The Effects of Streptozotocin-Diabetes and Food Deprivation on Electromyographic Activity in Urethane-Anesthetized, Hypothermic Rats

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ABSTRACT

The Effects of Streptozotocin-Diabetes and Food Deprivation on Electromyographic Activity in Urethane Anesthetized, Hypothermic Rats

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Diabetes mellitus and fasting are two conditions known to significantly reduce the ability to generate body heat during periods of acute cold stress. The primary purpose of this study was to determine if streptozotocin (STZ)-induced diabetes attenuates the shivering response in urethane anesthetized (1.5 g/kg) rats during an acute cold stress (10 ± 2°C). An additional group of STZ-diabetic and non-diabetic rats were used to examine shivering activity under identical cold stress conditions following a nocturnal (12 hour) fast. Shivering was assessed using the mean rectified EMG signal obtained from indwelling bipolar electrodes placed in the gluteus superficialis muscle as rectal temperature (T_{rec}) declined from 36 to 28°C. In the non-fasted controls, mean EMG activity showed a steady increase between a T_{rec} of 33 and 28°C and achieved peak shivering activity (7.81 ± 1.84 μV) at 29°C. Conversely, shivering was virtually absent during cooling (peak EMG activity: 0.49 ± 0.09 μV) in STZ-diabetic rats. The reduced shivering activity in diabetic animals could partly explain the shorter time to reach 28°C (STZ; 48.5 ± 1.5 vs Control; 136.5 ± 23.0 min; $p<.05$). Divergent trends in oxygen consumption (peak ΔVO_2: STZ; -2.73 ± 0.19 vs Control; +4.50 ± 0.50 ml O_2/min) were observed between controls and STZ-diabetics. Following the 12 hour fast, non-diabetic animals displayed significant reductions in EMG activity

(peak activity: 3.09 ± 1.35 μV), which was associated with an attenuated peak ΔVO_2 (+2.11 ± 0.36 ml O_2/min) and shorter time to reach 28°C (73.4 ± 4.2 min) compared with fed controls. The EMG activity, ΔVO_2, and length of cooling were not affected by fasting in STZ-diabetic animals. The results of this study demonstrate that: 1) shivering thermogenesis is severely depressed in STZ-diabetic rats as evidenced by the overall reduction in mean EMG activity and ΔVO_2, and 2) overnight fasting resulted in lower EMG and ΔVO_2 responses in control animals but had no effect on the cold-induced responses in STZ-diabetic rats.

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INTRODUCTION

Heat Conservation and Production

When mammals are placed in a cold environment, the rate of body heat loss increases due to a greater thermal gradient between the skin and the surrounding air. If heat loss exceeds the rate of heat production, hypothermia will ensue and survival of the animal will be jeopardized. The thermoregulatory system enables mammals to cope in a cold environment by monitoring body temperature fluctuations and initiating appropriate responses to prevent any significant drops in core temperature. An external cold stimulus is first sensed by the cutaneous thermosensitive neurons, located directly below the epidermal layer. Afferent information about the stimulus is sent back to the thermoregulatory centres of the spinal cord, brain stem and hypothalamus, which integrate the incoming signals and initiate appropriate responses to counteract heat loss (Simon, 1974). The thermoregulatory effectors consist of heat conserving and heat generating responses which act to decrease heat loss and increase heat production, respectively.

Heat conserving mechanisms are controlled by the sympathetic nervous system and include vasoconstriction of the cutaneous blood vessels and pilo erection of body hair. Peripheral vasoconstriction is the primary heat conserving mechanism in mammals, and acts to reduce heat transfer between the skin and the environment by decreasing blood flow to the skin. Pilo erection is another sympathetically-mediated cold defense mechanism which occurs in most mammals and involves contraction of

the piloerector smooth muscles attached to the base of hair follicles. When stimulated, these small muscles contract, bringing the hairs into an upright position. This allows the hairs to entrap a thick layer of air close to the skin which helps to insulate the animal and decrease convective heat loss to the environment. This response is not very important in humans, but it is an effective heat conservation method in fur-bearing animals, such as the rat.

Thermoregulatory heat generating responses can be classified as either non-shivering or shivering thermogenesis. Non-shivering thermogenesis (NST) increases heat production through an increase in the rate of cellular metabolism. This response is initiated by the activation of the sympathetic nervous system and occurs in most tissues of the body. The greatest portion of NST, however, occurs in brown adipose tissue (BAT) which is concentrated in the interscapular, perirenal, and axillary regions (Foster & Frydman, 1979). In fact, the maximal rate of NST in an animal was shown to be directly proportional to its total BAT mass. The importance of this tissue as a thermogenic source is undisputed and is evidenced by its very high oxidative capacity (Barnard & Skala, 1970) and rate of oxidative "uncoupling" (Shrago & Strieleman, 1987). The uncoupling of the oxidative phosphorylation process drastically reduces ATP synthesis, allowing BAT cells to continue oxidizing substrates for extended periods of time. Capacity for BAT thermogenesis varies greatly between species. BAT is abundant in hibernating animals and many rodents, and can contribute significantly to cold-induced thermogenesis (Shrago & Strieleman, 1987). In non-hibernating large animals, including humans, BAT is scarce and its functional capacity

for NST is controversial (Astrup, Bulow, Christensen, & Madsen, 1984).

Origin of Shivering Response

The majority of cold-induced thermogenesis in non-hibernating, non-acclimated animals, is derived from shivering activity in skeletal muscle. Shivering is an involuntary increase in activity and heat production in voluntary muscle. The primary centre responsible for initiating the shivering response is situated in the dorsomedial region of the posterior hypothalamus. This was established when electrical stimulation of this region was shown to elicit shivering in rats and cats (Stuart, Kawamura, & Hemingway, 1961; Halvorson & Thornhill, 1993), while lesioning of this area completely abolished shivering activity (Stuart, Kawamura, Hemingway, & Price, 1962). Stimulation of septum and midbrain regions of the CNS has also been shown to produce shivering activity in cats (Stuart et al., 1961) and goats (Andersson, 1957). Although these regions were able to elicit muscle activity, they were shown to be non-essential for shivering, as lesioning of the septum pellucidum (Stuart et al., 1962) and midbrain (Beaton, Leininger, & McKinley, 1943) left the cold-induced shivering response intact. Therefore, septum and midbrain areas appear to act as secondary control centres that either facilitate or inhibit the shivering signals generated by the primary centre in the dorsomedial region of the posterior hypothalamus.

The spinal cord has also been extensively studied as a possible site of thermal afferent integration and initiation of shivering. Increased electromyographic (EMG) activity was seen during cold-exposure in decerebrate cats (Stuart, Freeman, &

Hemingway, 1962), which demonstrates that the spinal cord is capable of integrating peripheral thermal afferents and initiating appropriate thermoregulatory responses. The spinal cord has also been shown to be a thermosensitive region by the fact that cooling the spinal canal elicited shivering in dogs (Jessen & Simon, 1971), guinea pigs (Bruck & Wunnenberg, 1970), monkeys (Chai & Lin, 1972), and rats (Lin, Yin, & Chai, 1972). When the hypothalamus was cooled or heated in addition to spinal cord cooling, shivering was shown to be augmented or inhibited, respectively (Jessen & Simon, 1971; Carlisle & Ingram, 1973). Thus the spinal cord acts as an additional thermoregulatory centre which can either reinforce or oppose the efferent signals generated by the posterior hypothalamic centre.

Signals generated from the hypothalamic centre for shivering descend caudally through the midbrain and pons to the rubro-spinal tracts in the lateral portion of the spinal cord and eventually synapse with alpha and gamma motoneurons in the anterior horns of the cord (Uprus, Gaylor, & Carmichael, 1935). The α-motoneurons of both the agonist and antagonist muscle groups are simultaneously stimulated so that muscle tension increases with no external work being accomplished. During the initial stages of shivering, motor units are recruited at different frequencies so that muscle tone increases but no coordinated tremor is observed. The tremor commonly associated with shivering is caused by an increase in the activity of the dynamic gamma motor fibres with a concomitant decrease in the static gamma motor fibres (Schafer & Schafer, 1973). This differential innervation of the intrafusal fibres creates an instability within the muscle spindle stretch reflex mechanism which produces the

oscillatory contractions. Perkins (1946) demonstrated the importance of muscle afferent fibres for shivering when cutting the dorsal roots of the spinal cord eliminated the tremor of shivering but retained the increased muscle tone.

Typically, shivering shows the strongest electrical activity within the muscles of the neck, shoulder girdle, thoracic wall, and thigh (Smith & Davidson, 1982; Tikuisis, Bell & Jacobs, 1991). Arm and lower leg muscles also exhibit some shivering activity as cooling becomes more intense, but EMG activity remains less than that of the larger, more centrally located muscles (Simon, 1974). This recruitment pattern during shivering may be beneficial for heat balance since much of the heat produced by the muscles of the periphery would be lost due to the smaller volume to surface area ratio (Precht, Christophersen, Hensel, & Larcher, 1973). Also, an increased blood flow to the peripheral muscles to support shivering would augment heat loss from the warm blood to the environment.

Substrate Availability and Utilization during Shivering

Intense shivering can result in a five-fold increase in basal metabolic rate, and can continue for hours if needed (Hemingway, 1963). During cold exposure, carbohydrate metabolism shows the greatest increase with that of lipids being much smaller. The importance of carbohydrates as a fuel source for thermogenesis was shown in rats (Depocas, 1962), dogs (Minaire, Vincent-Falquet, Pernod, & Chatonnet, 1973), and humans (Vallerand & Jacobs, 1989). Vallerand and Jacobs (1989) have shown that during cold exposure in humans, carbohydrate metabolism increased by

nearly 590%, whereas fat oxidation increased by only 63%. Both intracellular (glycogen) and circulating (blood glucose) carbohydrates were shown to be utilized in the thermoregulation of cold-exposed rats (Depocas, 1962; Depocas & Masironi, 1960) and dogs (Minaire et al., 1973). The importance of glycogen stores was evidenced in humans when subjects with low muscle glycogen levels exhibited a more rapid rate of cooling than those with normal muscle glycogen levels (Martineau & Jacobs, 1991). A sufficient level of circulating blood glucose also appears to be essential since insulin-induced hypoglycaemia inhibited shivering in cold exposed humans (Bennett, Gale, Green, MacDonald, & Walford, 1980). Carbohydrates are the primary fuel source for thermoregulatory heat production, however, lipids continue to contribute to overall energy expenditure in the cold. This was shown in humans, where the energy contributions from lipids and carbohydrates at rest were 59 and 18% respectively, whereas in the cold these ratios were 39 and 51%, respectively (Vallerand & Jacobs, 1989). Because lipids are used to a lesser degree and are normally stored in far greater quantities, any deficiency in shivering thermogenesis resulting from insufficient substrate supply would presumably result from an inadequate carbohydrate availability.

