Metabolic Effects of Steroid Hormones in Skeletal Muscle

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Abstract

Insulin resistance is a cornerstone of the metabolic abnormalities in abdominal obesity in both men and women, and a main location of this abnormality is muscle tissue. Since it might be considered a possibility that steroid hormones are important regulators of insulin sensitivity in muscle, and since there are several abnormalities in the secretions of steroid hormones in abdominal obesity, there is a need to determine the effects of steroid hormones on the regulation of insulin sensitivity in muscle. These abnormal hormonal secretions will probably exert an influence on the metabolic regulation of peripheral tissues.

After hormonal manipulation in rats, insulin sensitivity was followed by euglycemic, hyperinsulinemic glucose clamp measurements. An index of insulin-stimulated glucose transport was obtained in the white gastrocnemius (WG), extensor digitorum longus (EDL), red gastrocnemius (RG), and soleus (Sol) muscles after a bolus dose of 2-^{3}H-deoxyglucose (2-DOG) when steady state was obtained in the clamp measurements. Glycogen synthesis was measured similarly with ^{14}C-glucose as a labelled precursor after isolation of glycogen in the muscles mentioned, and in the liver. Maximal velocity and fractional velocity of the insulin-sensitive part of glycogen synthase (FV%) were measured in the muscles as well as muscle fibre composition and capillary density.

Female rats were given testosterone (T) in a low dose for 12 weeks to mimic the hyperandrogenic status of insulin-resistant women. This resulted in a marked decrease in insulin-stimulated glucose transport. T inhibited glycogen synthesis and 2-DOG transport in all muscles examined. T administration also resulted in changes in muscle morphology; the relative number of type 1 fibres decreased, whereas type 2 fibres increased. There was also a decrease in capillary density after T treatment. With exposure times between 24 h and 48 h, marked insulin resistance was found but only minor changes in muscle morphology.

In male rats, supraphysiological concentrations of T seem to have the same effect as in females, creating insulin resistance in muscle tissue. Low T levels induced by castration were also followed by a similar picture, reversible by T substitution to the levels of controls.

After cortisol exposure, female rats already showed diminished insulin sensitivity in the clamp measurements after 24 h. This was paralleled by a decrease in glycogen synthesis with a marked inhibition at FV%. No effects on the 2-DOG uptake were seen until after 48 h of exposure.

In conclusion, steroid hormones are important regulators of muscular insulin sensitivity. Our studies suggest that they exert this action probably mainly by influencing the regulation of glycogen synthesis.

Key words: Glucocorticoids, insulin resistance, skeletal muscle, steroid hormones, testosterone.

BAM 3 (2): 133-141, 1993

Although the statistical relationship between obesity and non-insulin dependent diabetes mellitus (NIDDM) is established since long, the potential association between obesity and cardiovascular disease (CVD) has been less clear, and controversial results have been reported [62]. Recent prospective epidemological studies have now indicated that only a subgroup of obesity is associated with CVD, namely those subjects who have excess adipose

tissue triglycerides accumulated in abdominal regions. Abdominal obesity is also a predictor of stroke, and more strongly associated with NIDDM than generalized obesity. In addition, established risk factors, such as elevated concentrations of plasma lipids and insulin, hypertension and smoking are all statistically associated with an abnormally high proportion of abdominally localized depot fat, particularly when accumulated in visceral depots [7].

The heterogenicity of human obesity has been apparent since long and different modes of subdivision have tentatively been applied. One of the earliest is that of Jean Vague [66], who distinguished the groups of android (male type) and gynoid (female type) types of obesity. Vague described the coupling of disease and risk factors to android obesity both in men and women. Android and abdominal (visceral) obesity are entities with a large overlap.

Abdominal obesity is associated with a number of endocrine aberrations. The hypothalamo-adrenal axis seems to be sensitive to stress at different levels [34] presumably resulting in frequent episodes of increased cortisol secretion. Furthermore, sex steroid hormone secretions are disturbed. Hyperandrogenicity in women has been established by several investigators [34, 45, 67]. Both men and women have disturbed secretions of sex-specific steroid hormones, such that men with visceral obesity have low testosterone (T) values. Women frequently do not ovulate regularly, and have low progesterone production. In addition, recent studies have shown that an inverse proportionality exists between concentrations of insulin-like growth factor I (IGF-I) and visceral contents of fat, suggesting that visceral obesity also is characterized by a relative deficiency of growth hormone (GH) secretion [50].

