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In vivo incorporation of tritium to measure lipogenesis in red skeletal muscle: significance of a nonlipogenic diet in LA/N-fa(k)("corpulent") and Zucker fa rat strains

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IN VIVO INCORPORATION OF TRITIUM TO MEASURE LIPOGENESIS IN RED SKELETAL MUSCLE: SIGNIFICANCE OF A NONLIPOGENIC DIET IN LA/N-*fa*^{k} (*"corpulent"*) AND ZUCKER *fa* RAT STRAINS

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ABSTRACT

Skeletal muscle lipogenesis in rodents may equal that of liver but its importance in the obese animals is not known. We evaluated the rate of fatty acid and triglyceride synthesis and storage content in red skeletal muscles, liver, inguinal fat, and retroperitoneal fat of the LA/N-*fa*^{k} *("corpulent")* and Zucker *fa* obese rat strains. Obese-lean pairs of both rat strains, weaned at 32 days, fed a nonlipogenic diet (54% starch for 21 days) were either dosed with 2 μCi ^3H-H_2O per gram of body weight and tested after 60 minutes for whole body ^3H-incorporation into fatty acids and triglycerides, or terminated for triglyceride storage content analysis. Fatty acid and triglyceride synthesis (microgram-atoms ^3H incorporation/hour/gram of tissue) and triglyceride storage content (milligrams/gram wet weight) for LA/N-*fa*^{k} *("corpulent")* obese rats were higher ($P < 0.05$) in gastrocnemius and vastus medius skeletal muscles. Triglyceride storage content was higher ($P < 0.05$) for obese versus lean animals in iliacus and psoas muscles, inguinal fat, and retroperitoneal fat. Liver fatty acid and triglyceride synthesis and storage content were

similar. Fatty acid and triglyceride synthesis and storage content for Zucker *fa* obese rats were higher ($P < 0.05$) in vastus medius muscles compared with lean littermates. Triglyceride synthesis in psoas, iliacus, and gastrocnemius muscles, and in liver was higher ($P < 0.05$) in obese versus lean rats. Obese rats had higher triglyceride storage content ($P < 0.05$) in soleus and gastrocnemius muscles, liver, inguinal fat, and retroperitoneal fat compared with lean littermates. Because skeletal muscle mass is several times that of liver and because obese rats synthesize significantly more lipids than their lean littermates, we suggest that skeletal muscle may play a substantial role in the development of obesity in both of these rat strains.

INTRODUCTION

Obesity should be viewed as a separate disease today. Statistics show that approximately 34 million adults are overweight with 12 million to be severely overweight (Kissebak et al., 1989). Obesity can be defined as being 20-50% over ideal body weight or moderately overweight with a body mass index greater than 30 kg/m^2 (Gray, 1989; Storlie & Jordan, 1984). Diseases such as diabetes, cardiovascular disease, endocrine disorders, and various cancers are further complicated by obesity.

How and why is the American population becoming obese? One way to look at this perplexing problem is to understand how the processes of lipogenesis and lipolysis play a role in select tissues affecting obesity. Obesity occurs when energy intake exceeds energy output. Isocaloric meals tend to generate different expenditures of energy depending upon whether carbohydrates, protein, or fat is the main part of the meal. Excess carbohydrates tend to be metabolized quickly while fats digested at a slower rate are more likely to be placed into adipose storage for later use.

Lipogenesis or fatty acid synthesis occurs primarily in liver

and adipose tissue. Fatty acids synthesized by the liver are secreted by the hepatocytes as circulating triglycerides, and transported in the blood as chylomicrons and very low density lipoproteins (Pullen et al., 1990). The majority of fatty acids are then taken up by adipocytes and esterified to glycerol-phosphate to form triglycerides. Triglycerides are stored in this manner in the adipocyte's lipid storage (Hirsh et al., 1989).

Lipolysis is the mobilization of stored triglycerides and their hydrolysis to glycerol by hormone sensitive lipase (Hirsh etal., 1989). Glycerol and free fatty acids are metabolized as energy sources (Houtsmuller, 1975).

In general, lipogenesis and lipolysis occur simultaneously. This results in fatty acid release and reesterification that is extremely sensitive to hormonal influence. Both the rates of fatty acid reesterification and release into the bloodstream are dependent upon glucose availability. The rate of lipogenesis is also related to the type of substrate consumed (Cawthorne & Cornish, 1979). The digestive product of dietary starch, glucose, is utilized via mitochondrial metabolism. Dietary sucrose enhances lipogenesis. One of the components of sucrose, fructose, bypasses the phospho-

fructokinase control point in glycolysis thus allowing fructose to flood the lipogenic pathway in the liver. This leads to enhanced fatty acid synthesis, esterification of fatty acids, and very low density lipoprotein secretion (Murray et al., 1993). Glucose, on the other hand, does not bypass the phosphofructo-kinase control point and thus is metabolized for fuel.

Red skeletal muscle tissue oxidizes fatty acids as the principal fuel for energy. Fatty acids are supplied to muscle tissue from the blood through diet, where they are transported either as free fatty acids bound to albumin or as triglycerides in the form of chylomicrons and very low density lipoproteins. If the fatty acids are not utilized, they combine with glycerol to form triglycerides which are then transported to intra- muscular triglyceride stores. Since fatty acids are the principal form utilized by muscle tissue, triglycerides are hydrolyzed to fatty acids prior to energy utilization by skeletal muscle tissue (Idell-Wenger & Neely, 1978).

Very little research has been published to date that specifically investigates the role of red skeletal muscle in lipogenesis. George and Naik (1958) conducted biochemical and histochemical studies on pigeon muscles finding greater stores of

triglyceride in red skeletal muscle as compared to white skeletal muscle. It was later proposed that oxidized fat not utilized during exercise must have come from intramuscular triglyceride stores (Issekutz & Paul, 1968) in agreement with the earlier work of George and Naik's 1958 study.

Later investigations have shown that red skeletal muscle contained a higher content of mitochondria in its fibers than white skeletal muscle, oxidized more intramuscular triglycerides, and used fatty acids as a source of acetyl-CoA (Carlson et al., 1966; Fröberg, 1971; Reitman et al., 1973; Friden et al., 1984; Holloszy & Coyle, 1984). Newsholme and Crabtree (1976) in particular suggested the existence of an acetyl-CoA - fatty acid - triglyceride cycle based upon the established high enzymatic potential of red muscle for fatty acid oxidation and the finding of high rates of lipogenesis. They also raised the possibility that this cycle could have implications for mechanisms involving both metabolic regulation and heat production. Also in 1976, Kannan and his coworkers published two significant papers on the potential role of lipogenesis in the muscular part of the carcass.

Working with mice, Kannan et al. (1976) assessed the

contribution of various tissues to the synthesis of fat from [U-^{14}C] glucose. They found that the muscular portion of the stripped carcass contained more than 75% of the total radioactivity in the fatty acids derived from ^{14}C-labeled glucose. However, the liver and epididymal fat pads combined only accounted for a small percentage of the remaining ^{14}C signal from this source.

Further findings by Kannan and coworkers (1976) included the discovery that a discrete fat body in the popliteal region of the leg could convert glucose carbon to fatty acids faster than the epididymal fat pads, suggesting that intermuscular fat pads may be responsible for the high rates of lipogenesis in the animal's body. The major portion of the label in popliteal fat at two and at six hours after ingestion of the glucose was in the fatty acid moiety of the triglycerides. The diacylglycerol pool was active at two hours post-ingestion, but by six hours its activity had faded, implicating its role as an intermediary in lipid metabolism. They concluded from these findings that fat cells within the musculature may well be major contributors in the *de novo* synthesis of fat from carbohydrates in the diet. They also suggested in a second paper on the work that fatty acids might even be exported from the

intermuscular fat to other fat tissues, possibly being mobilized almost as fast as they are formed (Kannan & Baker, 1976).

Hollands and Cawthorne (1981) investigated the possibility that the intramuscular fat pads might, as Kannan et al. (1976) proposed, account for the high rates of lipogenesis now commonly being reported in "the rest of the carcass". In order to investigate this proposal, these investigators divided the carcass into fourteen constituent parts and assessed the individual contribution of each part to the total fatty acid synthesis rate. In order to measure the total fatty acid synthesis irrespective of the carbon source, Hollands and Cawthorne (1981) used ^3H_2O incorporation. The mice were skinned, subcutaneous fat removed, and the musculature divided into the following: 1. the hindlimbs were dismembered adjacent to the gluteus maximus muscle; 2. the forelimbs were removed at the shoulder joint; 3. the head was removed; and 4. the neck muscles were left attached to the back musculature. The back region, therefore, consisted of the spine and the musculature of the back and shoulders.

Hollands and Cawthorne (1981) found that liver and adipose tissue accounted for only 22% and 7%, respectively, of the total

fatty acid synthesis rate, consistent with earlier research. The four other main sites of fatty acid synthesis in the rest of the carcass were the skin (13%), the head (15%), the gut, which was not stripped of adipose tissue (14%), and most significantly, the back region which contributed 21% of total fatty acid synthesis was found to be similar to liver (22%). However, they also found that the fatty acid synthesis rate of both the forelimbs and the hindlimbs together to account for only 2% of the total body fatty acid synthesis.

Since Kannan et al. (1976) had reported most of their newly synthesized fatty acids from the hindlimb in the popliteal fat pad, Hollands and Cawthorne (1981) carefully dissected out the muscles of the back region where they had detected so much fatty acid synthesis activity to assess the involvement of intermuscular fat pads. The investigators did not visually observe any fat pads in this region. Considering the relatively small contribution they detected in the hindlimbs which contain the popliteal fat pads, and the fact that no intermuscular fat pads were observed in the back region, Hollands and Cawthorne (1981) concluded that these fat pads were not significant in overall fatty acid synthesis. Therefore, the ^{3}H-labeled fatty acids found in the back region could either have

reflected the rate of *de novo* fatty acid synthesis in the muscle cells of the back, or could have arisen from the immigration of fatty acids from other sites of lipogenesis.

Haft (1973) showed that there was relatively little transport of newly synthesized triglycerides from liver to peripheral tissues during a one hour period. In the Hollands and Cawthorne study, if the ^{3}H-labeled fatty acids were immigrating into the back muscles, the time course of incorporation of ^{3}H into fatty acids would show a lag phase---low initial incorporation followed by an increasing gradient of ^{3}H-fatty acids in the cell lipid pool. With these data, Hollands and Cawthorne (1981) established that there was no lag phase in the time course. Indeed, the rate of ^{3}H-incorporation into fatty acid synthesis declined with advancing time. This suggested that fatty acids were synthesized within the muscles themselves. Decline in the fatty acid content with time led Hollands and Cawthorne (1981) to believe that rapid utilization or export of the newly synthesized fatty acids may be occurring at points later in the timed phase. A second element supporting muscle as the *de novo* fatty acid synthetic site centered focus on hepatocyte synthetic rates during the same time course. In the liver, fatty acid synthesis occurring

over the one hour time period showed a linear time course. They concluded that their reported rate of fatty acid synthesis in the back region was, therefore, "almost certainly an underestimate."

Addressing the issue of the high activity of the back region compared to the low activity of the hindlimb, Hollands and Cawthorne (1981) conducted additional studies and found that the rate of ^{3}H-incorporation into fatty acids in a predominantly red muscle of the psoas was 3 to 10 times greater than in a predominantly white muscle of the quadriceps femoris. The activity of enzymatic agents like acetyl-CoA carboxylase showed a similar time course. Predominantly red muscle within the skeletal musculature appears to be an important site for fatty acid synthesis. These data are highly consistent with earlier histochemical and biochemical findings that red skeletal muscles have a high density of mitochondria coupled with high oxidative potential compared with other skeletal muscle tissue types.

The work in our lab focuses upon obesity in rodent models and humans. During the last few years we have conducted enzymatic, molecular genetic, biochemical, and electron microscopic histochemical studies addressing mechanisms of inherited obesity

in the rodent strain, LA/N-fa^{cp}. It was a direct result of work in our lab that two prominent rodent obesity models, the Zucker 13M-fa with the fatty mutation and the LA/N-cp with the corpulent mutation, have been determined to possess single or multiple gene mutations at the same locus and have been redesignated at fa^{cp} (Kahle et al., 1996). However, the locus designation has not been modified to recognize the homologous positions of these two mutations. Therefore, LA/N-fa^{cp} will be referred to as LA/N-*fa^{k} ("corpulent")* (Kahle et al., 1997b).

Other work in our lab (Harapanahalli, 1993) has shown that obese animals can produce more citrate in predominantly red muscles than might be catabolized to CO_2 and coupled with the generation of ATP. This raised the possibility that the excess citrate may be translocated to the cytoplasm influencing ATP-citrate lyase activity, and in turn, the production of acetyl-CoA. Excess production of citrate in obese skeletal muscles might lead to increases in the acetyl-CoA moiety in the cytoplasm which would be routed into fatty acid synthesis. Harapanahalli (1993) also found that in sedentary obese animals, concentrations of both free fatty acids and triglycerides in the returning femoral venous circulation

were higher than in the arterial supply to the hindlimb. These data add additional support for the importance of skeletal muscle lipogenesis in obesity development and maintenance.

Recent electron microscopic histochemical work in our lab has provided anatomical evidence at the ultrastructural level for the role of skeletal muscle lipogenesis in obesity. Domaschko (1995) showed that satellite cells within predominantly red skeletal muscle from lean LA/N-*fa*^{k} *("corpulent")* rats demonstrate the expected morphology for mature, uninjured skeletal muscle: well-condensed chromatin, reduced cytoplasmic volume, and absent or poorly developed organelles. However, satellite cells within the same predominantly red skeletal muscles from obese LA/N-*fa*^{k} *("corpulent")* rats show a retention of cytoplasmic volume and well developed organelles, including more mitochondria. This indicates that in obese rats these cells are metabolically active, and possibly lipogenic.

Stated earlier in this paper, the nutrition of the LA/N-*fa*^{k} *("corpulent")* rats exerts a profound influence on the synthesis of lipids in the rat. Raney (1995) noted that feeding LA/N-*fa*^{k} *("corpulent")* rats a lipogenic diet increased the rate of fatty acid

synthesis in predominantly red skeletal muscle tissue and the liver while the rate of triglyceride synthesis was found to be higher in the red skeletal muscle tissue than the liver. Also, both fatty acid and triglyceride synthesis in both liver and red skeletal muscle tissue was found to be higher in obese LA/N-*fa*^{k} *("corpulent")* rats than in their lean littermates. Adipose tissue, however, exhibited similar rates of fatty acid and triglyceride synthesis in obese and lean animals.