Factors Affecting Shivering

Shivering is an involuntary response but, similar to respiration, it can be controlled to a certain extent through voluntary measures. Glickman, Mitchell, Keeton, and Lambert (1967) showed that even during extreme cold exposure (-29°C)

subjects were able to suppress shivering by voluntary relaxation of the muscle. This inhibition demonstrates that voluntary pathways retain greater influence over the α-motoneurons than pathways for the control of shivering.

Several other non-thermal stimuli, including cognitive tasks, muscle stretching and cardio-respiratory reflexes, have also been shown to either facilitate or suppress shivering activity during cold exposure. Performing mental arithmetic tasks, has consistently attenuated EMG activity in shivering subjects (Israel, Wittmers, Hoffman, & Pozos, 1993; Martin & Cooper, 1981; Klenow, Simmons, & Pozos, 1987). Inhibition of shivering by increased cortical activity is supported by studies in which shivering activity was abolished by electrical stimulation of various areas of the cerebral cortex in monkeys, cats, and dogs (Kaada, 1951). The mechanism by which the cortex exerts influence over shivering activity is not known but was suggested to be through a modulatory control over the primary shivering centre of hypothalamus (Klenow et al, 1987). The cerebral cortex, however, may also inhibit shivering via direct control over the shivering efferent pathway. The brainstem reticular formation has direct cortical connections and can influence muscle tone via gamma motoneuron activation (Carpenter, 1976). Stretching a muscle, on the other hand, was shown to increase EMG activity in a shivering animal (Burton & Bronk, 1937), and initiate shivering in a cooled but non-shivering cat (Kawamura & Kishi-Kinichi, 1956). This facilitation of shivering and generation of muscle tremor caused by muscle stretching is probably mediated through the muscle spindle stretch receptors (Schafer & Schafer, 1973). The influence of muscle length on shivering activity may explain the

oscillatory nature of shivering which parallels the respiratory cycle. For example, inspiration has been shown to have a facilitory effect on shivering in cats (Kawamura & Kishi-Kinichi, 1956), dogs (Hemingway, Forgrave, & Birzis, 1954), and humans (Blatteis, 1960), and may result from rhythmical stretching of the muscles of the thoracic cage.

In addition to cortical activity and mechanical stretch of the muscle, shivering intensity may also be affected by certain cardiovascular and respiratory reflexes. An increase in systemic arterial pressure has been shown to augment shivering activity, whereas carotid sinus pressures less than 70 mmHg resulted in inhibition of shivering (Ishii & Ishii, 1960). The relationship between arterial pressure and shivering is believed to be mediated through the arterial baroreceptors since cutting of the sinus nerve abolished the enhanced EMG activity that is typically observed when carotid sinus pressure is increased (Mott, 1963). Conversely, increased chemoreceptor stimulation elicited either by hypoxia (Mott, 1963; von Euler & Soederberg, 1958) or hypercapnia (Szegvari, Varnai, & Donhoffer, 1962) resulted in the attenuation of EMG activity. It is uncertain how baroreceptors and chemoreceptors regulate shivering activity, but it has been suggested that these afferent signals alter α-motoneuron activity primarily through stimulation of mid-brain regions (Mott, 1963).

Muscular shivering typically waxes and wanes in intensity. Part of this fluctuation is related to the respiratory rhythm, however periodic oscillations of a longer duration are also seen (Burton & Edholm, 1955). The combined influence of the preceding mechanisms may account for this additional fluctuation in shivering

activity.

Diabetes Mellitus and Environmental Cooling

The previous sections have outlined the physiological responses occurring in mammals during exposure to a cold environment. In healthy animals, these heat conserving and generating mechanisms would enable the animal to cope with most moderate cold stresses without suffering any serious decreases in body temperature. Some pathological conditions such as diabetes mellitus, however, impair certain effector response systems and decrease the thermoregulatory capacity of the animal. A decreased tolerance to cold exposure has been associated with diabetes in both humans (Scott, Bennett, & MacDonald, 1987; Scott, MacDonald, Bennett, & Tattersall, 1988) and animals (Kilgour & Williams, 1994;Macari, Ferro, & Eizirik, 1986; Shalaby, Yousef, & Dupre, 1989).

Human diabetes mellitus can be divided into insulin-dependant (type I) diabetes, characterized by an absolute insulin-deficiency, and a non-insulin dependant (type II) diabetes, characterized by a decreased insulin responsiveness to plasma glucose levels. Type I diabetes results from destruction of the insulin-secreting beta cells of the pancreas. Although the causes of Type I diabetes are not definite, there is a strong probability that destruction of the ß-cells results from a viral infection or an autoimmune reaction. Type II diabetes is often associated with obesity and results from a decreased responsiveness of the beta cells to increased blood glucose, and a relative decrease in the number of insulin receptors in target cells throughout the body

(Guyton, 1991). Physiological features common to both types of diabetes include; decreased glucose utilization by body cells resulting in hyperglycaemia in the range of 300-1200 mg/dl, increased mobilization and utilization of body lipid stores, and depletion of tissue protein. Directly or indirectly as a result of these diabetic complications, dysfunctions in several physiological systems occur, which could ultimately decrease an individual's capacity to regulate body temperature.

In order to effectively examine the problems associated with the diabetic condition, several different animal models of diabetes have been developed. Although none of the models exactly reproduce the full range of complications associated with human diabetes, many of these models display morphologic and metabolic alterations similar to the human condition. Among the most commonly used diabetic models in physiological research are the streptozotocin (STZ)- and alloxan-induced diabetic conditions. Both agents selectively destroy beta cells of the pancreas in a dose dependant manner. Standard diabetogenic doses for alloxan and streptozotocin are 40-45 mg/kg and 65 mg/kg, respectively (Bell & Hye, 1983). The diabetic conditions produced by alloxan and streptozotocin display similar metabolic changes which result directly or indirectly from the reduced insulin levels. As a direct consequence of the lower insulin levels, carbohydrate metabolism is severely attenuated due to the decrease in blood glucose uptake by the body. This results in a chronic, stable hyperglycaemic state in the range of 400 mg/dl or more (Shafrir, 1990). Although insulin is not required for glucose uptake by the liver, its presence normally stimulates glycogen synthetase activity which promote the formation of liver glycogen. In

addition, the lack of insulin stimulates glycogen phosphorylase activity in liver and skeletai muscle, further reducing glycogen levels in these tissues of diabetic animals (Miller, 1978). Lipid metabolism is also severely altered in chemically-induced diabetic animals. Mobilization and utilization of stored triglycerides is greatly enhanced, as evidenced by an increase in the activity of adipose tissue lipase (Shafrir, 1990), elevated plasma free fatty acid and triglyceride levels (Bar-On, Ronheim, & Eder, 1976), and increased mitochondrial fatty acid uptake in liver, kidney and skeletal muscle (Fogle & Bieber, 1979). Experimental diabetic animals also display a negative nitrogen balance caused by an increased proteolysis in muscle and other tissue, coupled with lower rates of protein synthesis (Smith, Wong & Gelfand, 1989). The amino acids generated from the increased protein catabolism fuel liver gluconeogenesis which is also enhanced by the lower insulin levels.

As a direct or indirect result of the hypoinsulinemia and altered metabolic functions, experimental diabetes is associated with functional and morphological changes in peripheral nervous, muscle (skeletal and cardiac), and vascular tissues. Functional alterations in these various tissues attenuate the capacity of the diabetic animal to regulate body temperature. Impaired thermoregulation in diabetic animals stems from deficiencies in both the heat conserving and thermogenic systems.

Thermoregulation in Experimental Diabetes: Influence on Heat Conservation

Diabetic animals have consistently shown attenuated pressor responses to both cold stress (Kilgour and Williams, 1994; Lucas, 1985) and catecholamine infusion

(Lucas, 1985). In a study by Lucas (1985), streptozotocin-induced (STZ) diabetic rats showed a slower rise and decreased maximal level of peripheral vascular resistance to exogenous norepinephrine, suggesting an insensitive vasoconstriction response of the cutaneous vasculature. In a study which examined the sensitivity of various arterioles to norepinephrine application, resistance vessels from STZ-diabetic rats showed an attenuated level of vasoconstriction (Morff, 1990). These findings suggest that one possible mechanism responsible for the increased heat loss in diabetic animals in the cold is a decreased sensitivity of the peripheral blood vessels to catecholamine stimulation, leading to decreased vasoconstriction. Deficits in pressor response, however, may also originate in the sympathetic nerve fibres innervating the blood vessels, as STZ-diabetic rats have shown reduced norepinephrine content of sympathetic nerve fibres and decreased vessel constriction in response to transmural nerve stimulation (Hart, Freas, McKenzie, & Muldoon, 1988). The decreased function of the sympathetic fibres in STZ-diabetic rats may be the result of a diabetic autonomic neuropathy (DAN) which results from structural, biochemical, and functional changes in autonomic nerve fibres, and has been shown to occur in chronically diabetic humans (Scott et al., 1988) and rats (Schmidt & Plurad, 1986). Impaired autonomic function and decreased vascular sensitivity to catecholamine stimulation would presumably cause the cold-stressed diabetic animal to display a greater skin blood flow and faster rate of heat loss. Evidence for this was shown when cold exposure (5°C) resulted in higher tail skin blood flows and larger decreases in core temperature in STZ-diabetic rats compared with controls (Shalaby et al.,

1989).

