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Oxidative capacity of liver and muscle from genetically obese rats

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OXIDATIVE CAPACITY OF LIVER AND MUSCLE FROM GENETICALLY OBESE RATS

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Oxidative capacity of liver and muscle from genetically obese rats

by

Gordon Mark Wardlaw

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Iowa State University
Ames, Iowa

1984

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INTRODUCTION

The obese Zucker rat has an inherited autosomal recessive obesity (12). The rat was a spontaneous mutant from a Sherman and Merck Stock M cross (12). Heterozygotes for the obesity gene appear normal. The Zucker rat obese syndrome is well-characterized, but the actual metabolic defects which are responsible for the obesity are unknown. This dissertation is designed to examine the role that the muscle and liver tissues may play in the etiology of the lower energy expenditure of this obese model.

The obese Zucker rat differs in many aspects from its nonobese counterpart. The obese rat exhibits hyperinsulinemia (14, 20), hyperphagia (23), hyperlipemia (11, 70), increased levels of nitrogen in the urine (14, 21), and increased rates of lipogenesis and lipid deposition (7, 11, 73). It also has larger adipose cells (46), a defective thermogenic response to cold (45) and diet (69, 79), an enlarged liver (15), and an increased adipose tissue lipoprotein lipase activity (34). Endocrine defects, in addition to hyperinsulinemia, include decreased serum levels of thyroxine (T_4), triiodothyronine (T_3) (79), growth hormone, and prolactin (56).

Norepinephrine synthesis is also depressed in the obese rat (5). The muscles of the obese rat are insulin-resistant (18, 19). Muscle growth in the male is compromised (64). The obese rat has a lower maintenance energy requirement than the nonobese rat (59) and a lower spontaneous activity (74).

It is evident from the above that the obese Zucker rat is quite different from its nonobese counterpart. The literature review which follows will examine many of the characteristics of the obese Zucker rat in more detail. Further, aspects of muscle metabolism which are germane to this dissertation are covered in the literature review. The individual papers in this dissertation also outline various aspects of Zucker rat metabolism and muscle metabolism. I have tried to reduce duplication between the literature review and the papers when possible. If a concept was covered sufficiently in the papers, it was not included in the literature review.

Explanation of dissertation format

This dissertation is written in the alternate style. It contains this introduction, a literature review, three papers for publication, and a final discussion.

I performed the majority of the laboratory work in the three papers. Dr. Murray Kaplan and Elaine Herink helped me measure hepatocyte oxygen consumption and Monica de Baca helped me determine the body composition of the rats which were exercised on the treadmill. In addition, Dr. Murray Kaplan helped me plan and analyse all of the experimental work presented in this dissertation.

LITERATURE REVIEW

The Zucker Rat

Hyperphagia

Hyperphagia, which begins at 4 weeks of age (73), is not necessary for development of the obese state in the Zucker rat (16). However, when fed ad libitum, hyperphagia allows the obese rat to gain weight and store lipid at such a rapid pace that the phenotypes can be visually identified at 5 weeks of age. Hyperinsulinemia is a main impetus behind this hyperphagia. When the obese rat is maintained in a normoinsulinemic state, the hyperphagia is reduced (14, 75). If insulin levels are then raised in the two phenotypes, the obese animals respond with a greater increase in food intake than the nonobese (14). Adrenalectomy also reduces hyperphagia in the obese rat, but it lowers insulin levels at the same time (78). So, it is not clear if adrenalectomy has a direct effect on hyperphagia, or just an indirect effect through insulin.

The obese rat does not convert dietary protein into lean body mass as efficiently as the nonobese rat (79). Radcliffe and Webster (63, 64) have suggested that, because of this inefficiency, the obese rat must

consume more food in order to receive the extra protein it needs. Hyperphagia is reduced when the protein concentration of the diet is increased (43, 63), but food consumption in the obese rat remains in excess of the nonobese even with a ration which contains twice the protein necessary for maximum lean body mass accretion in both rats (64). When given a chance to choose between three nutrient sources, namely a high carbohydrate ration, a high protein ration, and corn oil, the obese rat consumes more of the corn oil and the same amount of protein as the nonobese rat (13). If hyperphagia is a response to an increased need for protein, the obese animal should eat more of the high protein ration. So, the need for extra protein does not appear to be a major cause of the hyperphagia.

Cleary et al. (16) have suggested that elevated lipoprotein lipase levels in the adipose tissue pull too much lipid into storage and this forces the obese rat to eat more food to compensate. However, this increase in lipoprotein lipase activity precedes the hyperphagia by two weeks (30, 34, 73). Furthermore, the obese rat has hypertriglyceridemia by 2 weeks of age (11). Thus, before the hyperphagia begins, the obese rat already has

ample lipid available at all times for muscle and other tissues to utilize. In addition, lipid uptake and lipoprotein lipase activities are similar in the muscles of the obese and nonobese rats (58). As shown in fasting experiments, the obese can utilize lipid for fuel (25). It does not appear then that increased adipose tissue lipoprotein lipase activity is causing the hyperphagia.

Hyperinsulinemia

Hyperinsulinemia occurs at weaning in the obese rat, and Turkenkopf et al. (77) have presented evidence that hyperinsulinemia also may be present in the 21 day old preobese fetus. It is not possible, however, to identify phenotype in the fetus. These researchers used fetuses from a fa/fa male and fa/+ female cross, and fetuses from a +/+ x +/+ cross. The first cross should produce an equal amount of obese and nonobese fetuses, while the second cross should yield only nonobese fetuses. Plasma insulin in the fetuses from the first cross had a bimodal distribution. One group was normoinsulinemic when compared to the fetuses from the homozygote dominant cross (all nonobese). The other group was hyperinsulinemic by comparison. So, hyper-

insulinemia may already be present in the preobese fetus at birth, and is just suppressed by the high concentration of lipid in the dam's milk until weaning occurs.

Hyperinsulinemia is not a necessary condition for the manifestation of the obese state. The obese rat, when maintained in a normoinsulinemic state after streptozotocin or alloxan treatment, gains weight at a slower rate than the normal obese rat, and its food consumption is decreased (14, 75). However, the normoinsulinemic obese animal still maintains a greater percentage of body lipid than the nonobese rat (14, 75) and its elevated rates of hepatic lipogenesis remain intact (75).

Lipogenesis and lipid accretion

Increased hepatic lipogenesis is evident by 4 weeks of age in the obese rat (73). Peak rates of hepatic lipogenesis occur at 6 weeks, which coincides with the peak in the nonobese rat. However, peak rates are much higher in the obese rat in comparison to the nonobese (73). Increased hepatic lipogenesis in the obese rat is not normalized by pair feeding to the nonobese rat (8, 56), treadmill exercise (20), or adrenalectomy (78). The

liver of the obese rat also has a higher glycolytic activity than that of the nonobese. This directs metabolic flow to pyruvate, citrate, and then fatty acid synthesis, as well as provides glycerolphosphate for lipid esterification (57).

Some investigators suggest that the high serum insulin to glucagon levels create an environment in the obese animal which encourages lipogenesis, lipid esterification, and decreased lipid oxidation (2, 29). Normalizing insulin levels in the obese rat does not decrease the rates for hepatic lipogenic enzymes to nonobese levels (75). Hyperinsulinemia may play a role in the increased obese hepatic lipogenesis, but it is not obligatory. Further, in spite of serum insulin and glucagon level abnormalities, the obese liver is as sensitive to its respective lipogenic and lipolytic effects as the nonobese liver (2, 54, 76).

Hepatic lipogenesis is not as sensitive to the level of lipid availability in the obese rat as in the nonobese. Higher levels of lipid are needed to inhibit lipogenesis. Even with maximum inhibition, lipogenesis is still higher in the obese liver relative to that of the non-obese (2).

The obese rat has more body fat than the nonobese rat by 2 weeks of age (11). The obese rat also has hyperlipemia and increased lipoprotein lipase activity in the epididymal fat pad at this age (34). Increased lipid accretion in the obese rat occurs in spite of pair feeding to nonobese animals (8, 16), restriction of caloric intake to half that of the nonobese (21), maintenance of normoinsulinemia (75), adrenalectomy (78), thyroid hormone replacement (52), treatment with the ATP citrate lyase inhibitor (-)-hydroxycitrate (32), or treadmill exercise (20). The obese rat will synthesize and store more body lipid than the nonobese notwithstanding any nonlethal biochemical or physical attempt to stop it.

Thermogenesis

Body temperature is lower in obese rats than in nonobese rats at 16 days of age (31). Kaplan (45) has demonstrated that the 10 week old obese rat cannot increase its whole body oxygen consumption in response to cold. When given thyroid hormone replacement therapy, the obese rat has a normal cold-induced thermogenic response (52). Adrenalectomy of the obese rat also normalizes cold-induced thermogenesis (55). Marchington

et al. (55) suggest that adrenal steroid inhibition of intrascapular brown adipose tissue (IBAT) metabolism in the obese rat is in part responsible for this defective thermogenesis.

When injected with norepinephrine, the obese rat responds with a normal rise in whole body oxygen consumption (68). Levin et al. (51) and Rothwell et al. (68) suggest that the obese rat has an inadequate norepinephrine synthetic response to cold, which in turn contributes to its reduced cold-induced thermogenic response. Both groups indicate that norepinephrine-induced oxygen consumption is similar in the IBAT. The obese animals just do not make enough norepinephrine. Kasser and Martin (47) also have demonstrated that there is no defect in basal and norepinephrine-stimulated palmitate oxidation in the IBAT in obese rats at 5 weeks of age. They have further shown that the IBAT from obese animals weighs significantly more than that of the nonobese. However, Levin et al. (50) suggest that this difference in IBAT weight is due to white fat infiltration. They find fewer multiloculated cells and fewer high affinity β-adrenergic binding sites in the IBAT from obese rats than from nonobese rats.

Dietary-induced thermogenesis (DIT) is lower in the obese than in the nonobese rat (69). Rothwell and Stock (69) suggest that DIT has two components. Part of the increase in oxygen consumption after a meal is due to the digestion and absorption of food, and the other part is due to norepinephrine-stimulated IBAT oxygen consumption. This group has shown that β-adrenergic blockers significantly reduce DIT in nonobese rats, but have no effect on DIT in obese rats. They suggest that the reduction in DIT in the obese rats is due to a lack of the IBAT component (69). Young et al. (79) have demonstrated a lack of DIT in the obese rat in a different way than Rothwell and Stock. This group has diluted the protein content of the animals' diet with carbohydrate and showed that both phenotypes responded with an increase in food consumption. Serum T_3 levels increase in the nonobese rat in response to hyperphagia. However, serum T_3 levels do not increase in the obese, which indicates that these animals have defective DIT, possibly due to an inadequate response of T_3 to diet composition.

Metabolic efficiency

Using the body balance technique, Mowry and Hersh-

berger (59) have determined that the obese rat has a lower maintenance energy requirement than the nonobese rat. With this technique, the investigator feeds an animal different energy levels for periods of time and extrapolates back to the amount of energy which just allows for the maintenance of body weight. Cleary et al. (16) have demonstrated that even when pair fed to nonobese rats from weaning, as well as having their milk intake reduced before weaning, the increased metabolic efficiency of the obese rats allows them to gain more weight and weigh more at 33 weeks of age than the nonobese rats.

This increased metabolic efficiency in the obese rat may be in part due to its propensity for lipogenesis. Radcliffe and Webster (63) have shown that the energy cost for lipid and protein synthesis is 1.4 and 2.3 kilojoules of metabolizable energy per kilogram of tissue, respectively. The diversion of energy from lean body mass to lipid synthesis can then contribute to the obese rat's increased metabolic efficiency. Marchington et al. (55) suggest that the phenotypic difference in metabolic efficiency, as with thermogenesis, is in part due to adrenal steroid inhibition of IBAT metabolism. Adrenalectomy normalizes metabolic efficiency in the obese rat (55).

The phenotypic difference in metabolic efficiency has been erroneously linked to the decreased whole body oxygen consumption of the obese rat. On a whole animal (69) or per protein (44) basis, the obese and nonobese rats have similar rates of oxygen consumption. It is only when oxygen consumption is expressed on a body weight basis that the obese rat has a lower oxygen consumption. The obese rat has not only as much lean tissue as the nonobese rat (69), it also has much more lipid. The excess lipid adds weight to the animal but consumes very little oxygen. Thus, the obese animal ends up with a lower oxygen consumption per gram of body weight, but its lean tissue, which is what metabolic efficiency is based on, uses as much oxygen as in the nonobese animal. Similar oxygen consumption on a per protein basis actually has only been demonstrated for 6 month old Zucker rats (48). However, at approximately 12 weeks of age, whole body oxygen consumption (69) and total body protein (64) are phenotypically similar. Therefore, oxygen consumption on a per protein basis is probably phenotypically similar at 12 weeks.

Lean body mass and muscle mass accretion

Most studies of body composition of the Zucker rat measure lean body mass or total body protein rather than

muscle mass. Total body protein or lean body mass are not synonymous with muscle mass, especially in the obese Zucker rat. The larger liver of the obese rat, which is due to increased lipid (3, 10, 29) and protein deposition (15), can mask a deficit in muscle mass even when lean body mass or total body protein is phenotypically similar. If muscle mass is to be quantitated, it needs to be measured and not estimated from lean body mass or total body protein. Estimation of muscle mass can be made if "empty carcass" weight is determined. The "empty carcass" is defined as what remains of the animal after removal of skin and all subcutaneous fat, head, visceral organs, paws, and tail.

The obese rat has a decreased rate and efficiency of lean body mass accretion in comparison to the nonobese rat (24, 79). This has been demonstrated as early as 27 days of age (7). When injected with ^{14}C-amino acids, the obese rat converts half as much of the labeled amino acids to body protein and converts three times as much into lipid in comparison to the nonobese rat (24). Treadmill training improves nitrogen retention in the obese rat, but the obese rat is still less efficient in the conversion of dietary protein to body protein (20). When fed high

protein diets, obese rats gain similar amounts of lean body mass in comparison to their usual ration. Nonobese animals increase total lean body mass on the high protein diet (43). When the obese animal is maintained in a normoinsulinemic state, lean body mass accretion is increased, but the rate of accretion is still less than the nonobese rate (14).

"Empty carcass" weight, and therefore muscle mass, is phenotypically decreased in the male obese rat at 10 weeks of age (64). This is not true for females (64). Individual muscle weights are also lower in the male obese rat than in the nonobese rat (71). Pair feeding the obese to nonobese rats further exacerbates the difference in muscle mass (16).

Increased protein degradation in the obese rat may be primarily responsible for the lack of muscle growth (24), as evidenced by the increased levels of nitrogen (14) and 3-methylhistidine (25) in the urine. Further, the concentrations of protein, RNA, and DNA in the muscles of obese and nonobese rats are similar (71), as is the fractional rate of protein synthesis (53). Shapira et al. (71) suggest that the decreased bone growth and serum growth hormone levels also compromise muscle growth in

the obese rat. In addition, Czech et al. (19) suggest that the decreased muscle growth in the obese rat is partly due to muscle insulin resistance. This insulin resistance renders the muscles unresponsive to the anabolic effects of the hormone.

