patients could sustain exercise at the same absolute work load for the full hour.

The following results are from the prolonged exercise test. The respiratory exchange ratio was higher in the thyrotoxic state (0.88) than after treatment (0.83), indicating preferential carbohydrate oxidation. Muscle glycogen appears to be the major fuel: in thyrotoxic patients the glycogen content in muscle was reduced by 192 μmol/min per g dry wt. upon 60 min exercise compared with 120 μmol after treatment. Both before and after treatment glycogen was depleted from slow-twitch muscle fibres whereas work-induced glycogen depletion from fast-twitch muscle fibres was most pronounced in the thyrotoxic state. The high rate of glycolysis is also reflected in the doubling of the 10 min lactate concentration in all the thyrotoxic patients compared with the values obtained after treatment. No exercise-induced change in plasma free fatty acids was found in the thyrotoxic state and this was not changed by 3 months of treatment.

The mitochondrial enzyme pattern was complex. Cytochrome oxidase activity measured by succinate-stimulated oxygen uptake was low in the thyrotoxic state (one-third to one-half normal values) as was the V_max of citrate synthase and 3-hydroxy-acyl-CoA dehydrogenase in three of the patients; the remaining two displayed approximately normal mitochondrial enzyme activities. None of the measured enzyme activities was changed after treatment.

The pronounced glycolysis and lactate formation observed during the prolonged exercise can be understood on the grounds of the low mitochondrial enzyme activity of the muscle tissue. To what extent the low capacity of the respiratory chain, which was unchanged with treatment, can also explain the very low physical working capacity is difficult to assess.

It is concluded that, in contrast to findings in species other than man, elevated levels of thyroid hormones may not induce enhanced aerobic metabolic potential of skeletal muscle. The low capacity of certain mitochondrial enzymes affects the metabolic response to exercise and probably also explains the observed low physical working capacity of the thyrotoxic patient. It is not readily explained why a 3 month period of euthyroid state during medical treatment does not restore normal activities of mitochondrial enzymes and working capacity.

In the present study, we have used Triton extracts of human placenta membranes (1984) from either mammary tissue from lactating fed rats or from epididymal adipose tissue and contained no detectable intrinsic autophosphorylating activity at 0°C. SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1970). Immunoprecipitation of acetyl-CoA carboxylase, trypsin digestion and two-dimensional electrophoresis were performed as described by Brownsey & Denton (1982).

On addition of insulin and [7,25]P]ATP to Triton extracts an increased, tyrosine-specific phosphorylation of a protein of apparent M_r 95 000 was seen (Fig. 1) (see Avruch et al., 1982b; Pike et al., 1984). This was confirmed as the β-subunit of the insulin receptor by immunoprecipitation with antibody to the insulin receptor (a kind gift of Professor C. R. Kahn, Joslin Diabetes Center, Boston, U.S.A.). Phosphorylation at 0°C was maximal after 30–60 min. In the following 3–4 h considerable dephosphorylation occurred. Both rat adipose and mammary tissue acetyl-CoA carboxylase (subunit M_r 230 000) when added to such extracts of placenta membranes at 0°C were found to be phosphorylated in a cyclic AMP-independent manner, primarily on serine residues. The time course of phosphorylation was similar to that of the insulin receptor and was maximal between 30 and 60 min. As shown in Fig. 1, the extent and rate of phosphorylation could be increased on addition of insulin. This effect was variable; in a series of five separate experiments carried out essentially as in Fig. 1, the increases in phosphorylation of acetyl-CoA carboxylase after 30 min incubation with [7,25]P]ATP were 160, 460, 17, 125 and 10%, while corresponding increases in phosphorylation of the insulin receptor were 120, 230, 370, 50 and 140%. There was no significant phosphorylation of acetyl-CoA carboxylase by insulin receptor preparations, which had been partially purified by chromatography on wheat-germ lectin-sepharose.

**Insulin-activated acetyl-CoA carboxylase kinase in Triton extracts of human placenta membranes**

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Insulin not only promotes the dephosphorylation of several proteins such as glycogen, synthase, pyruvate dehydrogenase and triacylglycerol lipase, but promotes the increased phosphorylation of many others including acetyl-CoA carboxylase, ATP-citrate lyase and S_6-ribosomal protein (for reviews see Denton et al., 1981; 1984; Avruch et al., 1982a). These increases in phosphorylation occur mainly on serine residues. To account for them, Denton et al. (1981) argued that an early event in the mechanism of action of insulin might be the activation and possibly release, of a membrane-associated serine protein kinase. Subsequently the insulin receptor itself was found to exhibit an insulin-activated tryosine protein kinase capable of phosphorylating the receptor β-subunit and exogenous substrates such as histone (Kasuga et al., 1982; Van Obberghen et al., 1984). No physiological substrates other than the β-subunit itself have been identified.