The primary objective of the present study is to analyze the effects of a nonlipogenic diet (Michaelis et al., 1983) on the rate of lipogenesis in red skeletal muscle of obese vs. lean LA/N-*fa*^{k} *("corpulent")* rats. Because Raney (1995) was able to show that the role of predominantly red skeletal musculature did contribute to the development of lipogenesis in obese rodents on a lipogenic diet, we wish to test a more practical carbohydrate source, starch, shown by others (Bar-on & Stein, 1968; Ellwood et al., 1985; Michaelis et al., 1983; Sheehan et al., 1984) not to support excessive lipid synthesis, in order to further understand our hypothesis that skeletal muscle plays a major role in lipogenesis and possibly obesity development in the obese phenotype of our LA/N-*fa*^{k} *("corpulent")* obesity model.

Lipogenic and nonlipogenic diets have been compared in the literature in their ability to increase fatty acid and triglyceride synthesis. Using 54% starch as the carbohydrate source compared with 54% sucrose has produced fatty acid and triglyceride synthesis rates that are significantly lower compared with those data derived from sucrose diets (Bar-on & Stein, 1968; Gardner & Michaelis, 1979; Tulp et al., 1991; Yamini et al., 1991).

The secondary objective of this study is to compare the effects of the LA/N-*fa*^{k} *("corpulent")* rats on a nonlipogenic diet with a second obese rat model, the Zucker *fa* rat. In this study these rat models were chosen because they appear to be metabolically similar. Both strains have been found to be hypertriglyceidemic, hyperinsulinemic, hyperlipidemic, and hyperphagic (Ellwood et al., 1985; Argilés, 1989; Johnson et al., 1972; Zucker & Antoniades, 1972). Recently, Kahle, et al. (1997b) suggested that the *fa* and *fa*^{k} *("corpulent")* mutations are in the leptin receptor (*Lepr)* gene on different loci. This was found to be based on the production of *fa/f* compound mutants (Chua et al., 1996; Yen et al., 1997), mutation sequencing identification, and molecular mapping (Kahle et al., 1997a). The LA/N-*fa*^{k} *("corpulent")* rats along with other strains

carrying the *"corpulent"* mutation serve as a useful experimental model for disorders of carbohydrate utilization (Ellwood et al., 1985; Kahle et al., 1996; Michaelis et al., 1983). Both rat strains have also served as experimental animal models in numerous lipogenic and nonlipogenic studies (Ellwood et al., 1985; Michaelis et al., 1980; Sheehan et al., 1984).

MATERIALS AND METHODS

INTRODUCTION

The LA/N-*fa*^{k} *("corpulent")* rat is a congenic strain consisting of an outcross between a heterozygous (cp/+) animal to the homozygous normal (+/+) inbred strain (Hansen, 1983). The metabolic obesity in the LA/N-*fa*^{k} *("corpulent")* rat appears to involve an elevated capacity for pathways of glycolysis, gluconeogenesis, lipogenesis, and amino acid catabolism (Yamini et al., 1991).

The Zucker *fa* rat developed spontaneously from Sherman and Merck M rats (Zucker & Zucker, 1961) with the *fa* mutation appearing as autosomal recessive. This rat is considered hyperphagic, hyperlipidemic, and hyperinsulinemic (Argilés, 1989).

Animals for this particular study were maintained at 70° F on a 12 hour light/dark cycle that started on 0600 hours. Food was given *ad libitum* (Laboratory rodent diet 5001, P.M.I. Feeds, Inc.) and water bottles were refilled daily with fresh water. Cages and bedding were changed weekly. Potential animals for this study were visually inspected at 28 days of age. If obese animals were observed

in a litter, the litter was eartagged and weighed at 32 days of age. Obese animals were paired with their lean same-sex littermates and placed on a nonlipogenic diet for 21 days. Those animals not selected for the study were placed in the general population until time for breeding.

The research animals were then placed in a second room with a 12 hour light/dark cycle that began at 2100 hours. Each animal was placed in an individual metal hanging cage and allowed to feed and drink *ad libitum.* These animals were fed a nonlipogenic diet (54% carbohydrate from cornstarch; 20% protein from casein and lactalbumin; 16% fat from lard, beef tallow, corn oil, and hydrogenated coconut oil; 5.9% cellulose fiber; plus vitamin and mineral supplements) (Michaelis et al., 1983).

Water bottles were refilled daily. The animals were weighed every other day until sacrifice. Additional food was given at this time as well. Waste collection trays were lined with absorbent paper to allow fecal matter and wasted food to fall through the bottom of the cage. Fecal matter and wasted food were separated manually and each component was measured. The amount of wasted food, food leftover in the food bowl at the time of sacrifice, and the

amount of food given over a 21 day period determined the actual amount of food consumed by each individual rat. Animals were maintained on the nonlipogenic diet from 32 days of age to 53 days of age. At that point obese animals were visually significantly more fatty than their lean counterpart. All animals were sacrificed at 53 days of age.

I. FATTY ACID AND TRIGLYCERIDE SYNTHESIS STUDIES

On the day of sacrifice each animal was weighed and then injected intraperitoneally with 2.0 microCurie (μCu) of ^{3}H-H_2O per gram of body weight and allowed to remain sedentary in a glass holding vessel for 1 hour. After 1 hour the animal was administered 0.0001cc per gram of body weight with Nembutal™ (sodium pentabarbital) and exsanguinated by heart puncture. After exsanguination, the animal was euthanized by severe cervical dislocation. Eight specific tissues were surgically removed for this study. Skeletal muscle tissue (i.e. red fibers of psoas major, iliacus, soleus, red belly of the gastrocnemius, and vastus medius), the liver, and adipose depots (i.e. inguinal fat pads and retroperitoneal fat tissue) were isolated *in situ* . Each inguinal fat pad and

retroperitoneal fat from both sides of the body were weighed separately. All skeletal muscle tissues were taken from the left side of the animal's body except for the soleus which was taken from both sides of the body. The liver was removed as a whole unit and weighed. Precisely 0.100g of each tissue was weighed immediately after removal, minced into smaller pieces, and added to 400 μl of ethanolic KOH digest (1 volume 60% KOH:2 volumes 70% ethanol). This digest was vortexed periodically until the tissue completely dissolved. Female and male animals of both rat strains were used throughout this study.

II. DETERMINATION OF FATTY ACID SYNTHESIS

Exactly 2 hours after each tissue was placed in the ethanolic KOH digest, 200 μl of the digest was neutralized with 140 μl of concentrated HCL acid (pH = 1.65 - 2.0). This solution was vortexed and allowed to sit 26 hours. After 26 hours, the sample was vortexed and 1.0 ml of petroleum ether (b.p. = 40°-60°) was added in order to extract the fatty acids. Samples were then centrifuged for 20 minutes at 303G (1680 rpm), and 500 μl of the top petroleum ether layer which contained the fatty acids was transferred to a

scintillation vial containing 4.0 ml of Scintiverse II™ scintillation fluid and vortexed. These vials were allowed to sit overnight in the scintillation counter to allow any chemiluminsecence activity caused by ultraviolet radiation to dissipate. Samples were analyzed on an LKB Primo™ Scintillation Counter programmed to measure tritium counts using a sample window of 10 minutes. This window period is used most often for readings with the range of activity that we encounter using tritium because it reduces the counting error to ±2% (Marshall University College of Science Radiation Safety Specialist, Richard Petit, personal communication). All samples were counted in a single run along with a control blank of scintillation fluid which determined background radiation. Raw data for each sample was generated in the form of counts per minute (CPM). CPM are determined by the number of scintillations simultaneously received by opposite photomultiplier tubes within a defined period of time. However, CPM are not considered a viable unit for statistical analysis. Instead, disintegrations per minute (DPM) are used because they represent a more accurate picture of the disintegration events occurring throughout the photomultiplier tubes. DPM were calculated manually from CPM using a derived

efficiency (65%) as a correction factor. The following formula was used to calculate the DPM for each sample:

$$\text{DPM} = \frac{[\text{Sample Count Rate (CPM)}] - [\text{background (CPM)}]}{[65\% \text{ Efficiency}]}$$

DPM were then converted into microgram-atoms (μg-atoms) ^{3}H incorporation/hour by the following formula:

$$\frac{\text{SAMPLE DPM}}{1010 \text{ DPM}} = \mu\text{g-atoms } ^{3}H \text{ incorporation/hour}$$

III. DETERMINATION OF TRIGLYCERIDE SYNTHESIS

Of the remaining ethanolic KOH digest which had not been neutralized with HCl, 50 μl of the digest was added to 1.0 ml of petroleum ether and centrifuged for 20 minutes at 303G (1680 rpm). 500 μl of the top petroleum ether layer was then transferred to a vial containing 4.0 ml of scintillation fluid and measured for activity in the same manner as the sample vials for fatty acid synthesis analysis. All triglyceride synthesis readings were converted from CPM to DPM to μg-atoms ^{3}H incorporation/hour prior to statistical analysis.

IV. DETERMINATION OF TRIGLYCERIDE STORAGE CONTENT

Animals were rendered unconscious by forced inhalation of ethyl ether, exsanguinated by heart puncture, and euthanized by severe cervical dislocation. Skeletal muscle tissue, liver tissue, and adipose tissue (as described for fatty acid and triglyceride synthesis) were surgically removed for this experiment. All tissues were taken from both sides of the animal and a combined weight was recorded. Individual weights were recorded for each inguinal fat pad, each side of the retroperitoneal area, and the whole liver. All tissues were immediately frozen and preserved until homogenization. For homogenization, 0.100g of finely minced tissue was added to 2.0 ml of distilled, deionized water and homogenized by Tek-Mar Super Dispax Tissuemizer™. The homogenates were then centrifuged for 60 seconds at 567G (2300 rpm) to sediment larger particulate matter (primarily connective tissue). The pellet was then discarded. The supernatant of the homogenate was utilized for the determination of triglyceride storage content. Supernatant analysis was conducted at Cabell-Huntington Hospital in Huntington, WV, using a Kodak Ektachem 700 Analyzer. A 10 μl drop of each sample was placed onto a slide surface which contained a dry multi-

layered analytical element coated on a clear polyester support. Within the slide, enzymatic reagents reacted with the sample during a five minute incubation at 37°C, and the enzymatic assay was quantified by reflectance spectrophotometry at a wavelength of 540 nm (Spayd et al., 1978).

Triglyceride storage content determination begins with the dissociation of triglycerides from any lipoprotein complexes. Lipase then hydrolyzes triglycerides into glycerol which diffuses into the reagent layer and fatty acids. The reagent layer contains glycerol kinase, ATP, and magnesium chloride which facilitates glycerol phosphorylation. L-alpha-glycerolphosphate oxidase then oxidizes L-alpha-glycerolphosphate to dihydroxyacetonephosphate and hydrogen peroxide. Finally, peroxidase catalyzes the oxidation of a triarylimidazole leuco dye by the hydrogen peroxide which produces a dye whose density at 540 nm is proportional to the concentration of triglycerides in the sample (Spayd et al., 1978).

V. DETERMINATION OF GLUCOSE CONTENT

Tissues are extracted and homogenized as mentioned above in

the determination of triglyceride storage content. Glucose content determination occurs when the homogenate is spread evenly onto the slide. This permits the solute molecules to penetrate evenly into the underlying reagent layer. Using glucose oxidase as the catalyst, the oxidized glucose in the homogenate sample forms hydrogen peroxide and gluconate. Then in the presence of chromogens, peroxidase catalyzes an oxidative coupling dye intensity that is measured by reflected light at 540nm (Curme et al., 1978; Trinder, 1969).

VI. STATISTICAL ANALYSIS

Internal variation among animals of a treatment group (either obese or lean) was significant enough to overshadow individual variation between obese and lean littermates. For example, the individual fatty acid synthesis readings for liver tissue in lean animals ranged from 0.716 to 2.450 μg-atoms ^{3}H incorporation/hour. Internal variation also overshadowed the difference between the readings for a single pair: Lean liver = 2.450 μg-atoms ^{3}H incorporation/hour versus Obese liver = 6.657 μg-atoms ^{3}H incorporation/hour. All data were therefore evaluated using a paired

t-test which is capable of evaluating significant differences from several pairs of animals. Data with a two-tailed probability value of $P < 0.05$ were considered significant.

RESULTS

De novo synthesis of fatty acids and triglycerides, and triglyceride content in selected skeletal muscles, liver, and adipose depots are presented in Figures 1-3 (pp.31-36) and Tables 1-3 (pp.62-64) for the LA/N-*fa*^{k} *("corpulent")* rat and in Figures 4-6 (pp. 37-42) and Tables 4-6 (pp. 65-67) for the Zucker *fa* rat for both lean and obese phenotypes. Comparisons of these activities within phenotypes (i.e. obese versus obese and lean versus lean) between the LA/N-*fa*^{k} *("corpulent")* rat and the Zucker *fa* rat are presented in Tables 7-9 (pp. 68-70). Other measurements (e.g. diet consumption, body weight, fecal production, etc.) are presented for the LA/N-*fa*^{k} *("corpulent")* rat in Tables 10-17 (pp. 71-78) and for the Zucker *fa* rat In Tables 18-25 (pp. 79-86). All animals evaluated were fed a 54% starch diet (Michaelis *et al.*, 1983).

I. Phenotype Comparisons of the LA/N-*fa*^{k} *("corpulent")* Rat Strain

A. *De novo* synthesis of fatty acids

1. Obese LA/N-*fa*^{k} *("corpulent")* animals exhibited higher fatty acid synthesis rates in gastrocnemius ($P < 0.05$) and vastus

medius ($P < 0.01$) than their lean littermates. Fatty acid synthesis rates in both iliacus and soleus approached significance ($P < 0.07$ and $P < 0.09$, respectively) between obese and lean phenotypes. Fatty acid synthesis rates in the psoas major were similar in obese and lean animals.

2. The rates of fatty acid synthesis in liver tissue of obese animals approached significance ($P< 0.08$) over their lean littermates.