Thermoregulation in Experimental Diabetes: Influence on Brown Adipose Tissue

The only heat generating mechanism which has consistently been shown to be affected in experimentally diabetic animals is non-shivering thermogenesis within brown adipose tissue (BAT). The alterations precipitating the decrease in NST are numerous and originate within both the BAT and the sympathetic fibres innervating the tissue. The total mass of interscapular BAT from streptozotocin or alloxan diabetic rats has been shown to be reduced to 30-57% of control values (Yoshioka, Yoshida, Wakabayashi, Nishioka, & Kondo, 1989; Seydoux, et al., 1983; Macari et al., 1986). Seydoux et al. (1983) showed that the reduced mass of BAT in STZ-diabetic rats was due to cell atrophy, as protein and lipid contents were reduced to approximately 50% of control values, while no change in total DNA content was found. In addition to the decreased mass of BAT, oxygen uptake and heat production per unit weight of BAT was also shown to be reduced. Norepinephrine-stimulated oxygen uptake in isolated whole brown adipocytes from STZ-diabetics was found to be reduced to 58% of control values (Jamal and Saggerson, 1988). Yoshioka et al. (1989) showed that the lowered oxygen consumption of diabetic BAT tissue was not due simply to a reduced number of mitochondria, as oxygen consumption per milligram of mitochondrial protein was severely attenuated in diabetic rats compared with controls in response to a 4°C cold stress. In addition to the decreased oxidative capacity of brown adipocytes, interscapular BAT from diabetic rats showed a 56% lower rate of specific heat

production in response to catecholamine stimulation (Seydoux et al., 1983). The diminished heat generation was attributed to a tighter coupling of the oxidative phosphorylation process caused by a lower percentage of the uncoupling protein in the inner mitochondrial membrane. In addition to the detriments observed within BAT, sympathetic activation of this tissue has also been shown to be reduced. Using norepinephrine turnover rate as an indicator, sympathetic nerve activity in interscapular BAT of diabetic rodents was found to be greatly attenuated both at rest (Young, Einhorn, & Landsberg, 1982) and during cold exposure (Yoshioka et al., 1989). Due to the decreased oxidative and thermogenic capabilities of BAT, and the attenuated sympathetic activation, the heat generation from this tissue is severely depressed in diabetic animals.

Thermoregulation in Experimental Diabetes: Influence on Shivering

An additional form of heat production in mammals is shivering. Although this is the main thermogenic source in large animals and non-acclimated rodents, it has received very little attention in diabetic research. The main substrate utilized for shivering is carbohydrates, obtained from circulating plasma glucose and intramuscular glycogen stores. Normally, in the diabetic condition, low levels of insulin cause glucose uptake and glycogen storage by skeletal muscle to be severely depressed. The inability to utilize carbohydrates could presumably reduce the capacity for shivering thermogenesis in diabetic animals. However, Smith and Davidson (1982) showed that during a cold stress, glucose uptake into skeletal muscle was facilitated in mildly

(streptozotocin, 40 mg/kg) diabetic rats, as evidenced by decreases in plasma glucose. Conversely, severely diabetic (90 mg/kg) rats showed no glucose uptake in the cold, and even showed a rise in plasma glucose indicating that cold exposure exacerbated the hyperglycemic condition. The authors suggested that the markedly increased level of free fatty acids in the severely diabetic animals hindered glucose uptake, since oxidation of lipid substrates has been shown to inhibit carbohydrate utilization (Newsholme, 1979). Smith and Davidson (1982) attributed the enhanced glucose uptake in the milder diabetic state to shivering however, no quantitative measures of muscle activity were reported. Since these authors examined only mild and severe diabetic conditions, and found opposite results, it would be difficult to estimate how carbohydrate metabolism and shivering would be affected in moderately diabetic (65 mg/kg, streptozotocin) animals, which is a common level of STZ-induced diabetes used in research.

Examination of the shivering thermogenic response in diabetic animals does deserve merit. Apart from the relative deficit in carbohydrate utilization, several other abnormalities associated with the diabetic condition may contribute to reduced capacity for muscular shivering. STZ-diabetic rats have shown significantly lower activity levels for both oxidative (succinate dehydrogenase) and glycolytic (phosphofructokinase) enzymes in limb muscles (Ianuzzo & Armstrong, 1976). Considering the importance of carbohydrates for shivering, a reduction in glycolytic flux in skeletal muscle of diabetic animals may hinder shivering response. A lower aerobic capacity in diabetic rats could result in an earlier onset of fatigue during

prolonged muscular shivering.

Morphological changes such as decreased growth and progressive atrophy of skeletal muscle have also been shown to occur in STZ-diabetic rats. Kuehn, Dahlmann, Heath, and Kay (1988) examined the gastrocnemius muscle of STZ-diabetic rats at either 1,2,4, or 7 days post-STZ injection. These authors found progressive decreases in muscle weight and increases in the activity of inherent muscle protease enzymes. Smith, Wong, and Gelfand (1989) observed a 30% increase in total protein degradation in STZ-diabetic rats, with a specific 60% increase in catabolism of muscle myofibrils. The decreased muscle mass and amount of contractile proteins in diabetic rats would presumably lower the maximum contractile force produced by the skeletal muscles. This was conclusively shown by decreased maximal twitch tensions in soleus (Cameron, Cotter, & Robertson, 1991) and extensor digitorum longus muscles (Cameron, Cotter, & Robertson, 1990) of STZ-diabetic rats. Bell, Tikuisis, and Jacobs (1992) calculated the maximal intensity of shivering contraction to be approximately 20% of maximum voluntary contraction (MVC) in healthy subjects. They suggested that this relatively low intensity of muscle contraction acts to delay myoelectric fatigue during prolonged shivering. The relative intensity of diabetic skeletal muscle contraction during shivering has not been assessed. If the muscles of diabetic animals shiver at a percentage of MVC similar to that of normal animals, a lesser absolute contractile force for shivering would be generated leading to reduced thermogenesis. If a similar absolute muscle tension is elicited in the diabetic animal, a greater percentage of the maximum contractile capacity would

be recruited and fatigue of the muscle would occur sooner. Thus, the reduced maximal contraction of diabetic skeletal muscle could lead to either a lower rate of thermogenesis or an earlier onset of neuromuscular fatigue during shivering.

Neuropathy is frequently observed in both diabetic humans (Greene, Sima, Albers, & Pfeifer, 1990) and animals (Bell & Hye, 1983), and has been implicated as a contributing factor in many of the physiological abnormalities associated with the diabetic condition. The alterations which are most commonly observed with diabetic neuropathy are a slowing of the nerve fibre conduction velocity (Moore, Peterson, Felten, & O'Connor, 1980), and a degeneration of the axonal terminals (Jakobsen, 1976). The nerve fibres which are most commonly affected are the smaller, slower conducting sensory fibres (Greene et al., 1990), however deficits in motor nerve conduction velocity have also been observed (Greene, DeJesus, & Winegrad, 1975). The problems that these deficits may pose for thermoregulation are several-fold. First, a decrease in thermal perception, due to an increased threshold potential for the cold receptors could necessitate a greater external cold stimulus to elicit the same thermoregulatory response of a normal animal. Although it is difficult to assess thermal perception in diabetic animals, decreased thermal perception thresholds to cold are commonly observed in patients with diabetic neuropathy (Dyck, Karnes, & O'Brien, 1987).

Afferent fibres involved in the muscle stretch reflex have also been shown to be impaired in diabetic neuropathy. Diminished or absent deep tendon reflexes, especially the Achilles tendon reflex, are often observed in diabetic patients (Brown &

Greene, 1984). Since the muscle sensory nerves are necessary for the generation of normal shivering (Schafer & Schafer, 1973), impairment within these fibres may lead to a loss or attenuation of the tremor associated with muscular shivering. The motor nerve fibres, although affected to a lesser extent than sensory fibres, cannot be excluded as a possible site for the impairment of shivering in diabetic animals. A decrease in motor nerve conduction velocity may result in a decreased firing rate of muscle fibres during shivering. A reduction in firing frequencies of muscle fibres could lead to a reduction in the overall tension developed during shivering and lower level of heat production.

The metabolic and neuromuscular abnormalities previously outlined could potentially decrease the shivering ability of the diabetic animal. This would result in a greater susceptibility to hypothermia and death during exposure to an acute cold stress in a diabetic rat.

Purpose of the Study

The purpose of the present study was to compare the shivering response of non-diabetic and STZ-diabetic rats during progressive hypothermia in a cold environment (8-10°C). Shivering activity was assessed using intramuscular EMG recordings of the gluteus superficialis muscle.

Taking into account the importance of substrate availability, the effect of an overnight fast on shivering response in control and STZ-diabetic animals was also examined. The influence of the nutritive state on shivering response was proposed in

an attempt to identify whether the impairment of shivering in the diabetic animal was associated with alterations in substrate metabolism. Overnight fasting in healthy animals produces acute metabolic changes which are somewhat similar to the chronic alterations associated with the diabetic condition. Short-term fasting (12-16 hrs) in rats results in an almost complete depletion of liver glycogen stores with an increased mobilization and utilization of lipids (Smith & Davidson, 1982). Blood glucose levels are maintained at near normal values, however, glycolytic flux is severely inhibited due to the increased level of fatty acid oxidation (Newsholme, 1979). Fasting has also been shown to produce a decreased capacity for thermogenesis in rats (Wang, 1980), which can be overcome by feeding a mixture of substrates to the animal prior to cooling. Thus, the attenuated thermogenesis in the fasted rat was likely due to the lack of available carbohydrates, since free fatty acid levels are increased in the fasted state. Therefore, to determine if fasting in healthy animals and diabetic animals would result in a decrement in shivering response, an additional group of control and STZ-diabetic animals were tested following an overnight (12 hr) fast.