These abnormal hormonal secretions will probably exert an influence on the metabolic regulation of peripheral tissues. For example, the insulin resistance that follows an increased exposure of endogenous or exogenous glucocorticoids is well established. The mechanism and localization of this effect is, however, only partially known, as will be reviewed in a following section. The effects on metabolic regulation of sex steroid hormones are, surprisingly, known to a much lesser extent.

Insulin resistance is a cornerstone of the metabolic abnormalities in abdominal obesity in both men and women, and a main localization of this abnormality is muscle tissue [19, 37, 38]. Since it might be considered a possibility that steroid hormones are important regulators of insulin sensitivity in muscle, and since there are several abnormalities in the secretions of steroid hormones in abdominal obesity, there is a need to determine the effects of steroid hormones on the regulation of insulin sensitivity in muscle.

Insulin resistance in skeletal muscle

Skeletal muscle is an important target site for insulin since it represents 40-45% of total body weight and glucose is one of its major substrates. Non-insulin-dependent diabetes mellitus (NIDDM) is characterized by defects in insulin secretion and in tissue sensitivity to insulin. Muscle is generally believed to represent the principal site of insulin resistance in NIDDM. Once taken up by the muscle, glucose can be oxidized to carbon dioxide, converted to lactate, which is released into the blood, or stored as glycogen. The insulin resistance in NIDDM occurs in both oxidative and non-oxidative pathways, although the predominant defect apparently is in non-oxidative glucose metabolism [9, 15, 17, 20, 32, 48]. The reduced insulin-stimulated glucose disposal in NIDDM has been suggested to be due primarily to decreased rates of muscle glucose storage as glycogen [61]. The rate limiting key enzyme in this pathway is glycogen synthase [41, 47]. Muscle glycogen synthase is dephosphorylated and stimulated covalently by insulin [47, 68], and a close correlation between in vivo insulin-stimulated muscle glycogen synthase activity, and whole body nonoxidative glucose disposal has been demonstrated in normal subjects [15, 17, 47] and in patients with NIDDM [15, 17]. However, it is possible that decreased glucose storage could occur solely as a consequence of decreased glucose uptake, and that impaired activation of glycogen synthase is a secondary effect [15]. Another important question is whether the glycogen synthase defect is due to high concentrations of plasma free fatty acid values (FFA). The inhibitory effects of FFA on glucose uptake in muscle are well-known as the glucose-fatty acid cycle [56]. Chronic elevation of FFA might lead to the development of insulin resistance by promoting excess accumulation of glycogen and lipid stores in skeletal muscle [31].

In addition to cellular biochemical defects, the arrangement of capillaries and muscle fibers may play a role in determining in vivo insulin action, suggesting that diffusion distance from capillary to muscle cells and fiber type are of importance [24, 43, 44, 46]. It is well established that there is a wide variation in insulin-sensitive glucose uptake [28, 30] among muscles of various fiber types. Three metabolically distinct types of muscle fibers have been classified [13]. In humans, unlike the laboratory rat, there is a heterogenicity of the distribution and percentage of muscle fibers within a given muscle [13]. The three types of muscle fibers are known as fast-twitch glycolytic, (FG, type 2b), fast-twitch-oxidative-glycolytic (FOG, type 2a), and slow-twitch oxidative (SO, type 1). The fast-twitch, white (FG and FOG) and slow-twitch, red fibers (SO), differ markedly in their physiologic contractile properties [53], and the FOG, FG, and SO have unique biochemical profiles [6, 21] that predispose them to glycolysis and/or oxidative metabolism. The more oxidative (red) skeletal muscles demonstrate a higher degree of insulin sensitivity than the glycolytic (white) muscles. This is presumably due to differences in binding affinity and density of the insulin receptor [30, 36].

Glucocorticoids

Whereas the effects of insulin are anabolic, those of the glucocorticoids are largely catabolic [3, 57]. Glucocorticoid excess is associated with increased glucose production in liver [3], decreased peripheral glucose transport and

utilization [3, 18, 25, 51, 52, 65, 66], decreased protein synthesis and increased protein degradation in muscle [57]. The mechanism by which glucocorticoids antagonize the effects of insulin is poorly understood. Alterations in insulin binding to its receptor have been reported after administration of various glucocorticoids [3, 18, 25, 35, 66]. The results vary, however, with type of glucocorticoid [18], duration of administration [66], type of tissue studied [66], and whether the glucocorticoid is administered *in vivo* or *in vitro* [18, 25]. No consistent correlation between alterations in binding and altered insulin response has been observed in various hypercortisolemic states, leading to the conclusion that a postbinding mechanism is responsible for the insulin resistance [18, 25, 52]. In addition to different effects, glucocorticods may induce insulin resistance indirectly by increasing the levels of FFA.