3. No significant difference in fatty acid synthesis rates was exhibited between obese and lean animals for either inguinal fat or retroperitoneal fat tissues.

B. *De novo* synthesis of triglycerides

1. Obese animals exhibited higher triglyceride synthesis rates in gastrocnemius ($P < 0.05$) and vastus medius ($P < 0.05$) when compared to lean littermates. However, the obese and lean animals had similar triglyceride synthesis rates in the psoas major, iliacus, and soleus.

2. Obese animals exhibited a trend toward higher triglyceride synthesis rates ($P < 0.08$) in liver tissue when compared with lean animals.

3. Retroperitoneal fat tissue in obese animals had a higher triglyceride synthesis rate ($p < 0.05$) compared with the lean animals. Triglyceride synthesis rates in inguinal fat tissue were similar in obese and lean phenotypes.

C. Triglyceride storage content

1. Obese animals exhibited higher triglyceride storage content rates in the gastrocnemius ($P < 0.01$), vastus medius ($P < 0.01$), iliacus ($P < 0.01$), and psoas major ($P < 0.05$) compared with their lean littermates. Soleus tissue in obese animals had triglyceride storage content rates that approached significance ($P < 0.08$) compared with the lean animals.

2. Triglyceride storage content rates in the liver were found to be similar in obese and lean animals.

3. Obese animals had a higher triglyceride storage content in inguinal fat tissue ($P < 0.01$) and retroperitoneal fat tissue ($P < 0.05$) compared with lean animals.

II. Phenotype Comparisons of the Zucker *fa* Rat Strain

A. *De novo* synthesis of fatty acids

1. Obese Zucker *fa* animals had higher fatty acid

synthesis rates in the vastus medius ($P < 0.05$) as compared with their lean littermates.

2. Fatty acid synthesis rates in the psoas major, iliacus, soleus, and gastrocnemius skeletal muscles, liver, inguinal fat, and retroperitoneal fat were similar in obese and lean animals.

B. De novo synthesis of triglycerides

1. Obese animals had higher triglyceride synthesis rates in vastus medius ($P < 0.01$), and in psoas major, iliacus, and gastrocnemius ($P < 0.05$) than their lean littermates. Triglyceride synthesis rates in soleus tissue were similar in obese and lean animals.

2. Liver tissue in obese animals had a higher triglyceride synthesis rate ($P < 0.01$) than in lean animals.

3. Triglyceride synthesis rates in inguinal fat and retroperitoneal fat were similar in the obese and lean phenotypes.

C. Triglyceride storage content

1. Obese Zucker *fa* rats had a higher triglyceride storage content in gastrocnemius and vastus medius ($P < 0.01$), and in iliacus and soleus ($P < 0.05$) than lean Zucker *fa* rats.

2. The psoas major skeletal muscles had similar

triglyceride content rates in obese and lean rats.

3. Obese animals had a higher triglyceride storage content rate in liver ($P < 0.01$) compared with lean animals.

4. Inguinal fat ($P < 0.01$) and retroperitoneal fat ($P < 0.05$) tissues in obese animals had higher triglyceride storage content rates than their lean littermates.

III. Other Quantitative Measurements of the LA/N-fa^k ("corpulent") Rat Strain

A. Glucose content of the liver in LA/N-fa^k ("corpulent") rats was similar in obese and lean animals, yet approached significance ($P < 0.09$).

B. Obese LA/N-*fa^k ("corpulent")* animals had higher rates of diet consumption, fecal production, body weight at sacrifice, and body weight gained ($P < 0.01$) while on the nonlipogenic diet than their lean littermates.

C. Obese animals also had higher amounts of inguinal fat, retroperitoneal fat, and liver tissue ($P < 0.01$) present compared with lean animals.

IV. Other Quantitative Measurements of the Zucker *fa* Rat Strain

A. Glucose content of the liver in Zucker *fa* rats was similar in obese and lean animals, yet approached significance ($P < 0.08$).

B. Obese Zucker *fa* animals had higher rates of diet consumption, fecal production, body weight at sacrifice, and body weight gained ($P < 0.01$) while on the nonlipogenic diet than their lean littermates.

C. Obese animals had higher amounts of inguinal fat, retroperitoneal fat, and liver tissue ($P < 0.01$) present compared to their lean littermates.

Figure 1. ^{3}H-labeled fatty acid concentrations in selected skeletal muscles, liver, and adipose depots of LA/N *fa*^{k} *("corpulent")* rats at 60 minutes following intraperitoneal administration of $^{3}H_2O$. Obese *fa*^{k} *("corpulent")* rats exhibited higher concentrations in gastrocnemius ($P < 0.05$) and vastus medius ($P < 0.01$) tissues compared with lean littermates.

Data are presented as μg-atoms ^{3}H incorporation/hour. Significance is presented as * for $P < 0.01$ and ** for $P < 0.05$.

Figure 1

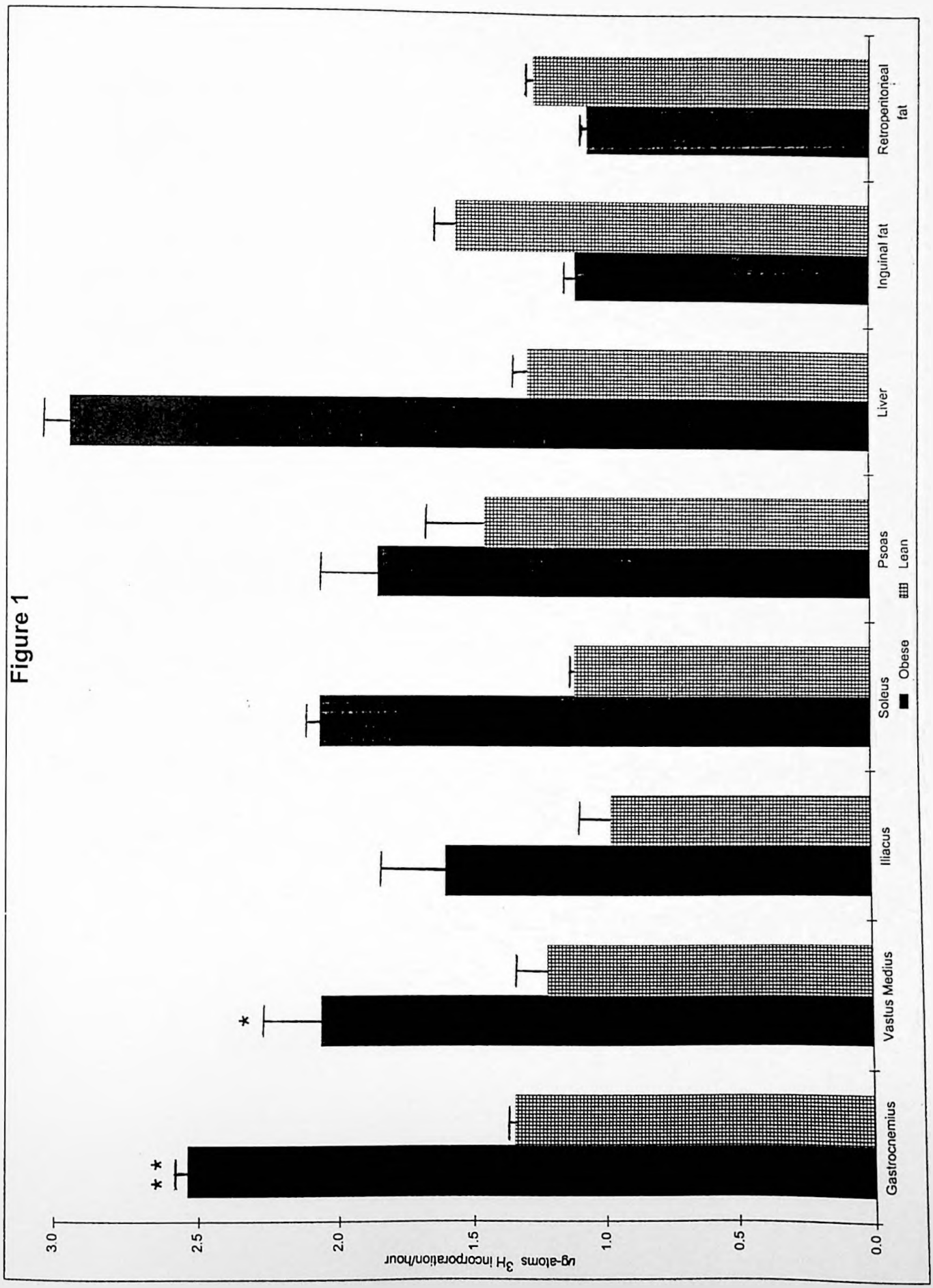

μg-atoms ³H incorporation/hour
3.0
2.5
2.0
1.5
1.0
0.5
0.0
* *
*
Gastrocnemius
Vastus Medius
Iliacus
Soleus
Psoas
Liver
Inguinal fat
Retroperitoneal fat
Obese
Lean

Figure 2. ^{3}H-labeled triglyceride concentrations in selected skeletal muscle, liver, and adipose depots of LA/N *fa^{k} ("corpulent")* rats at 60 minutes following intraperitoneal administration of $^{3}H_2O$. Obese *fa^{k} ("corpulent")* rats exhibited higher concentrations in gastrocnemius ($P < 0.05$), vastus medius ($P < 0.05$), and retroperitoneal fat ($P < 0.05$) tissues compared with lean littermates.

Data are presented as μg-atoms ^{3}H incorporation/hour. Significance is presented as ** for $P < 0.05$.

Figure 2

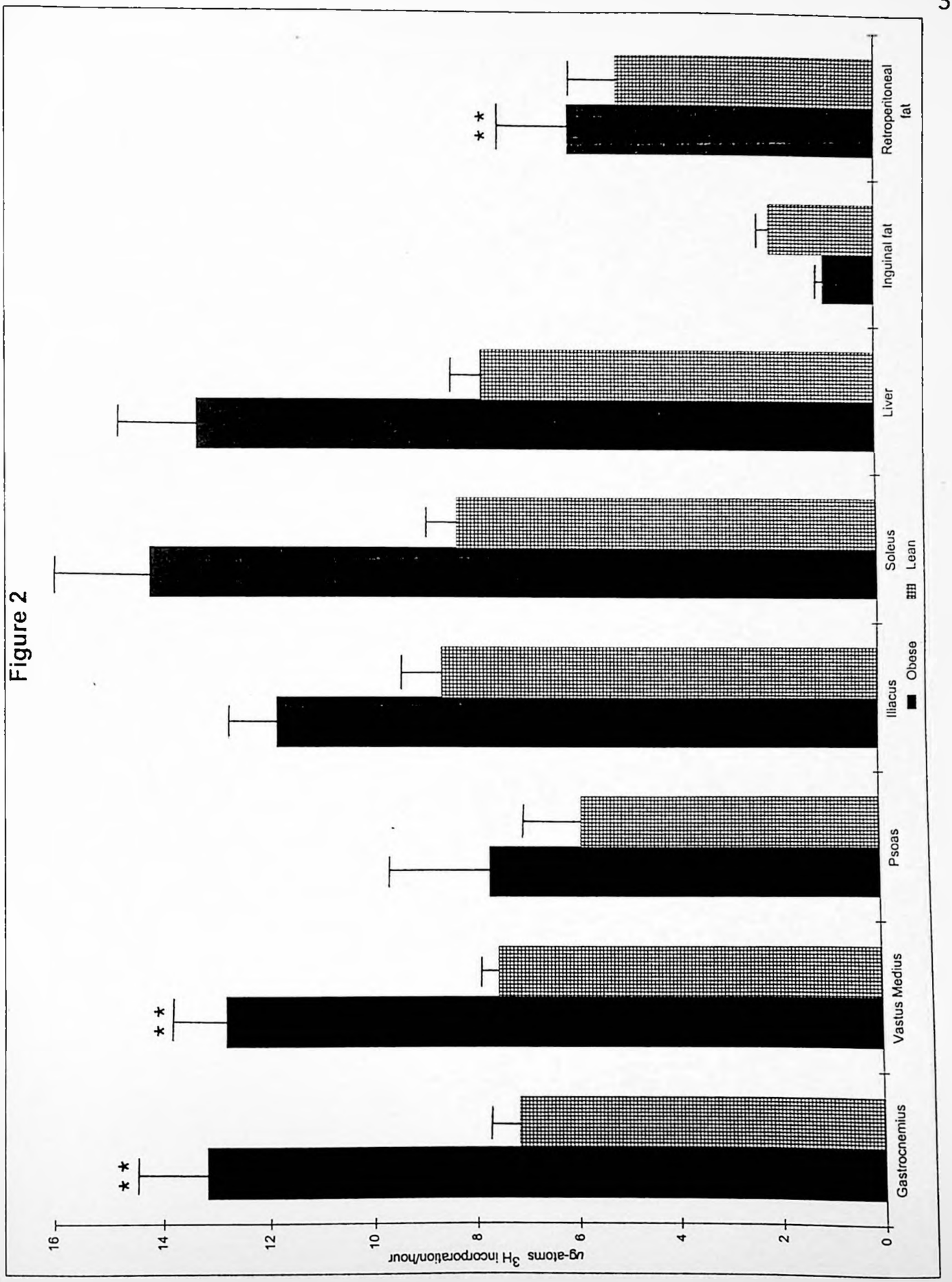

µg-atoms 3H incorporation/hour
0
2
4
6
8
10
12
14
16
**
**
**
Gastrocnemius
Vastus Medius
Psoas
Iliacus
Soleus
Liver
Inguinal fat
Retroperitoneal fat
Obese
Lean

Figure 3. Triglyceride storage concentrations in selected skeletal muscles, liver, and adipose depots of obese LA/N *fa*^{k} *("corpulent")* rats exhibited higher concentrations in gastrocnemius ($P < 0.01$), vastus medius ($P < 0.01$), iliacus ($P < 0.01$), psoas ($P < 0.05$), and retroperitoneal fat ($P < 0.05$) tissues compared with lean littermates.

Data are presented as milligrams/deciliter. Significance is presented as * for $P < 0.01$ and ** for $P < 0.05$.

