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Influence of environmental temperature on in vivo energy expenditure and in vitro ouabain-sensitive respiration in duodenal mucosa and liver in rats fed different levels of dietary fibre or protein

Einfluß der Umgebungstemperatur auf den In-vivo-Energieumsatz und die In-vitro-Quabain-sensitive Respiration der Duodenalmukosa und der Leber von Ratten bei einer Diät mit unterschiedlichem Faser- und Proteingehalt

Summary Seventy two Wistar rats were used in two repeat studies to investigate the effect of environmental temperature (18°C or 28°C) and increasing levels of dietary fibre (low, 68 g/kg DM; medium 110 g/kg DM; high, 157 g/kg DM) or protein (low, 91 g/kg DM; medium, 171 g/kg DM; high, 262 g/kg DM) on digestive tract, visceral organ size, energy metabolism, and respiration attributable to Na^+,K^+-ATPase activity in duodenal mucosa and liver. Total and ouabain-sensitive (a measure of Na^+,K^+-ATPase activity) O_2 consumption *in vitro* of tissues were measured polarographically using a Clark-style YSI biological O_2 monitor. Whole body heat production (*in vivo*) was measured using open-circuit respiration chambers. The weight of the visceral organs was higher in rats housed at 18°C than at 28°C. The empty weight of the small intestine, caecum, and colon increased as the level of dietary fibre increased (P 0.05). Heat production as a proportion of metabolizable energy was higher ($P < 0.05$) at 18°C than at 28°C in the first experiment but this difference was not significant in the second experiment. Rats fed the low protein diet had significantly higher ($P > 0.05$) heat production than those fed medium or high protein diets. Compared to 28°C, environmental temperature of 18°C caused an increased total and ouabain-sensitive O_2 consumption in duodenal mucosa. There was no significant effect of environmental temperature on total and ouabain-sensitive O_2 consumption in the liver. However, ouabain-sensitive O_2 consumption in liver was significantly higher (P 0.05) when rats were fed a low protein diet compared to the medium or high protein diet. Total and ouabain-sensitive O_2 consumption increased in duodenal mucosa of rats fed low level of dietary fibre compared to the medium or high dietary fibre diets. The *in vitro* results corresponded with the whole animal energy expenditure and O_2 consumption *in vivo*.

Zusammenfassung Die Wirkung der Umgebungstemperatur (18°C oder 28°C) und des Fasergehalts in der Diät (g je kg Trockensubstanz (TS) niedrig - 68, mittel - 110, hoch - 157) oder des Proteingehalts (g je kg TS niedrig - 91, mittel - 171, hoch - 262) auf den Verdauungstrakt, die Darmmasse, den Energieumsatz und auf die mit der Na^+, K^+-ATPase-Aktivität zusammenhängenden Respiration von Duodenalmukosa und Leber wurde bei 72 Wistar-Ratten in wiederholten Experimenten untersucht. Der Gesamte und Quabain-sensitive (ein Maß der Na^+, K^+-ATPase Aktivität) O_2-Verbrauch der Gewebe wurde in vitro polarographisch ermittelt (YSI-biologische Sauerstoff-Erfassung nach dem Clark-Meßprinzip). Die Wärmeproduktion (WP) intakter Tiere wurde über Respirationskammern mit offenem Gasaustausch erfaßt. Die bei 18°C gehaltenen Ratten wiesen im Vergleich zu 28°C eine höhere Darmmasse auf. Die Masse an leerem Dünndarm, Caecum und Colon stieg mit ansteigendem Fasergehalt in der Diät ($P < 0.05$). Die WP als Korrelat der umsetzbaren Energie war nur im 1. Experiment höher ($P < 0.05$) bei 18°C als bei 28°C. Bei niedriger Proteinstufe war die WP signifikant höher (P 0.05) als bei den anderen Stufen. Verglichen mit 28°C

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erzeugte 18°C einen ansteigenden Gesamt- und Quabain-sensitiven O_2-Verbrauch in der Duodenalmukosa. Die Leber reagierte nicht auf Temperaturunterschiede. Jedoch war ihr Quabain-sensitiver O_2-Verbrauch bei niedrigem Proteingehalt in der Nahrung höher ($P < 0.05$) als bei den anderen Varianten. Bei niedrigem Fasergehalt war der gesamte und Quabain-sensitive O_2-Verbrauch der Duodenalmukosa höher als bei den anderen Fasergehaltsvarianten. Die In-vitro-Ergebnisse stimmten mit der WP und dem O_2-Verbrauch intakter Tiere überein.