METHODS

Animal Care

Forty-three male Wistar rats (221.2 ± 1.6 grams) were obtained from Charles River Breeding Farms (St. Constant, Que.). Animals were housed individually, provided with free access to a standard dry laboratory rat chow and water, and maintained on a 12/12 hr light/dark cycle with an ambient temperature of 22 ± 2 °C. All rats were randomly assigned to one of four groups; control non-fasted (n=10), diabetic non-fasted (n=10), control fasted (n=11), and diabetic fasted (n=12).

Induction of Diabetes

Diabetes was induced by a single injection of streptozotocin (STZ; 65 mg/kg, Sigma) into the penile vein under methoxyflurane (Metofane, Pitmann-Moore) anesthesia. STZ was freshly dissolved in less than 1 ml of 0.9% saline containing sodium citrate (20 mmol/l, Ph=4.5). Control animals received an injection of citrate buffer. Diabetes was confirmed by decreased weight gain throughout the four week period, and elevated plasma glucose concentration at the time of testing (>400 mg/dl).

Pre-experimental Protocol

Animals were monitored weekly for weight gains. Testing occurred four weeks following the streptozotocin injection. The fasted groups of rats had food

removed 12-14 hours prior to testing. Animals were anesthetized with an intraperitoneal injection of urethane (1.5 g/kg, Sigma). Throughout the surgical preparation, rectal temperature was maintained between 36.5 and 37.0 °C using a Harvard homeothermic blanket (model 50-7053). After inserting an endotracheal tube (PE-240; Clay-Adams), the animal was ventilated with room air (90 breaths/min, minute ventilation ≈ 450 ml/kg/min) using a Harvard Rodent Ventilator (model 683). Expired gases were collected in 300 ml rubber bladders (Physabek, Model 162130) from the expiration port of the ventilator. A catheter (PE-50; Clay-Adams) was inserted into the left common carotid artery and connected to a pressure transducer (Lafayette, model 76626) and a physiograph (Lafayette, model 76102) for monitoring of heart rate and blood pressure. The catheter and transducer were filled with heparinized (200 units/ml) saline to prevent coagulation.

After exposing the gluteus superficialis muscle, two insulated fine wire platinum electrodes were separately threaded through the central part of the muscle (approx. 5mm apart) using 20 gauge stainless steel needles. A small portion (approx. 1mm) of the insulative covering was removed from the two recording electrodes approximately 1 cm from the ends so that electrical activity could be recorded form the centre of the muscle. The wires were silk-sutured at the entry and exit points of the muscle to ensure that the bared recording portion of the electrodes remained fixed at the same central location of the muscle throughout the experiment. Electrodes were prepared prior to each testing session and a test for conductivity was performed to ensure that the insulative covering had been sufficiently removed. A ground electrode

was placed subcutaneously and approximately 5 cm from the site of the recording electrodes.

Experimental Protocol

After the surgical preparation, a 15-20 minute stabilization period was used to establish baseline heart rate and blood pressure values. Following this period, the animal was removed from the heating blanket and placed on a wire mesh stand. The stand and the animal were placed inside a steel chamber (48 X 16 X 14 cm), which was submerged 12 cm into an ice bath. The top of the chamber was covered with insulative styrofoam, with spaces to allow for passage of the EMG leads, arterial catheter and the endotracheal tube. The ambient temperature inside the chamber rapidly declined and remained constant (10 $\pm$ 2°C) throughout the entire cooling session. Blood pressure, heart rate, EMG activity, and oxygen consumption measurements were recorded at every °C of T_{rec}, between 36 and 28°C. The experiment was terminated when T_{rec} reached 28°C.

Determination of Plasma Glucose

An arterial blood sample (< 0.5 ml) was collected from the animal prior to cooling and was immediately centrifuged (1,000 X g for 5 min.). Plamsa was removed and frozen for subsequent analysis. Plasma glucose concentration was determined spectrophotometrically (LKB, Model 4050) using a commercially available enzymatic assay (Synermed). Plasma from the diabetic animals was diluted two-fold

to ensure that concentration values would fall within the calibrated range of the assay.

Determination of Temperature Measurements

Rectal temperature (T_{rec}) was measured using a rectal probe inserted 5 cm beyond the anal sphincter. Chamber temperature was monitored using a 10 mm diameter thermistor (Yellow Springs Instrument (YSI), model 409B) placed directly adjacent to the animal. Using a YSI scanning telethermometer (model 47) both temperature readings were recorded in millivolts and later converted into thermal units using a previously calibrated (range 0-40°C) conversion factor.

Determination of Heart Rate and Blood Pressure

Systolic (SBP) and diastolic (DBP) blood pressures were determined by averaging 30 seconds of the upper and lower peak signals on the physiograph tracings. Mean arterial pressure (MAP) was calculated using the equation MAP = DBP + 0.3(SBP-DBP). Heart rate (HR) was calculated using the time between 20 consecutive systolic peaks and later converted into beats/minute.

Determination of Oxygen Consumption

Expired gases were collected continuously for 60 seconds from the export valve of the rodent ventilator. Oxygen and carbon dioxide contents of the gas were determined off-line using an O_2 analyzer (Applied Electrochemistry Inc., model S-3A) and an infrared CO_2 analyzer (model CD-3A), respectively. The absolute volume of

the one minute sample was determined by evacuating the bladder with a calibrated syringe. Oxygen consumption (VO_2) data was expressed as the absolute change from baseline value (ml/min).

Recording of the EMG Signal

Electrical activity from the recording electrodes was differentially amplified (X 10,000) using a DISA (model 15-C-01) bioelectric amplifier (bandpass 0.5-2000 Hz), and digitized using an analog-to-digital converter (Techmar Labmaster). Eight second intervals of EMG activity were saved on computer (IBM 386 PC), at a sampling rate of 1024 Hz, and later analyzed off-line. This sampling rate was chosen to ensure the recording of the entire spectrum of EMG activity, since the EMG of shivering has been previously shown to enter into the 600 Hz range (Hohtola, 1982).

Signal Processing

Raw EMG signals were analyzed using a signal processing software package (386-Matlab, Mathworks, Inc.). Signals were first submitted to a high-pass (cutoff 15 Hz), high-order (1024) digital filter to eliminate lower frequency artifacts produced by heart rate and respiratory movements. A narrow band-rejection filter (bandwidth 58-62 Hz, order 1024) was used to eliminate any extraneous 60 Hz noise. The filtered EMG signal was full-wave rectified and integrated over the entire 8 seconds. The integrated voltage was divided by the total number of points (n) to obtain a mean rectified EMG value (U_{mrv}), according to equation 1.

$$U_{mrv} = \frac{\int_{i=1}^{i=n} |Y_i|}{n} \quad \text{....eq. 1}$$

where Y_i is the voltage at each point (i) over the eight second aquisition. An example of the filtered EMG, a full wave rectification of the signal, and the area integration of the curve is illustrated in Figure 1.

Statistical Analysis

A three-way analysis of variance (ANOVA) with repeated measures was used to compare the two levels of treatment (control vs. STZ), the two levels of feeding (non-fasted vs. fasted), and the ten levels of T_{rec}. Simple two-way ANOVA's (treatment X feeding) were used for analyzing plasma glucose levels and total time of cooling. When the appropriate level of significance ($P < .05$) was obtained, Bonferroni corrected t-tests were used to identify differences between pairs of means for each dependant variable. Statistics were analyzed using the SYSTAT software package developed by Wilkinson (1989).

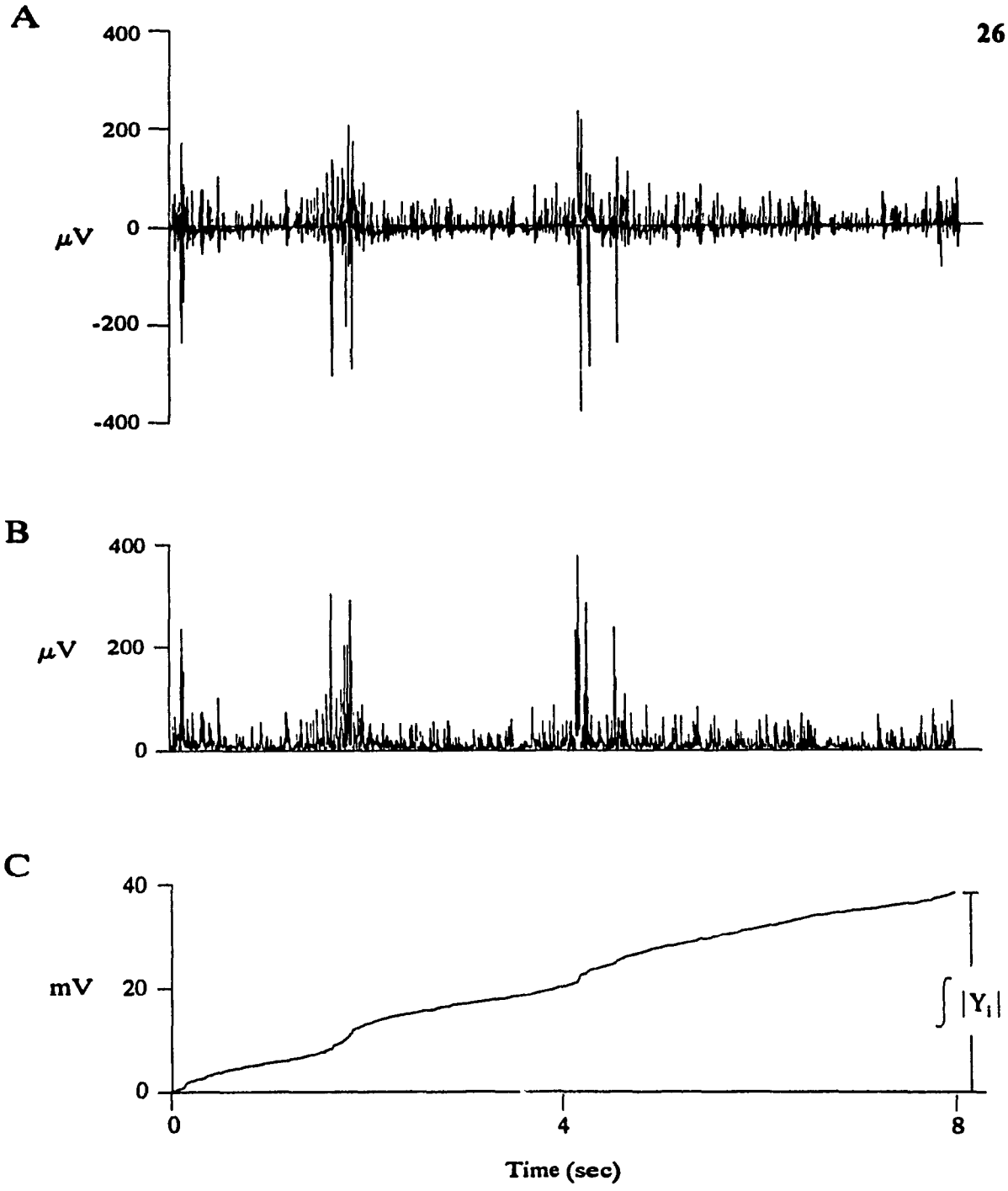

Figure 1. An illustration of a filtered EMG signal (A), a full wave rectified signal (B), and the integrated area under this curve over an eight second acquisition (C).