Figure 3

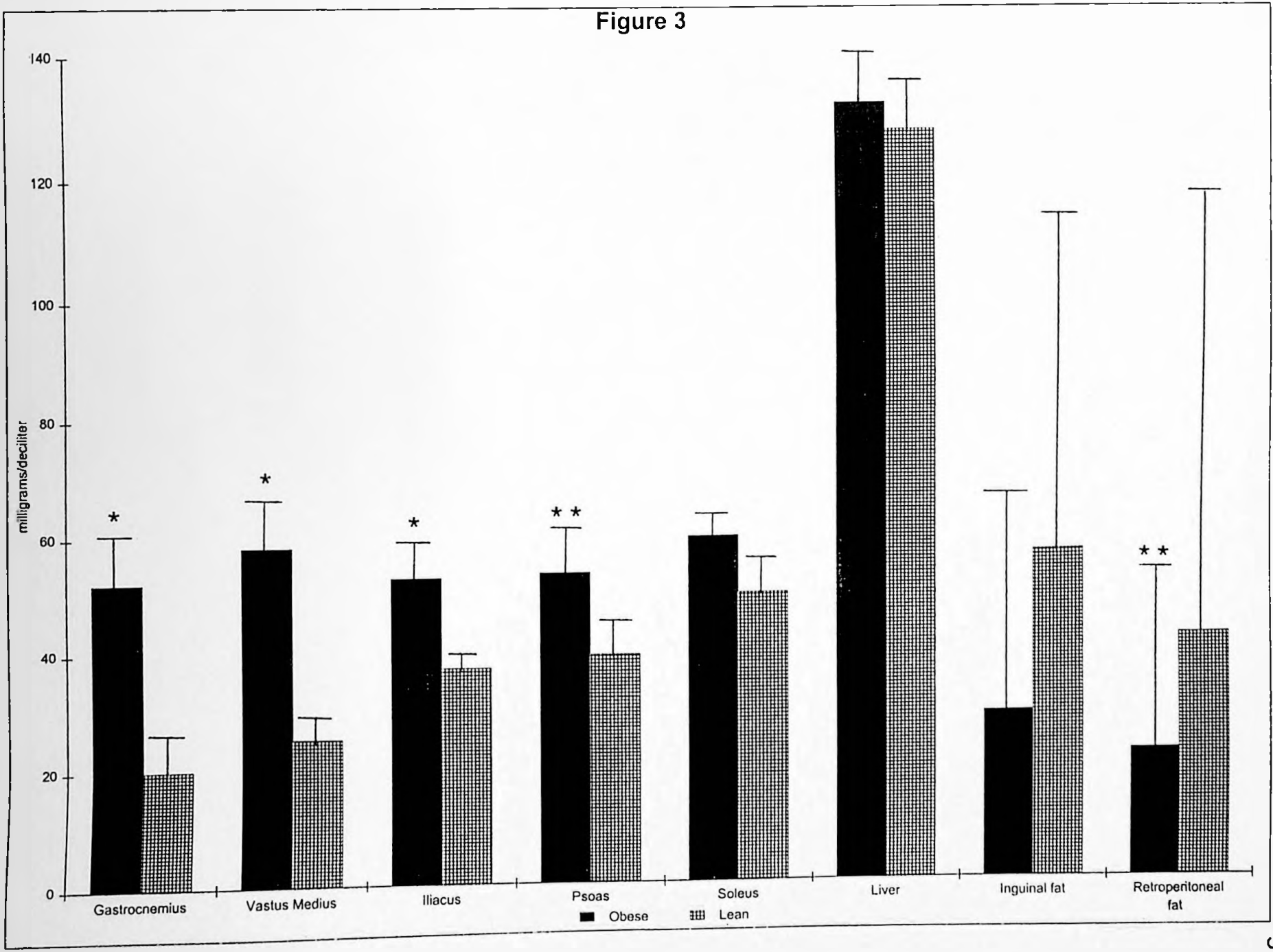

140
120
100
80
60
40
20
0
milligrams/deciliter
*
*
*
* *
* *
Gastrocnemius
Vastus Medius
Iliacus
Psoas
Soleus
Liver
Inguinal fat
Retroperitoneal fat
Obese
Lean

Figure 4. ^{3}H-labeled fatty acid concentrations in selected skeletal muscles, liver, and adipose depots of Zucker *fa* rats at 60 minutes following intraperitoneal administration of $^{3}H_2O$. Obese *fa* rats exhibited higher concentrations in vastus medius ($P < 0.05$) tissues compared with lean littermates.

Data are presented at μg-atoms ^{3}H incorporation/hour. Significance is presented as ** for $P < 0.05$.

Figure 4

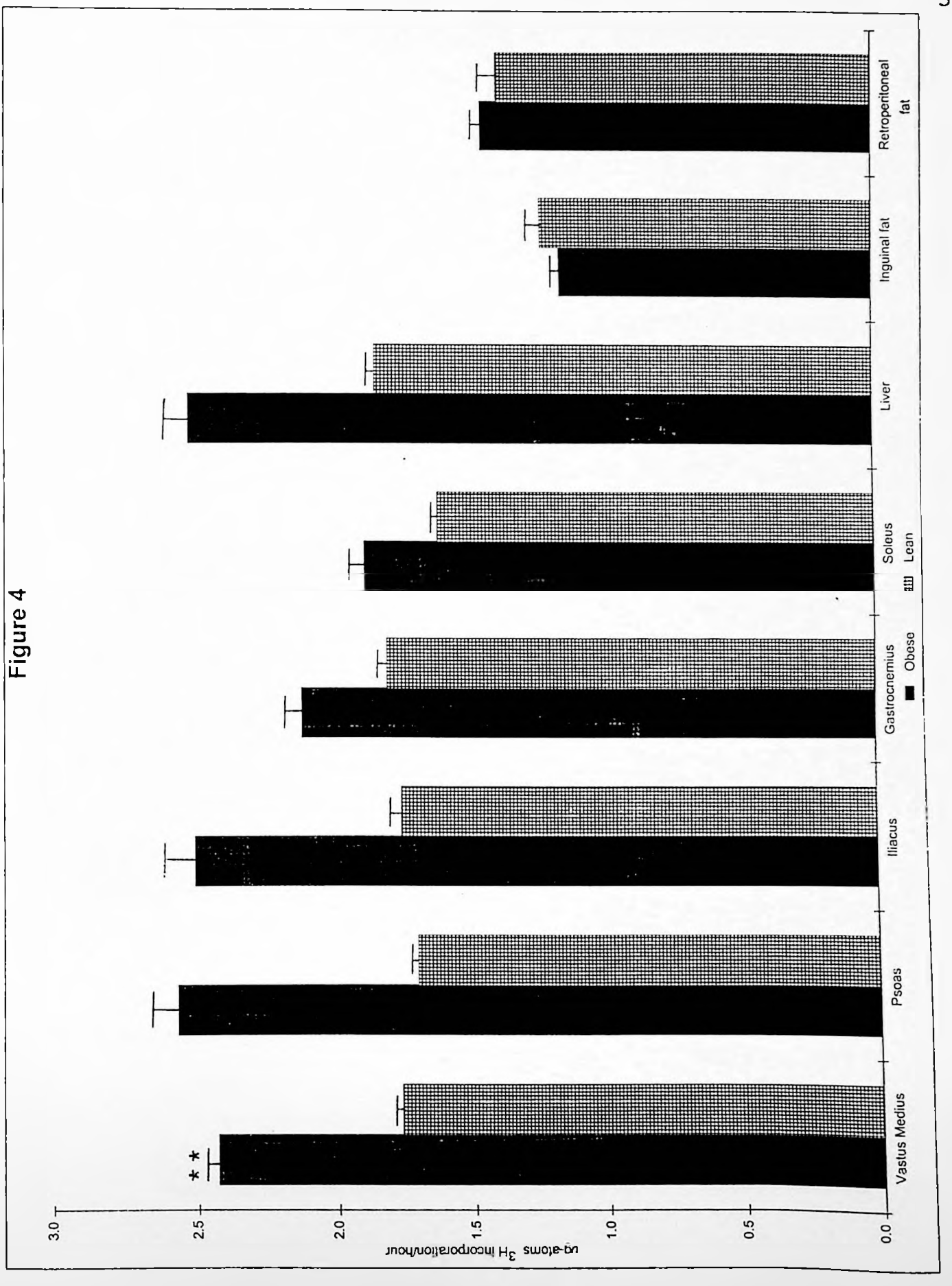
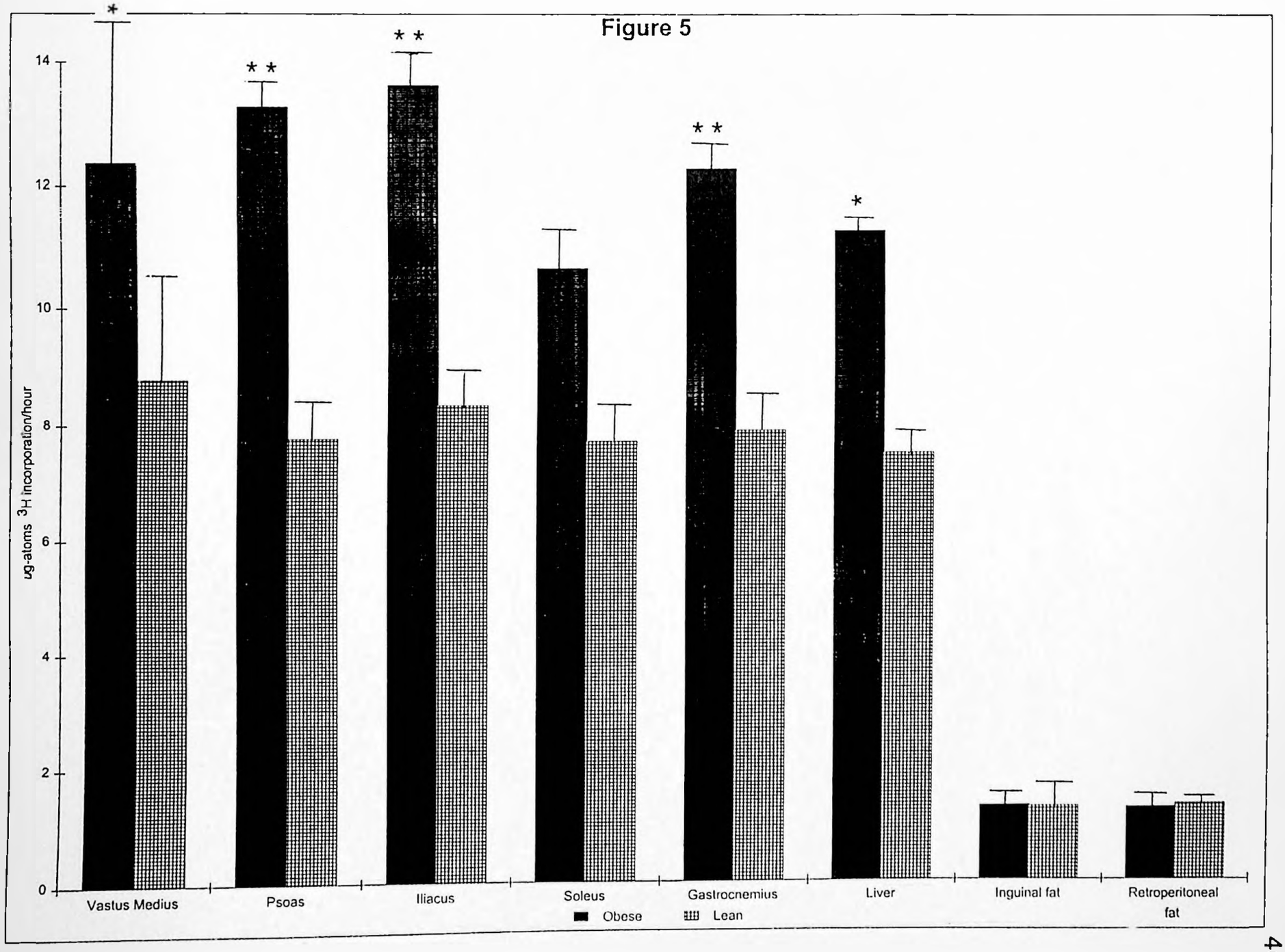

μg-atoms ³H incorporation/hour
3.0
2.5
2.0
1.5
1.0
0.5
0.0
* *
Vastus Medius
Psoas
Iliacus
Gastrocnemius
Soleus
Liver
Inguinal fat
Retroperitoneal fat
Obese
Lean

Figure 5. ^{3}H-labeled triglyceride concentrations in selected skeletal muscles, liver, and adipose depots of Zucker *fa* rats at 60 minutes following intraperitoneal administration of $^{3}H_2O$. Obese *fa* rats exhibited higher concentrations in vastus medius ($P < 0.01$), psoas ($P < 0.05$), iliacus ($P < 0.05$), gastrocnemius ($P < 0.05$), and liver ($P < 0.01$) compared with lean littermates.

Data are presented as μg-atoms ^{3}H incorporation/hour. Significance is presented as * for $P < 0.01$ and ** for $P < 0.05$.