Key words Environmental temperature - energy expenditure - ouabain-sensitive respiration - duodenal mucosa - liver - rats

Schlüsselwörter Umgebungstemperatur - Energieumsatz - Quabain-sensitive Respiration - Duodenalmukosa - Leber - Ratten

Introduction

Two major processes accounting for most of the cellular ATP utilization are Na^+,K^+-ATPase activity and protein synthesis (9, 23). These processes are very active in the gastrointestinal tract. Na^+,K^+-ATPase (the sodium pump) is a major contributor to energy required for maintenance (9). McBride and Milligan (1984) calculated that Na^+,K^+-ATPase activity could account for up to 61% of O_2 consumption in the duodenum of the lactating cow, while Rompala et al. (1987) reported that ovine jejunal O_2 consumption increased with the level of metabolizable energy intake. Na^+,K^+-ATPase dependent respiration is also induced in skeletal muscle of animals exhibiting elevated metabolic rate due to the physiological stress induced by cold ambient temperature (5). However, even for the same tissue there is very little agreement on the energy cost of Na^+,K^+-ATPase activity (for review see Clausen et al. 1991).

The contribution of Na^+,K^+-ATPase activity to energy expenditure has been investigated in many studies. However, there is little information on how environmental temperature and dietary fibre (DF) or dietary protein affect Na^+,K^+-ATPase dependent O_2 consumption in the gastrointestinal tract. In our earlier studies (7, 24) we observed that the size of the digestive tract was significantly increased when rats and pigs were fed high fibre diets and housed in a cold environment. Furthermore, whole body energy expenditure was increased when these animals were housed in a cold environment. However, in these studies measurements of tissue Na^+,K^+-ATPase activity dependent O_2 consumption was not made. It is plausible that diet-induced thermogenesis or cold-induced thermogenesis might be elevated in some active tissues, e.g., intestinal mucosa and liver when animals are housed at a cold environment.

The objectives of the present experiment were to study Na^+,K^+-ATPase activity dependent O_2 consumption in duodenal mucosa and liver in rats kept at different environmental temperatures and fed different levels of DF or dietary protein. The duodenal mucosa and liver were chosen for study because of the significant role of the gastrointestinal tract and liver in heat production and contribution to maintenance energy requirement.

Materials and methods

Animals and diets

Seventy-two male Wistar rats were used in two separate experiments. In each experiment, thirty-six rats with an initial live weight of 77-80 g were randomly allocated to six groups. The rats were kept individually in metabolic cages throughout the experiment. Each experiment was carried out according to a factorial design consisting of three levels of DF (low, 68 g/kg DM; medium, 110 g/kg DM; high, 157 g/kg DM) or dietary protein (low, 91 g/kg DM; medium, 171 g/kg DM; high, 262 g/kg DM) and two environmental temperatures, 18°C or 28°C, respectively. Pea fibre and fish meal were used as DF and protein source, respectively (Table 1).

Table 1 Main ingredients (%) and chemical composition of diets (g/kg dry matter)

	Experiment 1 (fibre)			Experiment 2 (protein)		
	Low	Medium	High	Low	Medium	High
Pea fibre	10.0	20.0	30.0	-	-	-
Fish meal	22.0	21.0	20.0	11.5	23.0	34.5
Protein	173	175	176	91	171	262
Dietary fibre	68	110	157	80	56	43

Daily food allowance was kept as close to ad libitum food intake as possible. Water was freely available and a 12 h (06:00-18:00) light-dark cycle was employed. The rats were kept at two different temperatures (18°C or 28°C) in controlled climatic rooms (7). Relative humidity was maintained at 60%. The same two temperatures were also maintained in the respiration chambers. Five days of adaptation were followed by four balance periods of 5 d duration. There was a 2 d interval between each balance period without collection of excreta.

Whole body respiration

Whole body gas exchange was measured with two open-circuit respiration chambers. Gas exchange measurements were done on groups of six rats fed the same diet and housed at either 18°C or 28°C and calculation of heat production on an individual basis was performed (24).