Muscle metabolism

Rates of palmitate (58) and glucose (18) oxidation are phenotypically similar in the soleus muscles from 6 week old Zucker rats. Glycolytic flux, 2-deoxyglucose uptake, rates of basal and insulin-stimulated glycogen synthesis (18, 19) and in vitro muscle oxygen consumption (18) also are similar in both phenotypes at this age in the soleus muscle. By ten weeks of age, the soleus muscle from obese rats is insulin resistant and exhibits a rightward shift in the insulin response curve for glycolytic flux, glucose oxidation, glycogen synthesis, and 2-deoxyglucose uptake (18, 19). Muscle insulin resistance also has recently been demonstrated in the 10 week old eviscerated obese carcass (72). Palmitate oxidation rates at 12 weeks are still phenotypically similar (58) as is muscle oxygen consumption in perfused hindquarters from 20 week old Zucker rats (48). Therefore, insulin resistance is the only known major

metabolic defect present in the muscles of the obese Zucker rat.

Response to fasting

When fasted, the obese animal decreases its rate of protein degradation and increases its rate of lipid oxidation more than the nonobese animal. The result of this phenotypic difference in lipid and protein metabolism is that the obese animal can fast for 60 days, while the nonobese can only fast for 9 days (25). The importance of this difference in response to fasting is questionable, but it does point out that the obese animal can oxidize lipid readily for its energy needs.

Skeletal Muscle

Classification of fiber types

Skeletal muscle fibers were originally classified by color, red or white. This classification was later refined and the categories became red-slow twitch, red-fast twitch, and white-fast twitch, as twitch time and color were used for classification criteria. This nomenclature was further refined and today we have fast twitch-glycolytic (FG), fast twitch-oxidative-glycolytic (FOG), and slow twitch-oxidative (SO) muscle fiber types

(61). This scheme uses twitch time and enzymatic profile to classify fibers. A fourth type of skeletal muscle fiber, which is a tonic rather than a twitch fiber, is also present in mammals. However, it does not exist in locomotory muscles (36).

Skeletal muscle fibers in a mammal are primarily slow twitch at birth, and in the rat differentiate into slow and fast twitch fibers by five weeks of age (17). Twitch time is an important characteristic of a muscle fiber, especially for slow twitch fibers. A slow twitch time allows for summation of fibers so tetanic contractions can be maintained at constant tension with a low metabolic cost (28). The actual twitch time and enzymatic profile of any fiber is determined by the pattern of electrical stimulation the fiber receives from its motoneuron. Skeletal muscles are composed of groups of fibers. Each group or motor unit is controlled by its individual motoneuron (9). Muscles are composed of many motor units and each muscle can be primarily one fiber type, or a mixture of fiber types. This depends on the variety of electrical signals sent via the motoneurons that control the individual muscle (37).

Muscle fibers primarily perform aerobic respiration since their high levels of ATP and citrate inhibit gly-

colysis (66). When necessary, the FG and FOG fibers have a great capacity for anaerobic respiration as well. The capacity for different types of respiration is a function of the muscle fiber's enzymatic profile (5). An entire muscle can be classified as either primarily glycolytic or oxidative from the relative concentrations of FG, FOG, and SO fibers that it contains. The SO and FOG fibers have similar activities of β-oxidation, TCA cycle, and oxidative phosphorylation enzymes. The FG and FOG fibers have similar activities of glycolytic enzymes. The SO fibers have little glycolytic capacity and the FG fibers have limited oxidative metabolic capacity (61). Enzyme activities allow the FOG fibers to respire either aerobically or anaerobically at high rates. Enzymatic profiles in muscle, as with twitch time, can be altered if the electrical pattern which is received by a fiber from its motoneuron is altered. Cross innervation of SO fibers with a FOG motoneuron results in an increase in glycolytic capacity and a reduction in oxidative capacity (5).

Consistent with their profiles of enzymatic activity, the FG, FOG, and SO fibers differ in their relative capacity to oxidize various substrates. The FOG and SO fibers oxidize pyruvate and palmitate at approximately the same rate, whereas the FG fibers only oxi-

dize palmitate half as readily as the other fibers (39). The FG fiber has a high capacity for α-glycerolphosphate oxidation relative to the other fibers, and this difference is more pronounced when FG and SO fibers are compared to each other. The high rate of α-glycerolphosphate oxidation in the FG and FOG fibers provides an alternate pathway to lactate dehydrogenase for NADH reoxidation during anaerobic metabolism (39).

This dissertation uses the soleus muscle for a model of SO fibers, and the extensor digitorum longus (EDL) and white portions of the medial gastrocnemius muscles for models of a combination of FG and FOG fibers. The rat has no muscle which is entirely FG or FOG. The soleus is composed of 84% SO fibers, whereas the EDL and gastrocnemius muscles contain 96% FG and FOG fibers (1). The FOG fibers in the EDL and gastrocnemius give these muscles oxidative as well as glycolytic capacity.

Muscle blood flow and oxygen consumption

Muscle blood flow is the metabolic throttle for muscle oxygen consumption and metabolism in small animals (9, 33). In vitro muscle oxygen consumption rates reported in this dissertation may not reflect in vivo rates since the muscles are necessarily isolated from their blood flow. In light of this potential difference between in vivo and in vitro muscle oxygen con-

sumption, it is important to examine the relationship between blood flow and oxygen consumption.

Blood flow in the cat soleus muscle is twice as great as in the gastrocnemius muscle at rest and three times as great during contraction (65). Since the EDL and gastrocnemius muscles are similar in twitch time and enzymatic profile, this same difference in blood flow probably holds for the soleus and EDL muscles as well. This difference in blood flow between slow and fast twitch muscles is not just due to the maintenance of posture since the difference remains when the animal is under anesthesia (65). Muscles with large proportions of both slow and fast twitch fibers probably have a dual circulation with different rates of blood flow to the different fiber types (28, 40).

The higher rate of blood flow is critical to the soleus muscle since it relies more on blood-borne nutrients and less on glycogen stores for its metabolic fuel (65). Further, since the soleus is a tonic muscle, rather than a phasic muscle such as the EDL, it must be able to sustain its tension. Therefore, it must receive a plentiful oxygen supply. Folkow and Halicka (28) have demonstrated that the vascular bed of the cat gastrocnemius can only keep the muscle oxygenated during contraction for 4-5 seconds. In this muscle, the extra

oxygen stored in myoglobin is very important. The soleus muscle has an ample vascular bed and blood flow to keep it oxygenated over its entire range of normal discharge. When blood flow to a fast twitch muscle is partially blocked, endurance decreases very little, while in the same circumstances endurance in the soleus is greatly affected (62).

Cross innervation of the rabbit EDL with the soleus motoneurons causes the EDL muscle to increase its capillary density and resistance to fatigue in only four days (41). This change takes place long before any enzymatic changes (42). This increase in capillary density allows the EDL muscle to extract more oxygen out of the blood, even if blood flow remains the same (41). Capillary density is six times greater in the rat soleus than in the gastrocnemius (67).

It appears then that in vitro oxygen consumption of the soleus and EDL muscles may not reflect their true relative in vivo rates since they probably don't receive the same amount of blood flow or have similar capillary densities. Blood flow in rat soleus and EDL muscles never has been compared directly, but it is safe to assume from the data presented that the capacity for oxygenation of muscle fibers will be greater in the soleus than in the EDL muscle.

The response of skeletal muscle to treadmill training

The leg muscles of the rat respond to treadmill training with an increase in mitochondrial number (38), mitochondrial enzyme activities (4, 38), capillary density (67), and other changes. The pace and duration of the exercise needs to be approximately 25 meters/minute for 90 minutes up a 5-10° grade to consistently see these results. The soleus and gastrocnemius muscles show the most rapid adaptation. The EDL muscle needs a much steeper grade and higher speeds (30 meters/minute) to experience a training response (26). Several investigators report that the degree of muscle response to exercise is primarily determined by the duration, rather than the pace of the training (23, 27, 35).

Training protocols vary. Muscle responses to treadmill training vary as well. Generally, muscles respond quantitatively as follows:

1.) Mitochondrial enzymes - citrate synthase activity increases 25-100%, succinate dehydrogenase activity increases 25-100%, and cytochrome oxidase activity increases 50-100% (4, 26, 27, 38)

2. Myoglobin increases approximately 80% (60)

3.) Total mitochondrial protein increases approximately 50% (38)

4.) Cytochrome c content increases approximately 40% (4)

5.) Mitochondrial respiration on a per protein basis increases by approximately 65% (26) or not at all (49). This depends on the intensity of the exercise.

The male rat further responds to treadmill exercise with a decrease in food intake and a lower rate of lean body mass accretion than the sedentary controls (23). Females do not decrease their food intake when they are trained on a treadmill and grow normally (22, 35).

PAPER I. OXYGEN CONSUMPTION AND OXIDATIVE CAPACITY OF MUSCLES FROM YOUNG OBESE AND NONOBESE ZUCKER RATS

Oxygen consumption and oxidative capacity of muscles from young obese and nonobese Zucker rats

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ABSTRACT

The contribution of muscle tissue to the increased metabolic efficiency of the obese (fa/fa) Zucker rat at 6 weeks of age was examined. In vitro oxygen consumption was similar in obese and nonobese soleus and extensor digitorum longus (EDL) muscles, whether the animals were in an ad libitum fed, fasted, or triiodothyronine-stimulated state. No phenotypic difference in in vitro oxygen consumption was seen when the muscles were preincubated with insulin. Pyruvate kinase, citrate synthase, succinate dehydrogenase, and cytochrome oxidase activities were phenotypically similar in the soleus and the EDL muscles. Phosphofructokinase and lactate dehydrogenase activities were increased in the obese soleus, while hexokinase activity was increased in the nonobese EDL. Mitochondrial and whole muscle homogenate respiration rates were similar in both phenotypes. The soleus and EDL muscles from the obese animals weighed less than those from the nonobese, but "empty carcass" weights were similar. Taken together these data suggest that muscle mass, muscle oxygen consumption, and muscle oxidative capacity are similar in 6 week old obese and nonobese rats. Therefore, defective energy metabolism in muscle probably does not contribute to the increased metabolic efficiency of the young obese rat.

KEY WORDS

Oxygen consumption, empty body weight, mitochondrial enzyme activities, glycolytic enzyme activities, mitochondrial oxygen consumption, muscle, obesity, triiodothyronine

INTRODUCTION

The obese Zucker rat, in comparison to the nonobese rat, exhibits hyperphagia (46), hyperinsulinemia (52), a decreased whole body oxygen consumption (29), a lower maintenance energy requirement (37), and decreased thermogenic responses to cold (29) and diet (56). The animal also has lower serum levels of growth hormone (34), thyroxine (T_4), and triiodothyronine (T_3) (56). The liver and adipose tissue of this rat show increased rates of de novo lipogenesis (46) and the adipose tissue is further characterized by hypertrophy and eventual hyperplasia (30). Increased lipid accretion in the obese animal is evident at one week of age (7), which, along with hyperinsulinemia and a decreased whole body oxygen consumption, make it one of the first metabolic abnormalities seen in the obese rat. This increased lipid accretion will take place in spite of food restriction (5, 13), treadmill training (14, 17), or the maintenance of normoinsulinemia (9, 49).

The male obese rat specifically has a lower rate of accretion for lean body mass (39), a decreased efficiency of protein gain (20), and stunted growth in certain skeletal muscles (43). This rat also has increased levels of nitrogen (9) and 3-methyl histidine (20) in its urine,

and oxidizes protein at a greater rate for energy production (20). Even if the concentration of protein in the diet is increased, the obese rat still cannot gain lean body mass at the rate of the nonobese rat (28).

Muscle contributes significantly to the rat's body composition. A lower muscle oxygen consumption or total muscle mass in the young obese rat could make an important contribution to the rat's increased metabolic efficiency. The purpose of this study was to determine if a phenotypic difference existed in muscle oxygen consumption, muscle oxidative capacity, or total muscle mass in the 6 week old male Zucker rat. In so doing, we wanted to outline the role of skeletal muscle, if any, in this phenotypic difference in metabolic efficiency.

MATERIALS AND METHODS

Animals

Six week old male obese and nonobese Zucker rats were obtained from the breeding colony at Iowa State University. The animals were fed a commercial rat ration (Ralston-Purina, St. Louis, MO) ad libitum and maintained at 24°C and 50% relative humidity. They were housed in wire bottom cages on a 12 hour light-dark cycle. When treated with triiodothyronine (T_3), the rats were injected i.p. with 3.1 ug T_3 per gram of body protein for 3 days. Controls received saline injections. Body protein was estimated from body weight as outlined by Dunn et al. (21).

"Empty carcass" weight and protein

Each rat was decapitated, skinned, and eviscerated. Then the paws, all visible fat deposits, and the tail were removed. We will refer to what remains of the carcass as the "empty carcass". This "empty carcass" was weighed and frozen. The frozen carcass was homogenized in distilled water, and a portion was lyophilized. The lyophilized samples were weighed and portions were taken for protein and lipid determination. Total "empty carcass" lipid was determined gravimetrically after extraction with chloroform:methanol (2:1 v/v), filtra-

tion over Na_2SO_4, and evaporation. Samples for the determination of "empty carcass" protein (approximately 250 mg) were digested at 420°C in 20 ml of concentrated sulfuric acid which contained 0.3% selenium dioxide. The ammonia that remained in the digest was determined spectrophotometrically by the method of Chaney and Marbach (11). The nitrogen concentration was multiplied by 6.25 to yield the protein concentration.

In vitro muscle oxygen consumption

Soleus and extensor digitorum longus (EDL) muscles were rapidly dissected after decapitation of the rat and tied taut at the tendons across a wire clip which was formed in the shape of a horsehoe. The muscles were incubated for 30 minutes in Krebs-Ringer bicarbonate (KRB) (18) media which contained only 1.25 mM calcium at 37°C in a gryrotory metabolic shaker and gassed continually with 95% O_2-5% CO_2. Substrates and hormones were added as indicated in the tables. The KRB media which contained palmitate was prepared as outlined by McNamara et al. (36). After 30 minutes of incubation oxygen consumption was determined at 37°C in 95% O_2-5% CO_2-saturated KRB media of the same composition that was used during the incubation, except in the case of assays

with palmitate. The bovine serum albumin (BSA) that was used in the preparation of the palmitate medium foamed excessively in the oxygen electrode chamber. In those assays, the muscles were incubated in the palmitate medium and oxygen consumption was determined in the KRB medium without added substrate. Leakage of oxygen from the electrode chamber (Yellow Springs Model No. 53) was minimized by the injection of mineral oil into the sample introduction slot. Oxygen content of the media was determined by the method of Robinson and Cooper (41). Muscle protein was quantified by the biuret method with BSA as a standard (32).

Mitochondrial oxygen consumption

Minced soleus and EDL muscle tissue from decapitated rats was digested for 7 minutes in 100 mM sucrose, 180 mM KCl, 50 mM tris (hydroxymethyl)aminomethane (Tris), 10 mM ethylenediaminetetraacetic acid (EDTA), 5 mM $MgCl_2$, 1 mM ATP, and 1% BSA, which contained 0.5% Nagarse (51). The concentration of muscle in the digest was 10%. The digest was then diluted to twice its volume in homogenizing media without Nagarse and homogenized by hand with a teflon and glass homogenizer. The digest was centrifuged at 600xg for 10 minutes and the supernatant was decanted

and again centrifuged at 600xg for 10 minutes. This second supernatant then was decanted and centrifuged twice at 14,000xg for 10 minutes. The mitochondrial pellet was suspended the first time in the homogenizing media without Nagarse, and finally was suspended in 250 mM sucrose, 2 mM EDTA and 20 mM Tris, pH 7.4. All procedures were done at $4^{o}C$.

