guidry, Banerjee & Burnett, 1963). In sheep, lipid absorption is almost completely abolished in the absence of bile (Heath & Morris, 1963). As discussed above, the lowered concentrations of cholesterol and phospholipid in the plasma of heat-stressed animals may imply a reduced production of these lipids in the liver and, if so, it is likely that formation and secretion of bile would also be reduced.

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