Gas exchange was measured for 22h each day on two consecutive days (on the second and third day during balance period). The concentration of oxygen, carbon dioxide, hydrogen, and methane as well as temperature, relative humidity, and air flow rate from each chamber were recorded automatically every second minute.

Biopsy procedures and tissue respiration

After the four week experimental period, the animals were killed by cervical dislocation, and duodenal mucosa and liver were obtained within 2-3 minutes after death. Duodenal mucosa was scraped with a glass microscope slide. Liver biopsies were approximately 4-5 mm long and 3-4 mm wide and less than 0.5 mm thick. Two biopsies were taken during each measurement. The samples were washed in ice-cold saline (154 mmol/L) and transferred to a gassed (O_2-CO_2; 95:5, v/v) M199 liquid medium (pH 7.4, Sigma Chemical Co., St. Louis, MO) on ice before respiration measurements. Mucosa and liver dry weights were determined by drying the sample in a vacuum drying oven at 55°C for 24 hours.

Rates of O_2 consumption were measured polarographically using a Clark-style YSI biological oxygen monitor (model 5300 O_2 electrode assembly, Yellow Springs Instruments, Yellow Springs, OH). Air-saturated M199 media (37°C, 5.02 mL O_2/mL) was used in all O_2 consumption measurements.

The ouabain-sensitive O_2 consumption is an estimate of the magnitude of Na^+,K^+-ATPase dependent O_2 consumption (4). This assay relies on the accepted high degree of specificity of ouabain as an inhibitor of Na^+,K^+-ATPase. For measuring ouabain-sensitive O_2 consumption, samples were placed in M199 media (37°C in the O_2 electrode incubation chamber) where after approximately 5 min of acclimation to chamber conditions, total (initial) O_2 consumption was determined for 5 min. Following the determination of O_2 consumption for 5 min, 100 mL ouabain was added to the medium (final concentration 10^{-4} mol/L) (15) through the add port with a microsyringe. The ouabain-insensitive O_2 consumption was determined for another 5 min. Ouabain-sensitive O_2 consumption (Na^+,K^+-ATPase dependent O_2 consumption) was calculated as the difference between total O_2 and ouabain-insensitive O_2 consumption. The percentage associated with Na^+,K^+-ATPase dependent O_2 consumption was calculated using the ratio of ouabain-sensitive O_2 consumption to total O_2 consumption.

Duplicate tissue samples were taken to measure ouabain-sensitive O_2 consumption in the first experiment. In the second experiment, one sample was used to measure ouabain-sensitive O_2 consumption and the other one was used to measure cycloheximide-sensitive O_2 consumption (results not shown), respectively. The size of the tissue samples used was such that the reduction in the O_2 content of the incubation medium during the 20-25 min procedure was not greater than 25% of its initial value (19).

Results and discussion

Thermal environment

The weight of the visceral organ and the empty gastrointestinal tract relative to the empty body weight was higher at 18°C ($P < 0.05$) than at 28°C (Table 2). The visceral organs such as liver, heart, kidney, and the digestive tract have a substantial impact on metabolic rate. Quantitating the relative importance of changes in weights of various organs on fasting heat production, point out that gut and liver weights are highly related to fasting heat production (10). Dry matter intake and HP of rats housed at 18°C were approximately 40% and 41% higher than of those rats housed at 28°C (Tables 3). Similar results were found in a previous study with rats kept at 16°C or 24°C (24). However, in this study rats were fed equal amounts of gross energy and HP increased by 28% when the environment temperature was changed from 28°C to 16°C. The increased size of the liver, heart, and the digestive tract in the rats kept at 18°C was likely due to an enhanced metabolic rate although an effect of increased food intake can not be excluded. It can be presumed that a cold environment causes visceral organ size to increase and this is one of the mechanisms animals use to acclimate to a cold environment.