RESULTS

Body Weight and Plasma Glucose

Table 1 shows the mean body weights of the four groups at the time of injection, during the four weeks following the injection, and at the time of testing. The STZ groups showed significantly reduced weight gains from weeks one to four compared with controls (significant main effect for treatment, $F(1,41)=152.98$, $p<.001$). No differences in weight gains were observed between the two control groups or the two STZ groups from the time of injection until week 4. Table 1 also shows the mean weights of each group prior to the cooling experiment. The STZ fasted group weighed significantly less than the STZ non-fasted group following the 12 hour fast ($p<.05$). However, the fasted control group showed no significant difference in weight compared with the non-fasted group at the time of testing.

Figure 2 shows the plasma glucose levels of the four groups at the time of testing. As expected, glucose levels following the four weeks were significantly higher in both STZ groups compared with their corresponding controls (STZ fasted, 620.0 ± 32.9 vs Control fasted, 239.8 ± 12.3 $mg{\cdot}dl^{-1}$; $p<.05$; STZ non-fasted, 571.3 ± 24.3 vs Control non-fasted, 234.2 ± 15.4 $mg{\cdot}dl^{-1}$; $p<.05$). The plasma glucose levels of the STZ fasted and Control fasted groups were not significantly different from the corresponding non-fasted groups.

	Body Weight (grams)					
Group	Day of Injection	Week 1	Week 2	Week 3	Week 4	Day of Testing
Control non-fasted	223.7 (3.5)	279.1 (5.9)	324.5 (5.4)	357.5 (5.4)	384.2 (6.1)	409.4 (8.0)
STZ non-fasted	224.5 (3.4)	244.2^{+} (4.8)	274.0^{+} (8.3)	285.1^{+} (10.0)	288.7^{+} (9.3)	291.4^{+} (11.5)
Control fasted	216.9 (2.4)	274.7 (1.6)	324.9 (4.5)	364.7 (6.3)	390.7 (5.8)	394.7 (7.0)
STZ fasted	217.9 (3.2)	243.8^{+} (3.6)	271.5^{+} (5.7)	288.4^{+} (6.7)	287.6^{+} (8.7)	253.9$^{+,\bullet}$ (6.8)

Table 1. Body weights (mean ± S.E.M.) of control and STZ-diabetic groups at the time of STZ injection, the subsequent four weeks, and on the day of testing. On the day of testing, fasted groups were weighed following 12 hours of total food restriction. Symbols indicate significant differences ($p < .05$) between corresponding control groups ($^{+}$) or corresponding non-fasted groups ($^{\bullet}$).

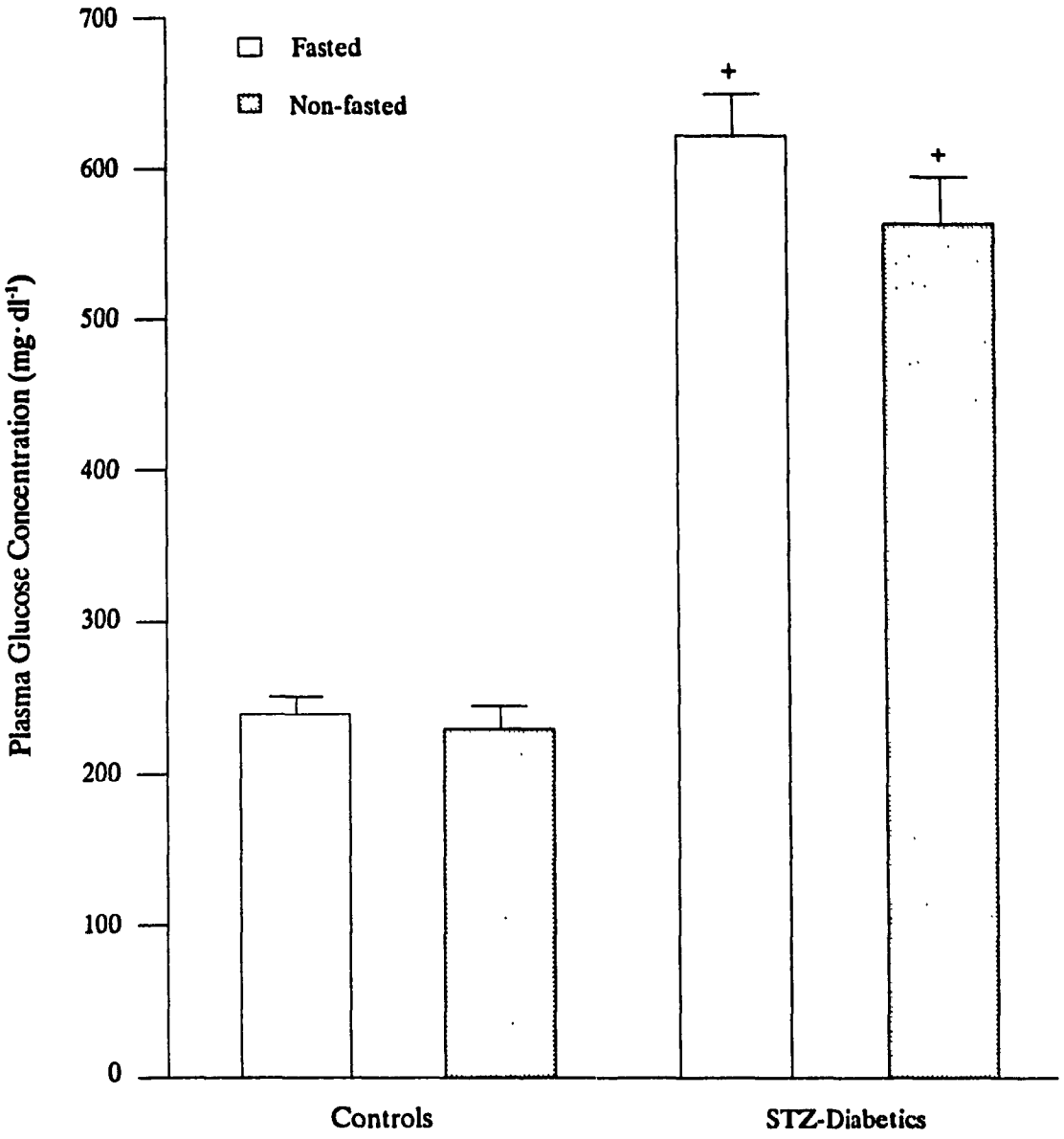

Figure 2. Plasma glucose concentrations (mean ± S.E.M.) of control and STZ-diabetic animals in both non-fasted and fasted conditions. Symbols indicate significant ($p < .05$) difference from corresponding control group (+).

Total Time of Cooling

The average length of time for each group to reach a T_{rec} of 28°C is shown in Figure 3. Diabetic animals cooled more rapidly than controls (significant main effect for treatment, $F(1,39)=23.99$, $p<.001$). Under the non-fasted conditions, STZ rats displayed a significantly reduced time to reach a T_{rec} of 28°C (STZ; 48.5 ± 1.5 vs Control; 136.5 ± 23.0 minutes; $p<.05$). Following 12 hours of fasting, STZ animals still cooled at a faster rate than the fasted control, reaching the final rectal temperature earlier (STZ; 43.9 ± 1.3 vs Control; 73.4 ± 4.2 minutes; $p<.05$). No significant differences were observed between the STZ fasted and non-fasted groups with respect to length of time reach T_{rec} of 28°C. Fasted controls, however, cooled more rapidly than the non-fasted controls, as their length of time to reach T_{rec} of 28°C was significantly lower ($p<.05$).

