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Obesity and insulin resistance: lessons learned from the Pima Indians

S Lillioja
University of Wollongong, lillioja@uow.edu.au

C Bogardus
National Institutes of Health, Arizona

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Abstract
Diabetes and obesity are epidemic in the Pima Indians of the Southwestern United States, and the prevalence of diabetes is increasing. The most likely link between obesity and diabetes is tissue insulin resistance. If obesity is defined as an excess of body fat, then it can only be accurately assessed by measurements of body composition and not by approximations such as body mass index or percent of ideal weight. To compare the metabolic data of individuals of varying size, an accurate measure of metabolic size is needed. Total body weight is not an appropriate means of comparing individuals since obese subjects have a greater proportion of nonmetabolizing mass (triglyceride). Body surface area shows a sex difference, and this may distort data if both sexes are present. From studies of metabolic rate we have determined that metabolic rate is indirectly proportional to the fat-free mass plus 18 kg, and we suggest that this weight can be equated with metabolic size. Glucose storage in skeletal muscle appears to be important in the disposal of an intravenous glucose load. Consistent with its role in glycogen storage, glycogen synthase enzyme is activated in proportion to the ability to dispose of glucose during a hyperinsulinemic, euglycemic clamp. The role of glycogen synthase is most notable at supraphysiological plasma insulin concentrations; and since glucose uptake at these insulin concentrations is highly familial independent of the degree of obesity, we suggest that there may be a specific genetic defect expressed in skeletal muscle that reduces insulin responsiveness in some subjects. The lack of correlation between 24 hour respiratory quotient measured in a metabolic chamber (a measure of the proportion of fat derived calories) and degree of obesity indicates that in obese Pima Indians insulin resistance is not due to an inhibition of glucose metabolism by free fatty acids (glucose-fatty acid-ketone cycle). Obesity is associated with an increase in fat-free mass almost kilogram-for kilogram with fat mass when compared to the lean state. A role for this increase in fat-free tissue in producing insulin resistance has been given insufficient attention in the past. With an increase in fat-free mass, muscle cells are hypertrophied and capillaries in muscle are more widely spaced. We propose that these biophysical changes in muscle mediate, at least in part, the effects of obesity to produce a reduction in insulin sensitivity and the abnormal kinetics of insulin action found in the obese. We suggest therefore that insulin resistance is a combination of a genetic defect and obesity-induced changes in the biophysical properties of skeletal muscle. These defects may in turn lead to the development of non-insulin-dependent diabetes mellitus.

Keywords
learned, indians, lessons, pima, resistance, insulin, obesity

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I. INTRODUCTION

Obesity is so frequently a feature of persons with non-insulin-dependent diabetes mellitus that Ethan A. H. Sims coined the team diabesity to describe this increasingly occurring syndrome. The reasons for the association of these two metabolic disorders are not fully known, but it is hypothesized to be a result of the association of obesity with insulin resistance, which is a common finding in subjects with non-insulin-dependent diabetes mellitus. In this brief report we will review data from studies performed with the cooperation of the Pima Indians of the Gila River Indian Community that are relevant to an increased understanding of the relationship of obesity and insulin resistance.

II. BACKGROUND AND EPIDEMIOLOGIC STUDIES

The Pima Indians are a relatively genetically homogeneous population whose ancestors have lived in the same arid environment in central Arizona for about 2,000 years. The current study population consists of over 4,000 individuals who are at least one-half Pima heritage, are at least 5 years of age, and reside on the Gila River Indian Reservation. They are examined every 2 years and the examination includes an oral glucose tolerance test (75 grams). Diabetes mellitus is diagnosed according to the 1985 WHO criteria at a survey examination or in the course of routine medical care. The diabetes in the Pima is not ketosis-prone, not associated with islet-cell antibodies, and is therefore, even in the young, entirely non-insulin-dependent diabetes mellitus. As late as 1940, only 21 Pima Indians were identified with diabetes and it was concluded that diabetes prevalence was similar in American Indians and the general population. Only since the 1950s has diabetes been reported as an unusually frequent disease among the Pimas. By the 1970s, 40% of the males aged 45–74 years and 68% of the females aged 55–64 years had diabetes (Figure 1), and between then and 1980 the prevalence rates continued to increase. In the mid-1970s the diabetes incidence rate, age- and sex-adjusted to the 1970 U.S. Caucasian population, was 26.5 ± 1.9 cases/per 1,000 person-years (rate ± S.E.), 19 times the rate in Rochester, Minnesota. The incidence rates increased further by the mid-1980s.

The Pimas are also commonly obese. The mean body mass indices—weight (kg) divided by height (meter)—of males and females exceed those of the U.S. population at all ages (Figure 2). Longitudinal studies showed that the incidence of diabetes increases with increasing body mass index. This observation was essential in demonstrating the pathophysiologic relevance of the high prevalence of obesity to the high prevalence of diabetes.

However, obesity is not the only risk factor for the development of diabetes mellitus among the Pimas. There are Pimas who are obese and do not have diabetes. Obesity is therefore not a sufficient abnormality to cause diabetes. Other risk factors for the development of diabetes among the Pimas, independent of degree of obesity, include duration of obesity, and parental diabetes status (Figure 3). The mechanisms of the association of obesity duration and parental diabetes with increased risk of diabetes are currently unknown, but insulin resistance has been hypothesized as the mechanism of the association of obesity with an increased risk of diabetes.
III. RELATIONSHIP BETWEEN OBESITY AND INSULIN RESISTANCE

Obesity has frequently been associated with insulin resistance, but few investigators have carefully examined the relationship of degree of obesity with insulin resistance in a large number of subjects representing a broad range of body composition. A major, and infrequently discussed, problem in such studies of obesity and insulin action is how to appropriately estimate degree of obesity, and, equally important, how to compare rates of insulin-mediated glucose disposal among subjects of different body sizes. In the past 5 years we have addressed these issues as part of our metabolic studies of the Pimas.