Mitochondrial oxygen consumption was determined immediately at $25^{o}C$ in the air saturated media of Max et al. (35), which contained 15 mM KCL, 30 mM KH_2PO_4, 25 mM Tris, 45 mM sucrose, 7 mM EDTA and 5 mM $MgCl_2$, pH 7.4. State 4a respiration was recorded at the start of the assay when only substrate was added to the mitochondria and media. State 3 respiration then was initiated with 320 uM ADP. State 4b respiration was recorded after the ADP that was added in State 3 was phosphorylated to ATP and the rate of oxygen consumption returned to that of State 4a. The Respiratory Control Index (RCI) was calculated by dividing the State 3 rate by the State 4b rate (10). The P:O ratio was calculated as described by Estabrook (22). The amount of ADP added in State 3 was determined spectrophotometrically at 259nm with 16.3 $mM^{-1} \cdot cm^{-1}$ as the extinction coefficient (44).

Oxygen content of the media was determined as before. Assays with palmitoylcarnitine and malate contained 2% BSA (53), and those with pyruvate and malate had 0.2% BSA added to the media. Mitochondrial protein was determined by the method of Lowry et al. (33), with the addition of 2 mg per tube of sodium deoxycholate.

Muscle enzyme activities

Muscles for the determination of glycolytic enzyme activities were homogenized with a ground glass homogenizer in 50 mM KCL, 20 mM Tris, 50 mM NaF, 10 mM dithiothreitol, 2 mM EDTA and 1 mM ATP, pH 8.0. The homogenate was centrifuged at 700xg for 10 minutes. The supernatant then was again centrifuged for 30 minutes at 40,000xg and this second supernatant was used for the enzyme assays.

Hexokinase (EC 2.7.1.1) was assayed as outlined by Frank and Fromm (24) with a glucose-6-phosphate dehydrogenase coupled system. Phosphofructokinase (EC 2.7.1.11) was assayed by the method of Dunaway et al. (19) with a coupled system of aldolase, triose phosphate isomerase, and α-glycerolphosphate dehydrogenase. Pyruvate kinase (EC 2.7.1.40) and lactate dehydrogenase (EC 1.1.1.27) were assayed as outlined by Bergmeyer (4).

The pyruvate kinase assay used a coupled lactate dehydrogenase system.

Muscle homogenates for mitochondrial enzymes were prepared with a ground glass homogenizer in a media that consisted of 100 mM KCL, 5 mM $MgCl_2$, 5 mM EDTA, 20 mM Tris, and 1 mM ATP, pH 7.4. The homogenate was divided into two parts. One part was frozen and thawed 3 times in a dry ice-acetone bath and centrifuged at 600xg for 10 minutes. The other part was centrifuged at 600xg for 10 minutes, decanted, and the supernatant again centrifuged at 1000xg for 10 minutes. The mitochondrial pellet was recovered by centrifugation of the 1000xg supernatant at 14,000xg for 10 minutes. The pellet was suspended in 250 mM sucrose, 20 mM Tris, and 2 mM EDTA, pH 7.4, and then frozen and thawed 3 times. Citrate synthase (EC 4.1.3.7) was assayed by the method of Srere (48) with 5,5' dithiobis(2-nitrobenzoate) as the sulfhydryl acceptor. Succinate dehydrogenase (EC 1.3.99.1) was assayed by the method of Singer and Kearney (45) with phenazine methosulfate and 2,6-dichloroindophenol as the artificial electrón acceptors. Cytochrome oxidase (EC 1.9.3.1) was assayed by the method of Wharton and Tzagoloff (55), except that a trace amount of sodium

dithionate was used to reduce the cytochrome C. The oxidation of reduced cytochrome C was followed at 550 nm in order to quantify enzyme activity. Mitochondrial enzyme activities were determined in both the 600xg supernatant and the 14,000xg pellet.

All steps in the preparation of muscles for enzyme assays were carried out at 4°C. All enzyme activities were determined at 25°C with a Gilford recording spectophotometer. Protein was determined by the method of Lowry et al. as before. Enzyme activities were proportional to enzyme concentration except for cytochrome oxidase, in which activity was proportional to the first order rate constant.

Whole homogenate muscle oxygen consumption

Whole homogenate muscle oxygen consumption was assayed with a modification of the method of Hooker and Baldwin (26). The muscles were homogenized with a ground glass homogenizer in 175 mM KCL, 20 mM Tris, and 2 mM EDTA, ph 7.4 (1 ml per 100 mg). An aliquot was diluted with an assay mix to a final concentration of 87.5 mM KCl 5 mM $MgCl_2$, 40 mM KH_2PO_4, 1 mM EDTA, 5 mM Tris, and 50 mM sucrose, pH 7.4. This contained 20 mg of muscle per ml. When exogenous substrates were used in the

assays, 10 mM NaF was added to prevent glycogen oxidation and glycolysis (26). All procedures were carried out at 4°C.

Oxygen consumption was determined in the air-saturated muscle assay preparation at 25°C after the addition of 2 mM ADP. Assays containing NaF only gave a linear respiratory response (State 3) for a few minutes, since they could not oxidize their glycogen stores. When the respiration rate slowed, which indicated the endogenous substrates were exhausted, exogenous substrates were added. These substrates were 9 mM pyruvate or 50 uM palmitoylcarnitine, both with 1 mM malate. Once all of the endogenous substrates were exhausted, we could be sure that the resumption and subsequent rate of State 3 respiration was entirely dependent on the exogenous substrate. Oxygen content of the final assay media was determined as before.

Chemicals

All substrates, hormones, and auxiliary enzymes were purchased from Sigma Chemical Co. (St. Louis, MO) except acetyl-COA was purchased from P-L Biochemicals (Milwaukee, WI). Other chemicals were reagent grade and were purchased from Fisher Chemical Co. (Fair Lawn, NJ).

Statistics

In vitro muscle oxygen consumption experiments were analysed by analysis of variance (ANOVA) at the Computer Center at Iowa State University. Main effects included phenotypes, hormone stimulation, and presence of substrate. Each muscle type was analysed separately. Significant main effects were identified and means within these main effects were tested with the t-test (47). The pooled mean square error was used for the estimate of experimental error. Other experiments were analysed with the t-test (47). A probability of 0.05 or less was taken to be statistically significant.

RESULTS

In vitro muscle oxygen consumption

In vitro soleus muscle oxygen consumption in the ad libitum fed obese rat was not significantly different from its nonobese counterpart (Table 1). In vitro oxygen consumption was higher in the nonobese EDL when no substrate was added to the KRB media, but this is the only instance in the EDL where oxygen consumption was phenotypically different. Analysis of variance of in vitro oxygen consumption of the EDL muscle with phenotype and the presence of substrate as the main effects showed a significant main effect for phenotype ($p < 0.02$). So, these data suggests that there was a phenotypic difference in in vitro EDL muscle oxygen consumption. However, it is unclear why rates were only significantly different when the assay did not contain added substrates.

Rats were fasted for one day in order to deplete glycogen stores, lower insulin levels, and remove any possible diet-induced thermogenic effects on muscle oxygen consumption. The resulting in vitro muscle oxygen consumption rates were similar for the obese and nonobese rats (Table 2). Table 3 combines rates from Table 1 and Table 2 to show specifically that the fasted

Table 1. In vitro muscle oxygen consumption in obese and nonobese Zucker rats

Substrate	Obese	Nonobese
	Soleus	
KRB	(14) 3.35 ± 0.25[a]	(10) 3.89 ± 0.29
KRB with 5mM glucose	(8) 3.73 ± 0.32	(10) 3.38 ± 0.29
KRB with 0.5mM palmitate and 1.5mM BSA	(8) 3.06 ± 0.32	(6) 3.30 ± 0.37
	EDL[b]	
KRB	(13) 3.66 ± 0.25	(12) 4.46 ± 0.26*
KRB with 5mM glucose	(14) 3.79 ± 0.25	(12) 4.47 ± 0.26
KRB with 0.5mM palmitate and 1.5mM BSA	(8) 3.85 ± 0.32	(7) 4.05 ± 0.35

[a]Values are Mean ± SEM with number in group in parentheses. See "Materials and Methods" for details of the incubations. Units are nmoles O_2/(mg protein x minute).

[b]ANOVA phenotype main effect $p < 0.02$.

*Significantly different from obese rate at $p < 0.05$ level.

Table 2. In vitro muscle oxygen consumption in fasted obese and nonobese Zucker rats

Substrate	Obese	Nonobese
	Soleus	
KRB	(5) 3.44 ± 0.59[a]	(4) 4.14 ± 0.66
KRB with 5mM glucose	(10) 3.12 ± 0.42	(6) 3.51 ± 0.54
KRB with 0.5mM palmitate and 1.5mM BSA	(5) 4.47 ± 0.59	(8) 3.78 ± 0.47
	EDL	
KRB	(6) 3.75 ± 0.54	(4) 4.89 ± 0.66
KRB with 5mM glucose	(13) 4.37 ± 0.37	(8) 3.92 ± 0.47
KRB with 0.5mM palmitate and 1.5mM BSA	(5) 5.68 ± 0.59	(8) 5.27 ± 0.47

[a]Values are Mean ± SEM with number in group in parentheses. See "Materials and Methods" for details of the incubations. Units are nmoles O_2/(mg protein x minute). Animals were fasted for 24 hours.

animals responded to palmitate in both muscles with a significant increase in oxygen consumption over ad libitum fed rates with palmitate (ANOVA, fed vs. fasted main effect $p < 0.02$). Comparison of the individual means indicates only the obese animals had a significant increase in oxygen consumption. This difference in response between the obese and nonobese rats was not significant. The rates for in vitro oxygen consumption in fasted verses fed rats when either glucose or no exogenous substrates were added to the KRB media were not significantly different.

The treatment of the rats with T_3 significantly increased soleus muscle oxygen consumption (ANOVA, hormone main effect $p < 0.001$) (Table 4). Both phenotypes responded similarly. The EDL showed a T_3-induced increase in oxygen consumption, but ANOVA indicated the response was not significant. The two substrates used in the T_3 experiments had no significant effect on oxygen consumption rates in either muscle. The saline-injected controls in this experiment had similar in vitro muscle oxygen consumption rates in comparison to noninjected controls from other experiments with identical conditions. Therefore, the saline

Table 3. In vitro muscle oxygen consumption of ad libitum fed and fasted obese and nonobese Zucker rats with palmitate as the substrate

	Ad Libitum Fed	Fasted
	Soleus	
Obese	(8) 3.06 ± 0.32[a]	(5) 4.47 ± 0.59*
Nonobese	(6) 3.30 ± 0.37	(8) 3.78 ± 0.47
	EDL	
Obese	(8) 3.85 ± 0.32	(5) 5.68 ± 0.59*
Nonobese	(7) 4.05 ± 0.35	(8) 5.27 ± 0.47

[a]Values are Mean ± SEM with number of observations in parentheses. Muscles were incubated for 30 minutes in KRB media which contained 0.5 mM palmitate and 1.5 mM BSA. Oxygen consumption was then determined in the KRB media alone. See "Materials and Methods" for details of the incubations. Units are nmoles O_2/(mg protein x minute). Animals were fasted for 24 hours. The effect of ad libitum fed vs. fasted condition on oxygen consumption was significant in both muscles (ANOVA $p < 0.02$).

*Significantly different from ad libitum fed rate at $p < 0.05$ level.

Table 4. Effect of triiodothyronine on in vitro muscle oxygen consumption in obese and nonobese Zucker rats

T_3	Obese	Nonobese
	5mM Glucose as Substrate	
	Soleus	
+	(6) 4.71 ± 0.40[a,b]	(6) 4.48 ± 0.40*
-	(8) 3.73 ± 0.32	(10) 3.38 ± 0.29
	EDL	
+	(6) 5.10 ± 0.40*	(5) 3.74 ± 0.44+
-	(14) 3.79 ± 0.25	(12) 4.47 ± 0.26

	0.5mM Palmitate plus 1.5mM BSA as Substrate	
	Soleus	
+	(6) 4.36 ± 0.40*	(6) 4.47 ± 0.40*
-	(8) 3.06 ± 0.32	(6) 3.30 ± 0.37
	EDL	
+	(6) 4.80 ± 0.40	(6) 4.84 ± 0.40
-	(8) 3.85 ± 0.32	(7) 4.05 ± 0.35

[a]Values are Means ± SEM with number of observations in parentheses. Units are nmoles O_2/(mg protein x minute).

[b]Hormone was injected i.p. for 3 days at 3.1 ug T_3 per gram of body protein. Oxygen consumption was measured on the fourth day. See "Materials and Methods" for details of the incubations. Hormone treatment significantly increased oxygen consumption in the soleus muscles (ANOVA main effect for hormone $p<0.001$).

*Significantly different from non-T_3 treated rate at $p<0.05$ level.

+Significantly different from obese rate at $p<0.05$ level.

and nonsaline-injected controls which were otherwise similarly treated were combined into one control group. Muscles incubated in the presence of 0.1 U/ml insulin and 5 mM glucose showed no difference in oxygen consumption compared to rates without insulin in both phenotypes (Table 5).

Regression of oxygen consumption on nonobese muscle weight in experiments when either glucose or no exogenous substrate were added to the KRB media resulted in a correlation coefficient of -0.21 for the EDL ($y=0.025x + 5.42$, $n=22$) and -0.493 for the soleus ($y=0.55 + 6.51$, $n=20$). These r values indicate that only 4% and 24% of the variation in oxygen consumption can be attributed to muscle weight in the EDL and soleus muscles, respectively.

Mitochondrial oxygen consumption

Mitochondrial respiration rates in State 4a, State 4b, and State 3 were not significantly different in obese and nonobese rats (Table 6). The RCI of all preparations indicated that they contained highly coupled mitochondria.

Whole homogenate muscle oxygen consumption

Whole homogenate muscle oxygen consumption was

Table 5. Effect of insulin on in vitro muscle oxygen consumption of obese and nonobese Zucker rats

Insulin	Obese	Nonobese
	Fed Ad libitum	
	Soleus	
+	(5) 3.17 ± 0.47[a]	(5) 3.82 ± 0.47
-	(8) 3.73 ± 0.32	(10) 3.38 ± 0.29
	EDL	
+	(5) 3.43 ± 0.47	(5) 4.14 ± 0.47
-	(14) 3.79 ± 0.25	(12) 4.47 ± 0.26
	Fasted One Day	
	Soleus	
+	(6) 3.77 ± 0.43	(7) 4.01 ± 0.40
-	(10) 3.12 ± 0.42	(6) 3.51 ± 0.54
	EDL	
+	(6) 4.38 ± 0.43	(11) 3.70 ± 0.32
-	(13) 4.31 ± 0.37	(8) 3.92 ± 0.47

[a]Values are Means ± SEM with number of observations in parentheses. Units are nmoles O_2/(mg protein x minute). Muscles were preincubated with 0.1 U/ml insulin and 5 mM glucose in KRB media for 30 minutes and oxygen consumption was measured in the same media. See "Materials and Methods" for details of the incubations.