Figure 5


ug-atoms ^{3}H incorporation/hour
0
2
4
6
8
10
12
14
*
* *
* *
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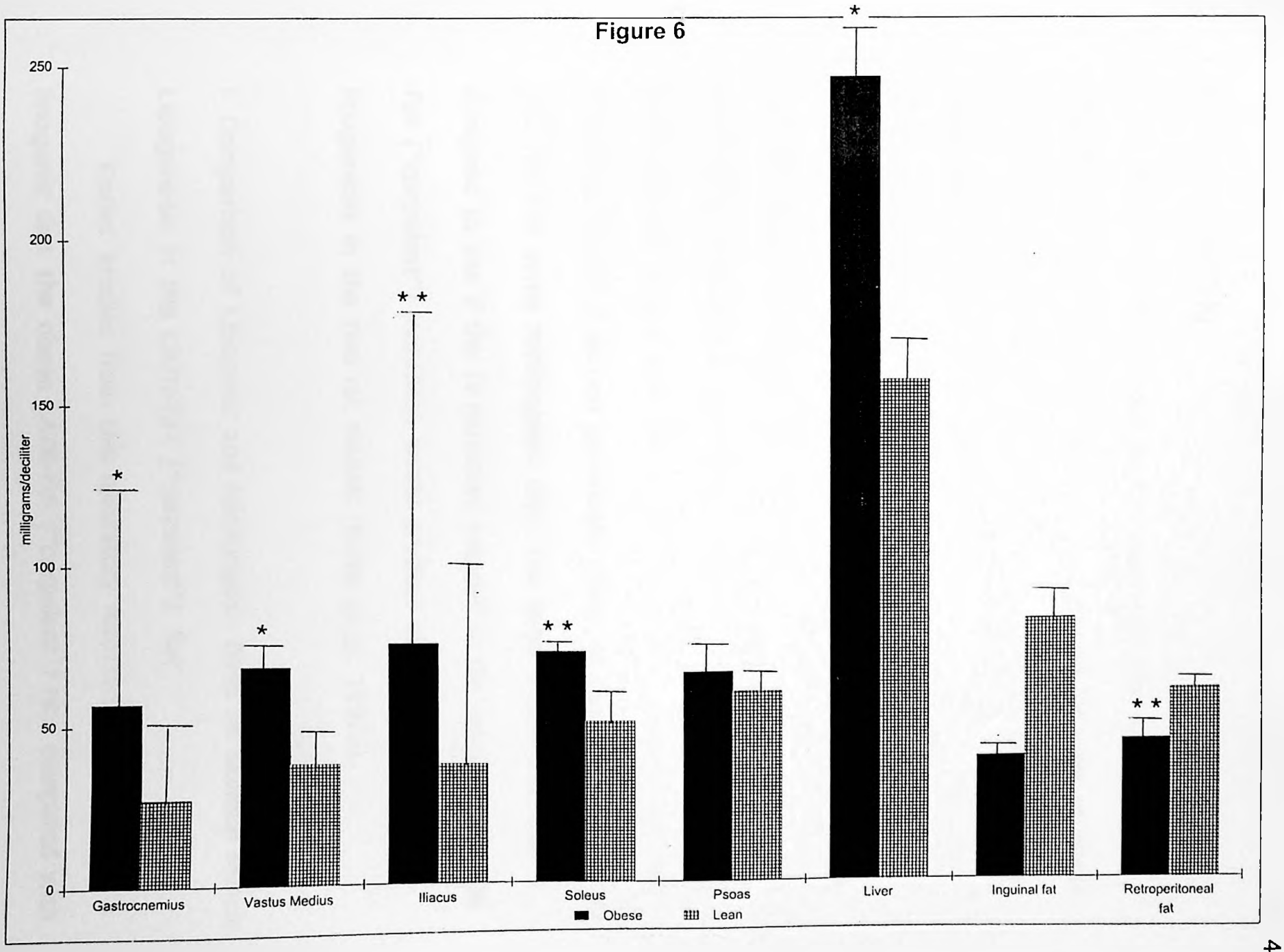
*
Vastus Medius
Psoas
Iliacus
Soleus
Gastrocnemius
Liver
Inguinal fat
Retroperitoneal fat
Obese
Lean

Figure 6. Triglyceride storage concentrations in selected skeletal muscles, liver, and adipose depots of obese Zucker *fa* rats exhibited higher concentrations in gastrocnemius ($P < 0.01$), vastus medius ($P < 0.01$, iliacus ($P < 0.05$), soleus ($P < 0.05$), liver ($P < 0.01$), and retroperitoneal fat ($P < 0.05$) tissues compared with lean littermates.

Data are presented as milligrams/deciliter. Significance is presented as * for $P < 0.01$ and ** $P < 0.05$.

Figure 6


250
200
150
100
50
0
milligrams/deciliter
*
* *
*
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Gastrocnemius
Vastus Medius
Iliacus
Soleus
Psoas
Liver
Inguinal fat
Retroperitoneal fat
Obese
Lean

DISCUSSION

Recent work from our lab has suggested that tissues from selected skeletal muscles, the liver, and inguinal fat pads of obese LA/N-*fa*^{k} *("corpulent")* rats on a lipogenic diet showed significant elevations in rates of lipogenesis when compared with their lean littermates (Kahle et al., 1997b; Raney, 1995). The present study explored the rate of lipogenesis that occurred in these tissues when the rat model for human obesity, the LA/N-*fa*^{k} *("corpulent")* rat, was fed a nonlipogenic diet replacing sucrose as the sole carbohydrate source with starch. This study also explored the rate of lipogenesis of a second genetically obese rat model, the Zucker *fa* rat, fed the same nonlipogenic diet. The latter experiment was designed to see if the *fa* mutation, mapped to the same locus as the *fa*^{k} *("corpulent")* mutation, would produce similar rates of lipogenesis in the two rat models (Kahle et al., 1997a).

I. Comparison of Lipogenic and Nonlipogenic Diets on Skeletal Muscle Lipogenesis in the LA/N-*fa*^{k} *("corpulent")* Rat

Earlier studies from this laboratory reported that when fed a lipogenic diet the obese LA/N-*fa*^{k} *("corpulent")* rats compared with

their lean littermates were found to have higher rates of fatty acid synthesis, triglyceride synthesis and triglyceride storage content in all skeletal muscles examined including the gastrocnemius, vastus medius, soleus, psoas and iliacus (Raney, 1995; Kahle et al., 1997b). The present work was able to show that dietary substitution of starch for sucrose in the above mentioned reports caused differences in the rates of lipid synthesis and storage in fewer muscles. Only the gastrocnemius and vastus medius tissues exhibited differences in rates of lipogenesis in obese compared with lean animals.

The red fibers of gastrocnemius and quadriceps group muscle tissues have been shown in earlier human studies to be more dependent on stored lipids than other skeletal muscles. Triglyceride stores in the red skeletal muscle fibers in the quadriceps femoris muscle (primarily in the vastus medius) utilized up to 70% of their intramuscular triglyceride stores during exercise, while white skeletal muscle fibers (more importantly the rectus femoris) are not dependent on these stores during exercise bouts (Reitman et al., 1973). In the same study, Reitman and coworkers reported that with exercise the soleus composed of intermediate fiber types showed a

far smaller dependence on its stored lipid with a 25% depletion in triglyceride content. This evaluation of the soleus may explain the lack of significant lipid metabolism in animals in the current study both in obese and lean rats. Staron et al. (1984) suggested that at least 40% of the variability of obesity may be related to the variation in muscle fiber type. Obese men on average in this study had a higher proportion of fast (white) fibers.

Even though the psoas major and iliacus muscles consist predominantly of red fibers, the rates of lipogenesis were found to be similar in both obese and lean rats on the nonlipogenic diet. These findings are contradictory to earlier literature which indicated that these postural muscles had lipogenic rates 3-10 times greater than in hindlimb muscles and that fatty acid/triglyceride cycling rates were similar in both postural and hindlimb muscles (Hollands & Cawthorne, 1981; Mattacks & Pond, 1988).

Liver tissue from obese LA/N-*fa*^{k} *("corpulent")* rats on the lipogenic diet had significantly higher rates of fatty acid synthesis, triglyceride synthesis, and triglyceride storage content analysis compared to lean littermates. In general, rats on a lipogenic diet had a greater liver size, weight, and elevated liver lipogenic enzymes

than rats on a nonlipogenic diet (Ellwood et al., 1985; Michaelis et al., 1983). *De novo* fatty acid synthesis in the liver was also greater in the obese phenotypes rather in the lean phenotypes while on the lipogenic diet (Berdanier, 1995).

Inguinal fat tissue from obese LA/N-*fak* *("corpulent")* rats on both diets had similar rates of lipogenesis as their lean counterparts. Diet in this case did not significantly increase the rates of fatty acid synthesis, triglyceride synthesis, or triglyceride content analysis in obese animals. Inguinal fat tissue weight was significantly higher ($P < 0.01$) in obese rats on both diets compared with lean littermates.

We also analyzed several related parameters which were characteristically different in the obese animals than in their lean littermates. Since we worked with very young animals, and subjected them to experimental manipulation of diet and light cycle for only three weeks, we considered it useful to document various characteristics of the LA/N-*fak* *("corpulent")* rat strain. Measurements including diet consumption, fecal production, weight gain, total body weight, inguinal fat pad weight, retroperitoneal fat weight, and whole liver weight were significantly ($P < 0.01$)

greater in obese animals than in their lean littermates. In summary, obese rats ate more than their lean siblings, produced more fecal matter, gained more weight, developed larger fat deposits and livers, and became more massive than their lean littermates, consistent with previous measurements (Berdanier et al., 1993; Shillabeer et al., 1992; Yamini et al., 1991; Michaelis et al., 1983). For the most part, our young animals sufficiently expressed obesity to be compared with previous work.

We also analyzed the glucose content of liver tissues extracted from rats on both diets. Obese rats on the lipogenic diet had a higher glucose content rate ($P < 0.05$) than lean rats while obese and lean rats on the nonlipogenic diet had similar glucose content rates ($P < 0.09$) (Table 10). This may be explained by the nature of the diets. The lipogenic diet has a more pronounced effect than the nonlipogenic diet because of its components (fructose and glucose). It has been suggested that fructose produces a more profound effect on hypertriglyceridemia than glucose (DaSilva et al., 1993). Fructose has also been shown to exhibit a greater tendency for conversion into lipids than glucose (Bar-on & Stein, 1968).

II. Comparison of LA/N-*fa*^{k} *("corpulent")* and Zucker *fa* Rat Strains on a Nonlipogenic Diet

This study also explored the rate of lipogenesis of two rat strains, the LA/N-*fa*^{k} *("corpulent")* rat and the Zucker *fa* rat, placed on the nonlipogenic diet. Based on three pieces of evidence, the production of *fa/fa*^{k}*("corpulent")* compound mutants, mutation sequencing identification, and molecular mapping (Kahle et al., 1997a), it is now clear that *fa* and *fa*^{k} *("corpulent")* are mutations in the same gene, the leptin receptor. The question addressed here: "Do the *fa* and *fa*^{k} mutations have similar effects on the rate of lipogenesis if representative rat strains are placed on the same diet?".

Lipogenic activity in skeletal muscle tissues of obese rats having either *fa* and *fa*^{k} *("corpulent")* mutation are affected. Two hindlimb skeletal muscles, gastrocnemius and vastus medius, of obese rats in both strains had higher rates of lipogenesis than their lean littermates. Peter & Fiehn (1971) suggested that increased lipogenesis may be due to increased neutral lipids (triglycerides and cholesterol) in the blood which then transports the neutral lipids to the tissues. López-Soriano et al. (1991) found that the rate of

lipogenesis in obese Zucker *fa* rats was significantly higher in vastus medius muscle by 2.6 fold compared with lean rats. In Harapanahalli (1993) free fatty acids and triglycerides were higher in venous drainage versus arterial supply in the hindlimb muscles of LA/N-*fa*^{k} *("corpulent")* rats. This research lends itself to supporting the hypothesis that fatty acid synthesis is occurring at high rates in both the gastrocnemius and the vastus medius in both rat models.

Liver tissue from obese LA/N-*fa*^{k} *("corpulent")* rats exhibited similar rates of fatty acid synthesis, triglyceride synthesis, and triglyceride storage content as lean rats. Liver tissue from obese Zucker *fa* rats had higher rates of triglyceride synthesis and storage content compared to lean rats, while obese and lean phenotypes exhibited similar fatty acid synthesis rates. Obese rats in both strains had greater liver weight and size compared to lean rats (Berdanier et al., 1993; Shillabeer et al., 1992; Michaelis et al., 1980). Activities of liver lipogenic enzymes also tend to be higher in obese versus lean rats (Ellwood et al., 1985; Michaelis et al., 1980).

In this particular study inguinal fat and retroperitoneal fat tissues were extracted and analyzed for lipogenesis in both rat

models. Inguinal fat tissue from obese LA/N-*fak* *("corpulent")* and Zucker *fa* rats had similar rates of fatty acid and triglyceride synthesis and high rates of triglyceride content compared to lean counterparts. Evaluation of this information must wait until future studies can detect the cell numbers analyzed in obese and lean adipose samples.

Retroperitoneal fat tissue from obese LA/N-*fak* *("corpulent")* rats had higher rates of triglyceride synthesis and triglyceride content and similar rates of fatty acid synthesis compared to lean rats. Retroperitoneal fat tissue from obese Zucker *fa* rats had high rates of triglyceride content yet similar rates of fatty acid and triglyceride synthesis compared to lean rats. Overall, inguinal fat and retroperitoneal fat tissue weights were greater in obese animals than lean animals of both rat models.

Measurements including diet consumption, fecal production, weight gain, total body weight, inguinal fat pad weight, retroperitoneal fat weight, and whole liver weight show that obese rats from both rat models had higher values for each of these measurements ($P < 0.01$) compared to lean counterparts. Again, the obese phenotypes of both rat models ate more than lean phenotypes,

excreted more fecal matter, gained more weight, developed larger fat deposits, and developed larger livers compared to the lean phenotypes (Berdanier et al., 1993; Yamini et al., 1991; Argilés, 1989; Michaelis et al., 1983).

We noted that any amount of glucose in adipose tissue or skeletal muscle was below that detectable by our analytical techniques. Glucose content of the liver, however, was measured in both rat models on the nonlipogenic diet. Obese animals had similar rates of glucose content compared to lean animals in both rat models, yet approached significance, ($P < 0.09$) in the LA/N-*fa*^{k} *("corpulent")* and ($P < 0.08$) in the Zucker *fa* rat strains.

CONCLUSION

In conclusion, we demonstrated that the rate of lipogenesis was seen only in selected skeletal muscles in obese versus lean rats on a nonlipogenic diet. Also, a lipogenic diet has a more profound effect on the amount of lipogenesis than a nonlipogenic diet. This data is consistent with the initial findings of Michaelis et al. (1983) that feeding sucrose resulted in greater body fatness and levels of serum triglyceride and free fatty acids than did feeding starch. However, since they did not analyze any skeletal muscle tissue in this study, the data was considered preliminary background material for our study.

A common thread did emerge in the LA/N-*fak* *("corpulent")* and Zucker *fa* rats. The hindlimb skeletal muscles, vastus medius and gastrocnemius, had the most marked fatty acid and triglyceride synthesis as well as triglyceride storage content among the selected skeletal muscles examined. These same muscles have marked lipid synthesis and storage features when the LA/N-*fak* *("corpulent")* rat was evaluated with either a diet designed to produce hyperlipogenic activity (54% sucrose), or one that does not

promote excessive lipogenesis (54% starch), as published by Michaelis and coworkers (1983). Any differences in the heterogenetic background of the two strains appears to be negligible.