A. Estimating Degrees of Obesity

The word "obesity" is derived from Latin, ob- over and edere- to eat. It is defined as an excess of body fat. How much excess of body fat mass is sufficient to label someone obese is arguable since it requires an arbitrary limit, or cut-off point, of a continuously distributed variable. Also, the definition of excess body fat necessitates expressing degree of obesity in relative rather than absolute terms. For example, a 7-foot, 100-kg male with a 15-kg fat mass (15% body fat) is leaner than a 4-foot, 50-kg female with a similar absolute body fat mass (30% body fat). Degree of obesity is therefore expressed as a fraction or percentage of the body mass that is fat. There are many methods available to assess percent body fat, including: densitometry underwater weighing, water dilution, total body K*, impedance, etc. Many, but not all, of these methods require specialized and/or expensive equipment. Densitometry is a method readily available to most hospital-based investigators. Subjects can be weighed underwater in the physical therapist's water immersion tank, and residual lung volume can be measured in the pulmonary lab. It is preferable to measure the residual lung volume while the subject is immersed in water—but if this is not possible, correction can be made to the residual lung volume measured in air—with small error.
Figure 2. Prevalence of obesity (from Knowler et al).
Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Males (n = 117)</th>
<th>Females (n = 96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>26 ± 1</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>1.71 ± 0.45</td>
<td>1.59 ± 0.51</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>98.2 ± 25</td>
<td>88.9 ± 2.0</td>
</tr>
<tr>
<td>Surface area (m²)</td>
<td>2.08 ± 0.02</td>
<td>1.90 ± 0.02</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>33.7 ± 0.8</td>
<td>35.0 ± 0.8</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>28 ± 1</td>
<td>39 ± 1</td>
</tr>
<tr>
<td>Fat-free body mass (kg)</td>
<td>68.2 ± 1.0</td>
<td>53.6 ± 0.9</td>
</tr>
<tr>
<td>Waist circumference (inches)</td>
<td>42.2 ± 0.7</td>
<td>42.0 ± 0.7</td>
</tr>
<tr>
<td>Thigh circumference (inches)</td>
<td>25.6 ± 0.3</td>
<td>26.5 ± 0.4</td>
</tr>
<tr>
<td>Waist/thigh</td>
<td>1.64 ± 0.02</td>
<td>1.59 ± 0.02</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>92 ± 1</td>
<td>94 ± 1</td>
</tr>
<tr>
<td>2-hr post-load glucose (mg/dl)</td>
<td>119 ± 2</td>
<td>136 ± 3</td>
</tr>
<tr>
<td>Fasting plasma insulin (μU/ml)</td>
<td>33 ± 2</td>
<td>44 ± 2</td>
</tr>
<tr>
<td>2-hr post-load insulin (μU/ml)</td>
<td>181 ± 16</td>
<td>246 ± 15</td>
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uU = microunits μU.

Unfortunately, many investigators estimate percent body fat from measures of body weight and height, rather than trying to directly assess body composition. One of the most commonly used estimates of percent body fat is the body mass index, or BMI. It is calculated as the body weight (in kg) divided by height (in meters) squared. It was first used by Quetelet over 100 years ago. However, he did not use BMI to estimate degree of obesity. As reviewed by Keys et al., Quetelet simply noted that weight/height² was more constant than W/H or W/H³ with increasing height.

Keys et al. were the first to coin the term body mass index and to assess its utility as an estimate of percent body fat. Keys and co-workers assumed that a good index of percent body fat should be independent of height—and BMI was. They then assessed the correlation of BMI with percent body fat as determined by densitometry.

The studies were performed on 180 college-aged males (aged 18–24) and an older group of 249 "executive" males (aged 49–59) in Minnesota. In the younger group, body density significantly correlated with BMI (r = −0.850), as it did in the older group (r = −0.666). BMI therefore satisfied the authors' criteria for a good index of percent body fat: It was independent of height and correlated significantly with percent body fat by densitometry. It was the preferred index since it also was better correlated with body density than W/H and W/H³. However, Keys was careful to point out that BMI was not significantly better correlated with body density than body weight. In the students, the correlation coefficient between body density and body weight was −0.777 (as compared to −0.850 for BMI); and in the executive men was −0.618 (as compared to −0.666).

We have also examined the relationships between BMI, body weight, and percent body fat by densitometry among nondiabetic Pima males and also among nondiabetic Pima females (Table I) (Figures 5 and 6). The relationship of fat mass and fat-free mass in a group of nondiabetic Pimas is also shown (Figure 4). Fat mass and fat-free mass both increase in obesity. It is apparent from Figure 5 that the relationship between BMI and percent body fat is not linear, as previously discussed by Garrow. (This is confirmed statistically since in the linear regression model the coefficient of the quadratic term is significant, independent of the coefficient of the linear function.) At the same BMI, women, on average, have a ~10% greater percentage of body fat compared to men. Among men, the linear correlation between percent body fat and BMI was 0.88 (p < 0.0001)—quite similar to the correlation observed by Keys et al. in similarly aged men. The correlation is improved by using a quadratic equation to relate the two variables (r = 0.91, p < 0.0001). Among Pima females, the correlation coefficients between percent body fat and BMI were 0.76 (p < 0.0001), using a linear relationship, and 0.79 (p < 0.0001) using a quadratic function.