EMG Activity

Figure 4 shows the mean rectified EMG values (U_{mrv}) of the four groups at baseline and as rectal temperature declined from 36 to 28 °C. There were no significant differences in U_{mrv} between any groups prior to cooling. The only group to display a significant increase in U_{mrv} during cooling was the non-fasted control group. The U_{mrv} of this group was significantly ($p<.05$) higher than baseline beyond a T_{rec} of 32°C, and demonstrated a peak value of 7.89 ± 1.8 μvolts at a T_{rec} of 29°C. Consequently, the U_{mrv} of the non-fasted control group was significantly higher than the non-fasted STZ group between a T_{rec} of 33 and 28°C ($p<.05$), and significantly

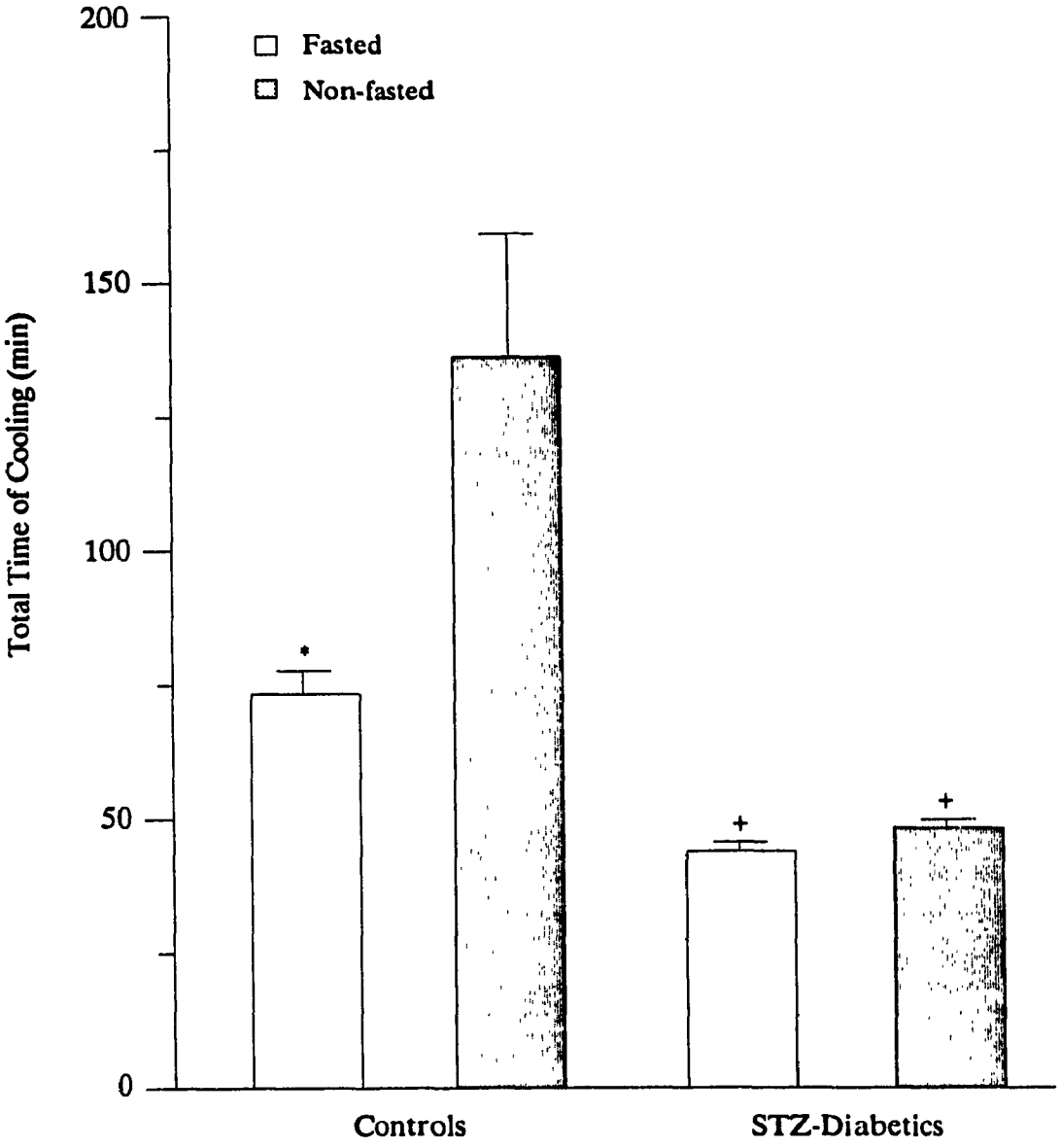

Figure 3. The length of time (mean ± S.E.M.) taken by each group to achieve a rectal temperature of 28°C. Symbols indicate significant ($p < .05$) differences from corresponding control group (+) or corresponding non-fasted group (*).

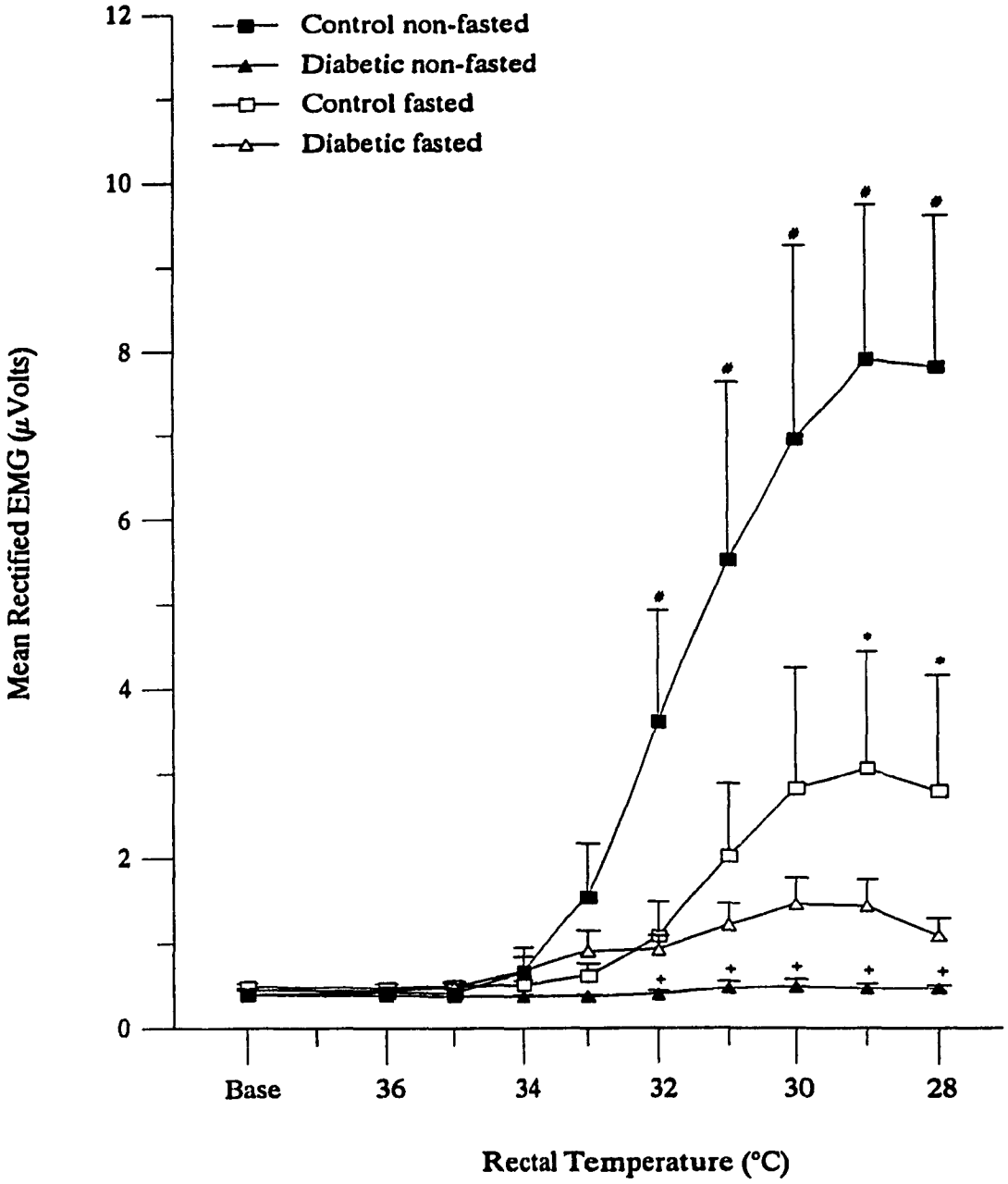

Figure 4. The influence of progressive hypothermia on the mean rectified EMG (mean ± S.E.M) in control and STZ-diabetic animals in both non-fasted and fasted conditions. Symbols indicate significant ($p < .05$) differences from corresponding control group (+), corresponding non-fasted group (*), or from baseline values (#).

higher than the fasted control group between a T_{rec} of 32 and 28°C ($p < .05$). The fasted control group showed a small increase in U_{mrv} during cooling, however there was no significant change from baseline due to the large variance associated with this group. The STZ fasted group also displayed a slight, but non-significant increase in U_{mrv} during cooling.

Oxygen Consumption

The change in oxygen consumption (ΔVO_2) from baseline during cooling is shown in Figure 5. All four groups experienced significant changes in oxygen consumption during the cold stress, however opposite trends were observed between the control and STZ groups. The non-fasted control group showed the largest rise in VO_2, being significantly increased from baseline beyond a T_{rec} of 34°C and reaching a peak ΔVO_2 of 4.51 $\pm$ 0.50 ml $O_2 \cdot min^{-1}$ at a T_{rec} of 31°C. The fasted control group showed a similar trend in VO_2 response being significantly increased from baseline beyond a T_{rec} of 32°C. However, the fasted controls displayed a significantly ($p < .05$) lower increase in VO_2 (ΔVO_2 peak = 2.11 $\pm$ 0.36 ml $O_2 \cdot min^{-1}$ at 31°C), compared with non-fasted controls.