Table 6. Mitochondrial respiration and degree of coupling in obese and nonobese Zucker rats

	State 4a	State 3	State 4b	RCI	P:O
	50mM Palmitoylcarnitine plus 1mM Malate				
	Soleus				
Obese	9.02±0.67[a]	126±12	14.5±1.7	9.3±0.9	2.98±0.05
Nonobese	7.80±0.48	135±9	11.6±0.9	11.9±1.2	3.03±0.12
	EDL				
Obese	6.82±0.96	74.8±7.4	12.7± 0.6	6.21±0.57	3.12±0.06
Nonobese	6.91±0.63	86.0±6.2	10.5±0.7	8.31±0.63*	3.27±0.08
	9mM Pyruvate plus 1mM Malate				
	Soleus				
Obese	8.05±0.74	119±13	11.5±0.8	10.3±0.7	3.19±0.05
Nonobese	7.16±0.53	120±11	10.5±0.5	11.5±0.8	3.33±0.09
	EDL				
Obese	6.79±0.57	114±14	10.7±0.4	10.9±1.4	3.41±0.10
Nonobese	7.37±0.68	132±7	10.4±0.6	12.1±0.7	3.41±0.10

[a]Values are Means ± SEM. Assays with palmitoylcarnitine had n=8 observations, and those with pyruvate had n=6 observations per group. Units are nAtoms O/(mg mitochondrial protein x minute) except for the RCI and P:O which are unitless.

*Significantly different from obese rat at $p < 0.05$ level.

similar for obese and nonobese rats with either pyruvate plus malate or palmitoylcarnitine plus malate as the substrate (Table 7). Note that the NaF used to inhibit glycogen oxidation had no effect on State 3 respiration when pyruvate plus malate was used as the substrate. The effect of NaF was not tested with palmitoylcarnitine because there was not enough muscle homogenate to do those assays. These whole homogenate data were expressed on a wet weight basis so we could use all the muscle for the assays. Since the percentage of wet weight of muscle as protein was similar for obese and nonobese rats (Table 10), we felt this was a reasonable way to express the data.

Glycolytic and mitochondrial enzyme rates

Overall, glycolytic enzyme activities were similar in obese and nonobese rats (Table 8). Exceptions included an increased hexokinase activity in the nonobese EDL and increased phosphofructokinase and lactate dehydrogenase activities in the obese soleus. Mitochondrial enzyme activities for citrate synthase, succinate dehydrogenase, and cytochrome oxidase did not differ phenotypically (Table 9).

Table 7. Whole homogenate oxygen consumption of muscles from obese and nonobese Zucker rats

Media	Obese	Nonobese
	Soleus	
Media	397 ± 45[a]	403 ± 42
Media with 9mM pyruvate plus 1mM malate	733 ± 27**	730 ± 33**
Media with 9mM pyruvate, 1mM malate, and 10mM NaF	727 ± 60	719 ± 44
Media with 50uM palmitoylcarnitine, 1mM malate, and 10mM NaF	580 ± 42*	603 ± 55*

	EDL	
Media	456 ± 47	488 ± 49
Media with 9mM pyruvate plus 1mM malate	556 ± 42	557 ± 51
Media with 9mM pyruvate, 1mM malate, and 10mM NaF	527 ± 41	477 ± 44
Media with 50uM palmitoylcarnitine, 1mM malate, and 10mM NaF	339 ± 23*	311 ± 42*

[a]Values are Means ± SEM for 8 observations per group. Units are nmoles O_2/(gram wet weight x minute).

*Significantly different from the rate of the media alone at $p < 0.05$ level.

**Significantly different from the rate of the media alone at $p < 0.01$ level.

Table 8. Glycolytic enzyme activities in muscles from obese and nonobese Zucker rats

	Hexokinase	Phospho-fructo-kinase	Pyruvate Kinase	Lactate Dehydro-genase
		Soleus		
Obese	(8)16.1±0.9[a]	(8)214±23	(8) 581±37	(8)1780±110
Nonobese	(8)14.8±0.6	(8)155±14*	(8) 500±29	(8)1470±60*
		EDL		
Obese	(8)18.9±0.9	(8)636±39	(8)1950±140	(8)4370±530
Nonobese	(9)22.4±1.1*	(9)629±35	(9)1840±150	(9)4540±550

[a]Values are Means ± SEM with number of observations in parentheses. Units are nmoles/(mg protein in 40,000xg fraction x minute).

*Significantly different from obese rate at $p < 0.05$ level.

Muscle and "empty carcass" weight

Soleus and EDL muscles weighed significantly less in the 6 week old obese rat (Table 10), but this phenotypic difference in muscle weights was not generalized over the entire carcass. "Empty carcass" weight and protein, which were used as measures of total muscle mass, were similar in obese and nonobese rats (Table 11).

Further, the percentage of muscle weight which was protein in the "empty carcass" and in the soleus and EDL muscles were similar for the obese and nonobese rats.

Table 9. Mitochondrial enzyme activities in muscles of obese and nonobese Zucker rats

	Citrate Synthase	Succinate Dehydrogenase	Cytochrome Oxidase
		Soleus	
		600xg Supernatant	
Obese	701 ± 40[a]	28.0 ± 1.1	14.8 ± 1.9
Nonobese	635 ± 34	29.2 ± 1.2	17.5 ± 1.7
		14,000xg Mitochondrial Pellet	
Obese	391 ± 38	161 ± 7	167 ± 13
Nonobese	390 ± 29	164 ± 5	176 ± 21
		EDL	
		600xg Supernatant	
Obese	492 ± 43	16.9 ± 2.2	9.6 ± 1.3
Nonobese	438 ± 32	20.3 ± 2.0	12.0 ± 1.4
		14,000xg Mitochondrial Pellet	
Obese	362 ± 26	132 ± 7	236 ± 25
Nonobese	392 ± 35	161 ± 12	219 ± 31

[a]Values are Means + SEM for n=8 animals. Units are nmoles/ (mg protein x minute).

Table 10. Muscle wet weight and muscle protein as a percentage of wet weight in obese and nonobese Zucker rats

	Wet Weight (mg)	$\left(\frac{\text{mg protein}}{\text{mg wet weight}}\right)$ 100
	Soleus	
Obese	(18) 42 ± 1[a]	(17) 21.0 ± 0.4
Nonobese	(17) 51 ± 1*	(17) 21.2 ± 0.4
	EDL	
Obese	(18) 44 ± 1	(17) 22.4 ± 0.4
Nonobese	(18) 51 ± 1**	(18) 21.8 ± 0.5

[a]Values are Means ± SEM with number of observations in parentheses.

*Significantly different from obese rat at $p < 0.01$ level.

Table 11. Live weight and "empty carcass" analysis of obese and nonobese Zucker rats

	Obese	Nonobese
Body weight (grams)	(10) 177±7[a]	145±3*
"Empty carcass" weight (grams)[b]	(10) 57.9±2.2	59.1±1.6
"Empty carcass" protein (grams)	(10) 9.32±0.46	9.72±0.38
Lipid free "empty carcass" weight (grams)	(10) 53.4±2.1	57.2±1.7
% "empty carcass" protein on a lipid free "empty carcass" wet weight basis	(10) 17.5±0.4	17.0±0.5
% "empty carcass" protein on a lipid free "empty carcass" dry weight basis	(9) 70.5±2.4	76.2±3.0

[a]Values are Means ± SEM with number of observations in parentheses.

[b]"Empty carcass" is defined as what remains after the head, tail, paws, pelt, viscera, and visible fat deposits are removed. What remains is primarily muscle tissue, connective tissue, and bone.

*Significantly different from obese values at $p < 0.01$ level.

DISCUSSION

In vitro muscle oxygen consumption was similar phenotypically in all cases except for the increased rate in the EDL muscles from ad libitum fed nonobese rats. The fact that the majority of in vitro rates were similar between the obese and nonobese rats indicates there probably is no overall phenotypic difference in muscle oxygen consumption. Treatment with T_3 in vivo significantly increased muscle oxygen consumption in the soleus, and it effected both phenotypes equally. This effect of T_3 on muscle oxygen consumption has been well-documented (54). Insulin in vitro had no effect on muscle oxygen consumption. Ruderman et al. (42) have shown that insulin increases oxygen consumption in the perfused rat hindquarter, but Kemmer et al. (31) did not find this same effect in a similar hindquarter perfusion. Crettaz et al. (15) also did not demonstrate an effect by insulin on muscle oxygen consumption with in vitro muscle strips. Insulin appears to have no effect on oxygen consumption in isolated muscles, and it is unclear whether it has an effect on perfused hindquarter oxygen consumption.

Soleus and EDL muscles were chosen for this study for

two reasons. These muscles are thin enough to allow for adequate diffusion of oxygen and substrate into them. The regression of oxygen consumption on muscle weight supports this conclusion. Over 75% and 95% of the variation in muscle oxygen consumption was due to factors other than muscle weight in the soleus and EDL muscles, respectively. Furthermore, in vitro oxygen consumption rates of these muscles equalled or exceeded those for perfused hindquarters (42, 54), muscle strips (15), or 30-40 mg soleus muscles (38). The maximum muscle weight used in this study was 64 mg. We found, however, that soleus and EDL muscles that weighed more than 100 mg, or muscles that were not stretched across a wire clip, had significantly lower oxygen consumption rates than the muscles in this study (data not shown). These larger muscles, or unstretched muscles, are not suitable for in vitro incubations.

Soleus and EDL muscles also were chosen for this study because they are models for slow and fast twitch muscles, respectively. The EDL muscle is composed of both oxidative and glycolytic muscle fibers while the soleus muscle is almost wholly composed of oxidative muscle fibers (1). The greater glycolytic capacity of

the EDL muscle is evident when glycolytic enzyme activities are compared. Activities for phosphofructokinase and pyruvate kinase, which are considered rate-limiting for glycolysis (40), are three times higher in the EDL than in the soleus muscle.

In vitro oxygen consumption was similar or greater for the EDL when compared to the soleus muscle in this study, but this may not be true in vivo. Blood flow in the rat is generally three times higher in slow twitch muscles when compared to fast twitch (27). Blood flow is the metabolic throttle for muscle oxygen consumption (6), especially in small animals (25). This difference in blood flow would probably give the soleus muscle a higher in vivo oxygen consumption in comparison to the EDL muscle.

A lack of a substrate effect on muscle oxygen consumption, as demonstrated in this study, is consistent with the work of others (3, 16, 38). The majority of palmitate taken up by the muscle in vitro or in vivo is stored in lipid pools and little is oxidized immediately when muscles are at rest (3, 16). However, in this study palmitate did stimulate oxygen consumption in muscles from fasted rats. It can be presumed that those muscles

from fasted rats were depleted of glycogen and partially depleted of lipid stores. Therefore, the muscles relied on exogenous substrates to a greater degree than is usual in in vitro preparations. It is not clear why these muscles from fasted rats increased their oxygen consumption over ad libitum fed rates with palmitate as the substrate. Glucose also is not oxidized to any significant extent by the muscle in vitro since it is not a major fuel for muscles at rest (12). Glucose utilization does increase if an in vitro muscle preparation is stimulated to contract (12).

State 3 mitochondrial respiration rates suggest that in both muscle types there is no phenotypic difference in muscle oxidative capacity. Whole homogenate oxygen consumption rates suggest there is no phenotypic difference in mitochondrial number in the EDL and soleus muscles. Furthermore, similar oxygen consumption rates with palmitoylcarnitine for obese and nonobese muscles in both mitochondrial and whole homogenate fractions indicate that there is no phenotypic difference in β-oxidation in either muscle type.

Mitochondrial enzyme activities further indicate that there is probably no phenotypic difference in

muscle oxidative capacity. In vitro enzyme activities as such do not necessarily represent in vivo activities. However, since mitochondrial respiration rates and enzyme activities lead to the same conclusion, these enzyme activities probably do accurately reflect in vivo muscle oxidative capacity.

The work of Crettaz et al. (15) with in vitro soleus muscle strips in 6 week old Zucker rats has shown no phenotypic difference in glycolytic flux, glycogen synthesis, 2-deoxy glucose uptake, and insulin sensitivity. We have further shown that glycolytic capacity, as determined from glycolytic enzyme activities, was similar phenotypically. Additionally, McNamara et al. (36) have shown that soleus muscles in these 6 week old rats oxidize ^{14}C-palmitate to $^{14}CO_2$ at similar rates. Kemmer et al. (31) also have demonstrated that oxygen consumption of the perfused hindquarter of approximately 20 week old obese and nonobese rats is not phenotypically different. The work of others, in conjunction with the studies reported here, indicate that no significant phenotypic difference in oxygen consumption or oxidative capacity for major muscle fuels exists in the muscles from young obese and nonobese Zucker rats.

"Empty carcass" weight data further indicate a lack of a difference in muscle mass between lean and obese rats at 6 weeks of age. The data of Radcliffe and Webster (39) demonstrate a significant difference in obese and nonobese "empty carcass" weights at 10 weeks of age in males, but not in females. The absence of a lower female "empty carcass" weight at 10 weeks of age suggests that, because both sexes exhibit a similar obese syndrome, the lower muscle mass in the males is probably not a major factor in the etiology of its obesity.

Muscle metabolism, as stated previously, is to some extent different in obese and nonobese rats. The obese rat has more nitrogen in its urine and is less efficient at converting dietary protein into body protein. This inefficiency, as shown recently by Castonguay et al. (8) does not drive the hyperphagia of the obese rat as had been previously suggested (39). Furthermore, these differences in muscle metabolism must be minor ones with respect to the factors which are responsible for the obesity in the Zucker rat since this obese rat will synthesize lipid even at the expense of the growth of lean body mass when its calorie intake is restricted (5, 13).

The obese animal cannot properly apportion its dietary intake in order to achieve balanced growth and this defect surely transcends any small differences in muscle metabolism seen in the obese rat. Differences in muscle metabolism in the Zucker rat, then, whether either primary or secondary in nature, do not appear to constitute an important early etiological factor for the obese state or contribute to the phenotypic difference in metabolic efficiency. It is possible, though, that muscle may contribute to the later phases of the obesity syndrome in the male rat, especially in terms of the defect in its growth as the obese rat ages.

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PAPER II. THE EFFECT OF TREADMILL TRAINING ON THE OXIDATIVE CAPACITY AND GROWTH OF MUSCLES FROM YOUNG OBESE AND NONOBESE ZUCKER RATS

The effect of treadmill training on the oxidative capacity and growth of muscles from young obese and nonobese Zucker rats

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ABSTRACT

This study was designed to determine if treadmill training could reverse the lower rate of muscle accretion in the male obese Zucker rat and expose a possible latent defect in muscle oxidative capacity. Muscle mass was significantly lower at 12 weeks of age in the sedentary obese rat as compared to the nonobese rat. Exercise significantly decreased muscle mass and body weight similarly in both phenotypes, but it decreased food consumption and body fat to a greater extent in the obese than in the nonobese rats. Activities of citrate synthase (CS), succinate dehydrogenase and cytochrome oxidase (CYTOX) in the soleus, extensor digitorum longus (EDL), and gastrocnemius muscles were generally similar in obese and nonobese rats in both the sedentary and maximally-exercised state. Exceptions were significantly higher rates of CS in the 800xg supernatant of the EDL and soleus muscles from sedentary obese rats. Rates of CYTOX were significantly higher in the 14,000xg pellet in the EDL and soleus muscles from those same rats. In addition, the soleus muscles from the exercised nonobese rats had higher CYTOX activities in the 14,000xg pellet. Mitochondrial respiration rates were phenotypically similar, and were significantly decreased

by exercise only in the nonobese EDL muscles. Taken together these data indicate that oxidative capacity per unit of muscle is not defective in the obese Zucker rat in either the sedentary or exercised states. Further, exercise does not reverse the defect in muscle mass accretion in the obese rat. The data also indicate that total muscle oxidative capacity is lower in the obese due to lower muscle weights. This may contribute to the energy efficiency, but it is not a primary lesion.