In the present study, we have used Triton extracts of human placenta membranes (which are particularly rich in insulin receptors) to explore the possible relationships between the receptor and serine protein kinase activity capable of phosphorylating added acetyl-CoA carboxylase.

Cude placenta membranes (Avruch et al., 1982b) and placental syncytiotrophoblast membranes (Smith et al., 1974) were prepared and 1% (v/v) Triton X-100 extracts centrifuged (60 000g for 30 min). Acetyl-CoA carboxylase was prepared essentially as described by Brownsey et al. (1984) from either mammary tissue from lactating fed rats or from epididymal adipose tissue and contained no detectable intrinsic autophosphorylating activity at 0°C. SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1970). Immunoprecipitation of acetyl-CoA carboxylase, trypsin digestion and two-dimensional electrophoresis were performed as described by Brownsey & Denton (1982).

Abbreviation used: SDS, sodium dodecyl sulphate.
Fig. 1. Effect of insulin on insulin receptor tyrosine kinase and acetyl-CoA carboxylase kinase activity in Triton extracts of human placenta membranes

Triton extracts of crude placenta membranes (approx. 400 μg of protein) were preincubated with or without insulin (1.1 μM) at 30°C for 15 min in 300 μl of 20 mM-Mops (4-morpholinepropanesulfonic acid) buffer, pH 7.4, containing 12 mM-MgCl2, 2 mM-MnCl2, 50 mM-NaF and 0.25 mM-dithiothreitol. Acetyl-CoA carboxylase (approx. 12 μg) was added, the reaction mixture cooled to 0°C and phosphorylation initiated by addition of [γ-32P]ATP (100 μM, approx. 500 c.p.m./pmol). Samples (60 μl) were taken at 5, 15, 30 and 60 min and terminated by the addition of 25 μl of a solution containing 20% (w/v) sucrose, 10% (w/v) SDS, 125 mM-Tris/Cl, pH 6.8, 1% 2-mercaptoethanol, boiled for 5 min and subjected to SDS/polyacrylamide-gel electrophoresis (10% gel). The Figure shows an autoradiograph of the dried gel.

Further studies explored the sites phosphorylated on acetyl-CoA carboxylase utilizing immunoprecipitation followed by trypsin digestion and two-dimensional phosho-peptide mapping. Incubations were carried out for 2 h in the presence of vanadate to obtain maximal phosphorylation. Both mammary and adipose tissue acetyl-CoA carboxylase were found to be phosphorylated on a number of sites. One of the major sites appeared to be the same as the site on the enzyme which exhibits increased phosphorylation in fat cells exposed to insulin (Brownsey & Denton, 1982). Insulin-dependent increases in the phosphorylation of this site was apparent but again variable and difficult to quantify.

These studies offer encouraging support for the idea that there may be a serine protein kinase closely associated with the insulin receptor and that this kinase can be activated when insulin binds to the receptor (Denton & Brownsey, 1983; Gazzano et al., 1983; Machicao et al., 1984). Further studies are needed to identify the basis of the variable effects of insulin in the system and then to explore the relationship between this serine protein kinase activity and that of the tyrosine protein kinase activity of the receptor itself.

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Vasopressin and glucagon activate key oxidative enzymes in rat liver by increasing intramitochondrial [Ca2+]

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Abbreviations used: PDH, pyruvate dehydrogenase; PDHε, active, non-phosphorylated pyruvate dehydrogenase; OGDH, oxoglutarate dehydrogenase.

In mammals there are three exclusively intramitochondrial enzymes which can be activated by increasing Ca2+ in the range 0.1–10 μM. They are pyruvate dehydrogenase (PDH) phosphate phosphatase, NAD+-isocitrate dehydrogenase and oxoglutarate dehydrogenase (OGDH) (see Denton & McCormack, 1980). Phosphatase activation leads to increased amounts of active, non-phosphorylated, PDH (PDHε); Ca2+ activates the other two dehydrogenases by decreasing their Kₘ values for three-D₃-isocitrate and oxoglutarate respectively. As these enzymes can be activated within intact mitochondria (from several different tissues, including liver; McCormack, 1985) by increases in extramitochondrial [Ca2+], these enzymes and their physiological behaviour offer an explanation for the stimulating actions of vasopressin (McComb et al., 1982; Hackman et al., 1984) which has been shown to stimulate the citric acid cycle in liver mitochondria.