Both STZ groups showed a progressive decline in VO_2 throughout cooling ($p < .05$), however the two groups did not differ from each other during the cooling period. The non-fasted STZ group was significantly lower than the corresponding control group beyond a T_{rec} of 35°C ($p < .05$), while the fasted STZ group showed a significantly lower VO_2 than its corresponding control group beyond a T_{rec} of 34°C

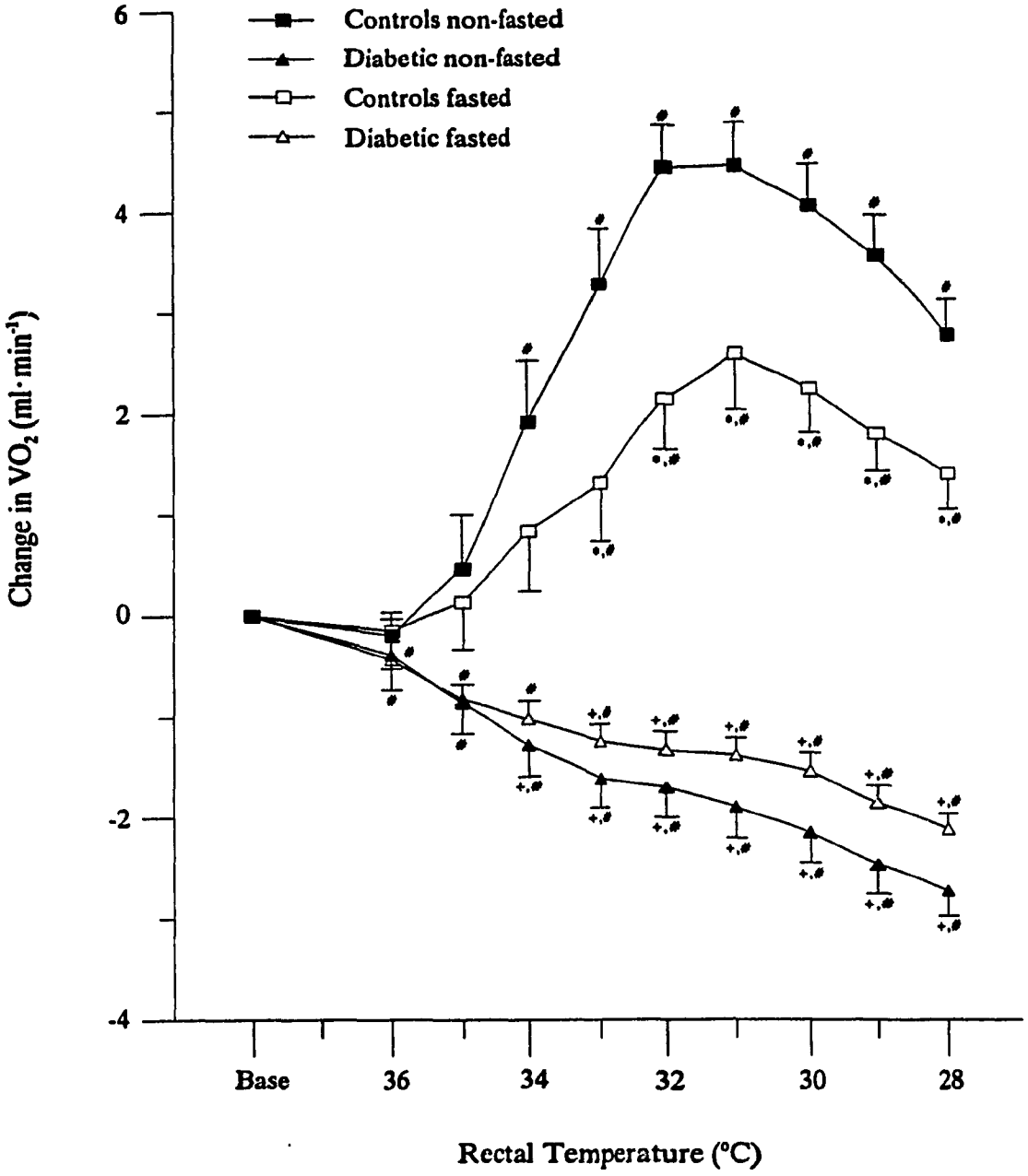

Figure 5. The relative changes in oxygen consumption (VO_2; mean ± S.E.M.) during cooling in fasted and non-fasted control and STZ-diabetic groups. Symbols indicate significant ($p < .05$) differences from corresponding control group (+), corresponding non-fasted group (*), or from baseline values (#).

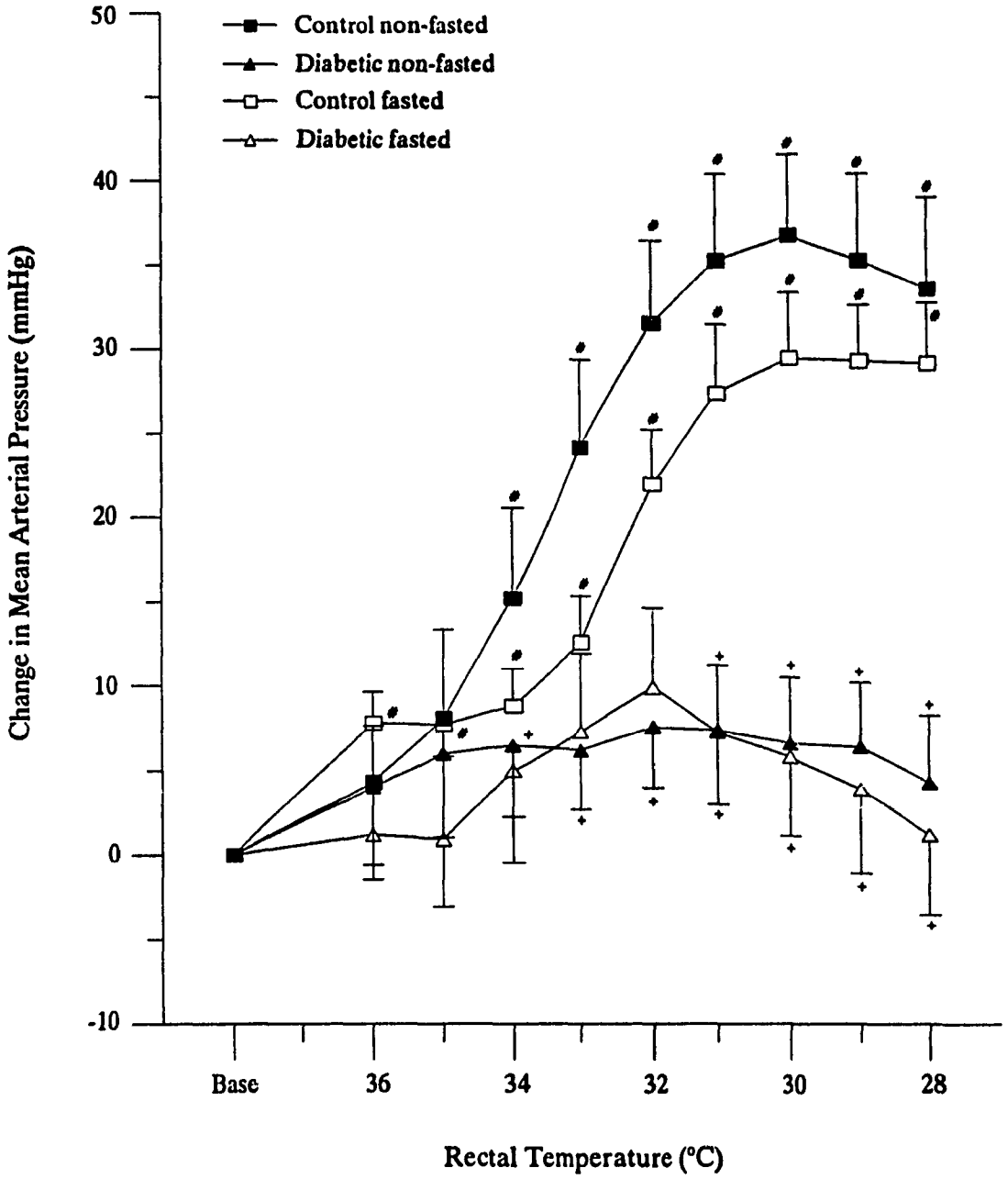

Figure 6. The relative changes in mean arterial pressure (MAP; mean ± S.E.M.) during cooling in fasted and non-fasted control and STZ-diabetic groups. Symbols indicate significant ($p < .05$) differences from corresponding control group (+), corresponding non-fasted group (*), or from baseline values (#).

($p < .05$).

Mean Arterial Pressure

The relative changes in mean arterial pressure (MAP) during the cold exposure are depicted in Figure 6. Both control groups showed significant ($p < .05$) increases in MAP throughout the cooling period, whereas the MAP responses of the two STZ groups did not differ significantly from baseline at any time during the cold stress. Non-fasted controls showed MAP responses that were significantly higher than baseline beyond a T_{rec} of 34° ($p < .05$), with a peak increase in pressure of 36.8 ± 4.8 mmhg at 29°C. The fasted controls showed a similar trend, with significantly increased pressures beyond a T_{rec} of 36° ($p < .05$), with a peak increase in pressure of 29.5 ± 3.9 mmhg at 29°C.

Although the two control groups were significantly higher than baseline beyond a T_{rec} of 34°C, no differences between groups were observed at any time during cooling. The non-fasted STZ animals showed significantly lower pressures than the non-fasted controls beyond a T_{rec} of 34°C ($p < .05$), while the MAP of the fasted STZ group was significantly lower than the fasted controls beyond a T_{rec} of 32°C ($p < .05$).

DISCUSSION

The results of this study clearly demonstrate that STZ-diabetic rats exhibit significantly attenuated EMG activity of the gluteus superficialis (GS) muscle during progressive hypothermia compared with control animals. This study represents the first documented account of a quantitative deficit in shivering response of diabetic animals to cold exposure. As expected, the well fed non-diabetic rats exhibited a progressive increase in EMG activity during the decline in rectal temperature (T_{rec}) from 36 to 28°C. The effect of a 12 hour nocturnal fast prior to cooling, had no effect on the shivering EMG response of diabetic animals. In non-diabetic animals, however, fasting caused a significant attenuation of EMG activity.

The original hypothesis of this study stated that STZ-diabetic rats would demonstrate reduced shivering activity leading to a more rapid rate of hypothermia than non-diabetic animals. The present findings support this hypothesis as diabetic rats cooled approximately three times as quickly as the well nourished non-diabetics. Previous studies have shown that conscious (Macari, Ferro, & Eizirik, 1986) and anesthetized (Kilgour & Williams, 1994) diabetic rats experience an accelerated hypothermic response to cold stress when compared to their respective control groups. The reduced shivering activity in diabetics may not be the only contributing factor to the more rapid decline in T_{rec} since reductions in non-shivering thermogenic (NST) capacity (Seydoux et al., 1983) and attenuated vasoconstrictor responses (Lucas, 1985) have also been observed in STZ-diabetic rats. However, shivering is a major form of

heat production in the unacclimatized animal (Hemingway, 1963), and an attenuation in shivering would presumably hinder the ability to maintain a stable core temperature during periods of cold stress.