REFERENCES

Argilés, J.M. 1989. *The obese Zucker rat: a choice for fat metabolism.* Prog. Lip. Res. 28: 53-66.

Bar-on, H., and Y. Stein. 1968. *Effect of glucose and fructose administration on lipid metabolism in the rat.* J. Nutr. 94: 95-105.

Berdanier, C.D. 1995. Advanced nutrition: macronutrients. CRC Press, London, England, pp. 223-232.

Berdanier, C.D., J.S. Pan, D.K. Hartle, and O.E. Michaelis, IV. 1993. *Glucose turnover in lean and obese rats of the SHR/N-cp and LA/N-cp strains.* Comp. Biochem. Physiol. 106B: 87-94.

Carlson, L.A., S.O. Froberg, and E.R. Nye. 1966. *Acute effects of nicotinic acid on plasma, liver, heart, and muscle lipids.* Acta. Med. Scan. 180: 571-579.

Cawthorne, M.A., and S. Cornish. 1979. *Lipogenesis in vivo in lean and genetically obese (ob/ob) mice fed on diets with a high fat content.* Inter. J. Obesity 3: 83-90.

Chua, S.C., D.W. White, X.S. Wu-Peng, S-M. Liu, N. Okada, E.E. Kershaw, W.K. Chung, L. Power-Kehoe, M. Chua, L.A. Tartaglia, and R.L. Leibel. 1996. *Phenotypes of fatty due to Gln269Pro mutation in the leptin receptor (Lepr).* Diabetes 45: 1141-1143.

Chung, W.K., L. Power-Kehoe, M. Chua, and R.L. Leibel. 1996. *Mapping of the OB receptor (OBR) to 1p in the region of non-conserved gene order from mouse and rat to human.* Genome Research 1996 6: 431-438.

Curme, H.G., R.L. Columbus, G.M. Dappen, T.W. Eder, W.D. Fellows, J. Figueras, C.P. Glover, C.A. Goffe, D.E. Hill, W.H. Lawton, E.J. Muka, J.E. Pinney, R.N. Rand, K.J. Sanford, and T.W. Wu. 1978. *Multilayer film elements for clinical analysis.* Clin. Chem.

24: 1335-1342.

DaSilva, L.A., O.L. DeMarcucci, and Z.R. Kuhnle. 1993. *Dietary polyunsaturated fats suppress the high-sucrose-induced increase of rat liver pyruvate dehydrogenase levels.* Biochim. Biophy. Acta 1169: 126-134.

Domaschko, D.W. 1995. *Ultrastructural comparisons of skeletal muscle morphologies from obese and lean LA/N-fa^{cp} rats.* Masters thesis, Marshall University.

Ellwood, K.C., O.E. Michaelis IV, J.J. Emberland, and S.J. Bhathena. 1985. *Hormonal and lipogenic and gluconeogenic enzymatic responses in LA/N-corpulent rats.* Proc. Soc. Exp. Bio. Med. 179: 163-167.

Friden, J., Sjostrom M., Ekblom B. 1984. *Muscle fibre type characteristics in endurance-trained and untrained individuals.* Eur. J. Appl. Physiol. 54: 266-271.

Froberg, S.O. 1971. *Effect of acute exercise on tissue lipids in rats.* Metab. Clin. Exp. 20: 714-720.

Gardner, L.B., and O.E. Michaelis, IV. 1979. *Serum insulin and glucagon and hepatic and adipose cyclic-AMP responses of Zucker rats fed carbohydrate diets ad libitum or in meals.* Nutr. Rep. Inter. 20: 845-854.

George, J.C., and R.M. Naik. 1958. *Relative distribution and chemical nature of the fuel store of the two types of fibres in the pectoralis major muscle of the pigeon.* Nature 181: 709-711.

Gray, D.S. 1989. *Diagnosis and prevalence of obesity.* Med. Clinics of NA 73: 1-13.

Haft, D.E. 1973. *Role of plasma triglyceride turnover in deposition of newly synthesized fatty acid in adipose tissue.* Horm. Metab. Res. 5: 449-453.

Hansen, C.T. 1983. *Two new congenic rat strains for nutrition and obesity research.* Fed. Proc. 42: 536.

Harapanahalli. S. 1993. *Mechanisms for lipogenesis in running muscles of the obese LA/N-cp^{fa} rat hindlimb and total lipid exchange in these tissues.* Masters thesis, Marshall University.

Hirsch, J., S.K. Fried, N.K. Edens, and R.L. Leibel. 1989. *The fat cell.* Med. Clinics of NA 73: 83-96.

Hollands, M.A., and M.A. Cawthorne. 1981. *Important sites of lipogenesis in the mouse other than liver and white adipose tissue*. Biochem. J. 196: 645-647.

Holloszy, J.O., and Coyle E.F. 1984. *Adaptations of skeletal muscle to endurance exercise and their metabolic consequences.* J. Appl. Physiol. 56: 831-838.

Houtsmuller, A.J. 1975. *The role of fat in the treatment of diabetes mellitus* In A.J. Vergroesen, ed. The role of fats in human nutrition. Academic Press, New York, pp. 231-302.

Idell-Wenger, J.A., and J.R. Neely. 1978. *Regulation of uptake and metabolism of fatty acids by muscle.* In J.M. Dietschy, A.M. Gotto, and J.A. Ontko, eds. Disturbances in lipid and lipoprotein metabolism. American Physiological Society, Bethesda, Maryland, pp. 269-284.

Issekutz, B., and P. Paul. 1968. *Intramuscular energy sources in exercising normal and pancreatectomized dogs.* Amer. J. Physiol. 215: 197-204.

Johnson, P.R., L.M. Zu, J.A. Cruce, and J. Hirsh. 1972. *Cellularity of adipose depots in the genetically obese zucker rat.* J. Lipid Res. 12: 706-714.

Kahle, E.B., K.G. Butz, S.C. Chua, E.E. Kershaw, R.L. Leibel, T.W. Fenger, C.T. Hansen, and O.E. Michaelis. 1997a. *The corpulent (cp)*

mutation maps to the same interval on (Pgm1-Glut1) rat chromosome 5 as the fatty (fa) mutation. Obesity Research (in press).

Kahle, E.B., K.G. Butz, R.L. Leibel, C.T. Hansen, S.J. Bhathena, and O.E. Michaelis IV. 1996. *Glucose homeostasis in three interstrains (LA/N-BN/CRL cp/cp; Zuc13M-BN/Crl fa/fa; and Zuc13M-LA/N-faΦ) of genetically obese rats* In E. Shafrir, ed. Lessons from animal diabetes. Birkhauser, Boston, Massachusetts, pp. 411-418.

Kahle, E.B., R. L. Leibel, D.W. Domascho, S.G. Raney, and K.T. Mann. 1997b. *Obesity genes and insulin resistance syndrome.* N.Y. Acad. Sci. (in press).

Kannan, R., and N. Baker. 1976. *Net changes in intermuscular fat before and during rapid lipogenic activation in mice.* Biochimica et Biophysica Acta 431: 233-240.

Kannan, R., D.L. Palmquist, and N. Baker. 1976. *Contribution of intermuscular fat to lipogenesis from dietary glucose carbon in mice.* Biochimica et Biophysica Acta 431: 225-232.

Kissebak, A.H., D.S. Freedman, and A.N. Peiris. 1989. *Health Risks of Obesity.* Med. Clinics of NA 73: 111-138.

López-soriano, F.J., N. Carbó, and J.M. Argilés. 1991. *Lipid metabolism in the obese Zucker rat.* Biochem. J. 274: 651-656.

Mattacks, C.A., & C.M. Pond. 1988. *Site-specific and sex differences in the rates of fatty acid/triglyceride substrate cycling in adipose tissue and muscle of sedentary and exercised dwarf hamsters (Phodopus sungorus).* Int. J. Ob. 12: 585-597.

Michaelis IV, O.E., K.C. Ellwood, J. Hallfrisch, and C.T. Hansen. 1983. *Effect of dietary sucrose and genotype on metabolic parameters of a new strain of genetically obese rat: LA/N-corpulent.* Nutr. Res. 3: 217-228.

Michaelis IV, O.E., D.J. Scholfield, L.B. Gardner, and S. Cataland. 1980. *Metabolic responses of Zucker fatty and lean rats fed carbohydrate diets ad libitum or in meals.* J. Nutr. 110: 1409-1420.

Murray, R.K., D.K. Granner, P.A. Mayes, and V.W. Rodwell. 1993. *Harper's Biochemistry.* 23rd ed. Appleton and Lange, Norwalk, Connecticut, pp. 212-219.

Newsholme, E.A., and B. Crabtree. 1976. Biochem. Soc. Symp. 41: 61-109.

Peter, J.R., and W. Fiehn. 1971. *Muscle lipids: composition of muscle composed predominantly of one fiber type and of subcellular fractions* In B.A. Kakulas, ed. Basic research in myology. American Elsevier Company, Incorporated, New York, New York, pp. 183-189.

Pullen, D.L., J.S. Liesman, and R.S. Emery. 1990. *A species comparison of liver slice synthesis and secretion of triacylglycerol from nonesterified fatty acids in media.* J. Anim. Sci. 68:1395-1399.

Raney, S.G. 1995. *The role of red skeletal muscle in lipogenesis and in the development of obesity: comparisons of lean and obese LA/N-fa^{cp}rats at six weeks of age.* M.Sc. Thesis, Marshall University, Huntington, West Virginia.

Reitman, J., K.M. Baldwin, and J.O. Holloszy. 1973. *Intramuscular triglyceride utilization by red, white, and intermediate skeletal muscle and heart during exhausting exercise.* P.S.E.B.M. 142: 638-631.

Sheehan, P.M., L.K. Reynolds, F.W. Thye, and S.J. Ritchey. 1984. *Blood glucose and plasma lipids of Zucker rats fed diets containing cornstarch or sucrose.* Nutr. Rep. Inter. 29: 1337-1344.

Shillabeer, G., J. Hornford, J.M. Forden, N.C.W. Wong, J.C. Russell, and D.C.W. Lau. 1992. *Fatty acid synthase and adipsin mRNA levels in obese and lean JCR;LA-cp rats: effect of diet.* J. Lipid Res. 33: 31-39.

Spayd, R., B. Bruschi, B.A. Burdick, G.M. Dappen, J.N. Eikenberry, T.W. Esders, J. Figueras, C.T. Goodhue, D.D. LaRossa, R.W. Nelson, R.N. Rand, and T.W. Wu. 1978. *Multilayer film elements for clinical analysis.* Clin. Chem. 24: 1348-1350.

Staron, R.S., R.S. Hikida, F.C. Hagerman, G.A. Dudley, and T.F. Murray. 1984. *Human skeletal muscle fiber type adaptability to various workloads.* J. Histochem. Cytochem. 32: 146-152.

Storlie, J., and H.A. Jordan. 1984. *Nutrition and exercise in obesity management.* Medical and Scientific Books, New York, pp. 97-98.

Trinder, P. 1969. *Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor.* Ann. Clin. Biochem. 6: 24.

Tulp, O.L., S.P. DeBolt, C.T. Hansen, and O.E. Michaelis, IV. 1991. *Effects of dietary carbohydrate and phenotypes on adipose cellularity in female SHR/N-cp rats.* Comp. Biochem. Physiol. 99A: 229-234.

Yamini, S., R.C. Staples, C.T. Hansen, and B. Szepesi. 1991. *Effect of dietary carbohydrate on liver and kidney enzyme activities and plasma amino acids in the LA/N-cp rat.* Inter. J. Obesity 15:189-203.

Yen, T.T., W.N. Shaw, and P.L. Yu. 1997. *Genetics of obesity in Zucker rats and Koletsky rats.* Heredity 38: 373-377.

Zucker, L.M., and H.N. Antoniades. 1972. *Insulin and obesity in the zucker genetically obese rat "fatty".* Endocrinology 90: 1320-1330.

Zucker, L.M., and T.F. Zucker. 1961. *Fatty, a new mutation in the rat.* J. Heredity. 52: 275-278.

APPENDIX

TABLE 1 - FATTY ACID SYNTHESIS ANALYSIS IN LA/N-*fa*^{k} (*"corpulent"*) RATS*

ANIMAL	Gastrocnemius	Vastus Medius	Iliacus	Soleus	Psoas	Liver	Inguinal Fat	Retroperitoneal Fat
Pair 1 - Ob	4.128	3.549	4.182	4.179	4.890	6.657	2.300	1.767
Pair 2 - Ob	1.615	1.782	1.767	1.794	2.011	2.300	0.625	0.685
Pair 3 - Ob	1.950	1.980	1.188	0.746	1.249	1.538	0.899	1.889
Pair 4 - Ob	2.864	2.087	1.142	1.371	1.721	2.475	1.493	1.432
Pair 5 - Ob	1.934	2.361	1.462	1.904	1.919	1.736	0.777	1.097
Pair 6 - Ob	2.727	2.056	2.391	2.513	1.813	3.016	0.701	0.792
Pair 1 - Ln	2.909	2.026	2.498	1.543	2.209	2.450	0.655	0.975
Pair 2 - Ln	0.990	1.066	0.975	1.468	0.823	0.823	0.396	0.807
Pair 3 - Ln	0.472	0.609	0.670	0.594	1.523	0.777	5.484	1.584
Pair 4 - Ln	1.203	1.188	1.005	1.417	1.417	0.716	2.254	1.691
Pair 5 - Ln	1.112	1.280	0.960	0.762	0.883	1.843	0.229	0.335
Pair 6 - Ln	1.417	1.295	1.188	0.975	1.356	1.264	0.487	1.081

MEAN	Gastrocnemius++	Vastus Medius+	Iliacus	Soleus	Psoas	Liver	Inguinal Fat	Retroperitoneal Fat
OBESE**	2.54±0.376	2.07±2.36	1.615±2.39	2.08±0.483	1.87±2.01	2.95±0.772	1.13±0.266	1.08±0.206
LEAN**	1.35±0.337	1.23±1.30	0.990±1.19	1.13±0.165	1.47±2.21	1.31±0.286	1.58±0.836	1.28±0.205

*Values are reported as μatg of $^{3}H\text{-}H_2O$/hour/gram of tissue.