The ouabain-sensitive respiration rate is an estimate of the magnitude of Na^+,K^+-ATPase sensitive respiration (4). This assay relies on the accepted high degree of specificity of ouabain as an inhibitor of Na^+,K^+-ATPase activity. The proportion of tissue O_2 consumption associated with Na^+,K^+-ATPase activity (ouabain-sensitive O_2 consumption) has been reported to range from 2 to 45% (16). The magnitude of this value is affected by a variety of physiological conditions. Higher ouabain dependent O_2 consumption in intestinal mucosa has been found in dairy cows and ranged from 35% (nonlactating) to 54% (during peak lactation). In sheep fed a diet high in digestible energy, the ouabain dependent O_2 consumption in intestinal mucosa was 61% (14, 15). In the present study, Na^+,K^+-ATPase dependent O_2 consumption ranged from 31 to 39% in the first experiment and from 26 to 36% in the second experiment. These results are in agreement with the results of previous studies in which Na^+,K^+-ATPase dependent respiration accounted for 35% of the total O_2 uptake of the intestinal mucosa in fed rats (11) but were higher than the Na^+,K^+-ATPase dependent respiration observed in gestating or lactating rats (19). The results also indicate that Na^+,K^+-ATPase dependent O_2 consumption in the duodenal mucosa is significantly increased in rats kept at 18°C.

Table 2 The influence of environmental temperature on visceral organ and empty gastrointesinal tract (g/kg empty body weight) in rats

	Experiment 1 (fibre)		Experiment 2 (protein)	
Temperature	18°C	28°C	18°C	28°C
Liver	45.9[a]	40.8[b]	48.5[a]	41.2[b]
Heart	3.4[a]	2.9[b]	3.2[a]	2.9[b]
Kidney	8.8	6.9	8.5[a]	6.9[b]
Stomach	5.4[a]	5.0[b]	5.2	5.1
Small intestine	29.4[a]	26.1[b]	27.6[a]	23.6[b]
Caecum	4.3[a]	3.5[b]	2.4	2.2
Colon	4.9[a]	4.4[b]	5.0	4.8

Mean values with different superscript letter in the same row for 18°C and 28°C within experiment are significantly different ($P < 0.05$).

Table 3 The influence of environmental temperature on whole body energy expenditure and duedenal mucosa and liver O_2 consumption (μL/mg dry tissue per h) of rats

Experiment 1 (fibre)			Experiment 2 (protein)	
Temperature	18°C	28°C	18°C	28°C
ME intake (kJ/d)	305[a]	223[b]	303 [a]	214 [b]
ME intake (kJ/kg$^{0.75}$d	1128[a]	831[b]	1165 [a]	838 [b]
HP (kJ/kg$^{0.75}$d	974[a]	674[b]	994 [a]	704 [b]
O_2 consumption (mL/100 g LW h)	284[a]	208[b]	278 [a]	181 [b]
Duodenal mucosa				
Total O_2 consumption	6.4[a]	4.7[b]	5.1 [a]	4.9 [b]
Ouabain inhibition (%)	39[a]	31[b]	36 [a]	26 [b]
Liver				
Total O_2 consumption	2.1	1.8	1.9	1.8
Ouabain inhibition (%)	18	16	25	27

In the small intestine, transport of amino acids and glucose is directly related to the activity of Na^+,K^+-ATPase (13, 16). In the present study, the substantial Na^+,K^+-ATPase dependent O_2 consumption corresponds to an increased metabolizable energy intake and higher whole body energy expenditure in in vivo measurements (Table 3). Either increased food intake or cold exposure induced endocrine response can enhance tissue Na^+,K^+-ATPase activity (9). Food intake has been shown to affect markedly intestinal and hepatic O_2 consumption and associated Na^+,K^+-ATPase activity.

Total tissue O_2 consumption rates in the liver observed in the present study were lower than the 3.0-3.2 mL/mg per h which were observed in rats housed at 6°C or 26°C (6). However, the contribution of O_2 by liver to whole body O_2 consumption was greater at 18°C than at 28°C due to the larger liver of rats housed at 18°C. There was no effect ($P < 0.05$) of environmental temperature on the Na^+,K^+-ATPase dependent O_2 consumption in the liver although the Na^+,K^+-ATPase dependent O_2 consumption was slightly increased when rats were kept in a cold environment. Guernsey and Stevens (6) found that the Na^+,K^+-ATPase dependent O_2 consumption was increased from 14% to 19% in rats when the temperature was decreased from 26°C to 6°C and point out that the Na^+,K^+-ATPase activity is a major component of the enhanced tissue thermogenesis in the liver of cold acclimated rats. The results from the present study show that the Na^+,K^+-ATPase dependent O_2 consumption was numerically highest when rats was kept at cold environment.