KEY WORDS

exercise, obesity, Zucker rat, muscle mass, mitochondrial enzyme activity, mitochondrial respiration

INTRODUCTION

The obese Zucker rat has hyperinsulinemia (6), hyperlipemia (5), increased rates of lipogenesis and lipid accretion (4, 11), and an increased metabolic efficiency (26) in comparison to the nonobese rat. Altered aspects of protein metabolism in the obese rat include a decreased rate of protein accretion (14), an increased level of nitrogen in the urine (6), stunted growth of certain skeletal muscles (29), and an increased oxidation of protein for energy needs (14). This obesity syndrome is not reversed by food restriction (4, 9), maintenance of normoinsulinemia (6), or treadmill training (11, 37).

We reported previously that muscle mass, in vitro muscle oxygen consumption, and muscle oxidative capacity is similar in obese and nonobese male rats at 6 weeks of age (36). In the 10 week old male obese Zucker rat, Radcliffe and Webster (27) demonstrated that muscle mass is significantly lower than in the nonobese rat. Part of this investigation was to determine whether muscle oxidative capacity was compromised in this older animal, concomitant with the decreased muscle mass. Muscle tissue could be contributing to the increased metabolic efficiency of the obese rat by virtue of its decreased mass and possible

decreased oxidative capacity.

We also wondered whether a latent phenotypic difference existed in muscle oxidative capacity which was not evident in the sedentary state. Treadmill training increases muscle oxidative capacity in rats (1, 20), and this stress may expose a possible latent defect in muscle oxidative capacity in the obese rat. The stress of treadmill training also has been shown to decrease food consumption and weight gain in male rats in comparison to sedentary controls (12). The purpose of this study then was to determine whether a phenotypic difference in muscle oxidative capacity existed in 12 week old sedentary and exercised male Zucker rats. Further, we wanted to quantify the effect of exercise on muscle mass, body weight, and food consumption in the obese male rat.

MATERIALS AND METHODS

Animals

Five week old male obese and nonobese Zucker rats were obtained from the breeding colony at Iowa State University. The animals were fed a commercial rat ration (Ralston-Purina, St. Louis, MO) ad libitum and maintained at 24°C and 50% relative humidity. They were housed in wire cages with external food hoppers on a 12 hour light-dark cycle. Weekly food consumption was determined for each rat. Crumbs and spilled food were collected every other day, weighed weekly, and subtracted from the apparent food consumption.

Treadmill training

Treadmill training of rats began at 5 weeks of age and lasted for 7 weeks. Initially, the rats ran 7 days per week at 10 meters per minute for 15 minutes up an 8° incline. One group of obese and nonobese rats, designated pair-ex, were pair-exercised to the capacity of the obese group. Speed and duration were gradually increased until during the last week of training the pair-ex group was running 20 meters per minute for 90 minutes up an 8° incline. A second group of nonobese rats, designated LEX, were exercised to their capacity. Speed, duration, and incline were gradually increased and during the last week these nonobese rats were running 24 meters per minute for 90 minutes up an 11° incline.

When necessary, both groups of rats were prodded with cardboard strips to keep them running. Electric shock was used sparingly. Six rats had to be removed from the experiment. One obese and two nonobese rats in the exercise group were removed because they were injured by the treadmill, and three obese sedentary controls because they developed respiratory infections. All exercised animals were rested 24 hours before they were sacrificed.

Carcass analysis

Each rat was decapitated, skinned, and eviscerated. Then the paws, all visible fat deposits, and the tail were removed. The remaining "empty carcass" was weighed and frozen. This frozen carcass was homogenized in distilled water, and a portion was lyophilized. The lyophilized samples were weighed and portions (approximately 250 mg) were taken for protein determination. These portions were digested at 420°C in 20ml of concentrated sulfuric acid which contained 0.3% selenium dioxide. The ammonia that remained in the digest was determined spectrophotometrically by the method of Chaney and Marbach (8). The nitrogen concentration was multiplied by 6.25 to yield the protein concentration.

To determine body fat, the organs, skin, and dissected fat from each animal were combined and homogenized in distilled water. A weighed portion was lyophilized, extracted with chloroform:methanol (2:1 v/v), filtered over Na_2SO_4,

and evaporated. The head and "empty carcass" were not extracted. Body fat was subtracted from body weight to calculate "fat free" body weight. Liver protein was assayed by the biuret method after the liver was homogenized in distilled water (22). Bovine serum albumin (BSA) was used as the standard.

Mitochondrial oxygen consumption

Soleus, extensor digitorum longus (EDL), and white portions of the medial gastrocnemius muscles were rapidly dissected and minced. These muscle samples were digested for 7 minutes in 100 mM sucrose KCl, 50 mM tris (hydroxymethyl) aminomethane (Tris), 10 mM ethylenediaminetetraacetic acid (EDTA), 5 mM $MgCl_2$, 1 mM ATP, and 1% BSA, which contained 0.5% Nagarse (34). The concentration of muscle in the digest was 10%. The digest was then diluted to twice its volume in homogenizing media without Nagarse and homogenized by hand with a teflon and glass homogenizer. The digest was centrifuged at 300xg for 10 minutes and the supernatant was decanted and again centrifuged at 800xg for 10 minutes. This second supernatant then was decanted and centrifuged twice at 14,000xg for 10 minutes. The mitochondrial pellet was suspended the first time in the homogenizing media without Nagarse, and finally was suspended in 250 mM sucrose, 2 mM EDTA and 20 mM Tris, pH 7.4. All procedures were done at $4^{o}C$.

Mitochondrial oxygen consumption was determined immediately at 25°C in the air-saturated media of Max et al. (25) which contained 15 mM KCl, 30 mM KH_2PO_4, 25 mM Tris, 45 mM sucrose, 7 mM EDTA and 5 mM $MgCl_2$ and 0.2% BSA, pH 7.4. Initially 125 uM ADP was added to exhaust endogenous substrates. When oxygen consumption returned to basal levels a mixture of 9 mM pyruvate plus 1 mM malate was injected into the sample through a port in the oxygen electrode chamber. The resulting State 4b respiration rate was recorded. State 3 respiration was then initiated with 320 uM ADP. State 4b respiration was recorded again after the ADP added in State 3 was phosphorylated to ATP. Only one substrate was assayed because the amount of mitochondrial material from each muscle was limited.

The Respiratory Control Index (RCI) was calculated by dividing the State 3 rate by the State 4b rate (7). The P:O ratio was calculated as described by Estabrook (15). The amount of ADP added in State 3 was determined spectrophotometrically at 259 nm with 16.3 $mM^{-1} \cdot cm^{-1}$ as the extinction coefficient (30). Oxygen content of the media was determined by the method of Robinson and Cooper (28). Mitochrondial protein was determined by the method of Lowry et al. (24), with the addition of 2 mg per tube of sodium deoxycholate.

Muscle enzyme activities

Soleus, EDL, and white portions of the medial gastroc-

nemius muscles were rapidly dissected. Muscle homogenates for mitochondrial enzymes were prepared with a ground glass homogenizer in a media that consisted of 100 mM KCl, 5 mM EDTA, 5 mM $MgCl_2$, 20 mM Tris, and 1 mM ATP, ph 7.4. The homogenate was divided into two parts. One part was frozen and thawed 3 times in a dry ice-acetone bath and centrifuged at 800xg for 10 minutes. The other part was centrifuged at 800xg for 10 minutes, decanted, and the supernatant again centrifuged at 800xg for 10 minutes. The mitochondrial pellet was recovered by centrifugation of the 800xg supernatant at 14,000xg for 10 minutes. The pellet was suspended in 250 mM sucrose, 20 mM Tris, and 2 mM EDTA, ph 7.4, and then frozen and thawed 3 times as before. Both enzyme fractions were stored at -80°C and assayed within 2 weeks. A quantitative isolation of mitochondrial enzymes in the 14,000xg pellet is not possible with differential centrifugation of muscle homogenates (1). The 800xg fraction, therefore, was used as a measure of total enzyme activity in the muscle.

Citrate synthase (EC 4.1.3.7) was assayed by the method of Srere (33) with 5,5' dithiobis (2-nitrobenzoate) as the sulfhydryl acceptor. Succinate dehydrogenase (EC 1.3.99.1) was assayed by the method of Singer and Kearney (31) with phenazine methosulfate and 2,6-dichloro-

indophenol as the artificial electron acceptors. Cytochrome oxidase (EC 1.9.3.1) was assayed by the method of Wharton and Tzagoloff (39), except that a trace amount of sodium dithionate was used to reduce the cytochrome c. The oxidation of reduced cytochrome c at 550 nm was followed in order to quantify enzyme activity.

All steps in the preparation of muscles for enzyme assays were carried out at 4°C. All enzyme activities were determined at 25°C with a Gilford recording spectrophotometer. Protein was determined by the method of Lowry et al. (24) as before. Enzyme activity was proportional to enzyme concentration except for cytochrome oxidase, in which activity was proportional to the first order rate constant.

Chemicals

All substrates, hormones, and enzymes were purchased from Sigma Chemical Co. (St. Louis, MO), except acetyl-COA was purchased from P-L Biochemicals (Milwaukee, WI). Other chemicals were reagent grade and were purchased from Fisher Chemical Co. (Fair Lawn, NJ).

Statistics

Data were analyzed with a 2x2 analysis of variance at the Iowa State University Computer Center. Phenotype and exercise were the main effects. Significant main effects

and interactions were identified and means within those main effects were compared with the t-test (32). The pooled mean square error was used as the estimate for experimental error. For many parameters, such as body weight, the mean values were similar for the LEX and the nonobese pair-ex animals. For those parameters, just one 2x2 ANOVA was used, which contained the sedentary controls and the pair-ex animals. When the LEX and nonobese pair-ex means differed significantly, two ANOVA were used. One 2x2 ANOVA was used for the pair-ex and sedentary animals, while another 2x2 ANOVA combined the sedentary animals with the obese pair-ex animals and the LEX group. This latter ANOVA represented animals who were exercised to their capacity, and was primarily used to identify phenotype x exercise interactions.

RESULTS

Body weight and food consumption

The obese rats, whether exercised or not, weighed significantly more than the nonobese (Table 1). Exercise significantly decreased final body weight similarly in both phenotypes. The obese rats consumed significantly more food during the seven week experimental period than the nonobese (Table 2). Exercise significantly decreased food consumption in both phenotypes, but the effect was greater on the obese rats (ANOVA phenotype x exercise $p < 0.01$).

Body composition

"Empty carcass" weight and "empty carcass" protein were significantly lower in the obese than in the nonobese rats (Table 3). Exercise significantly decreased both parameters similarly in the obese and nonobese rats. Body lipid was significantly greater in the obese rats in comparison to the nonobese (Table 3). Exercise significantly decreased body lipid in both phenotypes, but the effect was greater on the obese rats (ANOVA phenotype x exercise $p < 0.001$). "Fat free" body weight, which includes water, was similar for obese and nonobese rats (Table 3). Exercise significantly decreased "fat free" body weight in a similar fashion in both phenotypes.

Table 1. Body weight of exercised and sedentary obese and nonobese Zucker rats

Condition[a]	5 weeks of age	12 weeks of age
	Obese	
Sedentary (7)	145 ± 7[b]	446 ± 8
Pair-Ex (9)	144 ± 5	362 ± 7+
	Nonobese	
Sedentary (12)	113 ± 3*	316 ± 4*
Pair-Ex (11)	110 ± 4*	263 ± 8*,+
LEX (6)	111 ± 3*	252 ± 7*,+

[a]Sedentary refers to rats who received no treadmill training. Pair-Ex refers to a group of obese and nonobese rats who were exercised to the capacity of the obese group. LEX refers to a group of nonobese rats who were exercised to their capacity. Treadmill training began at 5 weeks of age and ended at 12 weeks of age. See "Materials and Methods" for details.

[b]Values are Means ± SEM with number of observations in each group in parentheses. Units are grams.

*Significantly different from obese rat at $p < 0.01$ level.

+Significantly different from sedentary group at $p < 0.01$ level.

Table 2. Food consumption of exercised and sedentary obese and nonobese Zucker rats

	Obese	Nonobese	p
Sedentary	(7) 1606 ± 39[a]	(12) 1093 ± 14	<0.01
Pair-Ex	(9) 1385 ± 20*	(11) 1001 ± 23*	<0.01
LEX		(6) 993 ± 21*	

[a]Values are Means ± SEM with number of observations in parentheses. Units are grams of food eaten during the seven week training period.

*Significantly different from sedentary group at $p < 0.01$ level.

Organ weights

The obese rats had significantly heavier livers and these livers contained significantly more protein than those of the nonobese (Table 4). Liver protein as a percentage of liver wet weight was similar phenotypically for the sedentary groups and for the animals exercised to their capacity. Exercise significantly decreased liver weight and liver protein in the obese rats, but not in the nonobese. This phenotypic difference in liver weight in response to exercise was significant (ANOVA phenotype x exercise $p < 0.02$).

Table 3. Carcass analysis of exercised and sedentary obese and nonobese Zucker rats

	Sedentary	Pair-Ex	LEX
		"Empty carcass" weight[a]	
Obese	(7)119.3±3.0[b]	(9) 99.9±2.9**,++	
Nonobese	(11)146.3±3.3	(12)125.9±4.1**	(6)117.8±4.1**,++
p	<0.01	<0.01	
		"Empty carcass" protein	
Obese	(6) 22.9±1.0	(7) 19.4±1.0*,+	
Nonobese	(10) 28.3±1.2	(10) 24.3±1.5*	(5) 24.3±1.2*,+
p	<0.05	<0.05	
		Body lipid	
Obese	(7)155.6±6.6	(9)116.9±2.8**,++	
Nonobese	(9) 16.7±1.2	(8) 11.3±1.3**	(6) 8.7±1.1**,++
p	<0.01	<0.01	

	"Fat free" body weight					
Obese	(7)286	±6	(9)246	±6**		
Nonobese	(9)296	±5	(8)255	±7**	(6)243	±7**
p	NS		NS			

[a]"Empty carcass" weight is defined as what remains after the head, tail, paws, pelt, viscera, and visible fat deposits are removed. What remains is primarily muscle tissue, connective tissue, and bone.

[b]Values are Means ± SEM with number of observations in parentheses. Units are grams.

*Significantly different from sedentary group at $p < 0.05$ level.

**Significantly different from sedentary group at $p < 0.01$ level.

+Obese pair-ex vs. LEX differ significantly at $p < 0.05$ level.

++Obese pair-ex vs. LEX differ significantly at $p < 0.01$ level.

Heart weight was significantly greater in the obese than in the nonobese rats (Table 4). Exercise decreased heart weight significantly in the nonobese, but had no effect on heart weight in the obese rat. When heart weight was expressed on a "fat free" body weight basis, the significant effect of exercise on it was evident in both phenotypes. Heart weight ratios were significantly increased in the obese and nonobese exercised rats and the effect was significantly greater in the obese animals (ANOVA phenotype x exercise $p < 0.001$).

Soleus and EDL muscle weights were significantly lower in the sedentary obese than in the sedentary nonobese rats (Table 5). Exercise reduced EDL and soleus muscle weights in both phenotypes, with the exception of the soleus muscles from the nonobese rats. Gastrocnemius muscle weights were not listed because only a sample of this muscle was dissected.