**Values are reported as Mean ±Standard Error of the Mean.

+Significant difference between obese and lean: $P < 0.01$.

++Significant difference between obese and lean: $P < 0.05$.

TABLE 2 - TRIGLYCERIDE SYNTHESIS ANALYSIS IN LA/N-*fa*^{k} (*"corpulent"*) RATS*

ANIMAL	Gastrocnemius	Vastus Medius	Psoas	Iliacus	Soleus	Liver	Retroperitoneal Fat	Inguinal Fat
Pair 1 - Ob	22.254	19.418	21.904	17.244	29.497	22.072	1.980	0.655
Pair 2 - Ob	7.708	8.439	0.731	7.906	10.871	8.561	0.716	11.059
Pair 3 - Ob	8.835	10.053	0.518	9.094	9.520	8.363	0.594	14.989
Pair 4 - Ob	18.218	16.603	1.584	16.100	14.653	20.640	0.899	8.195
Pair 5 - Ob	11.135	9.368	7.845	10.724	9.322	9.261	1.142	0.975
Pair 6 -Ob	10.982	13.130	14.014	10.236	11.653	11.333	0.807	0.899
Pair 1 - Ln	9.612	9.977	15.156	15.720	11.909	11.942	2.605	3.184
Pair 2 - Ln	3.534	4.859	1.188	5.118	6.974	4.859	1.127	6.245
Pair 3 - Ln	9.383	8.957	2.437	8.865	10.510	10.388	2.452	4.052
Pair 4 - Ln	6.550	7.951	2.772	8.058	8.317	6.763	3.549	13.679
Pair 5 - Ln	7.159	6.352	6.108	6.626	6.078	5.484	0.944	1.889
Pair 6 - Ln	6.870	7.449	8.149	7.799	6.504	7.951	1.904	1.782
MEAN	**Gastrocnemius+**	**Vastus Medius+**	**Psoas**	**Iliacus**	**Soleus**	**Liver**	**Retroperitoneal Fat+**	**Inguinal Fat**
OBESE**	13.19±2.349	12.84±1.795	7.77±3.56	11.88±1.57	14.25±3.149	13.37±2.57	1.02±0.206	6.13±2.52
LEAN**	7.18±0.905	7.59±0.746	5.97±2.12	8.70±1.50	8.38±0.962	7.90±1.14	2.10±0.400	5.14±1.83

*Values are reported as μatg of $^{3}H\text{-}H_2O$/hour/gram of tissue. +Significant difference between obese and lean: $P < 0.05$.

**Values are reported as Mean ± Standard Error of the Mean.

TABLE 3 - TRIGLYCERIDE CONTENT ANALYSIS IN LA/N-*fak* (*"corpulent"*) RATS*

ANIMAL	Gastrocnemius	Vastus Medius	Iliacus	Psoas	Soleus	Liver	Inguinal Fat	Retroperitoneal Fat
Pair 1 - Ob	82.0	89.0	74.0	76.0	61.0	177.0	69.0	89.0
Pair 2 - Ob	77.0	76.0	76.0	83.0	49.0	159.0	68.0	51.0
Pair 3 - Ob	63.0	40.0	67.0	74.0	71.0	133.0	42.0	29.0
Pair 4 - Ob	48.0	61.0	51.0	51.0	52.0	114.0	29.0	21.0
Pair 5 - Ob	50.0	75.0	61.0	79.0	76.0	119.0	30.0	26.0
Pair 6 - Ob	45.0	46.0	45.0	42.0	77.0	121.0	28.0	18.0
Pair 7 - Ob	36.0	48.0	38.0	28.0	44.0	104.0	22.0	13.0
Pair 8 - Ob	29.0	46.0	37.0	20.0	57.0	126.0	24.0	16.0
Pair 9 - Ob	40.0	44.0	28.0	31.0	52.0	138.0	18.0	22.0
Pair 1 - Ln	33.0	33.0	45.0	54.0	79.0	122.0	195.0	187.0
Pair 2 - Ln	28.0	40.0	48.0	66.0	36.0	117.0	152.0	139.0
Pair 3 - Ln	27.0	31.0	41.0	36.0	47.0	177.0	68.0	87.0
Pair 4 - Ln	25.0	30.0	38.0	38.0	54.0	149.0	57.0	41.0
Pair 5 - Ln	36.0	38.0	42.0	53.0	69.0	142.0	63.0	47.0
Pair 6 - Ln	14.0	16.0	35.0	29.0	48.0	110.0	36.0	42.0
Pair 7 - Ln	< 10.0	< 10.0	20.0	26.0	26.0	115.0	38.0	38.0
Pair 8 - Ln	17.0	24.0	34.0	35.0	41.0	132.0	21.0	29.0
Pair 9 - Ln	< 10.0	16.0	34.0	19.0	50.0	93.0	30.0	25.0

MEAN	Gastrocnemius+	Vastus Medius+	Iliacus+	Psoas++	Soleus	Liver	Inguinal Fat	Retroperitoneal Fat++
OBESE**	52.2±6.06	58.3±5.88	53.0±5.78	53.8±8.22	59.89±4.05	132.33±766	29.0±48.5	22.0±34.5
LEAN**	20.0±4.42	25.3±4.26	37.4±2.73	39.6±5.06	50.00±5.38	128.56±8.7	57.0±89.0	42.0±100.0

*Values are reported in milligrams per deciliter.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: P < 0.01.
++Significant difference between obese and lean: P < 0.05.

TABLE 4 - FATTY ACID SYNTHESIS ANALYSIS IN ZUCKER *fa* RATS*

ANIMAL	Vastus Medius	Psoas	Iliacus	Gastrocnemius	Soleus	Liver	Inguinal Fat	Retroperitoneal Fat
Pair 1 - Ob	1.615	0.944	0.701	1.021	1.356	1.295	0.518	0.366
Pair 2 - Ob	2.346	2.955	2.331	2.331	2.270	2.772	1.691	2.163
Pair 3 - Ob	2.833	2.935	2.589	2.376	1.630	3.808	1.219	1.813
Pair 4 - Ob	2.742	3.686	3.412	2.102	1.508	2.072	1.828	0.929
Pair 5 - Ob	2.620	2.650	3.503	2.818	2.711	2.711	1.264	1.051
Pair 1 - Ln	2.270	1.599	2.391	2.498	1.569	2.102	1.142	0.944
Pair 2 - Ln	1.736	1.874	1.950	1.813	1.828	1.813	1.782	1.249
Pair 3 - Ln	1.599	1.280	1.447	1.736	1.828	2.087	2.300	1.508
Pair 4 - Ln	1.828	2.239	1.813	1.493	1.736	1.874	0.228	1.432
Pair 5 - Ln	1.432	1.538	1.264	1.584	1.173	1.417	0.929	1.462

MEAN	Vastus Medius+	Psoas	Iliacus	Gastrocnemius	Soleus	Liver	Inguinal Fat	Retroperitoneal Fat
OBESE**	2.43±0.220	2.57±0.450	2.51±0.505	2.13±0.300	1.90±0.257	2.53±0.416	1.17±0.159	1.46±0.180
LEAN**	1.77±0.141	1.71±0.163	1.77±0.198	1.82±0.177	1.63±0.123	1.86±0.124	1.24±0.233	1.40±0.276

*Values are reported as μatg of ^3H-H_2O/hour/gram of tissue.

**Values are reported as Mean ±Standard Error of the Mean.

+Significant difference between obese and lean: $P < 0.05$.

TABLE 5 - TRIGLYCERIDE SYNTHESIS ANALYSIS IN ZUCKER *fa* RATS*

ANIMAL	Vastus Medius	Psoas	Iliacus	Soleus	Gastrocnemius	Liver	Inguinal Fat	Retroperitoneal Fat
Pair 1 - Ob	1.615	0.944	0.701	1.356	1.021	1.295	0.518	0.366
Pair 2 - Ob	2.346	2.955	2.331	2.270	2.331	2.772	1.691	2.163
Pair 3 - Ob	2.833	2.935	2.589	1.630	2.376	3.808	1.219	1.813
Pair 4 - Ob	2.742	3.686	3.412	1.508	2.102	2.072	1.828	0.929
Pair 5 - Ob	2.620	2.650	3.503	2.711	2.818	2.711	1.264	1.051
Pair 1 - Ln	2.270	1.599	2.391	1.569	2.498	2.102	1.142	0.944
Pair 2 - Ln	1.736	1.874	1.950	1.828	1.813	1.813	1.782	1.249
Pair 3 - Ln	1.599	1.280	1.447	1.828	1.736	2.087	2.300	1.508
Pair 4 - Ln	1.828	2.239	1.813	1.736	1.493	1.874	0.228	1.432
Pair 5 - Ln	1.432	1.538	1.264	1.173	1.584	1.417	0.929	1.462

MEAN	Vastus Medius+	Psoas++	Iliacus++	Soleus	Gastrocnemius++	Liver+	Inguinal Fat	Retroperitoneal Fat
OBESE**	12.37±12.8	13.27±0.835	13.62±1.25	10.66±1.38	12.28±0.754	11.28±0.427	1.30±0.229	1.26±0.322
LEAN**	8.79±10.30	7.77±1.56	8.35±1.24	7.73±1.42	7.91±1.275	7.53±0.844	1.28±0.356	1.32±0.104

*Values are reported as μatg of ^3H-H_2O/hour/gram of tissue.

**Values are reported as Mean±Standard Error of the Mean.

+Significant difference between obese and lean: $P < 0.01$.

++Significant difference between obese and lean: $P < 0.05$.

TABLE 6 - TRIGLYCERIDE CONTENT ANALYSIS IN ZUCKER *fa* RATS*

ANIMAL	Gastrocnemius	Vastus Medius	Iliacus	Soleus	Psoas	Liver	Inguinal Fat	Retroperitoneal Fat
Pair 1 - Ob	21.0	58.0	111.0	82.0	46.0	243.0	42.0	44.0
Pair 2 - Ob	33.0	47.0	76.0	83.0	84.0	261.0	42.0	66.0
Pair 3 - Ob	119.0	93.0	140.0	75.0	58.0	188.0	45.0	48.0
Pair 4 - Ob	43.0	77.0	188.0	92.0	137.0	250.0	51.0	50.0
Pair 5 - Ob	57.0	69.0	44.0	66.0	57.0	328.0	29.0	25.0
Pair 6 - Ob	67.0	74.0	43.0	69.0	32.0	397.0	15.0	23.0
Pair 7 - Ob	49.0	82.0	149.0	71.0	68.0	213.0	34.0	46.0
Pair 8 - Ob	70.0	66.0	68.0	74.0	82.0	199.0	66.0	69.0
Pair 9 - Ob	103.0	51.0	68.0	50.0	36.0	142.0	31.0	28.0
Pair 1 - Ln	27.0	30.0	25.0	22.0	39.0	215.0	99.0	77.0
Pair 2 - Ln	< 10.0	41.0	38.0	20.0	56.0	147.0	82.0	86.0
Pair 3 - Ln	27.0	71.0	36.0	96.0	99.0	199.0	47.0	38.0
Pair 4 - Ln	13.0	23.0	18.0	54.0	32.0	105.0	48.0	42.0
Pair 5 - Ln	21.0	41.0	150.0	50.0	80.0	136.0	89.0	66.0
Pair 6 - Ln	28.0	23.0	129.0	82.0	68.0	237.0	92.0	53.0
Pair 7 - Ln	38.0	28.0	54.0	45.0	71.0	131.0	157.0	69.0
Pair 8 - Ln	28.0	62.0	57.0	37.0	53.0	171.0	71.0	63.0
Pair 9 - Ln	20.0	23.0	25.0	57.0	46.0	76.0	63.0	50.0

MEAN	Gastrocnemius+	Vastus Medius+	Iliacus++	Soleus++	Psoas	Liver+	Inguinal Fat	Retroperitoneal Fat ++
OBESE**	57.0±78.2	68.6±4.98	76.0±142.3	73.6±3.98	66.7±10.69	246.8±25.6	39.4±4.85	44.3±5.56
LEAN**	27.0±28.0	38.0±5.92	38.0±75.0	51.4±8.39	60.4±7.06	157.4±17.6	83.1±11.13	60.4±5.33

*Values are reported in milligrams per deciliter.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: P < 0.01.
++Significant difference between obese and lean: P < 0.05.

TABLE 7 - COMPARISON OF FATTY ACID SYNTHESIS WITHIN PHENOTYPES*

A. LA/N-*fa*^{k} (*"corpulent"*) RATS

MEAN	Gastrocnemius++	Vastus Medius+	Iliacus	Soleus	Psoas	Liver	Inguinal Fat	Retroperitoneal Fat
OBESE**	2.54±0.376	2.07±2.36	1.615±2.39	2.08±0.483	1.87±2.01	2.95±0.772	1.13±0.266	1.08±0.206
LEAN**	1.35±0.337	1.23±1.30	0.990±1.19	1.13±0.165	1.47±2.21	1.31±0.286	1.58±0.836	1.28±0.205

B. ZUCKER *fa* RATS

MEAN	Vastus Medius+	Psoas	Iliacus	Gastrocnemius	Soleus	Liver	Inguinal Fat	Retroperitoneal Fat
OBESE**	2.43±0.220	2.57±0.450	2.51±0.505	2.13±0.300	1.90±0.257	2.53±0.416	1.17±0.159	1.46±0.180
LEAN**	1.77±0.141	1.71±0.163	1.77±0.198	1.82±0.177	1.63±0.123	1.86±0.124	1.24±0.233	1.40±0.276

*Values are reported as μatg of ^3H-H_2O/hour/gram of tissue.
**Values are reported as Mean ±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.
++Significant difference between obese and lean: $P < 0.05$.