We also analyzed the relationship between percent body fat and body weight (Figure 6). The relationship between percent body fat and body weight is similar to the relationship between percent body fat and BMI, in both males and females. Among Pima males, the correlation coefficient between percent body fat and body weight was 0.90 (p < 0.0001) (using a quadratic model), no
different than the correlation of percent body fat and BMI (0.91). Similarly, in females, these correlation coefficients were 0.79 and 0.79, respectively. Thus, similar to the findings of Keys et al., we could not demonstrate that the correlation between percent body fat and BMI was better than the correlation of percent body fat and body weight.

We are aware of no data that have found BMI to be significantly better correlated to percent body fat than body weight. Thus, the commonly held belief that BMI is a better estimate of percent body fat than body weight is not well-founded. Also, it is clear that at the same BMI, women are fatter. Finally, in either sex, the BMI, or body weight, accounts for only about two-thirds of the variance of percent body fat. Thus direct, rather than indirect, measures of body composition should be
used to assess body composition in metabolic studies. This is particularly necessary when attempting to perform correlation analyses between degree of obesity and other metabolic variables. Unnecessary error may be introduced by only estimating percent body fat from means of body height and weight. In large-scale, population-based studies, body composition studies may not be feasible due to the cost and/or time consideration. But certainly metabolic studies with small study samples involving comparisons of individuals of potentially differing body composition (e.g., male vs. female, diabetic vs. nondiabetic) cannot rely on estimates of body composition and should make use of direct measurements.

B. Normalizing Data on Insulin Action Among Lean and Obese Persons

In studies of the relationship between degree of obesity and insulin action, there is not only the problem of estimating body composition, but there is also the problem of how to compare rates of insulin-mediated glucose disposal between subjects with different body sizes. For example, during an insulin infusion as part of a hyperinsulinemic, euglycemic clamp, the absolute rate of glucose infusion to maintain euglycemia in a short, lean, normal glucose tolerant female may be 350 mg/minute. A similar, absolute glucose infusion rate may be observed in a tall, obese male with impaired glucose tolerance or diabetes mellitus. However, since the male has a greater metabolically active tissue mass, it is assumed that he should consume more glucose than the smaller female. Thus to compare the rates of insulin-mediated glucose disposal rate between these two subjects the mean rate of glucose disposal must be normalized to the size of the metabolically active tissue mass.

The best way to estimate metabolic body size is to directly measure the body metabolic rate using direct or indirect calorimetric techniques. This is rarely done. Most investigators estimate metabolic body size by calculating body surface area according to the formula of DuBois and DuBois. We have examined the relationship between body surface area and metabolic rate in a large group of nondiabetic Pima males and females living on our metabolic ward to examine how well body surface area correlates with metabolic body size. Resting metabolic rate was determined by indirect calorimetry in all subjects after they had lived on our metabolic ward for at least 7 days while consuming a weight-maintaining diet. Body surface area is significantly correlated with the resting metabolic rate in males ($r = 0.89, p < 0.0001$) and females ($r = 0.86, p < 0.0001$) (Figure 7A). However, as can be seen in the figure, the relationship is different between males and females. At any given body surface area, the resting metabolic rate is higher in males than in females ($p < 0.0001$). Thus, body surface area is probably a good estimate of metabolic body size and could be

![Figure 6. The relationship between percent body fat as determined by densitometry and body weight in 117 nondiabetic Pima males and 96 nondiabetic Pima females (see Table I for subject characteristics). In males (.), $r = 0.90, p < 0.0001$; and in females (X), $r = 0.79, p < 0.0001$.](image-url)
Figure 7A. The relationship between resting metabolic rate, as determined by indirect calorimetry, and body surface area in 117 nondiabetic Pima males and 96 nondiabetic Pima females (see Table I for subject characteristics). In males (•), \( r = 0.89, p < 0.0001 \); and in females (X), \( r = 0.86, p < 0.0001 \). The relationship is different between the sexes: \( p < 0.0001 \).

An alternative way to estimate metabolic body size is to calculate the fat-free body mass using body composition techniques such as densitometry. Similar to body surface area, the fat-free body mass is closely correlated with the resting metabolic rate in males \( (r = 0.87, p < 0.001) \) and females \( (r = 0.85, p < 0.0001) \) (Figure 7B). In this

\[
\text{RMR} = 1.4 \times \text{FFM} + 0.08 \quad \text{(giving a x axis intercept of } -17.7 \text{ kg)}.
\]
instance, however, the relationship between these two variables is similar in males and females. Thus, in estimating the metabolic body size, the fat-free body mass has an advantage over using body surface area, in that it is similar between sexes. Thus comparisons of normalized data can be done on data from mixtures of males and females if the fat-free body mass is used.

It must be remembered, however, that the relationships between metabolic rate and fat-free body mass has an intercept significantly different from zero. Unless this intercept is taken into account when estimating the metabolic body size from fat-free body mass, the metabolic body size will be underestimated in those with a smaller fat-free body mass in comparison to those with a larger fat-free body mass. Each investigator should establish the magnitude of this intercept in his/her own laboratory to make the necessary correction.

