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Oxygen Consumption and Oxidative Capacity of Hepatocytes from Young Male Obese and Nonobese Zucker Rats (42405)

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Abstract. The contribution of the liver to the increased metabolic efficiency of the obese rat (*fa/fa*) was examined. Oxygen consumption of isolated hepatocytes and isolated mitochondria, and hepatic activities of mitochondrial enzymes were measured. 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. Taken together, these data show that *in vitro* 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 lipid oxidation is similar in obese and nonobese rats. Therefore, the increased metabolic efficiency of the obese rat probably cannot be attributed to an intrinsic decreased hepatic oxidative capacity. Further, there is no defect in hepatic lipid oxidative capacity in the young obese rat.

The obese syndrome of the Zucker rat (*fa/fa*) is well characterized (1). Major abnormalities exhibited by the obese rat include hyperinsulinemia (2), hyperphagia (1), hyperlipemia (3), accumulation of excessive carcass lipid (1), mild hypothyroidism (4), depressed muscle growth (5), increased metabolic efficiency (6), and a depressed whole body O_2 consumption (7). The liver of the obese rat has increased lipid content (8–10), increased rates of lipid synthesis (9, 11), increased activities of key lipogenic enzymes (2, 12, 13), increased lipid esterification (10, 14, 15), and increased release of very low density lipoproteins (3). In addition, the hepatocytes of obese rats are larger (9, 15), probably representing increased lipid content. Elevated rates of hepatic lipogenesis remain in spite of pair-feeding to nonobese rats (12, 17) or treadmill exercise (2).

Some metabolic characteristics of this model of obesity are not well established. A decreased rate of lipid oxidation by hepatocytes of obese rats (10, 15, 16) is reported by some authors, but not by others (11). Triscari *et al.* (16), and Azain *et al.* (14) reported that that *in vitro* [^{14}C]palmitate oxidation to $^{14}CO_2$ is lower in hepatocytes from obese than from nonobese rats when the concentration of palmitate is 0.1 m*M* in the incubation media. However, Azain *et al.* (14) demonstrated that no difference in palmitate oxidation exists when the concentration is 2.0–3.0 m*M* in the incubation media. McCune *et al.* (11) demonstrated similar findings. [^{14}C]Oleate oxidation to $^{14}CO_2$ was 48% lower in the hepatocytes from obese rats at 0.5 m*M* oleate, but only 18% lower at 2 m*M* oleate. Therefore, it is not clear whether the capacity for hepatic fatty acid oxidation is different in obese and nonobese rats, or if this difference is artificially induced by specific experimental protocols. The isolated liver mitochondria provide a more direct method than the hepatocyte for determination of the capacity for lipid oxidation. Mitochondria can easily be exhausted of endogenous substrates. Oxygen consumption of the mitochondria then can be directly related to the capacity for lipid 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 mitochondrial oxidation of nonlipid substrates exists in the obese rat.

This study also examined whether the liver contributes to the increased metabolic efficiency (6). The liver is a highly oxidative organ. At rest, the liver accounts for approximately 20–30% of the total body O_2 consumption

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(18). Hepatic oxidative metabolism in young obese rats is not extensively studied. A possible impairment in hepatic oxidative capacity may contribute to the increased metabolic efficiency of the obese rat and possibly other types of obesity. Therefore, the possibility of impaired hepatic oxygen consumption deserves further exploration.

In past reports (10, 14–16) data were always expressed on a per hepatocyte basis. Data were not expressed on a per liver basis. Because liver weights are much heavier in the obese than in the nonobese rats (12), the expression of oxygen consumption on a total liver basis is probably more meaningful. In the present report, hepatocyte O_2 consumption is expressed on a total liver basis to help resolve apparent discrepancies in the literature.

Materials and Methods. *Animals.* Six-week-old obese and nonobese male 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* at 25°C and 50% relative humidity. They were housed in wire cages on a 12-h light–dark cycle.