The effects of corticosteroids on muscle wasting are well known. Changes in fiber composition also occur. In a recent study it was found that patients with Cushing's disease had a marked decrease in the percentage of red fibers and an increase in that of white muscle fibers [60]. Similar findings have been reported after administration of corticosteroids [16]. We recently studied the effects of cortisol on insulin sensitivity in muscle by utilizing the euglycemic glucose clamp in rats, after manipulation of the endocrine status [22]. Cortisol was then shown to exert a marked insulin resistance after only 24 hours exposure in moderate doses. This was associated with marked insulin resistance in muscle mainly at the level of glycogen synthesis and activity of the insulin sensitive glycogen synthase. No changes in muscle morphology or capillary density were found.

Androgens

The association of a hyperandrogenic state and glucose intolerance was first noticed by Achard and Thiers [1] in their description of "diabetes of bearded women" in 1921. Hyperandrogenicity and obesity usually appear in parallel in such syndromes. It has therefore been difficult to separate the potential roles of these two factors in the pathogenesis of diabetes. In recent studies strong correlations between plasma testosterone and insulin concentrations remain when only non-obese hyperandrogenic women have been examined, suggesting the possibility of a cause-effect relationship between androgens and insulin resistance in women [12, 59]. Although a close relationship between insulin resistance and hyperandrogenism is well established, it remains unclear which of these two abnormalities is the primary event. Some authors have the opinion that hyperandrogenism produces insulin resistance [14, 40]. But on the other hand, there is also evidence that hyperinsulinemia may act as a gonadotropic hormone, suggesting that androgen elevation is secondary to hyperinsulinemia [54]. It also seems possible from several previous reports that steroid hormones exert a strong influence on muscle fiber composition. Estrogen causes a diminution of fiber diameter [64]. The effects of excess androgens on glucose disposal may be mediated by specific androgen receptors in human skeletal muscle [55, 63]. Testosterone is likely to be the major androgen influencing skeletal muscle, since muscle has minimal 5α-reductase activity [5] which is in contrast to other androgen target tissues. Furthermore, chronic exposure to androgens in castrated animals increases the percentage of fast twitch muscle fibers [39].

In our experiments, female rats were, rendered slightly hyperandrogenic by administration of T [26, 27]. Studies were performed after 3 months, 2 weeks (unpublished experiments), 1 week, 48 and 24 h. Although minor signs of insulin resistance were found already after 24 h exposure, a marked influence was seen after 48 h (Fig. 1). Dose-response experiments showed that the resistance was found only at submaximal and not maximal insulin concentrations, suggesting that a decreased sensitivity rather than responsiveness was the effects of T exposure. This in turn would indicate effects at the level of the insulin receptor. In muscle, glycogen synthesis and the activity of glycogen synthase (GS) were severely inhibited. There was also some inhibition of glucose transport, as examined with 2-DOG radioactivity in muscle. Other potential inhibitors of insulin sensitivity such as plasma FFA or cortisol were not elevated, as well as glycerol, an index of lipolytic activity. Furthermore, estrogen levels were not elevated, and preliminary studies suggest that dihydro-T, is not active (A. Holmäng, unpublished). These studies then suggest that T by itself is exerting its effects resulting in severe muscular insulin resistance in female rats. Preliminary experiments suggest that the total number, and the function of the tyrosine kinase of the receptors are not