Muscle enzyme activities

Citrate synthase activity in the 800xg supernatant of the soleus muscle was greater in the sedentary obese than in the nonobese rats. Exercise increased citrate synthase activity and the maximum exercised-induced rates were phenotypically similar (Table 6). Mitochondrial citrate synthase activity in the soleus muscle was phenotypically

Table 4. Organ weights of exercised and sedentary obese and nonobese Zucker rats

	Sedentary	Pair-Ex	LEX
		Liver weight (g)	
Obese	(7)17.4±0.7[a]	(8)12.3±0.2**,++	
Nonobese	(8)10.2±0.4	(8)8.97±0.42	(6)9.04±0.19++
p	<0.01	<0.01	
		Liver protein (g)	
Obese	(7)3.74±0.32	(8)2.43±0.20**,+	
Nonobese	(7)2.14±0.11	(8)2.08±0.16	(6)1.81±0.06+
p	<0.01	NS	
		(g protein/g liver) 100	
Obese	(7)21.8±1.6	(8)19.0±0.9	
Nonobese	(7)21.4±0.8	(8)23.1±1.1	(6)20.0±0.3
p	NS	<0.01	

	Heart weight (mg)		
Obese	(7)969 ±27	(9)1006±14^{++}	
Nonobese	(12)876 ±20	(12) 785±20**	(6)785±20$^{**,++}$
p	<0.01	<0.01	
	(g heart weight/g "fat free" body weight) 1000		
Obese	(7)3.38±0.10	(9)4.12±0.11$^{**,++}$	
Nonobese	(9)2.84±0.09	(8)3.13±0.09*	(6)3.24±0.09$^{*,++}$
p	<0.01	<0.01	

[a]Values are Means ± SEM with number of observations in parentheses.

[*]Significantly different from sedentary group at $p < 0.05$ level.

[**]Significantly different from sedentary group at $p < 0.01$ level.

[+]Obese pair-ex vs. LEX differ significantly at $p < 0.05$ level.

[++]Obese pair-ex vs. LEX differ significantly at $p < 0.01$ level.

Table 5. Muscle weights from sedentary and exercised obese and nonobese Zucker rats

	Sedentary	Pair-Ex	LEX
		Soleus	
Obese	(10) 109 ± 3[a]	(8) 100 ± 2[*,+]	
Nonobese	(11) 118 ± 2	(10) 111 ± 6	(6) 115 ± 4[+]
p	<0.05	NS	
		EDL	
Obese	(9) 128 ± 2	(8) 111 ± 4[**]	
Nonobese	(9) 150 ± 4	(8) 131 ± 5[**]	(6) 119 ± 6[**]
p	<0.01	<0.01	

[a]Values are Means ± SEM with number of observations in parentheses. Muscle weights from the left and right side of each animal were averaged and that average was used as the muscle weight for the animal. If the left and right side muscles differed by more than 25 mg, data from that rat were not included. A difference of that magnitude indicated one of the muscle pair was not completely dissected. Units are mg.

[*]Significantly different from sedentary weight at $p < 0.05$ level.

[**]Significantly different from sedentary weight at $p < 0.01$ level.

[+]Obese pair-ex and LEX differ significantly at $p < 0.01$ level.

similar in the sedentary animals. Exercise significantly increased mitochondrial activity and maximum rates were similar for the obese and nonobese rats. Citrate synthase activities in the soleus muscles from the nonobese rat demonstrated a graded response in both muscle fractions with increased exercise intensity. This graded response also frequently existed in other muscles and enzymes from the nonobese rat.

Succinate dehydrogenase activities in both soleus muscle fractions were similar for obese and nonobese rats in sedentary and exercised states (Table 6). Exercise significantly increased succinate dehydrogenase activity in the 800xg supernatant only in the soleus muscles from nonobese rats. Exercise significantly increased mitochondrial activity of succinate dehydrogenase similarly in the soleus muscles from both phenotypes.

Cytochrome oxidase activity in the 800xg supernatant was phenotypically similar in the soleus muscles from sedentary and exercised rats (Table 6). Exercise significantly increased activity in the 800xg supernatant only in the soleus muscles from the nonobese rats. Mitochondrial activity of cytochrome oxidase in the soleus muscle was significantly higher in the sedentary obese than in the nonobese rats. Exercise significantly increased mitochondrial cytochrome oxidase activity only in the nonobese rats and

Table 6. Mitochondrial enzyme activities of soleus muscles from exercised and sedentary obese and nonobese Zucker rats

	Sedentary	Pair-Ex	LEX
		800xg supernatant	
		Citrate synthase	
Obese	(7)512 ± 39[a]	(9)799 ± 46**	
Nonobese	(11)402 ± 23	(10)696 ± 40**	(6)777 ± 32**
p	<0.05	<0.05	
		Succinate dehydrogenase	
Obese	(7)22.2 ± 3.1	(9)25.5 ± 4.5	
Nonobese	(12)16.8 ± 1.5	(10)29.1 ± 3.5**	(6)31.5 ± 3.6**
p	NS	NS	
		Cytochrome oxidase	
Obese	(7)9.63 ± 2.68	(9)9.70 ± 1.91	
Nonobese	(12)6.24 ± 1.03	(10)11.0 ± 1.6*	(6)13.6 ± 1.1**
p	NS	NS	

14,000xg mitochondrial pellet

Citrate synthase

Obese	(7)209 ± 19	(9)335 ± 36**	
Nonobese	(10)187 ± 12	(10)262 ± 29*	(6)315 ± 20**
p	NS	<0.05	

Succinate dehydrogenase

Obese	(7)99.6 ± 9.4	(9)155 ± 7**	
Nonobese	(10)95.9 ± 9.2	(9)132 ± 12**	(6)169 ± 17**
p	NS	NS	

Cytochrome oxidase

Obese	(7)104 ± 8	(8)133 ± 13+	
Nonobese	(9)63.4 ± 4.3	(10)105 ± 14*	(6)196 ± 14**,+
p	<0.05	NS	

[a]Values are Means ± SEM with number of observations in parentheses. Units are nmoles/(mg protein x minute).

*Significantly different from sedentary rate at $p < 0.05$ level.

**Significantly different from sedentary rate at $p < 0.01$ level.

+Obese pair-ex and LEX differ significantly at $p < 0.01$ level.

the maximum rates were also significantly higher in the nonobese than in the obese rats. This phenotypic difference in mitochondrial activity in response to exercise was significant ($p < 0.001$).

Citrate synthase activity in the 800xg supernatant of the gastrocnemius muscle was phenotypically similar in sedentary and maximally exercised animals (Table 7). Exercise significantly increased citrate synthase activity in the 800xg supernatant in the gastrocnemius muscles only from nonobese rats. Mitochondrial activities for citrate synthase were phenotypically similar. Exercise resulted in no change in activities.

The 800xg supernatant and mitochondrial activities for succinate dehydrogenase in the gastrocnemius muscle were phenotypically similar in the sedentary animals (Table 7). Exercise had no significant effect on succinate dehydrogenase activity in this muscle. The 800xg supernatant and mitochondrial activities for cytochrome oxidase were phenotypically similar in the gastrocnemius muscle (Table 7). Exercise had no significant effect on cytochrome oxidase activity in the 800xg supernatant from this muscle, but exercise significantly increased mitochondrial activity in the nonobese rat. This phenotypic difference in the response of cytochrome oxidase to exercise in the mitochondrial fraction was not significant.

Table 7. Mitochondrial enzyme activities of gastrocnemius muscle from exercised and sedentary obese and nonobese Zucker rats

	Sedentary	Pair-Ex	LEX
		800xg supernatant	
		Citrate synthase	
Obese	(7)217 ± 40[a]	(9)257 ± 31	
Nonobese	(12)154 ± 13	(9)184 ± 17	(6)237 ± 18*
p	NS	<0.05	
		Succinate dehydrogenase	
Obese	(7)9.47 ± 1.19	(7)12.3 ± 1.9	
Nonobese	(11)6.07 ± 0.80	(10)7.26 ± 1.87	(6)8.51 ± 0.68
p	NS	<0.05	
		Cytochrome oxidase	
Obese	(7)4.58 ± 0.61	(9)5.75 ± 1.13	
Nonobese	(12)3.99 ± 0.69	(10)4.74 ± 1.22	(6)5.97 ± 0.93
p	NS	NS	

	14,000xg mitochondrial pellet		
	Citrate synthase		
Obese	(7)160 ± 23	(9)163 ± 17	
Nonobese	(10)147 ± 26	(10)129 ± 15	(6)124 ± 13
p	NS	NS	
	Succinate dehydrogenase		
Obese	(7)60.2 ± 11.0	(9)57.4 ± 4.5	
Nonobese	(10)49.0 ± 4.7	(10)47.6 ± 5.2	(6)52.8 ± 5.6
p	NS	NS	
	Cytochrome oxidase		
Obese	(7)76.9 ± 12.2	(9)79.2 ± 9.7	
Nonobese	(10)56.8 ± 6.5	(10)58.5 ± 9.6	(6)95.7 ± 7.7**
p	NS	NS	

[a]Values are Means ± SEM with number of observations in parentheses. Units are nmoles/(mg protein x minute).

*Significantly different from sedentary rate at $p < 0.05$ level.

**Significantly different from sedentary rate at $p < 0.01$ level.

The 800xg supernatant and mitochondrial activities in the EDL muscles were phenotypically similar for most of the enzymes that were tested (Table 8). Exceptions were a significant phenotypic difference for both citrate synthase activity in the 800xg supernatant, and for cytochrome oxidase activity in the mitochondrial fraction. In both instances, activity was higher in the obese animal. Exercise had no significant effect on enzyme activity in the EDL muscle.

Mitochondrial respiration

Mitochondrial State 3 respiration was similar for obese and nonobese rats in both the sedentary and exercised states (Table 9, 10, and 11). This respiration rate represents the maximum respiratory capacity of the mitochondria (7). In the EDL muscle, exercise significantly decreased State 3 respiration in the nonobese rat (Table 11). State 3 respiration was decreased by exercise in the obese EDL muscle, but not significantly. This phenotypic difference in response to exercise was not significant. Exercise had no significant effect on State 3 respiration in the soleus (Table 9) or gastrocnemius muscle (Table 10). Respiratory Control Indexes in all muscles indicated the isolated mitochondria were tightly coupled.

Work output

During the last week of training, the obese pair-ex rats had an average work output on the treadmill of

Table 8. Mitochondrial enzyme activities of EDL muscles from exercised and sedentary obese and nonobese Zucker rats

	Sedentary	Pair-Ex	LEX
	800xg supernatant		
	Citrate synthase		
Obese	(7)475 ± 12[a]	(9)400 ± 42	
Nonobese	(12)363 ± 16	(10)361 ± 25	(6)353 ± 37
p	<0.05	NS	
	Succinate dehydrogenase		
Obese	(7)20.1 ± 2.1	(9)18.1 ± 1.8	
Nonobese	(12)16.7 ± 2.1	(10)17.6 ± 2.8	(6)19.9 ± 1.8
p	NS	NS	
	Cytochrome oxidase		
Obese	(7)5.68 ± 1.23	(9)8.78 ± 2.0	
Nonobese	(12)6.99 ± 1.43	(10)7.30 ± 1.1	(6)10.1 ± 1.7
p	NS	NS	

14,000xg mitochondrial pellet			
Citrate synthase			
Obese	(7)249 ± 24	(8)187 ± 23	
Nonobese	(10)197 ± 19	(9)176 ± 22	(6)179 ± 7
p	NS	NS	
Succinate dehydrogenase			
Obese	(7)95.7 ± 8.8	(9)79.5 ± 6.7	
Nonobese	(10)99.4 ± 7.9	(9)92.4 ± 4.5	(6)82.5 ± 6.2
p	NS	NS	
Cytochrome oxidase			
Obese	(7)147 ± 13	(8)122 ± 12	
Nonobese	(10)113 ± 8	(9)95.3 ± 10.6	(6)95.7 ± 7.7
p	<0.05	NS	

[a]Values are Means ± SEM with number of observations in parentheses. Units are nmoles/(mg protein x minute). Exercise had no significant effect on enzyme activity.

90.7 ± 1.8 kg·meters·day^{-1} (mean $\pm$ SEM), as calculated by the method of Fruth and Gisolf (17). The nonobese rats had an output of 65.9 ± 2.0 when they were pair-exercised to the obese and 104 ± 3 when they were exercised to their capacity. All of these values differ significantly from each other ($p < 0.01$).

Table 9. Mitochondrial respiration and degree of coupling of soleus muscles from exercised and sedentary obese and nonobese Zucker rats

	Sedentary	Pair-Ex	LEX
	State 3 oxygen consumption		
Obese	(7)68.4 ± 13.7[a]	(9)60.8 ± 4.3	
Nonobese	(12)66.7 ± 9.3	(10)55.6 ± 7.8	(6)41.2 ± 7.2
	State 4b oxygen consumption		
Obese	(7)4.43 ± 1.15	(9)4.58 ± 0.99	
Nonobese	(12)4.74 ± 1.30	(10)4.77 ± 1.28	(6)4.37 ± 0.80
	Respiratory Control Index		
Obese	(7)24.7 ± 5.2	(9)18.1 ± 3.2	
Nonobese	(12)18.9 ± 2.6	(10)15.8 ± 2.0	(6)12.3 ± 1.4
	F:O		
Obese	(7)3.67 ± 0.07	(9)3.44 ± 0.16	
Nonobese	(12)3.60 ± 0.05	(10)3.48 ± 0.11	(6)3.34 ± 0.08

[a]Values are Means ± SEM with number of observations in parentheses. Units are natoms O/(mg protein x minute) with 9mM pyruvate plus 1 mM malate as the substrates. No obese vs. nonobese means differ significantly and exercise had no significant effect on oxygen consumption.

Table 10. Mitochondrial respiration and degree of coupling of gastrocnemius muscles from exercised and sedentary obese and nonobese Zucker rats

	Sedentary	Pair-Ex	LEX
	State 3 oxygen consumption		
Obese	(7)63.4 ± 5.5[a]	(8)46.7 ± 7.6	
Nonobese	(11)49.4 ± 5.5	(10)36.7 ± 3.7	(6)38.1 ± 7.9
	State 4b oxygen consumption		
Obese	(7)4.01 ± 1.23	(8)3.61 ± 1.51	
Nonobese	(11)4.30 ± 1.50	(10)2.64 ± 4.2	(6)2.04 ± 1.8
	Respiratory Control Index		
Obese	(7)27.6 ± 7.3	(8)22.5 ± 4.0	
Nonobese	(11)19.7 ± 2.8	(10)22.9 ± 4.2	(6)18.2 ± 1.8
	P:O		
Obese	(7)3.45 ± 0.07	(8)3.18 ± 0.13	
Nonobese	(11)3.38 ± 0.07	(10)3.25 ± 0.12	(6)3.03 ± 0.07

[a]Values are Means ± SEM with number of observations in parentheses. Units are natoms O/(mg protein x minute) with 9mM pyruvate plus 1mM malate as the substrates. No obese vs. nonobese differ significantly and exercise had no significant effect on oxygen consumption.