Hepatocyte isolation. The rats were killed by decapitation at 0900 hr. The livers were rapidly dissected and 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.* (19) because it avoids the use of anesthesia that is required by the *in vivo* perfusion methods (20). The liver was perfused by syringe via the portal vein with 20 ml of ice-cold calcium-free Krebs–Ringer bicarbonate medium (KRB) (21), pH 7.4, which contained 0.05% collagenase, 0.1% hyaluronidase, 5 m*M* glucose, 1 m*M* pyruvate, and 20 m*M* 4(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), and gassed with 95% O_2–5% CO_2. The liver was then sliced by hand with a Stadie–Riggs microtome (A. H. Thomas, Philadelphia, Pa.) which was mounted on ice to reduce cellular metabolism and the oxygen requirement during this part of the procedure. The slices were incubated in two 250-ml flasks that each contained 10 ml of the perfusion medium at 37°C under an atmosphere of 95% O_2–5% CO_2 in a gyrotory metabolic shaker. After 30 min of incubation, calcium was added to the medium to a final 1.25 m*M* concentration, and the incubation was continued for another 30 min.

The digested liver was poured over a 250-μm nylon screen into a 100-ml plastic beaker on ice. The screen was rinsed with 30 ml KRB without digestive enzymes. The material that passed through the screen was centrifuged at 120*g* for 1 min. The supernatant was aspirated and discarded. The pellet was suspended in cold KRB medium, which contained 20 m*M* Hepes and 1.25 m*M* calcium (KRB–Hepes). This was then centrifuged at 50*g* for 1 min. The supernatant was again removed, and the pellet was resuspended in the KRB–Hepes medium. The final centrifugation was again at 50*g* for 1 min. After the supernatant was removed, the pellet was resuspended in 7.0 ml of the KRB–Hepes medium. The cells were counted in a hemocytometer chamber. This hepatocyte count was used in subsequent calculations. Cell viability, which routinely exceeded 90%, was determined by the exclusion of 0.6% trypan blue. Although we did not routinely count nonparenchymal cells, very few were observed. Howard *et al.* (19) report that this method yields almost entirely parenchymal cells with high glycogen and potassium contents, and high respiratory activity that is coupled. The values of hepatocyte oxygen consumption reported by us are consistent with reports of others for isolated hepatocytes (19, 20, 22), liver slices (23, 24), and perfused livers (22) when data from various reports are recalculated to the identical mode of expression. The range of calculated values range from approximately 13 to 25 nmole/min/10^6 cells. Our values reported here are within this range, depending on the substrate used. This assures us of the good integrity of the isolated hepatocytes.

Hepatocyte oxygen consumption. Oxygen consumption was measured with a Clark-type oxygen electrode at 37°C. Approximately 1 $\times$ 10^6 cells/ml were added to the electrode chamber and brought up to volume with KRB–Hepes media. When sodium palmitate was used as a substrate, 1% fatty acid-free bovine serum albumin (BSA) was added to the medium. 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 linear base-

line oxygen consumption rate was first recorded. Substrates were subseqently introduced through the introduction port with a syringe, and the resulting rate of oxygen consumption was recorded. The substrates used were 5 m*M* pyruvate, 5 m*M* glucose, and 1 and 3 m*M* palmitate. Sodium 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 suspended cells was assayed by the method of Lowry *et al.* (25) 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 assumed that the liver cells accounted for almost all the protein in the liver. Total liver protein was divided by the protein per hepatocyte to yield total cells per liver. We are unaware of any other studies which attempt to account for total liver metabolism when isolated hepatocytes are used. Oxygen consumption per hepatocyte was multiplied by the number of hepatocytes per liver to obtain oxygen consumption per liver. Oxygen content of the O_2-saturated media was determined by the method of Robinson and Cooper (26).

Liver mitochondrial oxygen consumption. Liver samples were homogenized by hand with a Teflon and glass homogenizer in 250 m*M* sucrose, 2 m*M* ethylenediaminetetraacetic acid (EDTA), and 20 m*M* Tris(hydroxymethyl)aminomethane (Tris), pH 7.4 (27). The homogenate was centrifuged at 600*g* for 10 min. The superantant was saved, and the pellet was resuspended in the sucrose medium and centrifuged again at 600*g* for 10 min. The two supernatants then were combined and centrifuged at 9000*g* for 10 min. This speed was used in order to maximize mitochondrial yield and mitochondrial quality without also trapping broken mitochondrial membranes. The supernatant was aspirated, and the pellet was resuspended in the sucrose medium to a volume of 0.5 ml per gram of liver sample. All procedures were carried out at 4°C.