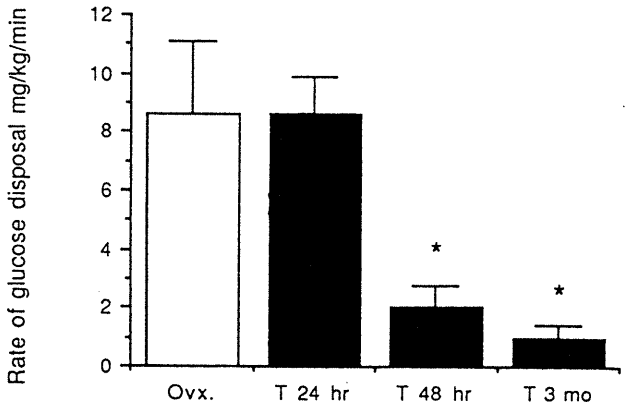

*Figure 1. Whole body glucose disposal during hyperinsulinemic, euglycemic clamp measurements in oophorectomized rats (OVX) and oophorectomized rats treated with testosterone (T) for 24 and 48 h and 3 mo. Euglycemic clamp measurements were performed at ~7mmol/l concentrations of glucose and insulin concentrations of 169+10 and 177± 13 μU/ml [not significant(NS)] for control rats and testosterone-treated rats, respectively. *p < 0.05. From Holmäng et al. [26]. © Am. J. Phys. 1992.*

involved (A. Holmäng, unpublished). This does not exclude aberrations in insulin receptor traffic between intracellular and extracellular pools, however. The total number of glucose transporter 4 is not changed either (A. Holmäng, unpublished), and the kinetics of glucose transports are currently being studied.

In summary, the results obtained suggest that T in female rats exerts a rapid and dramatic effect to produce muscular insulin resistance. Whether the primary locus of effect is at the level of the insulin receptor, glucose transport or at a post-receptor site such as insulin regulation of GS can not be decided as yet, although the GS system seems to be most markedly inhibited. The muscle morphology changed with a decreased number of type I fibers and an elevated number of type II, particularly type II b fibers (fig. 2 a, b). Furthermore, capillarization was severely diminished (fig. 3 a, b). This occurred apparently already after 48 h exposure, albeit to a small extent. The effects of T on insulin resistance and fiber composition and capillarization can at present not be separated in time. More precise and direct methods are needed for this purpose.

In male rats castration was followed by a severe insulin resistance [23]. Substitution with T to control values normalized insulin sensitivity completely, while excess doses again produced insulin resistance. The main site of action seemed to be on glycogen synthesis and GS in muscle. In combination with other studies [34] the data suggest similar morphological changes in muscle as after administration of T to female rats. These studies then show that administration of T to females and males to sex-specific excess is followed by muscular insulin resistance and similar morphological changes in muscle. Interestingly, deficiency of T is also associated with muscular insulin resistance in males, as suggested by the castration-substitution experiments. In males it thus appears that a window of normal T values is followed by optimal muscular insulin sensitivity. In female rats this window is set very low, and it may well be that the lower limit can not be defined.

Muscle morphology

Changes in muscle morphology followed the intervention studies where insulin resistance was produced. This was the case with excess T in both sexes, and was of the same character, namely a decrease of type I and an increase of type II, particularly type II b fibers. Muscles with a high proportion of type 1, highly oxidative, slow twitch fibers are reported to be insulin sensitive, and to have a high density of insulin receptors, while the reverse seems to be the case with type II, glycolytic, fast twitch fibers, particularly type II b fibers [30, 10]. One might then speculate that the change in muscular fiber composition actually caused the insulin resistance because of a transformation of muscle to a state with a lower density of insulin receptors. However, in preliminary experiments in hyperandrogenic rats insulin receptor density was not decreased, in spite of the changes in muscle fiber composition (A. Holmäng, unpublished). This finding opened up the possibility of the reverse chain of events, hyperinsulinemia causing the muscle fiber shift. This is apparently more likely from another aspect. Several unrelated conditions such as physi-