Table 11. Mitochondrial respiration and degree of coupling of extensor digitorum longus muscles for exercised and sedentary obese and nonobese Zucker rats

	Sedentary	Pair-Ex	LEX
	State 3 oxygen consumption		
Obese	(7)67.7 ± 10.4[a]	(9)44.5 ± 6.0	
Nonobese	(12)57.9 ± 8.9	(10)39.6 ± 3.4	(6)32.0 ± 4.9*
	State 4b oxygen consumption		
Obese	(7)3.41 ± 1.02	(9)3.31 ± 1.01	
Nonobese	(12)6.21 ± 2.54	(10)2.79 ± 0.56	(6)1.29 ± 0.14
	Respiratory Control Index		
Obese	(7)27.1 ± 6.07	(9)21.0 ± 4.5	
Nonobese	(12)19.6 ± 3.6	(10)19.6 ± 3.5	(6)26.6 ± 5.7
	P:O		
Obese	(7)3.53 ± 0.11	(9)3.29 ± 0.20	
Nonobese	(12)3.63 ± 0.13	(10)3.46 ± 0.15	(6)3.59 ± 0.06

[a]Values are Means + SEM with number of observations in parentheses. Units are natoms O/(mg protein x minute) with 9mM pyruvate plus 1mM malate as the substrates. No obese vs. nonobese means differ significantly.

*Significantly different from sedentary rate at $p < 0.05$ level.

DISCUSSION

Body weight and food consumption were significantly reduced by exercise in the obese and nonobese rats. Male rats of other strains respond similarly to exercise (13). However, in spite of an average weight loss of 84 grams, the exercised obese male rats in this study still contained over seven times more body lipid than the sedentary nonobese rats. Female rats, whether obese (3, 37) or nonobese (12), 18, 35), respond to exercise with no change or slight changes in body weight and food consumption.

The obese rats could not run as fast as the nonobese rats. This was probably due to their increased body weight, rather than due to a defect in muscle metabolism. When pair-weighed to nonobese rats, the obese rats can run at a pace of 26 meters per minute for 60 minutes (11). In addition, the calculated workloads were not very different for both maximally-exercised phenotypes in this experiment.

"Empty carcass" weight and "empty carcass" protein in both the sedentary and exercised rats were significantly reduced in the obese in comparison to the nonobese. This phenotypic difference in muscle accretion, which has also been shown by others (27), may contribute to the increased metabolic efficiency of the obese rat. It takes almost

twice as much energy to synthesize muscle tissues as it does lipid (27). Therefore, if the obese rat makes lipid rather than muscle, less energy need be expended. Further, this decreased muscle mass will account for less total energy utilization on a whole animal basis. In both instances, the energy saved by the obese rat is available and can be diverted into lipid synthesis.

Enzymatic activities in the 800xg supernatant were either similar or higher in the obese in comparison to the nonobese rat. Maximum exercise-induced activities in this fraction were always similar between the two phenotypes. In general, mitochondrial enzyme activities were either phenotypically similar or higher in the obese animal. The exception was the increased mitochondrial cytochrome oxidase activity in the soleus muscles from the maximally exercised nonobese rats. This enzyme activity data, in conjunction with the mitochondrial respiration data, clearly demonstrate that the muscles from obese rats respond normally to exercise. Therefore, there appears to be no latent defect in muscle oxidative capacity in the young obese rat.

In most instances in this study, when enzyme activity in the 800xg supernatant was increased by exercise, mitochondrial enzyme activity also was increased. It is not

clear in these cases if enzyme activity was increased because mitochondrial activity was increased, or because both mitochondrial activity and total mitochondrial number were increased. In the gastrocnemius muscles from nonobese rats, citrate synthase activity in the 800xg supernatant was significantly increased by exercise, but mitochondrial activity was unchanged. These data suggest that exercise increased the number of mitochondria in the gastrocnemius muscle.

The gastrocnemius muscle did not show consistent increases in enzyme activity with exercise. This lack of consistency is unusual for this muscle (20). This may be because we only sampled the white portion of the gastrocnemius muscle. We took this sample because we wanted to see the response of fast twitch fibers to exercise and knew from a pilot study that the fast twitch EDL muscle was not recruited enough by our exercise protocol to experience an exercise effect. It appears that the fast twitch portion of the gastrocnemius muscles also was not recruited to any significant extent. Fast twitch fibers are recruited after the slow twitch fibers, primarily to increase speed or force of contraction (38). As an animal adapts to treadmill training, fast twitch fibers are no longer used as fre-

quently. To keep fast twitch fibers in constant use, intervals of very high speed running can be added to the training protocol (2), or the treadmill incline can be raised to approximately 19° (19).

State 3 mitochondrial respiration was unchanged with exercise in the gastrocnemius and soleus, and decreased in the nonobese EDL muscle. This lack of an increase in State 3 respiration with exercise has been reported by Krieger et al. (21), but Farrar et al. (16) report significant increases in State 3 respiration with exercise. In this latter study, rats were exercised at 30 meters/minute for 2 hours, which was a much harder exercise protocol than we could achieve in our study. Since the increased mitochondrial activities for citrate synthase, succinate dehydrogenase, and cytochrome oxidase in the soleus muscle in this study did not produce a significant increase in State 3 respiration, none of these enzymes can be considered rate-limiting for mitochondrial respiration. Lemasters and Sowers (23) suggest that the activity of mitochondrial ATP-ADP translocase is the rate-limiting step for mitochondrial respiration.

We conclude that the muscles from the obese and nonobese rats have similar oxidative capacities per unit of muscle, but less total oxidative capacity due to less total muscle mass. Exercise can reduce body weight and body lipid

accretion in the obese rat, but this rat still remains obese and the defect in muscle accretion remains. Exercise may improve glucose tolerance (3, 10), but it cannot normalize lipid or muscle accretion in the obese rat.

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PAPER III. OXYGEN CONSUMPTION AND OXIDATIVE CAPACITY OF HEPATOCYTES FROM YOUNG OBESE AND NONOBESE ZUCKER RATS

Oxygen consumption and oxidative capacity of hepatocytes from young obese and nonobese Zucker rats

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ABSTRACT

Oxygen consumption of cells and isolated mitochondria, and mitochondrial enzyme activities, were measured in livers from obese and nonobese Zucker rats. The purpose was to determine if the liver plays a role in the increased metabolic efficiency of the obese rat. Hepatocyte oxygen consumption was similar in the obese and nonobese rats for all substrates tested. Mitochondrial respiration also was similar in both phenotypes for all substrates tested. Activities of citrate synthase, succinate dehydrogenase, and cytochrome oxidase were similar for obese and nonobese rats. The activity of mitochondrial α-glycerolphosphate dehydrogenase was lower in the obese rats. Taken together, these data show that hepatic oxygen consumption and oxidative capacity are similar in obese and nonobese rats. Rates of mitochondrial respiration with palmitoylcarnitine further show that the capacity for hepatic β-oxidation is similar in obese and nonobese rats. Therefore, the increased metabolic efficiency of the obese rat probably cannot be attributed to a decreased hepatic oxidative capacity.

KEY WORDS

hepatocyte oxygen consumption, mitochondrial respiration, mitochondrial enzyme activity, obesity, liver, Zucker rat

INTRODUCTION

The obese syndrome of the Zucker rat (fa/fa) has been well-characterized (6). Major abnormalities exhibited by the obese rat included hyperinsulinemia (8), hyperphagia (14), hyperlipemia (23), excessive carcass lipid accumulation (14), mild hypothyroidism (35), depressed muscle growth (24), and an increased metabolic efficiency (21). The liver of the obese rat has an increased lipid content (2, 4, 11), increased rates of lipid synthesis (4, 20), and key lipogenic enzymes (8, 27), increased lipid esterification (1, 11, 17), and increased release of very low density lipoprotein (23), larger cells (4, 17), and a possible decreased rate of lipid oxidation (11, 17, 30), compared with the nonobese rat. Elevated rates of hepatic lipogenesis remain in spite of pair-feeding to nonobese rats (3, 18), or treadmill exercise (8).

This study addresses two aspects of hepatocyte metabolism in the obese Zucker rat. First, lipid oxidation to CO_2 and ketone bodies is depressed in the obese rat, but it is not clear if this is because β-oxidation is depressed. Triscari et al. (30) and Azain and Martin (1) have shown that in vitro ^{14}C-palmitate oxidation is

lower phenotypically in hepatocytes from obese rats when the concentration of palmitate is 0.1 mM in the incubation media. However, Azain and Martin (1) have demonstrated that no phenotypic difference in palmitate oxidation exists when the concentration is 2.0-3.0 mM in the incubation media. It seems that capacity for β-oxidation is not different in obese and nonobese rats when certain experimental conditions are met. We believe that isolated liver mitochondria provide a more direct method than the hepatocyte for the measurement of β-oxidation. Mitochondria can easily be exhausted of endogenous substrates. Oxygen consumption of the mitochondria then can be directly related to the capacity of β-oxidation when a carnitine-lipid ester is used as an exogenous substrate. Furthermore, rates of respiration can be compared with a variety of different substrates to determine if defective hepatic oxidation of nonlipid substrates exists in the obese rat.

The second aspect of hepatocyte metabolism we address is the determination of in vitro hepatocyte oxygen consumption in the Zucker rat. It is not known which tissue or tissues account for the increased metabolic efficiency in the obese rat. The liver is a highly

oxidative organ, and we wondered if a difference in liver cell oxygen consumption existed between young obese and nonobese rats. If so, the liver could play an important role in the increased metabolic efficiency of the obese rat.

The purpose of this study, then, was to determine if a phenotypic difference existed in hepatocyte oxygen consumption. We further wanted to determine if β-oxidation was depressed in the obese rat. Finally, we wanted to see if a phenotypic difference existed for hepatic oxidation of substrates other than fatty acids.

MATERIALS AND METHODS

Animals

Six week old male obese and nonobese Zucker rats were obtained from the breeding colony at Iowa State University. The animals were fed a commercial rat ration (Ralston-Purina, St. Louis, MO) ad libitum and maintained at 24°C and 50% relative humidity. They were housed in wire cages on a 12-hour light-dark cycle.

Liver cell oxygen consumption

Livers were rapidly dissected from decapitated rats, rinsed in cold 0.9% saline, blotted, weighed, and placed in a petri dish on ice. A small piece of liver was saved for protein determination. Liver cells were isolated by a modification of the method of Howard et al. (12) because this method yielded cells with a high respiration rate. The liver was perfused with 20 ml of ice cold calcium-free Krebs-Ringer bicarbonate media, pH 7.4 (KRB) (9), which contained 0.05% collagenase, 0.1% hyaluronidase, 5 mM glucose, 1 mM pyruvate, and 20 mM n-2-hydroxyethylpiperazine-n'2-ethanesulfonic acid (HEPES). This was slowly injected into the portal vein with a syringe. The liver was then sliced by hand with a Stadie-Riggs microtome (A. H. Thomas, Philadel-

phia, PA) mounted on ice. The slices were incubated in two 250 ml flasks that contained 10 ml each of the perfusion media. This incubation was done at 37°C under at atmosphere of 95% O_2-5% CO_2 in a gyrotory metabolic shaker. After 30 minutes of incubation, calcium was added to the media to a final 1.25 mM concentration, and the incubation was continued for another 30 minutes.

The digested liver was poured over a 250 um nylon screen into a 100 ml plastic beaker on ice. The material that passed through the screen was centrifuged at 120xg for 1 minute. The supernatant was aspirated and the pellet was suspended in cold KRB media, which contained 20 mM HEPES and 1.25 mM calcium chloride (KRB-HEPES). This was then centrifuged at 50xg for 1 minute. The supernatant was again removed, and the pellet was resuspended in the KRB-HEPES media. The final centrifugation was again at 50xg for 1 minute. After the supernatant was removed, the pellet was resuspended in 7.0 ml of the KRB-HEPES media. The cells were counted in a hemocytometer chamber. Cell viability, which routinely exceeded 90%, was determined by the exclusion of 0.6% trypan blue. During the entire isolation, the cells and all media were kept at 4°C.

Oxygen consumption was measured with a Clark-type oxygen electrode at 37°C. Approximately 1×10^6 cells/ml were added to the electrode chamber and brought up to volume with KRB-HEPES media. When palmitate was used as a substrate, 1% bovine serum albumin (BSA) was added to the media. The medium and cells were saturated with 95% O_2 - 5% CO_2, and the sample introduction port on the oxygen electrode was sealed with mineral oil. A baseline oxygen consumption rate was first recorded. Substrates were subsequently introduced through the introduction port with a syringe, and the resulting rate of oxygen consumption was recorded. The substrates used were 5 mM pyruvate, 5 mM glucose, 10 mM DL-α-glycerolphosphate, 5 mM succinate, and 1 and 3 mM palmitate. Palmitate was heated in distilled water to 65°C to dissolve it and then was injected at that temperature with a preheated syringe.

Protein in the liver slices and cells was assayed by the method of Lowry et al. (16) with the addition of 2 mg per tube of sodium deoxycholate. To express oxygen consumption on a whole-liver basis, the total protein in the liver was estimated from the liver sample protein concentration. We then assumed that the liver

cells accounted for all of the protein in the liver, and in turn divided the total liver protein by the protein per liver cell to yield total cells per liver. Liver cell oxygen consumption was multiplied by the number of cells in the liver to give oxygen consumption per whole-liver. The assumption we made that the liver cells represented all the liver, although not entirely correct, was necessary to express the data on a whole-liver basis. Oxygen content of the O_2-saturated media was determined by the method of Robinson and Cooper (22).

Liver mitochondrial oxygen consumption

Liver samples were homogenized by hand with a teflon and glass homogenizer in 250 mM sucrose, 2 mM ethylendiaminetetraacetic acid (EDTA), and 20 mM tris (hydroxymethyl) aminomethane (Tris), pH 7.4 (13). The homogenate was centrifuged at 600xg for 10 minutes. The supernatant was saved, and the pellet was resuspended in the sucrose media and centrifuged again at 600xg for 10 minutes. The two supernatants then were combined and centrifuged at 9000xg for 10 minutes. The supernatant was aspirated, and the pellet was resuspended in the sucrose media to a concentration of 0.5 ml per gram of liver sample. All procedures were carried out at $4^{\circ}C$.

Oxygen consumption of the mitochondria was determined at 25°C in the air-saturated media of Max et al. (19) which contained 15 mM KCl, 30 mM KH_2PO_4, 25 mM Tris, 45 mM sucrose, 7 mM EDTA, and 5 mM $MgCl_2$, pH 7.4. Initially, 125 uM ADP was added to exhaust endogenous substrates and then exogenous substrates were added to the incubation chamber as before. A State 4b respiration rate was then obtained (7). This was followed by a larger addition of 320 uM ADP, which initiated State 3 respiration. State 4b respiration again followed after all the ADP was phosphorylated to form ATP. Substrates used in the assays were 9 mM pyruvate plus 1 mM malate, 50 uM palmitoylcarnitine plus 1 mM malate, 9 mM succinate plus 4 uM rotenone, and 9 mM DL-α-glycerolphosphate plus 4 uM rotenone. Assays with palmitoylcarnitine contained 2% BSA (32), while the other assays had 0.2% BSA in the media.

The Respiratory Control Index (RCI) was calculated by dividing the State 3 rate by the State 4b rate (7). The P:O ratio was calculated as described by Estabrook (10). The amount of ADP, which was added in State 3, was determined spectrophotometrically at 259 nm with an extinction coefficient of 16.3 $mM^{-1} \cdot cm^{-1}$ (25). Oxygen

content of the media and mitochondrial protein concentrations were determined as before.