Oxygen consumption of the mitochondria was determined at 25°C in the air-saturated medias of Max *et al.* (28), which contained 15 m*M* KCl, 30 m*M* KH_2PO_4, 25 m*M* Tris, 45 m*M* sucrose, 7 m*M* EDTA, and 5 m*M* $MgCl_2$, pH 7.4. Initially, 125 μM 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 (29). This was followed by a larger addition of 320 μM 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 m*M* pyruvate plus 1 m*M* malate, 50 μM palmitoylcarnitine plus 1 m*M* malate, and 9 m*M* succinate plus 4 μM rotenone. Assays with palmitoylcarnitine contained 2% fatty acid-free BSA (30), while the other assays had 0.2% fatty acid-free BSA in the medium.

The respiratory control index (RCI) was calculated by dividing the State 3 rate by the State 4b rate (29). The P:O ratio was calculated as described by Estabrook (31). The amount of ADP added in State 3 was determined spectrophotometrically at 259 nm with an extinction coefficient of 16.3 mM^{-1} cm^{-1}. The oxygen content of the medium and mitochondrial protein concentrations were determined as before.

Hepatic enzyme activities. Preparations for the assay of mitochondrial enzyme activities were slightly different than those for the mitochondrial respiration studies. Here the goal was to recover as much enzymatic activity as possible. Liver samples were homogenized in the sucrose medium used before. An aliquot of this homogenate was frozen and thawed three times in a dry ice and acetone bath in order to release the membrane-bound mitochondrial enzymes. This aliquot, which represents a whole homogenate fraction, was then centrifuged at 600*g* for 10 min to remove debris and unbroken cells. The 600*g* superantant was stored at −80°C until assayed. No sample was stored longer than 2 weeks. Without centrifugation the whole homogenate fraction contributed so much turbidity to the succinate dehydrogenase and cytochrome oxidase assays that accurate determinations were not possible. Another aliquot from the original homogenate, not subjected to repeated freeze-thawing, was centrifuged at 600*g* for 10 min. The supernatant was then again centrifuged at 600*g* for 10 min. The resulting supernatant was then centrifuged at 14,000*g* for 10 min,

and the mitochondrial pellet was resuspended in the sucrose media. This speed was chosen in order to maximize mitochondrial yield. 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. Preliminary studies indicated 90% recovery of succinate dehydrogenase with these procedures.

Citrate synthease (EC 4.1.3.7) was assayed by the method of Srere (32) with 5,5′ dithiobis (2-nitrobenzoate) as the sulfhydral acceptor. Succinate dehydrogenase (EC 1.3.99.1) was assayed by the method of Singer and Kearney (33) 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*. Protein in the mitochondrial fraction and 600*g* 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 Company (St. Louis, MO), except acetyl-CoA, which was purchased from P-L Biochemicals (Milwaukee, Wisc.). Collagenase and hyaluronidase were purchased from Worthington Diagnostic Systems (Freehold, N.J.). Other chemicals were reagent grade and purchased from Fisher Chemical Company (Fair Lawn, N.J.).

Statistics. The effect of substrate on *in vitro* liver cell oxygen consumption was analyzed with a paired *t* test when possible (35). Possible phenotype × substrate interactions for the addition of BSA and glucose to the hepatocyte assays were analyzed by analysis of variance (ANOVA). All other comparisons in this study were made with the *t* test. A probability of 0.05 or less was taken to be statistically significant.

Results. *Liver weight and composition.* Liver weight and total liver protein were significantly higher in the obese rats (Table I). The amounts of protein per 10^6 cells, percentages of wet weight as protein, and total number of cells were similar in the livers from obese and nonobese rats.

Liver cell oxygen consumption. Liver cell oxygen consumption was similar in obese and nonobese rats when data were expressed on either a cellular (Table II) or a whole liver basis (Table III). These rates were similar to those obtained by the *in vivo* perfusion method (20). The addition of glucose or pyruvate per se had no significant effect on hepatocyte oxygen consumption. Addition of palmitate did significantly increase hepatocyte oxygen consumption. Glucose produced a significant

TABLE I. LIVER WEIGHT, PROTEIN, AND CELL NUMBER IN OBESE AND NONOBESE ZUCKER RATS

	Obese	Nonobese	*P*
Body weight (g)	186 ± 1 (17)[a]	149 ± 1 (19)	<0.001
Liver weight (g)	8.68 ± 0.81 (8)	6.46 ± 0.29 (9)	<0.01
Liver protein (g)	1.89 ± 0.07 (8)	1.42 ± 0.09 (8)	<0.01
$\frac{\text{g protein}}{\text{liver}}$ (100)	22.0 ± 0.5 (8)	21.7 ± 0.7 (8)	NS
$\frac{\text{mg protein}}{10^6 \text{ cells}}$	3.51 ± 0.30 (8)	3.12 ± 0.27 (8)	NS
$\frac{\text{cells} \times 10^6}{\text{g wet weight}}$	71.3 ± 6.9 (8)	67.5 ± 7.6 (8)	NS
$\frac{\text{cells} \times 10^6}{\text{liver}}$	591 ± 81 (8)	473 ± 53 (8)	NS

[a] Values are means ± SEM with number of observations in parentheses. NS, not significant.