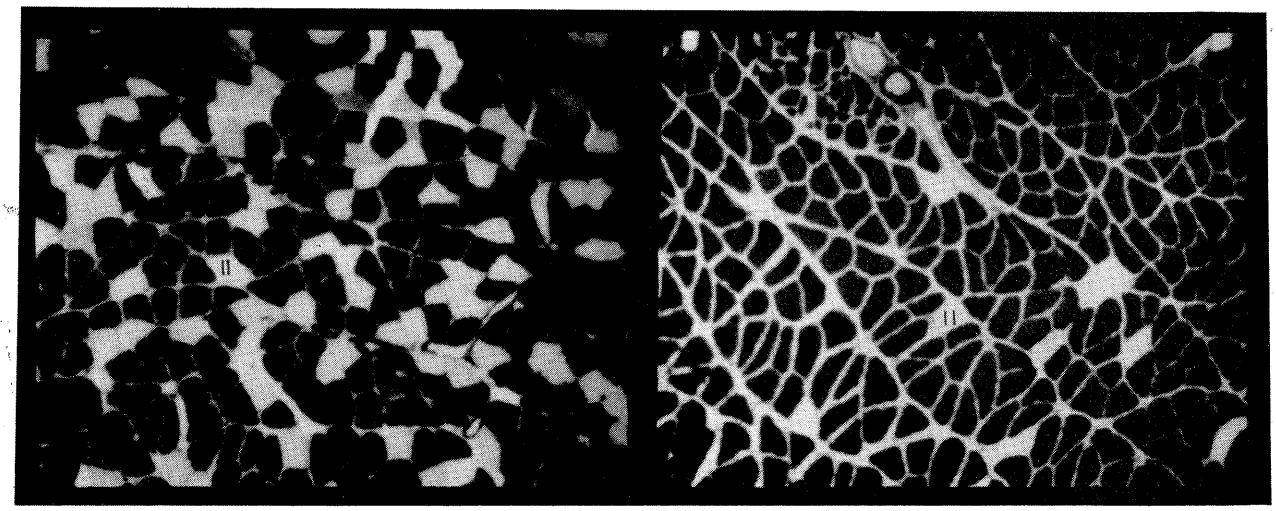

Figure 2. Photomicrographs of 10-μm transverse sections of soleus muscle before (a) and after (b) testosterone treatment of oophorectomized rat. Muscles are stained for myofibrillar ATPase after preincubation at pH 4.3. Type 1 fibers appear dark; type 2 fibers appear white. Magnification x200. From Holmäng et al. [27]. © Am. J. Phys. 1991.

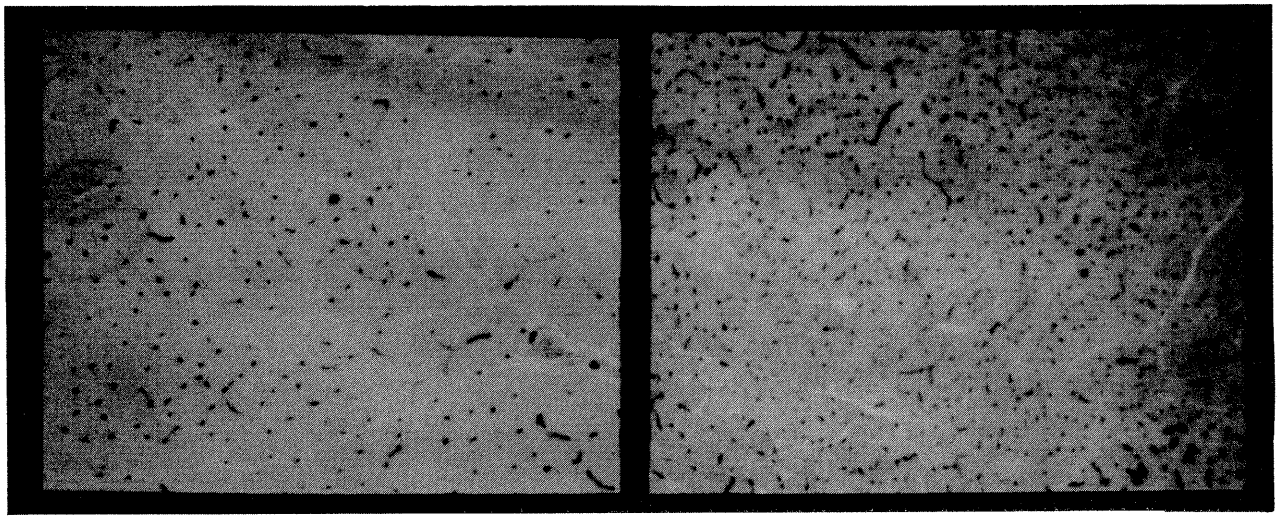

Figure 3. Capillaries in soleus before (a) and after (b) testosterone treatment of oophorectomized rat. Capillary phosphatase staining. Magnification x200 (a) and x400 (b). From Holmäng et al. [27]. © Am. J. Phys. 1991.

cal inactivity [8], visceral obesity [43], NIDDM [42] and several steroid hormone abnormalities are followed by the same muscle composition change, namely a low type I / type II ratio of fibers. It may well be that the common denominator of these conditions, the insulin resistance with hyperinsulinemia, is the primary factor.