In summary, to best normalize rates of insulin-mediated glucose disposal, it is preferable to estimate the metabolic body size by measuring the metabolic rate of the body. Since this may not always be possible, the next best estimate of metabolic body size is: fat-free body mass + 17.7 kg. The 17.7 kg corrects for the intercept in the relationship between fat-free body mass and resting metabolic rate (at least in Pima Indians). Lastly, surface can be used—but only when analyzing relationships within one sex. The only exception to these guidelines would be if the metabolic rate were increased or decreased due to disease processes. In this situation the metabolic rate does not accurately reflect metabolic body size. For example, this complication obtains when studying subjects with untreated diabetes mellitus and marked fasting hyperglycemia. In this situation, it is probably best to use the metabolic body size as estimated by: fat-free body mass + 17.7 kg.

IV. RELATIONSHIP BETWEEN DEGREE OF OBESITY AND INSULIN ACTION

The relationship between degree of obesity and in vivo insulin action has been studied in a large group of nondiabetic male and female Pima Indians (Table I). Degree of obesity was estimated by densitometry and in vivo insulin action was measured using a two-step, hyperinsulinemic, euglycemic clamp technique. In vivo insulin action, at physiologic plasma insulin concentrations is expressed as either mg/kjoule (Figure 8A) or as mg/minute × (kg − ffm + 17.7 kg) (Figure 8B). As seen in the figures, there is a significant, negative, nonlinear relationship between degree of obesity and in vivo insulin action at these physiologic insulin concentrations, as previously described in a smaller group of Pima males.21 Note that at a

![Figure 8A](image-url)
percent body fat less than \(~30\%\), there is a decline in insulin action with increasing obesity, whereas above a percent body fat of \(~30\%\) this relationship does not exist.

At maximally stimulating plasma insulin concentrations there is a significant, negative linear relationship between degree of obesity and insulin action in males (Figure 9A and B). The relationship is much weaker at these higher insulin concentrations than at physiologic insulin concentrations.

It is important to realize that degree of obesity only accounts for \(~36\%\) of the variance of insulin action at physiologic insulin concentrations; and even less, \(~15\%\), at maximally stimulating insulin concentrations. As we have published elsewhere,\(^{22}\) there is a significant familial, and possibly

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**Figure 8B.** The relationship between insulin-mediated glucose disposal rate during the hyperinsulinemic, euglycemic clamp and percent body fat determined by densitometry in 213 nondiabetic Pima males and females \((r = -0.61, p < 0.0001)\). The rate of insulin-mediated glucose disposal is normalized to the fat-free body mass plus 17.7 (see text). The mean plasma insulin concentration was 126 \(\pm 3\) uU/ml and the plasma glucose concentration was 95 \(\pm 0\) mg/dl.

**Figure 9A.** The relationship between maximal insulin-stimulated glucose disposal rate during the hyperinsulinemic, euglycemic clamp and percent body fat in 213 nondiabetic Pima males and females \((r = -0.36, p < 0.0001)\). The rate of insulin-mediated glucose disposal is normalized to the metabolic rate as measured using indirect calorimetry and is expressed as mg/kjoule. The mean plasma insulin concentration was 1981 \(\pm 35\) uU/ml and the mean plasma glucose concentration was 95 \(\pm 0\) mg/dl.
genetic, component that accounts for a significant portion of the variance of in vivo insulin action not accounted for by degree of obesity (Figure 10).

V. BODY FAT DISTRIBUTION AND INSULIN RESISTANCE

Jean Vague reported many years ago that subjects with non-insulin-dependent diabetes mellitus have a more centralized distribution of body fat compared to nondiabetics.23 This was subsequently confirmed by Feldman et al.24 In more recent years, Evans et al.25 have reported that women with truncal or upper-body distribution of body fat are more insulin-resistant than equally obese women with predominantly lower body obesity. We have assessed the relationship between truncal versus lower body obesity and total body fat and insulin resistance in a large group of nondiabetic Pima Indian males and females. Body fat distribution was estimated by measuring the supine, waist circumference at the level of the umbilicus. This was divided by the thigh circumference immediately below the gluteal fold.

The relationship between waist/thigh circumference (WTC) and percent body fat in Pima males and females is shown in Figure 11. In males, WTC is linearly, closely correlated with percent body fat ($r = 0.71, p < 0.0001$). Conversely, in Pima females WTC is poorly correlated with percent body fat ($r = 0.22, p < 0.03$). Thus, knowing that WTC in Pima males provides little more information than knowing percent body fat, and vice versa. In Pima females, WTC is nearly independent of degree of obesity, so that knowing the WTC may add further information regarding relationship between body fat and other metabolic variables.

In Pima males, WTC is not significantly correlated with in vivo insulin action independent of degree of obesity. This is true both at physiologic and at maximally-stimulating insulin concentrations. In females, WTC is significantly correlated with insulin action at physiologic ($p < 0.04$) and maximally-stimulating insulin concentrations ($p < 0.02$), independent of degree of obesity. The reason(s) for the association of central obesity with reduced insulin action is (are) unknown. In particular, it is not established whether they are causally related to each other or only are both secondary to a pathogenic third factor.

VI. MECHANISMS OF INSULIN RESISTANCE

A. Glucose Oxidation and Storage

Felber et al.26 and Boden et al.27 demonstrated that diabetic subjects have reduced storage of glucose after an oral glucose load. We compared the rates of oxidation and storage of glucose during the euglycemic clamp in lean and obese subjects with normal glucose tolerance.28 There was a wide range of insulin resistance in the group. In
Figure 10. Insulin action at supraphysiological insulin concentrations (Max M) by family. Glucose uptake was adjusted for individual variations in age, sex, and percent body fat. (*) Individual adjusted Max M. (—) Mean Max M for family. (□) All members of a particular family enclosed by this box. Notice that there is a much larger variation between families than within families ($p \leq 0.0001$).