TABLE II. HEPATOCYTE OXYGEN CONSUMPTION FROM OBESE AND NONOBESE ZUCKER RATS ON A CELLULAR BASIS

Media	Obese	Nonobese	*P*
KRB alone	13.3 ± 1.5 (8)[a]	11.3 ± 1.9 (9)	NS
KRB plus 5 m*M* glucose	14.9 ± 1.6 (8)	10.4 ± 0.8 (9)	<0.05
KRB plus 5 m*M* pyruvate	14.0 ± 2.3 (8)	12.9 ± 2.2 (7)	NS
KRB plus 1% BSA and 1 m*M* palmitate	24.4 ± 2.3** (8)	20.8 ± 1.4** (9)	NS
KRB plus 1% BSA and 3 m*M* palmitate	19.7 ± 2.7 (8)	17.4 ± 1.7* (9)	NS

[a] Values are means ± SEM with number of observations in parentheses. Units are nmole $O_2/10^6$ cells/min. NS, not significant.

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

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

phenotypic difference in hepatocyte oxygen consumption (Table II and III). The obese hepatocytes had a higher rate of glucose-induced oxygen consumption than nonobese hepatocytes. Due to variability, the difference in the obese and nonobese responses to glucose relative to KRB alone was not significant.

Liver enzyme activities. Enzyme activities for citrate synthase, succinate dehydrogenase, and cytochrome oxidase were similar in obese and nonobese rats in both the 600*g* supernatant (which represents a whole homogenate fraction) and the 14,000*g* mitochondrial pellet (Table IV).

Liver mitochondrial respiration. Maximum mitochondrial respiration rates (State 3) were similar for the nonobese and obese rats (Table V). Respiratory Control Indexes, P:O ratios, and State 4b respiration rates were also similar in both phenotypes for any substrate used. The respiratory control indexes show that the assays contained well-coupled mitochondria.

Discussion. Oxygen consumption in the isolated hepatocytes was generally similar in the obese and nonobese rats, which indicates an absence of an intrinsic defect in hepatocyte oxygen consumption in the obese Zucker rat. Glucose oxidation was significantly higher in the obese animal. One would expect a similar result with pyruvate as a substrate because it is the end of the glycolytic pathway prior to oxygen utilization. Oxygen consumption with exogenous pyruvate was not different between phenotypes. This discrepancy probably exists because the effect of glucose may be primarily a statistical one. With no exogenous sub-

TABLE III. HEPATOCYTE OXYGEN CONSUMPTION FROM OBESE AND NONOBESE ZUCKER RATS ON A WHOLE LIVER BASIS

Media	Obese	Nonobese	*P*
KRB alone	7.72 ± 1.39 (8)[a]	5.15 ± 0.86 (8)	NS
KRB plus 5 m*M* glucose	8.65 ± 1.56 (8)	4.76 ± 0.80 (8)	<0.05
KRB plus 5 m*M* pyruvate	8.11 ± 1.46 (8)	5.87 ± 0.98 (8)	NS
KRB plus 1% BSA and 1 m*M* palmitate	14.0 ± 2.2* (8)	9.83 ± 1.50* (8)	NS
KRB plus 1% BSA and 3 m*M* palmitate	11.3 ± 1.8 (8)	8.22 ± 1.26 (8)	NS

[a] Values are means ± SEM with number of observations in parentheses. Units are μmole O_2/whole liver/min. NS, not significant.