In order to test this possibility rats have been exposed to chronic hyperinsulinemia, with controlled secretion of hormones with antiinsulin effects. This was followed by a similar muscle fiber change as in the hyperinsulinemic, insulin resistant conditions mentioned above (fig. 4 a, b). The resulting effect on insulin sensitivity was an increase rather than a deterioration. These results suggest that hyperinsulinemia is contributing to the shift in muscle fiber composition seen after steroid hormones rather than the reverse sequence of events [25]. Muscle fiber composition changes in conditions of insulin resistance may therefore be a consequence rather than a cause of insulin resistance and hyperinsulinemia. Capillarization of muscle was also low after administration of T in excess. This has also been reported in several insulin resistant conditions including physical inactivity [4], abdominal obesity [43], NIDDM [42] as well as in our studies after exposure of excess of steroid hormones. Again the question of cause-effect is of

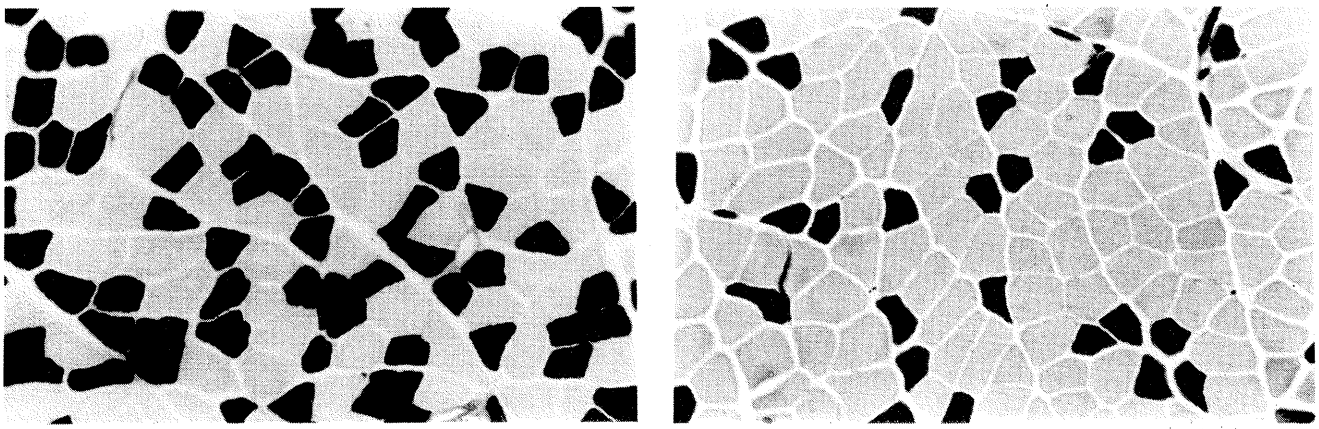

Figure 4. Fiber composition in the soleus muscle of controls (a) and adrenalectomized rats treated with insulin, cortisol, and propranolol (b). Staining for myofibrillar ATPase at pH 10.3. Type 1 fibers appear white; type 2 fibers appear dark. Magnification x328. From Holmäng et al. [25]. © American Diabetes Association, Inc. 1993.

interest. There is much evidence available to suggest that muscle capillaries are involved in the regulation of muscular insulin sensitivity. First, the effects of insulin on glucose transport in muscle are much more closely related to the lymphatic (presumably mirroring extracellular) concentrations of insulin than those occurring in plasma [69]. The equilibration of extravascular concentrations of insulin is slower than in circulation, and does not reach the concentrations in plasma [29, 69]. These data suggest a capillary barrier for insulin transport from circulation to its active site at the level of the muscle cell. Furthermore, the insulin space in muscle is larger than that of inulin, a molecule of the same size, again suggesting a barrier and, in addition, a pool of stored insulin in the capillaries [24]. Insulin binds to capillary endothelium [11] and capillary binding has previously been suggested by indirect methods utilizing measurements of arteriovenous differences of insulin concentrations [58].

These data, taken together, strongly suggest that capillary endothelium is binding insulin in an intermediary pool, and that this might be rate-limiting for the resulting insulin concentrations near the insulin receptor, the insulin effector in muscle. It is therefore apparently logical that a diminution of the capacity of capillary endothelium to bind insulin in conditions with a relative scarcity of muscle capillaries would be followed by an apparent decrease of insulin effects on muscle, labelled insulin resistance. The low capillarization in the conditions mentioned above including hyperandrogenicity may in this way at least contribute to the creation of insulin resistance.