Figure 11. The relationship between waist/thigh circumferences and percent body fat as determined by densitometry in 117 nondiabetic Pima males (●) and 96 nondiabetic Pima females (X). In males, $r = 0.71$, $p < 0.0001$ and in females, $r = 0.22$, $p < 0.03$. 
subjects with the lowest glucose uptake rates during hyperinsulinemia, there was almost no glucose storage. This added to the observations of Thiebaud et al.29 and Jacot et al.30 in lean young men whose glucose storage was never less than 55% of glucose uptake. Over the range, from those subjects with the greatest in vivo insulin resistance to those with the least insulin resistance, storage made a progressively greater contribution to glucose uptake. This study suggested that even in subjects with normal glucose tolerance, storage has a critical role in distinguishing between those who are insulin resistant and those who are not. Since other studies have shown that under the conditions of a clamp there is little hepatic uptake but significant peripheral glucose uptake,31-34 these studies emphasize the role of skeletal muscle glucose storage in determining insulin resistance.

B. The Role of Fatty Acid Oxidation in Regulating Glucose Uptake

It has been proposed that increased availability of free-fatty acids or ketones for oxidation may be responsible for an inhibition of carbohydrate metabolism in muscle, thus producing a reduction in glucose tolerance. The concept has been extended to suggest that one role of insulin is to control glucose uptake by controlling the rate of release of fatty acid from adipose tissue.35-40

As we have noted, glucose disposal has components of both glucose oxidation and glucose storage. There is good evidence for a role of lipid oxidation in the regulation of the oxidative component, and we find strong correlations of glucose oxidation and lipid oxidation rates. (Such correlations are not simply due to the method of calculating lipid and carbohydrate oxidation, since for each individual the respiratory quotient and total oxygen consumption vary independently. We have discussed this in more detail elsewhere.40) As recently noted, nonoxidative glucose disposal might have several components—lactate formation and muscle glycogen formation.41 The lack of correlation and lipid oxidation with storage40 could be due to there being opposing actions of lipid oxidation on components of nonoxidative glucose disposal. Alternatively, the lack of correlation could be due to fatty acids not affecting nonoxidative disposal at all.

There is some suggestion, however, that ketones may promote glycogen formation.36 There are several conflicting reports about the effect of fatty acids on glucose storage. In one report, maintaining basal FFA concentrations during hyperinsulinemia reduced oxidation, but did not affect storage.42 Similar conclusions were drawn from a study examining the effect of age on substrate disposal.43 In another report, storage was reduced by fatty acid infusion44; in this study, however, subjects did not serve as their own controls, and fatty acid levels were raised to levels higher in general than basal levels.

Furthermore, a biochemical basis for a role of lipid oxidation in regulating glucose storage is lacking. On the other hand, a correlation of pyruvate dehydrogenase activity with glucose oxidation rates, and of glycogen synthesis activity with glucose storage rates has recently been demonstrated.41 In conclusion, it seems that the components of glucose uptake, namely glucose oxidation and storage, are probably regulated by different mechanisms. If this is true, then the glucose fatty acid–ketone cycle may at best have only a partial role in producing insulin resistance.

Finally, we have recently shown that the proportion of 24 hour calorie expenditure derived from fat (24 hr R.Q.) is not correlated with degree of obesity (% fat). Therefore the insulin resistance found in obese Pima Indians is not due to the glucose–fatty acid–ketone cycle.44A

C. Glucose Disposal and Muscle Glycogen Synthase

Since current evidence suggests a crucial role for skeletal muscle in insulin-mediated glucose disposal,31-34 and since glucose storage is also an important component of glucose disposal, it is logical to examine the role of glycogen synthesis and its regulatory enzyme, glycogen synthase, in insulin action in muscle. That whole body uptake of glucose can reasonably be assumed to reflect muscle glucose metabolism comes from the observation that forearm or leg glucose disposal is well-correlated with whole body glucose disposal,32-34 and that during intravenous glucose infusion the liver takes up little glucose33 but the muscle does take up glucose.31

There is now considerable evidence in both Pima Indians and Caucasians that glycogen synthase enzyme from vastus lateralis is activated by insulin infusions, and activated in proportion to the ability of insulin to stimulate glucose uptake during the euglycemic clamp or related techniques (Figure 12).41,45-51 For example, we showed that
after glycogen-depleting exercise, the rate of glucose uptake and storage was correlated with the proportion of active enzyme present prior to insulin infusion. We subsequently showed that in a group of normal and diabetic subjects without exercise, glucose uptake and storage were correlated with muscle glycogen synthase activity after insulin stimulation and with the change in muscle glycogen synthase activity during insulin stimulation. We then measured the effect of the induction of insulin resistance by overfeeding on glycogen synthase. Overfeeding produced a reduction in the percent of active glycogen synthase.45

Taken together, these data indicate an important relationship between the regulation of glycogen synthase enzyme and in vivo insulin action. Further studies have indicated that glucose-6-phosphate levels in muscle fall rather than rise with the activation of glycogen synthase, and that increasing glucose uptake by increasing plasma glucose concentrations does not in itself increase glycogen synthase.50,51 These studies indicate that activation of glycogen synthase is dependent on insulin stimulation, rather than glucose uptake per se. Future work, aimed at determining the biochemical step at which defects in activation of glycogen synthase occur, will help to determine the specific biochemical defects in insulin resistance. Given that glucose uptake at supraphysiological insulin concentrations is highly familial22 and the major role of glucose storage and glycogen synthase activation at these insulin concentrations, it seems possible that a specific genetic defect that determines insulin resistance in many individuals may be located at a step between the insulin receptor and glycogen synthase activation.