Table 4 The influence of dietary fibre (experiment 1) on whole body energy expenditure and duodenal mucosa and liver O_2 consumption (μL/mg dry tissue per h) of rats

Dietary fibre (%)	6.8	11.0	15.7
ME intake (kJ/d)	261	263	268
ME intake (kJ/kg$^{0.75}$d)	1015 [a]	975 [b]	949 [b]
HP (kJ/kg$^{0.75}$d)	867 [a]	812 [b]	763 [b]
O_2 consumption (mL/100 g LW h)	263 [a]	238 [b]	237 [b]
Duodenal mucosa			
Total O_2 consumption	6.9 [a]	4.6 [b]	5.2 [ab]
Oubain inhibition	(%)	363	139
Liver			
Total O_2 consumption	2.2 [a]	1.8 [b]	1.7 [b]
Oubain inhibition	(%)	161	717

Dietary fibre effect

The weight of the stomach, small intestine and caecum was increased ($P < 0.05$) as the level of fibre increased (Fig. 1). When dietary fibre replaced digestible carbohydrate the protein content remained constant. As a fraction of DF is resistant to digestive enzymes, the digestibility of DF was very low at the terminal ileum of pigs (7). In contrast, starch and sugars are almost completely digested at the end of the small intestine. This may partly explain the increased total tissue O_2 consumption and ouabain-sensitive O_2 consumption in duodenal mucosa of rats fed low level of DF (Table 4). Although DF is considered to be responsible for a heavier small intestine in rats (24, 25), crypt increased height and proliferative activity in the proximal and mid small intestine of rats (1). The total tissue O_2 consumption and ouabain-sensitive O_2 consumption did not increase with increasing levels of DF. A study with rats also showed that the consumption of DF increases substrate oxidation by isolated colonocytes but not distal small intestinal enterocytes (12). Other studies with rats have confirmed that gut size increases in response to consumption of a fibrous diet (25, 1).

Total tissue O_2 consumption of liver and ouabain-sensitive O_2 consumption was higher when rats were fed the

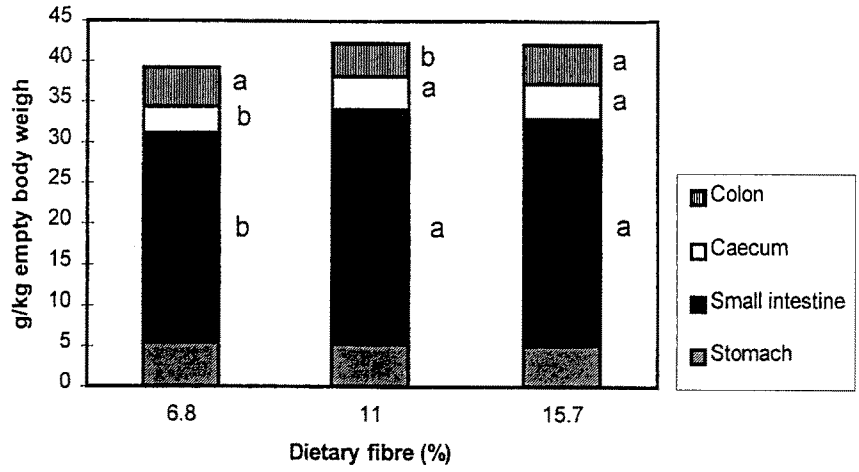

Fig. 1 The influence of dietary fibre (DF) on weight of the empty gastrointestinal tract in rats.

low level of DF than when fed a medium level of DF. The results correspond with the higher whole animal energy expenditure (Table 4). In the present study, a similar amount of ME was consumed by rats fed either the low or high level of DF, but the ratio of retained energy/ME increased and more energy was retained as fat when DF increased. The utilization of ME for fat deposition is higher than for protein deposition (3). This may partly explain that high level of DF have a low total tissue O_2 consumption and ouabain-sensitive O_2 consumption in liver of rats.