Oxygen consumption is a measure of overall metabolic activity and is directly related to thermogenic activity (Burton & Edholm, 1955). In the present study, significant changes in VO_2 were demonstrated by both STZ-diabetic and control animals. However, divergent trends were observed as control animals showed increases in VO_2, while STZ-diabetics exhibited a progressive decline from baseline VO_2. The reduced VO_2 response in the STZ-diabetics supports the findings of Trayhurn (1979) who showed reduced oxygen consumptions of diabetic mice at low ambient temperatures. The steady drop from baseline VO_2 in diabetics suggests that these animals were unable to initiate any thermogenic activity in response to this cold stress. The different trends in VO_2 exhibited by controls and diabetics paralleled the changes in mean EMG activity suggesting that the attenuated muscular shivering in diabetics was the main reason for the lack of thermogenic activity. However, the difference in VO_2 response between controls and STZ-diabetics could possibly result from a greater NST activity in control animals. Although shivering is the main thermogenic source in non-acclimatized rats, NST has been shown to contribute to overall cold-induced thermogenesis (Shrago & Strielman, 1987). The reduced capacity for NST in diabetic animals may partially account for the lower oxygen uptakes observed in this study.

A neural relationship between cardiovascular reflexes and shivering activity has

already been well established (Ishii & Ishii, 1960; Mott, 1963). Mott (1963) demonstrated that a lower carotid artery pressure leading to reduced baroreceptor stimulation was associated with a simultaneous decrease in EMG activity. In the present study, control animals exhibited a significant increase in MAP that was maintained until the end of the cooling period. Conversely, STZ-diabetic animals showed no change from baseline MAP during the cold stress, which supports previous findings of attenuated pressor responses in diabetic rats during cold stress (Kilgour & Williams, 1994) and catecholamine infusion (Lucas, 1985). The reduced MAP response of STZ-diabetic animals would presumably lead to a depressed baroreceptor stimulation, which could be a contributing factor to the lower EMG activity observed in these animals. However, the mechanism by which baroreceptor stimulation alters EMG activity has not been identified. It would therefore be premature to conclude that the lack of shivering in diabetic animals results directly from the attenuated pressor responses.

In addition to the depressed cardiovascular responses, several other factors not assessed in this study may contribute to the attenuation of the cold-induced EMG activity in the STZ-diabetic animal. A reduction in cold sensation has commonly been observed in diabetic humans due to neuropathy of the small thermal afferent fibres (Dyck et al., 1987). A diminished sensation of the cold stimulus in STZ-diabetic animals may decrease the threshold temperature for the onset of shivering or reduce the magnitude of the thermoregulatory response. Morphological and functional changes have been observed in skeletal muscle of STZ-diabetic animals, and may also

contribute to diminished shivering. Observations of reduced muscle mass (Kuehn et al., 1988), twitch tension, maximal rate of relaxation, and increased time to peak twitch tension (Cameron et al., 1990) indicate that the functional of diabetic skeletal muscle is depressed. In addition, afferent nerve fibres involved in the skeletal muscle stretch reflex have been shown to be impaired in diabetic patients (Brown & Greene, 1984). Since muscle sensory nerves are involved in the generation of shivering (Schafer & Schafer, 1973), deficits in these fibres may hinder the shivering response of diabetic animals. Furthermore, STZ-diabetic animals commonly exhibit decreased conduction velocities in motor nerve fibres (Moore et al., 1980), which may attenuate the frequency at which motor units are recruited during shivering. It is uncertain whether these alterations in muscle and peripheral nerve function would diminish the shivering response of diabetic animals, however, the possibility should not be excluded.

Altered substrate utilization of STZ-diabetic animals may additionally inhibit the cold-induced shivering response. Carbohydrates are the main substrate utilized for muscular shivering, and adequate muscle glycogen (Martineau & Jacobs, 1991) and plasma glucose levels (Bennett et al., 1980) have been shown to be necessary for normal shivering activity. Since STZ-diabetic animals exhibit reduced muscle glycogen levels (Shafrir, 1990) and lower glucose uptake and utilization (Smith and Davidson, 1982), it is possible that reduced carbohydrate metabolism in these animals contributes to the attenuated EMG response.

At the present time, the causes for the reduced shivering activity in STZ-

diabetic animals is not known. Future studies should focus on the sensory nervous system, central commanr. of thermoregulation, and neuromuscular activation of specific skeletal muscles, since deficits in the shivering response may originate at any of these points. It is evdient that this is a complex problem and that each portion of the afferent and efferent pathway must be investigated separately.

In the second portion of this study, the effect of fasting was adressed by exposing the STZ-diabetic and control rats to the same cold stress following a nocturnal (12 hour) fast. In control animals, fasting resulted in a significantly reduced mean EMG activity, which was associated with a smaller increase in VO_2 and a more rapid induction of hypothermia. This finding supports those of Wang (1980) who observed a reduction in total heat production in cold-exposed rats following an overnight fast. This author attributed the reduced thermogenic capacity in the fasted rats to decreased availability of carbohydrates, since feeding the animal one hour prior to cooling reversed the fasting effects. Fasted STZ-diabetic animals showed no significant differences in mean EMG or ΔVO_2 compared with the non-fasted diabetic animals. This suggests that overnight food deprivation in STZ-diabetic rats does not alter cold-induced responses, which were already severely depressed in the non-fasted state. Since the mean EMG activity was reduced in the fasted controls, it is possible that the reduction in shivering in diabetic animals originates from a lack of carbohydrate utilization. However, changes in plasma glucose and muscle glycogen levels were not measured in this study and the extent to which carbohydrate metabolism was suppressed in the animals cannot be assessed.

Results from this study demonstrate that the greater susceptibility to hypothermia commonly observed in diabetic animals is due in part to a diminished shivering response. The cause of the attenuated muscular activity in STZ-diabetic rats is still unclear, and may result from several factors including reduced cardiovascular responsiveness, diminished thermal sensitivity, and altered muscle and peripheral nerve function. A lack of carbohydrate utilization appears to contribute to the reduced shivering of diabetic animals since an attenuated EMG response was also observed in fasted non-diabetic rats.

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APPENDIX

Source Tables of Analysis of Variance

Table 1

Body Weight

Source	SS	DF	MS	F
Between Subjects				
Treatment	266695.76	1	266695.76	152.98 ***
Feeding	1421.12	1	1421.12	0.81
Treatment X Feeding	489.22	1	489.22	0.28
Error	67992.07	39	1743.39	
Within Subjects				
Time	477338.53	5	95467.71	421.87 ***
Time X Treatment	117044.49	5	23408.90	103.44 ***
Time X Feeding	6787.49	5	1357.50	5.99 **
Time X Treatment X Feeding	1166.08	5	223.21	1.03
Error	44127.31	195	226.29	

* p < .05
** p < .01
*** p < .001

Greenhouse-Geisser Epsilon = 0.5257

Table 2

Plasma Glucose

Source	SS	DF	MS	F
Between Subjects				
Treatment	1297889.02	1	1297889.02	213.31 ***
Feeding	7415.67	1	7415.67	1.22
Treatment X Feeding	4687.85	1	4687.85	0.77
Error	225127.78	37	6084.53	

* $p < .05$
** $p < .01$
*** $p < .001$

Table 3

Total Time of Cooling

Source	SS	DF	MS	F
Between Subjects				
Treatment	37108.39	1	37108.39	23.99 ***
Feeding	12120.44	1	12120.44	7.84 **
Treatment X Feeding	9233.91	1	9233.91	5.97 *
Error	60330.87	39	1546.94	

* $p < .05$
** $p < .01$
*** $p < .001$

Table 4

Electromyographic Activity (EMG)

Source	SS	DF	MS	F
Between Subjects				
Treatment	351.27	1	351.27	10.20 **
Feeding	58.98	1	58.98	1.71
Treatment X Feeding	177.05	1	177.05	5.14 *
Error	1343.29	39	34.44	
Within Subjects				
T_{rec}	537.42	9	59.71	15.33 ***
T_{rec} X Treatment	383.38	9	42.60	10.93 ***
T_{rec} X Feeding	82.81	9	9.20	2.36
T_{rec} X Treatment X Feeding	140.30	9	15.59	4.00 *
Error	1367.36	351	3.90	

* $p < .05$
** $p < .01$
*** $p < .001$

Greenhouse-Geisser Epsilon = 0.1689

Table 5

Change in Oxygen Consumption

Source	SS	DF	MS	F
Between Subjects				
Treatment	1061.75	1	1061.75	166.81 ***
Feeding	43.18	1	43.18	6.78 *
Treatment X Feeding	95.63	1	95.63	15.02 ***
Error	235.51	37	6.36	
Within Subjects				
T_{rec}	64.72	8	8.09	23.87 ***
T_{rec} X Treatment	265.55	8	33.19	97.96 ***
T_{rec} X Feeding	9.03	8	1.13	3.33 *
T_{rec} X Treatment X Feeding	28.34	8	3.54	10.45 ***
Error	100.30	296	0.34	

* $p < .05$
** $p < .01$
*** $p < .001$

Greenhouse-Geisser Epsilon = 0.3511

Table 6

Change in Mean Arterial Pressure

Source	SS	DF	MS	F
Between Subjects				
Treatment	42989.03	1	42989.03	32.10 ***
Feeding	162.36	1	162.36	0.12
Treatment X Feeding	4335.26	1	4335.26	3.24
Error	49555.57	37	1339.34	
Within Subjects				
T_{rec}	12334.97	8	1541.87	37.51 ***
T_{rec} X Treatment	8994.25	8	1124.28	27.35 ***
T_{rec} X Feeding	145.31	8	18.16	0.44
T_{rec} X Treatment X Feeding	975.13	8	121.89	2.97 *
Error	12165.50	296	41.10	

* p < .05
** p < .01
*** p < .001

Greenhouse-Geisser Epsilon = 0.4482