The studies of the effects of chronic hyperinsulinemia mentioned above support the importance of capillarization for regulation of insulin resistance. In these experiments an increased insulin sensitivity (Fig. 5) and muscular capillary density were closely parallel phenomena (Fig. 6 a, b). Furthermore, these results suggest that insulin may in

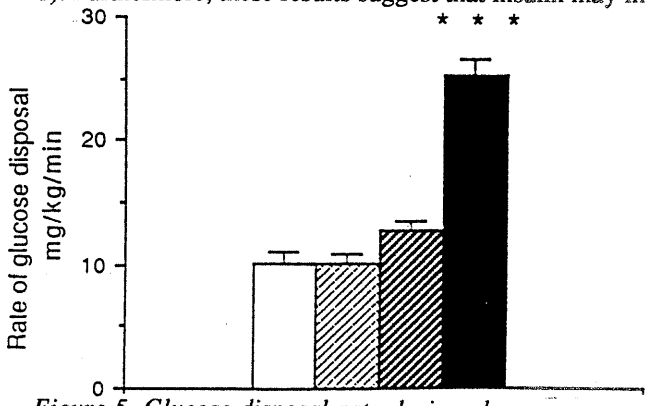

*Figure 5. Glucose disposal rate during clamp measurements in control rats (open column), saline treated rats (light, striped column), adrenalectomized rats treated with cortisol and propranolol (dark, striped column) and adrenalectomized rats treated with insulin, cortisol and propranolol (black columns). ***p > 0.001, ANOVA. Means ± SEM. From Holmäng et al. [25]. © American Diabetes Association, Inc. 1993.*

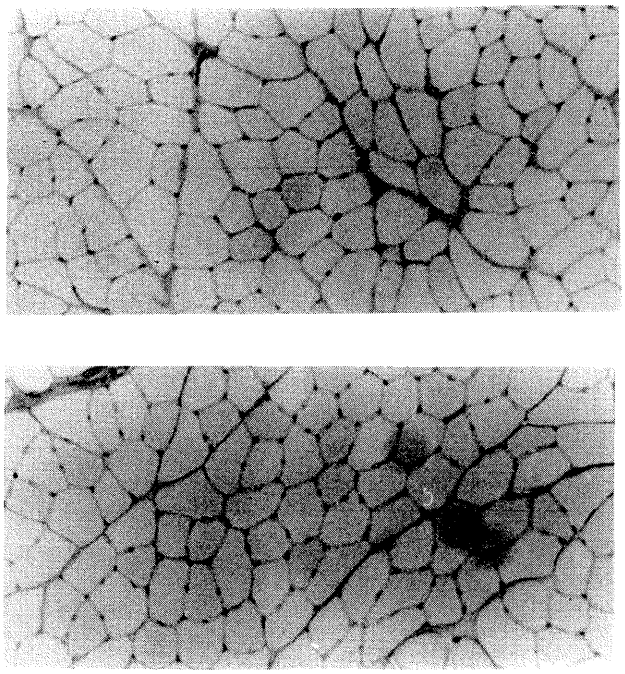

Figure 6. Capillaries in the soleus muscle of controls (a) and adrenalectomized rats treated with insulin, cortisol and propranolol (b). Magnification x164. From Holmäng et al. [25]. © American Diabetes Association, Inc. 1993.

fact be a regulatory factor of capillary density [25]. Other studies have shown that insulin enhances the multiplication of isolated endothelial cells [33].

In contrast to the muscle fiber composition changes noted in the studies summarized here, the associated changes of capillary density in muscle may well be a regulatory factor for insulin sensitivity of muscle. Further studies of the regulation of capillary density and function seem to be of considerable importance for the understanding of the regulation of insulin resistance. For example, it has already been shown that physical training, followed by increased muscular capillarization in normal individuals, is not inducing capillary growth in patients with NIDDM [2]. Furthermore, visceral obesity, a precursor condition to NIDDM, is characterized by a low density of muscle capillaries. Capillary abnormalities are prevalent, sometimes already at the debut of NIDDM and constitute major clinical problems in the microangiopathies of several vital organs in NIDDM. The regulation of capillary growth and function is therefore a problem of central importance for understanding of insulin resistance in NIDDM and its precursor states, such as visceral obesity.

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