Table 5 The influence of dietary protein (experiment 2) on whole body energy expenditure and duodenal mucosa and liver O_2 consumption (μL/mg dry tissue per h) of rats

Dietary protein (%)	9.1	17.1	26.2
ME intake (kJ/d)	232 ^{b}	271 ^{a}	273 ^{a}
ME intake (kJ/kg$^{0.75}$d)	1078 ^{a}	987 ^{b}	940 ^{c}
HP (kJ/kg$^{0.75}$d)	996 ^{a}	788 ^{b}	763 ^{c}
O_2 consumption (mL/100 g LW h)	267 ^{a}	216 ^{b}	205 ^{b}
Duodenal mucosa			
Total O_2 consumption	4.2	5.6	5.1
Oubain inhibition (%)	34	32	28
Liver			
Total O_2 consumption	1.4 ^{b}	2.1 ^{a}	2.0 ^{a}
Oubain inhibition (%)	27 ^{a}	18 ^{b}	23 ^{ab}

Dietary protein effect

The size of the liver and kidney increased ($P < 0.05$) with increasing dietary protein level. The length of small intestine, cecum, and colon were also enhanced by increasing dietary protein level (26) but the relative weight decreased ($P < 0.05$) with increasing dietary protein level (Fig. 2). The total relative weight of the digestive tract also decreased (P 0.05) as the level of dietary protein increased. These results are in agreement with the reported increases in organ masses of other animals fed high levels of dietary protein (18, 20). Increasing organ size, as previously discussed could result in higher maintenance requirements (10). Total tissue O_2 consumption rates in the liver obtained in the present study (Table 5) are in agreement with those obtained in previous studies with rats fed either a high or low protein diet during gestation and lactation: 1.4 to 2.2 mL/mg per h (19), but lower than the 3.0-3.2 mL/mg per h which were observed in rats housed at 6°C or 26°C (6). The proportion of tissue respiration associated with Na^+,K^+-ATPase activity in the liver ranged from 18 to 27% in rats fed different levels of dietary protein. The results are considerably lower than other published data for the liver. In sheep, McBride and Milligan (15) found 37 to 45% of tissue respiration was associated with Na^+,K^+-ATPase activity. The lower contribution of Na^+,K^+-ATPase activity to tissue respiration reported in this study may be attributed to differences in the composition of the incubation medium (19).

Although total tissue O_2 consumption of liver was higher in rats fed medium or high level of dietary protein than in rats fed low level of dietary protein, there was no difference ($P < 0.05$) in ouabain-sensitive O_2 consumption. The observed increase in ouabain inhibition of total tissue O_2 consumption in the present experiment suggests that feeding either a low or a high protein diet has an effect on Na^+,K^+-ATPase activity in the liver. Nutritionally unbalanced diets usually result in larger increases in heat production (defined as diet-induced thermogenesis) and therefore of energetic efficiencies than when the diets are well balanced in nutrient composition (22). Increased protein intake may result in a reduction in energy utilization (8, 17). In addition, energetic efficiency is decreased in rats fed a low protein diet

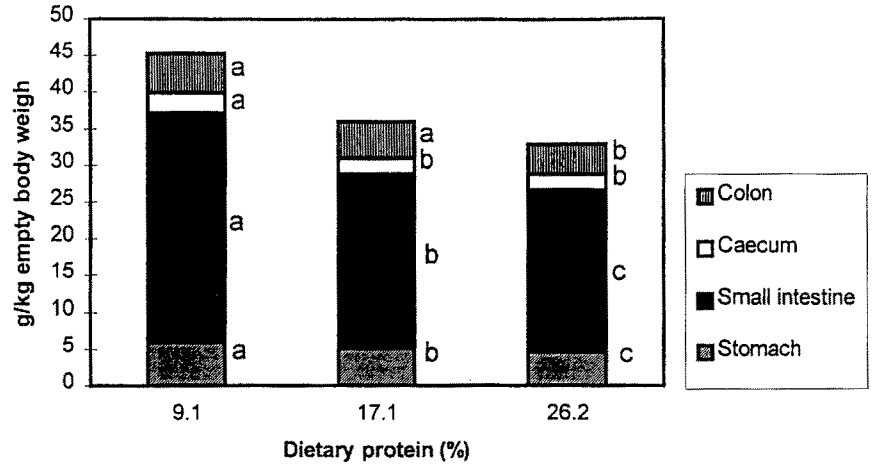

Fig. 2 The influence of dietary protein on weight of the empty gastrointestinal tract in rats.

(22). There are similarities and close relationships between diet-induced thermogenesis and nonshivering thermogenesis which are exhibited by animals housed in a cold environment. The results from the present study support that Na^+,K^+-ATPase activity can be responsible for diet-induced thermogenesis by low or high level of dietary protein.

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