D. Insulin Action and Muscle Fiber Type

There is good evidence for a relationship between insulin action and muscle fiber type in animals. Fibers that differ by staining and contractile properties (twitch speed) show differences in oxidative capacity, capillary supply, tyrosine kinase activity, and insulin stimulated glucose uptake.52-60 In animals, fibers of different twitch characteristics differ in their insulin sensitivity and responsiveness, although the mechanism is unknown. We have demonstrated a correlation of fiber type and glucose uptake at physiological and supraphysiological insulin concentrations (for percent type I fibers: $r = 0.29, p < 0.02, r = 0.29, p < 0.03$, respectively; for IIB fibers: $r = -0.38, p < 0.003, r = -0.32, p < 0.01$, respectively) (Figure 13).61 Whether this is a direct effect, or an effect of an association with obesity or capillary density, is not certain since in our data both obesity (Figure 14) and capillary density correlated with fiber type (for type I fibers: $r = -0.32, p < 0.01, r = 0.39, p < 0.002$, respectively; for type IIB fibers: $r = 0.32, p < 0.02, r = -0.27, p < 0.04$, respectively) (findings illustrated in Figure 14B). However, since fiber type may be inherited,62 the findings might also provide a mechanism for the familial dependence of insulin action.22

E. Insulin Action and Muscle Capillary Density

Several human studies have indicated that muscle cell size and muscle capillary supply are
associated with changes in fasting insulin concentrations and glucose tolerance.\textsuperscript{52-55} We have investigated the relationship of muscle capillary density in vastus lateralis and in vivo insulin action measured by the euglycemic hyperinsulinemic clamp.\textsuperscript{61} We found the capillary density very significantly correlated with insulin-mediated glucose uptake at physiological (Figure 15) and supra-physiological plasma insulin concentrations ($r = 0.63$, $r = 0.47$, $p \leq 0.0001$ for both). Capillary density also correlated significantly with fasting glucose (Figure 16) and fasting insulin concentration ($r = -0.46$, $r = -0.47$, $p \leq 0.0001$ for both). Capillary density was lower in the most obese ($r = -0.59$, $p = 0.0001$) (Figure 17) (illustrated in Figure 14B) and muscle cell size was greater ($r = 0.39$, $p < 0.002$).

These data may be interpreted in several ways. Capillary density is correlated with oxidative capacity of muscle cells.\textsuperscript{52,53,59,60} These correlations therefore might be due to a correlation between some biochemical mechanism associated with oxidative capacity and insulin action, but there is currently no evidence to support this
contention. An alternative explanation is that since oxidative capacity and twitch characteristics are correlated, they perhaps the induction of slow twitch genes also induces some biochemical changes that lead to alteration in insulin insensitivity (such as tyrosine kinase activity). Since the correlation of capillary density and insulin action was stronger than those of fiber type and insulin action in our data, this is not a wholly satisfactory explanation for the findings.

Reduced capillary density might indicate that blood flow to skeletal muscle is reduced. At rest not all capillaries have significant flow, and anatomical studies do not permit an assessment of which or how many capillaries are in use. Substrate uptake into tissues is regulated both by

**Figure 14B.** A cross-section of skeletal muscle. This illustrates the following: fiber size correlates with obesity (r = 0.39, p < 0.002). Capillary density negatively correlates with obesity (r = −0.59, p ≤ 0.0001). Percent of type 1 fibers was lower and type 2B greater in the obese (r = −0.32, p < 0.01, r = 0.32, p < 0.02, respectively). (Percent type 2A fibers was not correlated with % fat or W/T ratio.) Capillaries/mm² correlated with percentage of type 1 fibers (r = 0.39, p < 0.002) and negatively with % type 2B fibers (r = −0.27, p < 0.04). Drawing is similar to an actual microscopic preparation except that capillary diameter is not drawn to scale.

**Figure 15.** Relationship of glucose uptake at submaximally stimulating insulin concentrations and capillary density. The relationship is nonlinear and therefore plotted on a log scale for M (r = 0.63, p < 0.0001). X, Indians; +, Caucasians.
blood flow and its ability to diffuse into the tissue. A low extraction ratio, i.e., a small or modest decrease of insulin or glucose concentration between artery and vein, is direct evidence that diffusion, and not blood flow, may be primarily limiting their uptake. Recently, James et al. reported that blood flow was not a factor in determining insulin resistance in rats. We suggest therefore that a possible explanation for the association of insulin resistance with capillary density relates to biophysical changes in muscle that result from enlarged muscle cells and greater diffusion distances from capillaries to tissues. The following interpretations of capillary density are based on

the principle first proposed by Krogh—that since capillaries are aligned parallel to muscle fibers, each capillary supplies a cylinder of tissue. This cylinder varies in radius with the capillary density.

With enlargement of muscle cells, the surface to volume ratio of cells is decreased. Therefore, to obtain equivalent insulin action or glucose uptake per unit muscle mass, the insulin action/concentration or glucose uptake per unit cell surface area must be increased. Therefore, enlargement of muscle cells irrespective of the capillary supply will reduce insulin action.

With greater inter-capillary distances, there is a greater distance for insulin or glucose to diffuse
to all parts of muscle cells. Diffusion of insulin or glucose over 50U to 100U—the distance between capillaries—would be rapid in simple aqueous solutions. The cleft between muscle fibers may, however, be very narrow (71–0.1U),73 and this could possibly slow the rate of diffusion of insulin.