TABLE 8 - COMPARISON OF TRIGLYCERIDE SYNTHESIS WITHIN PHENOTYPES*

A. LA/N-*fa*^{k} (*"corpulent"*) RATS

MEAN	Gastrocnemius+	Vastus Medius+	Psoas	Iliacus	Soleus	Liver	Retroperitoneal Fat+	Inguinal Fat
OBESE**	13.19±2.349	12.84±1.795	7.77±3.56	11.88±1.57	14.25±3.149	13.37±2.57	1.02±0.206	6.13±2.52
LEAN**	7.18±0.905	7.59±0.746	5.97±2.12	8.70±1.50	8.38±0.962	7.90±1.14	2.10±0.400	5.14±1.83

B. ZUCKER *fa* RATS

MEAN	Vastus Medius+	Psoas+	Iliacus++	Soleus	Gastrocnemius++	Liver+	Inguinal Fat	Retroperitoneal Fat
OBESE**	12.37±12.8	13.27±0.835	13.62±1.25	10.66±1.38	12.28±0.754	11.28±0.427	1.30±0.229	1.26±0.322
LEAN**	8.79±10.30	7.77±1.56	8.35±1.24	7.73±1.42	7.91±1.275	7.53±0.844	1.28±0.356	1.32±0.104

*Values are reported as μatg of ^3H-H_2O/hour/gram of tissue.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.
++Signlficant differene between obese and lean: $P < 0.05$.

TABLE 9 - COMPARISON OF TRIGLYCERIDE CONTENT WITHIN PHENOTYPES*

A. LA/N-*fa*^{k} (*"corpulent"*) RATS

MEAN	Gastrocnemius+	Vastus Medius+	Iliacus+	Psoas++	Soleus	Liver	Inguinal Fat	Retroperitoneal Fat++
OBESE**	52.2±6.06	58.3±5.88	53.0±5.78	53.8±8.22	59.89±4.05	132.33±7.66	29.0±48.5	22.0±34.5
LEAN**	20.0±4.42	25.3±4.26	37.4±2.73	39.6±5.06	50.00±5.38	128.56±8.7	57.0±89.0	42.0±100.0

B. ZUCKER *fa* RATS

MEAN	Gastrocnemius+	Vastus Medius+	Iliacus++	Soleus++	Psoas	Liver+	Inguinal Fat	Retroperitoneal Fat++
OBESE**	57.0±78.2	68.6±4.98	76.0±142.3	73.6±3.98	66.7±10.69	246.8±25.6	39.4±4.85	44.3±5.56
LEAN**	27.0±28.0	38.0±5.92	38.0±75.0	51.4±8.39	60.4±7.06	157.4±17.6	83.1±11.13	60.4±5.33

*Values are reported in milligrams per deciliter.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.
++Significant difference between obese and lean: $P < 0.05$.

TABLE 10

GLUCOSE CONTENT OF LIVER IN LA/N-*fa*^{k} (*"corpulent"*) RATS*

ANIMALS+	Obese Liver	Lean Liver
Pair 1	99.0	105.0
Pair 2	129.0	122.0
Pair 3	125.0	89.0
Pair 4	111.0	96.0
Pair 5	101.0	111.0
Pair 6	95.0	63.0
Pair 7	98.0	71.0
Pair 8	100.0	119.0
Pair 9	122.0	69.0
MEAN**	108.9±4.40	93.9±7.42

*Values are reported in milligrams per deciliter.
**Values are reported as Mean±Standard Error of the Mean.
+Difference between obese and lean animals approached significance ($P < 0.09$).

TABLE 11

TOTAL AMOUNT OF NONLIPOGENIC DIET CONSUMED BY LA/N-*fa*^{k} (*"corpulent"*) RATS*

ANIMALS+	OBESE	LEAN
PAIR 1	330.15	218.37
PAIR 2	323.98	210.07
PAIR 3	422.75	228.90
PAIR 4	378.21	217.71
PAIR 5	422.43	255.85
PAIR 6	436.11	190.30
PAIR 7	291.75	240.28
PAIR 8	248.10	200.96
PAIR 9	391.30	258.54
PAIR 10	304.97	229.88
PAIR 11	371.30	261.80
PAIR 12	403.68	259.46
PAIR 13	473.37	211.17
PAIR 14	476.61	265.13
PAIR 15	449.32	269.20
MEAN**	381.6±17.84	234.5±6.69

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.

TABLE 12

FECAL PRODUCTION IN LA/N-*fa*^{k} (*"corpulent"*) RATS ON A NONLIPOGENIC DIET*

ANIMALS+	OBESE	LEAN
Pair 1	39.72	22.99
Pair 2	38.73	22.34
Pair 3	49.19	23.46
Pair 4	50.25	23.51
Pair 5	45.25	26.36
Pair 6	46.36	17.96
Pair 7	34.96	28.37
Pair 8	29.97	22.15
Pair 9	46.80	28.71
Pair 10	40.25	22.73
Pair 11	47.88	28.07
Pair 12	44.89	28.15
Pair 13	51.02	22.57
Pair 14	57.46	26.60
Pair 15	50.73	28.26
MEAN**	44.9±1.825	24.8±0.826

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.

TABLE 13

TOTAL BODY WEIGHT OF LA/N-*fa*^{k} (*"corpulent"*) RATS AT SACRIFICE*

ANIMALS+	OBESE	LEAN
Pair 1	168.83	120.32
Pair 2	178.94	125.02
Pair 3	238.05	128.23
Pair 4	212.41	132.90
Pair 5	235.25	173.92
Pair 6	219.46	110.30
Pair 7	142.03	135.40
Pair 8	141.37	113.06
Pair 9	223.63	165.22
Pair 10	167.03	147.55
Pair 11	211.73	162.75
Pair 12	220.75	166.69
Pair 13	251.80	121.80
Pair 14	273.98	170.21
Pair 15	256.78	171.01
MEAN**	209.5±10.62	143.0±6.01

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.

TABLE 14

TOTAL BODY WEIGHT GAINED BY LA/N-*fa*^{k} (*"corpulent"*) RATS ON A NONLIPOGENIC DIET*

ANIMALS+	OBESE	LEAN
Pair 1	127.64	68.90
Pair 2	116.75	65.36
Pair 3	152.15	58.05
Pair 4	131.76	61.62
Pair 5	168.55	104.36
Pair 6	158.15	62.00
Pair 7	100.58	80.56
Pair 8	104.14	65.29
Pair 9	151.48	97.21
Pair 10	109.72	93.41
Pair 11	152.82	104.61
Pair 12	149.89	95.69
Pair 13	182.67	62.33
Pair 14	202.48	98.70
Pair 15	186.93	101.26
MEAN**	146.4±8.02	81.3±4.73

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.

TABLE 15

COMBINED INGUINAL FAT PAD WEIGHT OF LA/N-*fa*^{k} (*"corpulent"*) RATS AT SACRIFICE*

ANIMALS+	OBESE	LEAN
Pair 1	1.89	0.25
Pair 2	1.55	0.17
Pair 3	2.62	0.23
Pair 4	1.96	0.23
Pair 5	4.35	1.03
Pair 6	2.85	0.16
Pair 7	2.01	0.44
Pair 8	0.99	0.12
Pair 9	3.31	0.65
Pair 10	2.63	0.59
Pair 11	3.22	0.71
Pair 12	3.39	0.91
Pair 13	2.93	0.27
Pair 14	4.78	0.69
Pair 15	5.00	0.84
MEAN**	2.899±0.2998	0.486±0.0789

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.

TABLE 16

WHOLE RETROPERITONEAL FAT WEIGHT OF LA/N-*fa*^{k} (*"corpulent"*) RATS AT SACRIFICE*

ANIMALS+	OBESE	LEAN
Pair 1	1.89	0.25
Pair 2	1.71	0.29
Pair 3	2.41	0.23
Pair 4	1.88	0.18
Pair 5	3.71	0.57
Pair 6	2.29	0.20
Pair 7	0.66	0.07
Pair 8	0.82	0.15
Pair 9	2.30	0.51
Pair 10	1.78	0.25
Pair 11	2.34	0.39
Pair 12	2.67	0.45
Pair 13	3.14	0.24
Pair 14	3.19	0.39
Pair 15	3.05	0.49
MEAN**	2.256±0.2184	0.311±0.0380

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.

TABLE 17

WHOLE LIVER WEIGHT OF LA/N-*fa*^{k} (*"corpulent"*) RATS AT SACRIFICE*

ANIMALS+	OBESE	LEAN
Pair 1	7.39	4.83
Pair 2	7.47	5.71
Pair 3	8.58	5.16
Pair 4	8.87	5.08
Pair 5	8.64	6.49
Pair 6	8.84	3.96
Pair 7	5.54	5.84
Pair 8	6.53	5.52
Pair 9	9.31	9.53
Pair 10	7.13	7.45
Pair 11	8.98	8.38
Pair 12	9.37	6.64
Pair 13	10.66	4.70
Pair 14	11.64	9.14
Pair 15	10.50	8.14
MEAN**	8.63±0.420	6.44±0.445

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.05$.

TABLE 18

GLUCOSE CONTENT OF LIVER IN ZUCKER *fa* RATS*

ANIMALS+	OBESE LIVER	LEAN LIVER
Pair 1	164.0	115.0
Pair 2	153.0	63.0
Pair 3	217.0	62.0
Pair 4	115.0	157.0
Pair 5	31.0	61.0
Pair 6	120.0	81.0
Pair 7	134.0	87.0
Pair 8	87.0	89.0
Pair 9	97.0	47.0
MEAN**	124.2±17.5	84.7±11.3

*Values are reported in milligrams per deciliter.

**Values are reported as Mean±Standard Error of the Mean.

+Difference between obese and lean animals approached significance ($P < 0.08$).

TABLE 19

TOTAL AMOUNT OF NONLIPOGENIC DIET CONSUMED BY ZUCKER *fa* RATS*

ANIMALS+	OBESE	LEAN
Pair 1	449.24	244.99
Pair 2	342.50	214.81
Pair 3	456.88	321.48
Pair 4	494.68	280.47
Pair 5	472.17	315.85
Pair 6	420.07	252.90
Pair 7	420.94	233.28
Pair 8	449.52	269.74
Pair 9	403.80	223.43
Pair 10	422.54	247.53
Pair 11	416.77	225.16
Pair 12	410.78	250.52
Pair 13	394.86	237.00
Pair 14	393.16	245.66
MEAN**	420.5±449.5	246.6±269.7

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.

TABLE 20

FECAL PRODUCTION IN ZUCKER *fa* RATS ON A NONLIPOGENIC DIET*

ANIMALS+	OBESE	LEAN
Pair 1	44.03	21.13
Pair 2	31.27	18.98
Pair 3	43.75	28.72
Pair 4	48.74	24.88
Pair 5	46.82	28.38
Pair 6	42.05	21.77
Pair 7	41.97	22.53
Pair 8	45.41	26.48
Pair 9	41.52	22.10
Pair 10	40.13	21.25
Pair 11	38.71	18.78
Pair 12	25.85	22.20
Pair 13	37.71	21.68
Pair 14	36.96	21.89
MEAN**	40.4±1.630	22.9±0.825

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.

TABLE 21

TOTAL BODY WEIGHT OF ZUCKER *fa* RATS AT SACRIFICE*

ANIMALS+	OBESE	LEAN
Pair 1	264.23	151.42
Pair 2	205.53	133.25
Pair 3	279.10	212.48
Pair 4	275.54	166.28
Pair 5	263.31	195.28
Pair 6	245.51	147.28
Pair 7	267.05	146.94
Pair 8	280.48	183.39
Pair 9	260.58	150.35
Pair 10	236.61	150.07
Pair 11	233.15	135.05
Pair 12	236.10	142.63
Pair 13	227.71	135.24
Pair 14	222.04	159.40
MEAN**	249.8±6.26	157.8±6.36

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.

TABLE 22

TOTAL BODY WEIGHT GAINED BY ZUCKER *fa* RATS ON A NONLIPOGENIC DIET*

ANIMALS+	OBESE	LEAN
Pair 1	175.25	81.10
Pair 2	150.67	80.61
Pair 3	189.56	146.53
Pair 4	201.62	114.91
Pair 5	173.54	137.16
Pair 6	163.75	80.37
Pair 7	193.45	83.22
Pair 8	185.68	115.08
Pair 9	166.52	92.97
Pair 10	162.75	70.11
Pair 11	162.18	77.21
Pair 12	165.23	83.87
Pair 13	159.94	78.09
Pair 14	155.75	86.97
MEAN**	165.9±185.7	83.5±114.9

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.

TABLE 23

COMBINED INGUINAL FAT PAD WEIGHT OF ZUCKER *fa* RATS AT SACRIFICE*

ANIMALS+	OBESE	LEAN
Pair 1	3.96	1.00
Pair 2	1.56	0.70
Pair 3	5.06	2.21
Pair 4	4.55	1.07
Pair 5	3.36	1.39
Pair 6	1.75	0.49
Pair 7	4.49	0.81
Pair 8	5.35	1.45
Pair 9	5.12	0.84
Pair 10	3.87	0.58
Pair 11	2.57	0.40
Pair 12	2.76	0.40
Pair 13	2.72	0.46
Pair 14	2.84	0.68
MEAN**	3.615±4.55	0.755±1.07

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.

TABLE 24

WHOLE RETROPERITONEAL FAT WEIGHT OF ZUCKER *fa* RATS AT SACRIFICE*

ANIMALS+	OBESE	LEAN
Pair 1	3.92	0.64
Pair 2	1.64	0.38
Pair 3	3.69	1.14
Pair 4	3.43	0.67
Pair 5	4.70	0.91
Pair 6	2.46	0.47
Pair 7	2.40	0.20
Pair 8	2.55	1.00
Pair 9	2.84	0.34
Pair 10	2.31	0.58
Pair 11	2.31	0.48
Pair 12	2.15	0.41
Pair 13	2.80	0.51
Pair 14	2.92	0.59
MEAN**	2.675±3.430	0.545±0.670

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.

TABLE 25

WHOLE LIVER WEIGHT OF ZUCKER *fa* RATS AT SACRIFICE*

ANIMALS+	OBESE	LEAN
Pair 1	11.30	4.99
Pair 2	8.51	5.22
Pair 3	11.84	8.22
Pair 4	11.83	6.89
Pair 5	10.79	6.60
Pair 6	10.93	6.05
Pair 7	11.97	6.07
Pair 8	12.25	8.02
Pair 9	11.20	6.50
Pair 10	9.13	6.20
Pair 11	9.75	5.99
Pair 12	11.08	6.89
Pair 13	11.20	6.53
Pair 14	9.75	7.09
MEAN**	10.82±0.301	6.52±0.241

*Values are reported in grams.
**Values are reported as Mean±Standard Error of the Mean.
+Significant difference between obese and lean: $P < 0.01$.

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EPILOGUE

Never give up on what you really want to do. The person with big dreams is more powerful than one with all the facts.

Anonymous