Liver enzyme activities

Liver samples for mitochondrial enzyme activity determinations were homogenized in the sucrose media used before. An aliquot of this homogenate was frozen and thawed three times in a dry ice and acetone bath. This aliquot, which represents a whole homogenate fraction, was then centrifuged at 600xg for 10 minutes, and the supernatant was stored at -80°C until assayed. No sample was stored longer than 2 weeks. Another aliquot from the original homogenate was centrifuged at 600xg for 10 minutes. The supernatant was then again centrifuged at 600xg for 10 minutes. The resulting supernatant was then centrifuged at 14,000xg for 10 minutes, and the mitochondrial pellet was resuspended in the sucrose media. This mitochondrial fraction was frozen and thawed three times as before, stored at -80°C, and assayed within 2 weeks. All procedures in the preparation of the enzyme fractions were carried out at 4°C.

Citrate synthase (EC 4.1.3.7) was assayed by the method of Srere (29) with 5,5' dithiobis (2-nitrobenzoate) as the sulfydryl acceptor. Succinate dehydrogenase (EC 1.3.99.1) and ∝-glycerolphosphate dehydrogenase

(EC 1.1.99.5) were assayed by the method of Singer and Kearney (26) with phenazine methosulfate and 2,6-dichloroindophenol as the artificial electron acceptors. Cytochrome oxidase (EC 1.9.3.1) was assayed by the method of Wharton and Tzagoloff (34), except that a trace amount of sodium dithionate was used to reduce the cytochrome C. Activity was monitored by following the oxidation of reduced cytochrome C at 550nm. Protein in the mitochondrial fraction and 600xg supernatant was assayed as before. All rates of enzyme activity were proportional to enzyme concentration, except cytochrome oxidase, which was proportional to the first order rate constant.

Chemicals

All substrates were purchased from Sigma Chemical Co. (St. Louis, MO) except acetyl-COA was purchased from P-L Biochemicals (Milwaukee, WI). Collagenase and hyaluronidase were purchased from Worthington Diagnostic Systems (Freehold, NJ). Other chemicals were reagent grade and purchased from Fisher Chemical Co. (Fair Lawn, NJ).

Statistics

The effect of substrate on in vitro liver cell oxygen consumption was analysed with a paired t-test

when possible (28). Possible phenotype x substrate interactions for the addition of BSA and glucose to the hepatocyte assays were analysed by analysis of variance (ANOVA) at the Iowa State University Computer Center. All other comparisons in this study were made with the t-test (28). A probability of 0.05 or less was taken to be statistically significant.

RESULTS

Liver weight and composition

Liver weight and liver protein were significantly increased in the obese rats (Table 1). The amount of protein per 10^6 cells, percent of wet weight as protein, and total number of cells were similar in the livers from obese and nonobese rats.

Table 1. Liver weight, protein, and cell number in obese and nonobese Zucker rats

	Obese	Nonobese	p
Wet weight (g)	(8)8.68 ± 0.81[a]	(9)6.46 ± 0.29	<0.01
Total protein (g)	(8)1.89 ± 0.07	(8)1.42 ± 0.09	<0.01
$\frac{\text{g protein}}{\text{g liver}}$ (100)	(8)22.0 ± 0.5	(8)21.7 ± 0.7	NS
$\frac{\text{mg protein}}{10^6 \text{ cells}}$	(8)3.51 ± 0.30	(8)3.12 ± 0.27	NS
$\frac{\text{cells} \times 10^6}{\text{g wet weight}}$	(7)71.3 ± 6.9	(8)67.5 ± 7.6	NS
$\frac{\text{cells} \times 10^6}{\text{liver}}$	(7)473 ± 53	(8)591 ± 81	NS

[a]Values are Means ± SEM with number of observations in parentheses.

Liver cell oxygen consumption

Liver cell oxygen consumption was similar in obese and nonobese rats when expressed either on a cellular basis (Table 2) or on a whole-liver basis (Table 3). Addition of 1% BSA significantly increased liver cell oxygen consumption in both phenotypes, and the ANOVA indicated that the animals responded similarly. The addition of glucose, pyruvate, or palmitate had no significant effect on liver cell oxygen consumption. The addition of succinate and α-glycerolphosphate significantly increased liver cell oxygen consumption. The addition of succinate was especially striking because it increased oxygen consumption over 5-fold in both phenotypes. On a cellular basis, only the addition of glucose produced a significant phenotypic difference in liver cell oxygen consumption. Due to variability, the difference in the obese and nonobese responses to glucose relative to KRB alone was not significant. On a whole-liver basis, the addition of glucose and succinate produced a significant phenotypic difference in oxygen consumption. In all of these cases, hepatocyte oxygen consumption was higher in the obese rat.

Liver enzyme activities

Enzyme activities for citrate synthase, succinate

Table 2. Hepatocyte oxygen consumption from obese and nonobese Zucker rats on a cellular basis

Media	Obese	Nonobese	p
KRB alone	(8)13.3±1.5[a]	(9)11.3±1.9	NS
KRB plus 1% BSA	(8)19.5±1.8**	(9)18.4±3.2**	NS
KRB plus 5mM glucose	(8)14.9±1.6	(9)10.4±0.8	<0.05
KRB plus 5mM pyruvate	(8)14.0±2.3	(7)12.9±2.2	NS
KRB plus 10mM DL-∝-glycerolphosphate	(8)18.0±1.6*	(9)18.8±2.5*	NS
KRB plus 5mM succinate	(8)93.0±15.8**	(8)65.8±8.1**	NS
KRB plus 1% BSA and 1mM palmitate	(8)24.4±2.3**	(9)20.8±1.4**	NS
KRB plus 1% BSA and 3mM palmitate	(8)19.7±2.7	(9)17.4±1.7*	NS

[a]Values are Means ± SEM with number of observations in parentheses. Units are nmoles O_2/(10^6 cells x minute).

*Significantly different from KRB alone rate at $p < 0.05$ level.

**Significantly different from KRB alone rate at $p < 0.01$ level.

Table 3. Hepatocyte oxygen consumption from obese and nonobese Zucker rats on a whole liver basis

Media	Obese	Nonobese	p
KRB alone	(8) 7.72±1.39[a]	(9) 5.15±0.86	NS
KRB plus 1% BSA	(8) 11.2±1.8**	(9) 8.70±1.33*	NS
KRB plus 5mM glucose	(8) 8.65±1.56	(9) 4.76±0.80	<0.05
KRB plus 5mM pyruvate	(8) 8.11±1.46	(7) 5.87±0.98	NS
KRB plus 10mM DL-α-glycerolphosphate	(8) 10.4±1.9*	(9) 8.55±1.43*	NS
KRB plus 5mM succinate	(8) 54.0±9.7**	(8) 30.0±5.0**	<0.05
KRB plus 1% BSA and 1mM palmitate	(8) 14.0±2.2*	(9) 9.83±1.50*	NS
KRB plus 1% BSA and 3mM palmitate	(8) 11.3±1.8	(9) 8.22±1.26	NS

[a]Values are Means ± SEM with number of observations in parentheses. Units are umoles O_2/(whole liver x minute).

*Significantly different from KRB alone rate at $p < 0.05$ level.

**Significantly different from KRB alone rate at $p < 0.01$ level.

dehydrogenase, and cytochrome oxidase were similar in obese and nonobese rats in both the 600xg supernatant (which represents a whole homogenate fraction) and the 14,000xg mitochondrial pellet (Table 4). The activity of α-glycerolphosphate dehydrogenase was significantly greater in the nonobese rat than in the obese rat.

Liver mitochondrial respiration

Maximum mitochondrial respiration rates (State 3) were similar for the nonobese and obese rats (Table 5). Respiratory Control Indexes, P:O ratios, and State 4b respiration rates were also similar for both phenotypes within any substrate. The Respiratory Control Indexes show that assays with pyruvate plus malate, palmitoylcarnitine plus malate, and succinate plus rotenone contained well-coupled mitochondria. The assays with α-glycerolphosphate plus rotenone were not uncoupled. The Respiratory Control Index is low because this substrate was poorly oxidized by the mitochondria. The State 4 respiration rate seems to represent the maximum oxidative capacity of the mitochondria for this substrate.

Table 4. Liver enzyme activity rates from obese and nonobese Zucker rats

Enzyme	Obese	Nonobese	p
	600xg supernatant		
Citrate synthase	(7)19.7±1.8[a]	(9)20.3±1.3	NS
Succinate dehydrogenase	(7)8.25±0.68	(9)9.84±0.53	NS
Cytochrome oxidase	(7)21.5±2.4	(9)25.8±2.4	NS
	14,000xg mitochondrial pellet		
Citrate synthase	(8)87.3±9.0	(8)74.5±3.5	NS
Succinate dehydrogenase	(8)75.8±2.6	(8)72.9±3.2	NS
Cytochrome oxidase	(8) 164±7	(8) 159±10	NS
α-glycerolphosphate dehydrogenase	(8)4.25±0.20	(8)5.34±0.19	<0.01

[a]Values are Means ± SEM with number of observations in parentheses. Units are nmoles/(mg protein x minute).

Table 5. Substrate dependent liver mitochondrial respiration and degree of coupling from obese and nonobese Zucker rats

Genotype	State 4b	State 3	R.C.I.	P:O
	Pyruvate plus malate (9mM:1mM)			
Obese	4.35±0.46[a]	21.1±2.6	4.8±0.3	2.62±0.11
Nonobese	4.42±0.30	17.5±2.5	4.1±0.6	2.70±0.11
	Palmitoylcarnitine plus malate (50uM:1mM)			
Obese	6.84±0.46	50.6±6.8	7.2±0.7	2.42±0.12
Nonobese	7.13±0.68	40.0±5.6	6.0±0.9	2.32±0.04
	Succinate plus rotenone (9mM:4uM)			
Obese	14.1±0.9	112 ± 6	8.0±0.2	1.62±0.05
Nonobese	15.2±0.7	102 ± 5	6.8±0.4	1.62±0.05
	DL-α-glycerolphosphate plus rotenone (9mM:4uM)			
Obese	5.65±0.47	7.14±0.95	1.3±1	1.43±0.04
Nonobese	6.00±0.64	7.94±0.49	1.4±0.1	1.38±0.07

[a]Values are Means ± SEM for n=8 observations in each group. No obese vs. nonobese means differ significantly. Units are nmoles O_2/(mg protein x minute).

DISCUSSION

Oxygen consumption in the isolated hepatocytes was generally similar in the obese and nonobese rats. On a cellular basis, hepatocyte oxygen consumption was significantly higher in the obese animal when glucose was used as the substrate. One would expect pyruvate to have an effect similar to glucose, but it did not. This discrepancy exists because the effect of glucose was primarily a statistical one. With no exogenous substrates present, hepatocyte oxygen consumption was slightly higher, although not significantly higher, in the obese animals. The addition of glucose decreased oxygen consumption slightly in the nonobese animals and increased oxygen consumption slightly in the obese animals. This in turn made the nonsignificant phenotypic difference in oxygen consumption statistically significant. However, without a parallel response with pyruvate or significant phenotype x substrate interaction, the real significance of this effect of glucose is questionable.

Mitochondrial respiration rates were phenotypically similar for all substrates tested. Mitochondrial respiration also was very substrate-dependent. State 3

respiration, which represents the maximum oxygen consumption rate of the mitochondria, could not be sustained without the addition of exogenous substrates. This link between mitochondrial oxygen consumption and the oxidation of exogenous substrates gave us a direct way for measuring rates of substrate oxidation. The oxidation of palmitoylcarnitine was not depressed in the obese rat liver because State 3 mitochondrial respiration rates with this substrate were similar for both phenotypes. This has been recently demonstrated in older female Zucker rats as well (5). Lipid oxidation is lower in hepatocytes from obese animals when incubated with 0.1 mM palmitate (1, 30). As shown in this study, however, a phenotypic difference in β-oxidation does not contribute to this difference in lipid oxidation.

Succinate greatly stimulated respiration in both liver mitochondria and isolated cells. Van Rossun (33) has determined that most of the succinate added to liver slice incubations is oxidized to fumarate and malate and then released into the incubation media. Alpha-glycerolphosphate stimulated hepatocyte respiration, but was poorly utilized by the isolated mitochondria.

Pyruvate had no effect on hepatocyte respiration, but was readily oxidized by the mitochondria. These data collectively indicate that no defect exists in liver mitochondrial oxidation of pyruvate, palmitoylcarnitine, succinate or α-glycerolphosphate in the obese rat. Triscari et al. (31) have suggested that, based on oxidation to $^{14}CO_2$, pyruvate and succinate oxidation is depressed in the hepatocytes from obese animals. However, the oxidation rates between phenotypes in their report are not very different. Furthermore, the use of ^{14}C-labeled substrates for the measurement of substrate oxidation is problematic since it is unclear how much of the label is taken up into the hepatocyte, how much is incorporated into glycogen, and how much is recycled and then oxidized to CO_2 by the hexose monophosphate shunt. Our method is a much more direct measure of substrate oxidation and probably better represents the true potential for cellular oxidation of the substrates.

Activities of the rate-limiting oxidative enzymes citrate synthase, succinate dehydrogenase, and cytochrome oxidase were similar for both phenotypes. These enzyme activities support the finding that no difference exists in the capacity for maximum mitochondrial

respiration. The rates for α-glycerolphosphate dehydrogenase were depressed in the obese rat. This difference may simply reflect the lower levels of serum T_3 and T_4 in the obese rat (35), since the activity of this flavin-linked mitochondrial enzyme is thyroid hormone-sensitive (15). It may also be of no consequence because α-glycerolphosphate was poorly oxidized by the mitochondria and the respiration rates were similar for obese and nonobese rats.

It is evident, as covered in the introduction, that certain aspects of liver metabolism differ in obese and nonobese rats. However, hepatic oxygen consumption and oxidative capacity are not phenotypically different. The liver does not seem to be a major contributor to the increased metabolic efficiency of the obese rat. Furthermore, differences in hepatic lipid oxidation in obese and nonobese rats are not caused by a difference in the capacity for β-oxidation.

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GENERAL DISCUSSION AND CONCLUSIONS

Muscle oxidative capacity, as measured by enzyme activities and mitochondrial oxygen consumption, was phenotypically similar in 6 and 12 week old male Zucker rats. The response of muscle to treadmill training further demonstrated that there was no latent defect in muscle oxidative capacity in the obese rat. In vitro muscle oxygen consumption was also similar in 6 week old obese and nonobese rats. The only phenotypic difference in muscle metabolism demonstrated in this dissertation was a depressed rate of muscle accretion in the obese rat. This defect may contribute to the increased metabolic efficiency of the obese rat since on a whole animal basis the muscle tissue will use less energy. However, the importance of this defect to the etiology of the obese syndrome is questionable as it is neither present in the obese female rat nor in the earliest phases of the development of the obesity.

Liver oxidative capacity was phenotypically similar in the 6 week old Zucker rats, as was hepatocyte oxygen consumption. The obess rats had larger livers which contained more protein than that of the nonobese rats. Since the oxidative capacity and oxygen consumption were phenotypically similar, the liver probably also does not contrib-

ute to the increased metabolic efficiency of the obese rat.

Exercise ameliorated, but did not normalize, the hyperphagia and excess lipid accretion of the obese rat. This rat has a great propensity for lipid accretion, and it is clear from the literature that it will synthesize excess lipid in spite of all attempts to stop it.

This dissertation does not constitute a breakthrough in the understanding of the etiology of Zucker rat obesity. However, it does rule out a difference in liver and muscle oxygen consumption and oxidative capacity as significant contributors to the obese syndrome. In this regard, this dissertation has furthered the understanding of obesity in the Zucker rat. In addition, because of this work, future investigators should be able to choose more fruitful organs or metabolic systems to study in an effort to determine the major etiological factors for this obese syndrome.

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