More important than just the diffusion distance, however, is the effect that an increased distance between capillaries might have on the ability of insulin to equilibrate within the muscle tissue. Two factors are involved in this equilibration: (1) when blood insulin concentration changes, insulin must pass through capillary walls and deep into interfiber clefts; and (2) muscle cell membrane is presumably constantly taking up insulin and degrading it. Capillaries in skeletal muscle are unfenestrated,74 hence they present a barrier between blood plasma and cell wall, especially for larger molecules such as insulin.

There are several lines of evidence that suggest that insulin penetrates the capillary wall slowly and equilibrates in tissues slowly. In spite of active transport of insulin through the capillary wall,75 current evidence suggests that diffusion of insulin through the capillary wall is the same or worse than insulin a molecule of similar size.76,77 On insulin infusion, arterial insulins are not matched by equivalent peaks in limb lymph and the lymphatic peaks are delayed 30–45 minutes (Figure 18).78,79 Glucose peaks in limb lymph are not delayed like this. Neither is there this much delay in insulin appearance in lymph draining the liver (where capillaries are fenestrated), nor in thoracic duct lymph.78,79 In the basal state, limb lymph insulin concentrations are apparently lower than arterial concentrations.78,79 Since lymphatics do not drain interfiber areas,80 the differences between arterial insulin and deep muscle insulin may be even more marked than lymphatic insulin indicates.

Studies of insulin kinetics81 have indicated that insulin equilibrates slowly (greater than 60 minutes) with a nonvascular compartment. Glucose uptake closely follows the calculated insulin concentration in this compartment which was thought to represent interstitial space in muscle and adipose tissue (Figure 19).81 These two lines of evidence indicate that diffusion of insulin into skeletal muscle is sufficiently slow to have important physiological effects.

If muscle cell membranes are able to take up and degrade insulin more rapidly than the diffusion of insulin from capillary to interstitial space and down between fibers, then the following principles apply at steady state: (1) there is a concentration drop of insulin across the capillary wall; (2)
with reduced capillary density, each capillary has a larger volume of tissue and greater area of cell membrane to supply—and the insulin concentrations drop is likely to be greater; (3) there will be a further concentration drop of insulin between interstitial space adjacent to the capillary and the most distance parts of the muscle cell; (4) this drop will be exaggerated with greater diffusion distances; (5) if so, the overall effect is that the average insulin concentration around a muscle cell with a poorer capillary supply will be reduced even at steady state (Figure 20). We propose that the correlations of fasting glucose, fasting insulin, and insulin resistance measurements with capillary density may be explained by these mechanisms.

Our discussion thus far has been concerned with the effect of capillary density on insulin access to muscle. We now wish to extend this to suggest that insulin action is reduced in obese subjects because lower capillary density results in reduced access of insulin to the muscles of obese subjects. Obese subjects have an increased size of visceral cells, and several studies also indicate muscle cells are enlarged in the obese. This increase in muscle fiber size is not simply due to increased tissue fat which is found in only small amounts in skeletal muscle. Fat-free mass increases with obesity (Figure 4); and since muscle cells do not multiply, they must hypertrophy. The number of capillaries per fiber was not increased in obesity in our data, so that obese subjects had lower capillary densities (r = -0.59, p ≤ 0.0001). We propose therefore that at least some of the insulin resistance associated with obesity is explained by the effects of capillary density on insulin action.

There are several other observations that might be explained by an association of obesity with reduced capillary density. We have reported that obesity explains much less of the variability of insulin action at supraphysiological plasma insulin concentration (~19%) than at lower plasma insulin concentrations (~43%). This might be because very high insulin concentrations overwhelm the effects of capillary density on insulin diffusion and the effect of decreased surface to volume ratio of muscle cells on insulin action.

Several studies have observed a delay in onset of insulin action in obese subjects. Prager et al. noted that the onset of insulin action was delayed in the obese. Higher insulin concentrations in both

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**Figure 19.** Computer-derived estimates of insulin levels in model compartments for primed-continuous infusions of insulin. Compartment 3 is a large and slowly equilibrating compartment that may represent interstitial fluid of muscle and adipose tissue. Note that glucose uptake follows the insulin concentration in this compartment not the plasma compartment (compartment 1). (Redrawn from Sherwin RS, Kramer KJ, Tobin JD, Insel PA, Liljenquist JE, Berman M, and Andres R, J Clin Invest 1974.)
Figure 20. A demonstration of the theoretical fall in hormone or substrate concentrations from capillary to deep in the interfiber space. The scale on the y axis is given solely for illustrative purposes. The scale on the x axis represents the extremes in our data and the two curves represent (1) high capillary density and small diffusion radius (25 μ), and (2) low capillary density and large diffusion radius (45 μ). The figure is designed to show the possible effect of capillary wall on insulin concentrations and the possible effect of the interfiber space on concentrations. The relative proportions of the capillary wall or interfiber space effect are not known and the absolute values are not known. The figure is also designed to show the possible effects of decreased capillary density. Such as occurs in obesity. Future research needs to be directed at determining if these gradients occur. Support for this analysis is given by reports of whole-body insulin kinetics in obese subjects. In obese subjects at steady state, tissue insulin concentrations are reduced relative to plasma insulin concentrations compared to lean subjects (26% reduction of tissue insulin in the obese for the same plasma insulin).\textsuperscript{47} mf, muscle fiber; w, width of interfiber space. Cc, capillary insulin concentration; Co, insulin concentration immediately outside capillary; Cr, concentration of insulin at radial distance R; Rc, capillary radius; Rt, radius of tissue cylinder. The development of this mathematical model is based on the principles that insulin diffuses from the capillary and is taken up and degraded by muscle cell membrane. We are grateful to Dr. Timothy Secomb of the Department of Physiology at the University of Arizona in Tucson for his theoretical analysis of possible insulin gradients in muscle.
Figure 21. Time course of glucose uptake in lean and obese subjects at four insulin infusion rates: -15, 40, 120, and 1,200 mU/m²/minute. The insulin infusions were started at a constant rate (i.e., not primed as in Figure 18), but T1/2 to reach an insulin steady state was the same in lean and obese. Note the delayed activation of glucose uptake in the obese and the fact that higher insulin infusions appear to overcome this delay. (Redrawn from Prager R, Wallace P, and Olefsky JM, J Clin Invest, 78:472–481, 1986 by permission of the American Society for Clinical Investigation.) Compare this figure with Figure 19. These data suggest reduced penetration of insulin into skeletal muscle in obesity. Furthermore, kinetic analyses suggest that in the obese at steady state tissue insulin concentrations are reduced at any given plasma insulin concentration compared to lean subjects.87