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

TABLE IV. LIVER ENZYME ACTIVITY RATES FROM OBESE AND NONOBESE ZUCKER RATS

Enzyme	Obese	Nonobese	*P*
	600*g* supernatant		
Citrate synthase	19.7 ± 1.8 (7)[a]	20.3 ± 1.3 (9)	NS
Succinate dehydrogenase	8.25 ± 0.68 (7)	9.84 ± 0.53 (9)	NS
Cytochrome oxidase	21.5 ± 2.4 (7)	25.8 ± 2.4 (9)	NS
	14,000*g* mitochondrial pellet		
Citrate synthase	87.3 ± 9.0 (8)	74.5 ± 3.5 (8)	NS
Succinate dehydrogenase	75.8 ± 2.6 (8)	72.9 ± 3.2 (8)	NS
Cytochrome oxidase	164 ± 7 (8)	159 ± 10 (8)	NS

[a] Values are means ± SEM with number of observations in parentheses. Units are nmole/mg protein/min. NS, not significant.

strates 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. The result was a statistically significant phenotypic difference in oxygen consumption when glucose is used as a substrate. However, without a parallel difference with pyruvate or a significant phenotype × substrate interaction, a real difference with glucose is questionable. The addition of palmitate approximately doubled the hepatocyte oxygen consumption above control values. Plomp *et al.* (36) reported similar findings upon addition of either octanoate or oleate to the incubation of hepatocytes. Because the increase was prevented by oligomycin and carboxyatractyloside and total adenine nucleotide ratios were maintained, these authors (36) attributed the increased rate to mitochondrial respiration.

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. Oxidation of palmitoylcarnitine was similar in mitochondria from the obese and nonobese hepatocytes as evidenced by State 3 mitochondrial respiration rates. While this work was in progress, Brady and Hoppel (37) reported that the rates of mitochondrial respi-

TABLE V. SUBSTRATE-DEPENDENT LIVER MITOCHONDRIA OXYGEN CONSUMPTION FROM OBESE AND NONOBESE ZUCKER RATS

Phenotype	State 4b	State 3	RCI	P:O
	Pyruvate plus malate (9 m*M*: 1 m*M*)			
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 (50 μM:1 m*M*)			
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 (9 m*M*:4 m*M*)			
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

[a] Values are means ± SEM for $n = 8$ observations in each group. No obese vs nonobese means differ significantly. Units are nmole O_2/mg protein/min.

ration were similar in older obese and nonobese female Zucker rats. Our work supports the findings of Brady and Hoppel, and extends them to both male rats and younger rats. Lipid oxidation is reported to be lower in hepatocytes from obese animals when incubated with 0.1 m*M* palmitate (14, 16), as determined by [^{14}C]palmitate oxidation to $^{14}CO_2$, but not consistently when the fatty acid concentration was raised to 2.0–3.0 m*M* (14). However, as shown in our study and by Brady and Hoppel (37) a phenotypic difference in the potential for mitochondrial lipid oxidation does not contribute to this difference in lipid oxidation as measured by $^{14}CO_2$ production at low levels of substrate.

The rates of mitochondrial respiration indicate that the capacity for hepatic oxidation of pyruvate, palmitoylcarnitine, and succinate is similar for obese and nonobese rats. Triscari *et al.* (38) suggested that based on oxidation of ^{14}C-labeled substrates to $^{14}CO_2$, pyruvate and succinate oxidation are depressed in the hepatocytes from obese animals. The differences in oxidation rates between phenotypes in their report (16) are not very great. In addition, their data were expressed (16) per weight of cells that were dried. Because obese hepatocytes are larger (9, 15) and contain more fat, this represents fewer cells in the dried weight. This may skew the data. To avoid this problem we expressed oxygen consumption on both per cell and per liver bases. 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. The use of isolated mitochondrial preparations avoids some of these problems. The mitochondrial data, taken collectively with our hepatocyte respiration data, probably better represent the true potential for cellular oxidation of the substrates. Our data for mitochondrial respiration rates with pyruvate and succinate are consistent with those of Brady and Hoppel (37). Activities of the oxidative enzymes citrate synthase, succinate dehydrogenase, and cytochrome oxidase were similar for both phenotypes. These enzyme activities further support the conclusion that hepatocytes from obese and nonobese rats have similar capacities for maximum mitochondrial respiration.

Difference in hepatic lipid oxidation in obese and nonobese rats which are noted in the literature (11, 14, 16) are not caused by an intrinsic difference in the capacity for mitochondrial lipid oxidation, and therefore must simply reflect specific *in vitro* effects. Hepatic oxidative capacity is intrinsically identical in obese and nonobese Zucker rats. The liver probably does not account for the depressed oxygen of the obese Zucker rat (7) because hepatocyte oxygen consumption is not depressed, although various physiological stresses and endocrine alterations might alter hepatic oxidation of substrates.

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