the lean and the obese accelerated the onset of insulin action, suggesting that higher concentrations could overcome the kinetic defect (Figure 21). A comparison of Figures 19 and 21 suggests insulin’s equilibration in muscle is delayed in the obese. Parenthetically insulin action is not delayed in obese subjects in the liver—an organ with fenestrated capillaries even though glucagon does not suppress as well in the obese.86 Doeden et al.86 also noted a delayed onset of action of insulin in obese subjects, the effect of which was most noticeable while changing plasma insulin concentrations.

Finally, studies of whole-body insulin kinetics suggest that in obese subjects the steady-state insulin concentration in tissue is reduced relative to plasma in obese subjects compared to lean subjects. The obese subject’s tissue insulins are 26% lower than in lean subjects at the same plasma insulin concentration.87 Qualitatively this is what we have predicted might occur if capillary density is reduced (Figure 20). All of these studies suggest that in obese subjects the insulin penetration into skeletal muscle is reduced and its equilibration in muscle may be slowed. We propose that this might be an effect that is due to reduced capillary supply to skeletal muscle in obese subjects.

We conclude that the correlation of insulin resistance and basal insulin and glucose levels with capillary density may be a result of biophysical restraints on glucose and insulin access to cells. Furthermore, the effects of obesity on capillary density may partially explain the relationship of insulin resistance and obesity, explain the poor correlation of obesity and insulin action at supraphysiological plasma concentrations of insulin, and explain the delayed onset of action of insulin in the obese. Finally, the development of insulin resistance and slightly elevated plasma glucose concentrations that signal the pancreas that insulin resistance is present might initiate a vicious cycle of pancreatic glucose insensitivity, further hyperglycemia, and noninsulin-dependent diabetes mellitus (see Ref. 22).

VII. SUMMARY

Diabetes and obesity are epidemic in the Pima Indians of the Southwestern United States, and the prevalence of diabetes is increasing. The most
likely link between obesity and diabetes is tissue insulin resistance.

If obesity is defined as an excess of body fat, then it can only be accurately assessed by measurements of body composition and not by approximations such as body mass index or percent of ideal weight.

To compare the metabolic data of individuals of varying size, an accurate measure of metabolic size is needed. Total body weight is not an appropriate means of comparing individuals since obese subjects have a greater proportion of nonmetabolizing mass (triglyceride). Body surface area shows a sex difference, and this may distort data if both sexes are present. From studies of metabolic rate we have determined that metabolic rate is directly proportional to the fat-free mass plus 18 kg, and we suggest that this weight can be equated with metabolic size.

Glucose storage in skeletal muscle appears to be important in the disposal of an intravenous glucose load. Consistent with its role in glycogen storage, glycogen synthase enzyme is activated in proportion to the ability to dispose of glucose during a hyperinsulinemic, euglycemic clamp. The role of glycogen synthase is most notable at supraphysiological plasma insulin concentrations; and since glucose uptake at these insulin concentrations is highly familial independent of the degree of obesity, we suggest that there may be a specific genetic defect expressed in skeletal muscle that reduces insulin responsiveness in some subjects.

The lack of correlation between 24 hour respiratory quotient measured in a metabolic chamber (a measure of the proportion of fat derived calories) and degree of obesity indicates that in obese Pima Indians insulin resistance is not due to an inhibition of glucose metabolism by free fatty acids (glucose–fatty acid–ketone cycle).

Obesity is associated with an increase in fat-free mass almost kilogram- for kilogram with fat mass when compared to the lean state. A role for this increase in fat-free tissue in producing insulin resistance has been given insufficient attention in the past. With an increase in fat-free mass, muscle cells are hypertrophied and capillaries in muscle are more widely spaced. We propose that these biophysical changes in muscle mediate, at least in part, the effects of obesity to produce a reduction in insulin sensitivity and the abnormal kinetics of insulin action found in the obese. We suggest therefore that insulin resistance is a combination of a genetic defect and obesity-induced changes in the biophysical properties of skeletal muscle.

These defects may in turn lead to the development of non-insulin-dependent diabetes mellitus.

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References

9. Knowler WC, Pettitt DJ, Savage PJ, and Bennett PH:


41. Mandarino LJ, Wright KS, Verify LS, Nichols J, Bell JM, Koltermann O, and Bock-Nielsen H: Effects of insulin infusion on human skeletal muscle pyruvate dehydrogenase, phosphofructokinase, and glyco-


71. James DE, Burleigh KM, Storlien CH, Bennett SP,


